Systemic Excretion of Benzo(a)pyrene in the Control and Microsomally Induced Rat: The Influence of Plasma Lipoproteins and Albumin as Carrier Molecules

Helen P. Shu and Edwin N. Bymun

ABSTRACT

In vitro studies have previously indicated that benzo(a)pyrene distributes primarily into the plasma lipoprotein fraction when incubated with whole plasma. Hydroxylated metabolites of benzo(a)pyrene distribute increasingly into the albumin fraction as the degree of metabolite hydroxylation increases. This report assesses the influence of plasma lipoproteins and albumin as carriers for benzo(a)pyrene on carcinogen excretion in the control and microsomally induced rat. Male Sprague-Dawley rats cannulated in the bile duct received i.v. injections of radioiodinated benzo(a)pyrene noncovalently bound to the very-low-density, low-density, or high-density lipoproteins, respectively. Values for excretion of benzo(a)pyrene complexed with rat lipoproteins were 39.6 ± 9.7 (S.D.), 24.6 ± 1.3, and 21.2 ± 8.8% for very-low-density, low-density, and high-density lipoprotein, respectively. Values for excretion of benzo(a)pyrene complexed with rat or human lipoproteins were comparable. These data suggest that the transport molecule can effect a 2-fold difference in benzo(a)pyrene excretion under conditions of the present study. We infer that metabolism of the plasma lipoprotein molecules determines, in part, the extent of benzo(a)pyrene excretion.

Cumulative biliary excretions of benzo(a)pyrene complexed with rat lipoproteins were 39.6 ± 9.7 (S.D.), 24.6 ± 1.3, and 21.2 ± 8.8% for very-low-density, low-density, and high-density lipoprotein, respectively. Values for excretion of benzo(a)pyrene complexed with rat or human lipoproteins were comparable. These data suggest that the transport molecule can effect a 2-fold difference in benzo(a)pyrene excretion under conditions of the present study. We infer that metabolism of the plasma lipoprotein molecules determines, in part, the extent of benzo(a)pyrene excretion.

INTRODUCTION

A large fraction of environmental carcinogens to which humans are exposed are lipid soluble. The fate of absorbed carcinogens includes uptake by target or nontarget tissues, storage in body fat, or elimination via the bile or urine. For carcinogens which require metabolism prior to excretion, whether retention or elimination predominates is determined to a large degree by the extent of metabolism of the chemical. Thus, the relatively low rate of metabolism of polychlorinated hydrocarbons leads to their accumulation in body fat and biomagnification in the food chain (9). On the other hand, the relatively high rate of metabolism of the polycyclic aromatic hydrocarbons results in their effective removal from the body (3). The rate of systemic excretion can be artificially increased by increasing the metabolism of the chemical compound. Thus, excretion in rat bile of metabolites of benzo(a)pyrene or 3-methylcholanthrene is accelerated by pretreatment of the animals with microsomal enzyme inducers which stimulate drug metabolism and is retarded by pretreatment with agents which inhibit drug metabolism (5, 6, 11).

This report evaluates the role of the carrier molecule in the disposition and excretion of lipid-soluble carcinogens. Previous studies have shown that benzo(a)pyrene preferentially distributes into the lipoprotein fractions of plasma when plasma is incubated with benzo(a)pyrene in vitro. Of the benzo(a)pyrene in the lipoprotein fractions of a normal lipoproteinemic plasma, the LDL binds 60%, the VLDL binds 30%, and the HDL binds 10% (13). Metabolites of benzo(a)pyrene also bind to plasma lipoproteins, although the extent of binding decreases as the degree of benzo(a)pyrene hydroxylation increases (14, 15). The albumin fraction binds benzo(a)pyrene metabolites in increasing amounts as the degree of hydroxylation of the metabolites increases (14, 15). These in vitro studies primarily imply the plasma lipoproteins in benzo(a)pyrene transport and albumin in benzo(a)pyrene metabolite transport. Specifically, this report examines the significance of plasma lipoproteins and albumin as transport molecules in benzo(a)pyrene excretion. We also compare excretion of lipoprotein-bound benzo(a)pyrene in the microsomally induced rat with that in the control rat.

MATERIALS AND METHODS

[7,10-14C]Benzo(a)pyrene (specific activity, 60.7 μCi/μmol) and [G-3H]benzo(a)pyrene (specific activity, 27 Ci/mmol) were purchased from Amersham Corporation, Arlington Heights, Ill. Benzo(a)pyrene-hydroxylated metabolites were provided by the National Cancer Institute Chemical Carcinogen Reference Repository (specific activities: 3-3H)hydroxybenzo(a)pyrene, 466 μCi/μmol; [1H]benzo(a)pyrene-trans-7,8-dihydriodiol, 386 μCi/μmol; [3H]benzo(a)pyrene-4,5-epoxide, 282 μCi/μmol). Benzo(a)pyrene samples were handled in foil-covered
vessels in subdued light. The benzo(a)pyrene and metabolites were periodically assessed for degradation by thin-layer chromatography on heat-activated silica gel in benzene:ethanol (9:1). Bovine serum albumin (Fraction V Powder) was purchased from Sigma Chemical Company, St. Louis, Mo.; Aroclor 1254 was purchased from Analabs, North Haven, Conn.

**Plasma Lipoprotein Preparation.** Human and rat blood were drawn from overnight-fasted subjects into heparinized (600 units/100 ml) tubes. Successive centrifugation at 200 x g for 10 min and 400 x g for 30 min removed cellular components to yield plasma. Sequential ultracentrifugal flotation of plasma at increasing density yielded plasma lipoproteins (7). Isolated lipoprotein fractions were dialyzed before use against 0.15 M NaCl:0.01% EDTA, pH 7.4. Lipoprotein concentration was calculated from the protein concentration (8) assuming standard percentage composition for phospholipid, free and esterified cholesterol, and triglyceride (1, 12).

**Benzo(a)pyrene Binding to Plasma Fractions.** Benzo(a)pyrene or its metabolites were noncovalently bound to isolated plasma lipoprotein fractions or to albumin as follows. The labeled compound under study was dissolved in acetone and dried on the glass surface of the incubation vessel under a stream of nitrogen. To the vessel well was added 0.5 ml of ethanol:Protosol (2:1; New England Nuclear, Boston, Mass.) and bleached with 0.5 ml of 30% hydrogen peroxide, the pH was adjusted with NaOH solution, and the blood was counted in PCS scintillation counting fluid (Amersham Corporation, Arlington Heights, Ill.). Radioactivity in the aliquot of blood was converted to radioactivity per rat, assuming that rat blood volume represents 9.3% of body weight. These data are expressed as a percentage of the injected protein ratio ranged from 1 to 190 (see Table 1). In homologous studies, rats received injections of benzo(a)pyrene bound to rat lipoproteins; in heterologous studies, rats received injections of benzo(a)pyrene bound to human lipoproteins.

* Blood samples (0.1 ml) were withdrawn periodically for radioactivity measurement. The blood was solubilized by heating for 1 hr at 60° with 0.5 ml of ethanol:Protosol (2:1; New England Nuclear, Boston, Mass.) and bleached with 0.5 ml of 30% hydrogen peroxide, the pH was adjusted with NaOH solution, and the blood was counted in PCS scintillation counting fluid (Amersham Corporation, Arlington Heights, Ill.). Radioactivity in the aliquot of blood was converted to radioactivity per rat, assuming that rat blood volume represents 9.3% of body weight. These data are expressed as a percentage of the injected dose.

**Carcinogen Excretion Studies.** Male Sprague-Dawley rats weighing 300 to 400 g and fasted overnight were anesthetized with urethane (1.2 g/kg i.p.). Body temperature was maintained throughout the study with lamps. For bile collection, the common bile duct was cannulated with PE-10 tubing through an abdominal incision. To facilitate carcinogen injection and blood sampling, a heparinized PE-50 cannula was placed in the jugular vein. In initial studies, animals received injections of benzo(a)pyrene bound to lipoproteins singly. In later studies, animals received injections sequentially (within 1 min) in the jugular vein with pairs of lipoprotein fractions bound with either [14C]benzo(a)pyrene or [3H]benzo(a)pyrene. In studies involving benzo(a)pyrene metabolites, animals received sequential injections of [14C]benzo(a)pyrene and [3H]metabolites bound to albumin. Data obtained from the dual-label experiments did not differ significantly from data obtained in early single-label experiments. The use of dual labels permitted each animal to act as its own control. The concentration of lipoprotein injected ranged from 0.004 to 0.3 nmol/rat (3 to 250, 1 to 75, and 0.2 to 16 μg protein for VLDL, LDL, and HDL, respectively) (see Table 1). Typical lipoprotein protein concentrations in these rats are 0.036, 0.067, and 0.36 mg/ml plasma or approximately 9, 145, and 1000 pmol/rat for VLDL, LDL, and HDL, respectively. 4 The concentration of albumin injected was 41 or 62 nmol/rat; albumin concentration in the rat is approximately 3 μmol/rat. The concentration of carcinogen injected ranged from 0.007 to 10 nmol/rat (1.8 to 2.5 μg). The benzo(a)pyrene:lipoprotein ratio ranged from 1 to 190 (see Table 1). In homologous studies, rats received injections of benzo(a)pyrene bound to rat lipoproteins; in heterologous studies, rats received injections of benzo(a)pyrene bound to human lipoproteins.

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP × (nmol/</td>
<td>0.01</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>BP/LP</td>
<td>46.2</td>
<td>45.9</td>
<td>44.2</td>
</tr>
<tr>
<td>% excreted</td>
<td>0.013</td>
<td>0.013</td>
<td>0.039</td>
</tr>
<tr>
<td>% excreted</td>
<td>34.0</td>
<td>32.8</td>
<td>30.4</td>
</tr>
<tr>
<td>BP × (nmol/</td>
<td>0.013</td>
<td>0.013</td>
<td>0.039</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>BP/LP</td>
<td>46.2</td>
<td>45.9</td>
<td>44.2</td>
</tr>
<tr>
<td>% excreted</td>
<td>0.013</td>
<td>0.013</td>
<td>0.039</td>
</tr>
<tr>
<td>% excreted</td>
<td>34.0</td>
<td>32.8</td>
<td>30.4</td>
</tr>
</tbody>
</table>

H. P. Shu and E. N. Bymun, unpublished observations.

| BP, benzo(a)pyrene; LP, lipoprotein. |
|--------|--------|--------|--------|
| Expressed as cumulative excretion. | Mean ± S.D. |
infusion pump at the rate of 1.5 ml/hr. The animals were given additional urethane as needed during the experiment.

**Microsomal Enzyme Induction.** Rats were induced by the i.p. injection of polychlorinated biphenyl, Aroclor 1254, (500 mg/kg) dissolved in peanut oil 4 days prior to experimentation. Aroclor 1254 is a potent inducer of the forms of cytochrome P-450 found in both phenobarbital- and 3-methylcholanthrene-treated animals and stimulates the metabolism of chemicals for which these 2 are the prototypes (10). Rats treated with this protocol exhibited significant increases in liver weight, liver protein, and cytochrome P-450 concentration but had plasma transaminase levels comparable to those of controls. 6

**RESULTS**

Radioactive benzo(a)pyrene bound to isolated plasma lipoprotein classes or to albumin were injected in equimolar amounts i.v. into rats. The level of radioactivity in the blood and output into the bile were monitored over time. Chart 1 shows the blood level of radioactivity in 3 animals receiving i.v. injections of benzo(a)pyrene bound to human VLDL, LDL, and HDL. A rapid decline in the percentage of the injected dose of radioactivity occurs in the first 10 min followed by a period of slower decline. By 120 min, approximately 10% of the injected radioactivity remains in the blood.

Chart 2 shows the cumulative biliary excretion of benzo(a)pyrene bound to different classes of human lipoproteins. One rat received a sequential injection of [3H]benzo(a)pyrene:VLDL and [14C]benzo(a)pyrene:LDL, and another rat received [3H]benzo(a)pyrene:VLDL and [14C]benzo(a)pyrene:LDL. Cumulative biliary excretion of benzo(a)pyrene in both animals increases rapidly during the first hr. Between the first and second hr, excretion begins to slow. By 6 hr, excretion has slowed substantially and approaches zero. The extent of benzo(a)pyrene excretion is related to the lipoprotein molecule to which it is bound. Excretion of carcinogen bound to VLDL is approximately 2-fold higher than that bound to LDL. Similarly, excretion of benzo(a)pyrene bound to LDL is greater than that bound to HDL. Since pairs of benzo(a)pyrene:lipoprotein complexes were injected in the same animal, differences seen in carcinogen excretion can be attributed to differences in how the animals handle the lipoprotein carriers.

To confirm that lipoproteins are indeed responsible for the differences observed in the extent of benzo(a)pyrene excretion, more animals received injections of pairs of lipoprotein bound to benzo(a)pyrene. Table 1 presents data on individual rats receiving injections of different combinations of lipoprotein pairs to which benzo(a)pyrene had been noncovalently bound. The amount of lipoprotein and the benzo(a)pyrene:lipoprotein ratio injected from rat to rat were selected for their variety. However, for any given animal, equimolar amounts of lipoprotein and comparable benzo(a)pyrene:lipoprotein ratios were selected. The concentration of lipoproteins injected ranged from 0.004 to 0.3 nmol/rat, depending on the experiment. The concentrations of circulating lipoprotein in the control rat are approximately 0.009, 0.145, and 1 nmol/rat for the VLDL, LDL, and HDL, respectively. 6 The ratio of benzo(a)pyrene:lipoprotein injected ranged from 1 to 190. As can be seen from Table 1, neither the range in lipoprotein concentration nor the benzo(a)pyrene:lipoprotein ratio used in these studies appeared to affect materially the level of benzo(a)pyrene excretion in the bile. Similarly, benzo(a)pyrene excretion did not appear to be affected by the injection of different combinations of lipoprotein pairs when human lipoproteins were used. More variation was observed when rat lipoproteins were used.

Data in Table 1 are presented in Chart 3. Biliary excretion of benzo(a)pyrene appears to be related to the plasma lipoprotein carrier. Cumulative biliary excretions of the injected benzo(a)pyrene complexed with human lipoproteins (Chart 3, left) and 41.0 ± 4.8% (S.D.) with VLDL, 32.3 ± 1.3% with LDL, and 27.2 ± 5.7% with HDL. Excretion of VLDL-bound benzo(a)pyrene is significantly higher than that of LDL-bound (p ≤ 0.005) or that of HDL-bound (p ≤ 0.005) benzo(a)pyrene.
studies also indicate increases in the degree of binding to the albumin fraction as the extent of hydroxylation of benzo(a)-pyrene increases. Biliary excretion of benzo(a)pyrene metabolites was examined by monitoring the biliary output of rats receiving i.v. injections of albumin-bound metabolites.

$^3$H-metabolites of benzo(a)pyrene possessing varying degrees of hydroxylation were each noncovalently bound to albumin as described in “Materials and Methods.” Pairs of $^3$H-metabolite:albumin and [$^{14}$C]benzo(a)pyrene:albumin complexes were injected i.v. into rats cannulated in the bile duct. Cumulative biliary excretions of the $^{14}$C and $^3$H labels were monitored over a 6-hr period and were plotted for each carcinogen in Chart 4 against the degree of hydroxylation of the carcinogen. Excretion of albumin-bound benzo(a)pyrene (28.0 ± 2.7%) (Chart 4) is comparable to that observed with rat LDL (24.6 ± 7.7%) and rat HDL (21.2 ± 8.8%) described in Chart 3. Excretion levels of benzo(a)pyrene metabolites are higher than that of benzo(a)pyrene and increase with the increasing degree of hydroxylation. Excretions of 3-hydroxybenzo(a)pyrene, benzo(a)pyrene 7,8-dihydrodiol, and benzo(a)pyrene 4,5-epoxide are 39.8 ± 0.5, 46.9 ± 2.5, and 49.8 ± 1.2%, respectively. These findings suggest that when other factors are held constant, metabolites of albumin-bound benzo(a)pyrene are excreted to a greater extent than is albumin-bound benzo(a)pyrene; the more polar metabolites are excreted to a greater extent than the less polar.

### Table 2

**Benzo(a)pyrene excretion in Aroclor-treated rats**

<table>
<thead>
<tr>
<th>Lipoprotein fraction</th>
<th>Control</th>
<th>Aroclor</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL</td>
<td>39.6 ± 9.7</td>
<td>23.4 ± 9.6</td>
</tr>
<tr>
<td>LDL</td>
<td>24.6 ± 7.7</td>
<td>18.6 ± 8.5</td>
</tr>
<tr>
<td>HDL</td>
<td>21.2 ± 8.8</td>
<td>14.0 ± 4.0</td>
</tr>
</tbody>
</table>

*Excretion into the bile over the 6-hr collection period.

The above results were obtained in heterologous experiments in which human lipoproteins were injected into the rat. Species specificity of the lipoprotein carrier was examined by homologous experiments in which rats received injections of benzo(a)pyrene complexed with rat lipoproteins. Chart 3 (right) shows that cumulative biliary excretions at 6 hr for benzo(a)pyrene complexed with rat VLDL, LDL, and HDL are 39.6 ± 9.7, 24.6 ± 7.7, and 21.2 ± 8.8%, respectively. The excretion values obtained with rat lipoproteins are very similar to those obtained with human lipoproteins. Again, benzo(a)pyrene excretion via transport by rat VLDL is significantly higher than that by LDL ($p \leq 0.005$) or by HDL ($p \leq 0.005$). Unlike the human data, the rat data exhibit more variability. However, the general trend is the same whether rat or human lipoproteins are used.

Aroclor administration increases the activity of microsomal enzymes which metabolize xenobiotic chemicals. Examination of benzo(a)pyrene excretion in Aroclor-pretreated rats allows us to assess the effect of microsomal enzyme induction on biliary excretion of lipoprotein-bound benzo(a)pyrene. Excretions of benzo(a)pyrene bound to rat VLDL, LDL, and HDL in Aroclor-treated rats over a 6-hr period were 23.4 ± 9.6, 18.6 ± 8.5, and 14.0 ± 4.0%, respectively (Table 2). Excretions of the corresponding benzo(a)pyrene in the untreated control rats were 39.6 ± 9.7, 24.6 ± 7.7, and 21.2 ± 8.8% for the VLDL, LDL, and HDL, respectively. Carcinogen excretion did not increase in the Aroclor-pretreated rats, as might be expected from the increased capability of these animals for benzo(a)-pyrene metabolism.

In vitro binding studies indicate a greater distribution of hydroxylated benzo(a)pyrene metabolites than of benzo(a)-pyrene into the albumin fraction of plasma (14, 15). These
DISCUSSION

When benzo(a)pyrene is incubated with human plasma, approximately 95% associates with the plasma lipoproteins, while 5% associates with the remainder of the proteins composed mainly of albumin (13). The major carrier of benzo(a)pyrene among the normolipoproteinemic plasma lipoproteins is the LDL with approximately 60%, followed by the VLDL with 30%, and the HDL with 10%. While benzo(a)pyrene distributes mostly into the lipoprotein fraction of plasma, a shift in distribution occurs for benzo(a)pyrene metabolites from the lipoprotein fraction into the albumin fraction (14, 15). The extent of metabolite binding to the albumin fraction increases as the degree of hydroxylation of metabolite increases (14, 15). These in vitro studies imply that plasma lipoproteins may function in the transport of benzo(a)pyrene in the circulation and that albumin may function in the transport of benzo(a)pyrene metabolites. The present study was undertaken to answer the question whether interactions observed in vitro between plasma lipoproteins or albumin- and lipid-soluble carcinogens have a biological consequence. Radiolabeled benzo(a)pyrene or benzo(a)pyrene metabolites noncovalently bound to different classes of plasma lipoproteins or to albumin were injected i.v. into rats cannulated in the bile duct. Appearance of the label in the bile was taken as indication of carcinogen excretion; the nature of the excreted label was not determined. The data imply that the macromolecular carcinogen carriers can indeed influence the extent of carcinogen excretion.

Biliary excretion of benzo(a)pyrene bound to the VLDL (39.6 ± 9.7%) is nearly twice as much as that bound to the LDL (24.6 ± 7.7%) or HDL (21.1 ± 8.8%). Similar excretion values were observed whether corresponding human or rat lipoproteins were used as the transport molecule, although the rat lipoproteins produced more variation in the data. Benzo(a)pyrene excretion in animals pretreated with the microsomal enzyme inducer Aroclor (23.4 ± 9.6% for VLDL, 18.6 ± 8.5% for LDL, and 14.0 ± 4.0% for HDL) was somewhat less than that in the corresponding controls. Excretion of albumin-bound benzo(a)pyrene metabolites increased as the degree of hydroxylation of the metabolites increased (28.0 ± 2.7% for benzo(a)pyrene; 39.8 ± 0.5% for 3-hydroxybenzo(a)pyrene; 46.9 ± 2.5% for benzo(a)pyrene 7,8-dihydropyridol; and 49.8 ± 1.2% for benzo(a)pyrene-4,5-epoxide).

We have assumed that appearance of the label in the bile represents excretion of metabolized benzo(a)pyrene. Indirect evidence supports the validity of this assumption. The use of dual labels in the same animal shows differential excretion of the benzo(a)pyrene labels depending on the nature of the carrier molecule. If unmetabolized benzo(a)pyrene were passing through into the bile due to, for example, trauma or urethan treatment, excretion for both labels in the same animal would be expected to be the same. Furthermore, reproducibility of the excretion data from animal to animal and with injection of different combinations of lipoprotein pairs also argue that excretion is fairly specific for the carrier molecule.

Differential excretion of benzo(a)pyrene by the VLDL, LDL, and HDL carriers may be due, in part, to differences in metabolism of the lipoprotein classes. The liver synthesizes and secretes lipoproteins into, as well as clears lipoproteins from, the circulation (2). The rate that lipoproteins are taken up by the liver for degradation depends on the plasma half-life of the lipoprotein class. Plasma half-life is approximately 10 min for rat VLDL and 11 hr for rat HDL (2). Consequently, VLDL is taken up by the liver at a higher rate than is HDL. Benzo(a)pyrene transported by the VLDL would be expected to be cleared by the liver at a higher rate than that transported by the HDL.

Vauhkonen et al. (16), who injected benzo(a)pyrene: chylomicron complexes into the rat, also observed a relationship between chylomicron metabolism and disposition of benzo(a)pyrene. The disappearance of benzo(a)pyrene from plasma paralleled the catabolism of chylomicrons. Of the benzo(a)pyrene remaining in plasma during the initial phase of disappearance, approximately 50% was associated with albumin and 50% was associated with the VLDL. The kinetics of benzo(a)pyrene appearance in the albumin fraction was comparable to that for fatty acids; the investigators suggested that simultaneous transfer of benzo(a)pyrene with fatty acids from chylomicrons to albumin occurred during triglyceride hydrolysis.

Studies of benzo(a)pyrene complexed with different classes of plasma lipoproteins involve studies in which the carcinogen is constant but the carrier molecules are varied. These studies suggest that metabolism of the carrier molecule may contribute to differences in extent of carcinogen excretion. In contrast to these studies, examination of albumin-bound metabolites investigates the extent of excretion of different carcinogens as a function of the same transport molecule. In this instance, metabolism of the carrier molecule is expected to be the same for all benzo(a)pyrene metabolites examined. Differences seen in the extent of excretion may be attributed to differences in how the metabolites interact with their target sites. The increased excretion observed with increasing metabolite hydroxylation may result from several possibilities. First, the metabolites with a greater degree of hydroxylation require fewer steps for conversion into conjugated forms required for excretion. Thus, their chances for forming reactive species which bind covalently to hepatic macromolecules are less. Also, the less-hydroxylated derivatives may undergo partial metabolism and rerelease into the circulation instead of releasing into the bile. Finally, the more hydroxylated derivatives may preferentially bind to the albumin and thus remain in the circulation instead of binding to cellular membranes of extrahepatic tissues. This would be especially true for benzo(a)pyrene 7,8-dihydropyridol and benzo(a)pyrene-4,5-epoxide in light of the high affinity of these metabolites for albumin (14, 15).

For lipid-soluble carcinogens which require metabolism prior to excretion, the activity of metabolic enzymes is indisputably an important determinant of carcinogen excretion. It has been shown that systemic excretion of benzo(a)pyrene metabolites or 3-methylcholanganthrene can be elevated by pretreatment of rats with microsomal enzyme inducers which stimulate drug metabolism and can be depressed by pretreatment with agents which inhibit drug metabolism (5, 6, 11). In accordance with these studies, when we examined excretion of lipoprotein-bound benzo(a)pyrene in Aroclor-treated rats, we expected to see enhanced benzo(a)pyrene excretion in the microsomal induced rats. Unexpectedly, the extent of benzo(a)pyrene excretion was less in the induced animals than in the controls. Of the benzo(a)pyrene injected into the control rats, 60% of the VLDL-bound, 75% of the LDL-bound, and 79% of the HDL-bound chemical were not excreted over 6 hr, the period over
which most of the carcinogen was excreted. Of the benzo(a)pyrene injected into the Aroclor-pretreated rats, 77% of the VLDL-bound, 81% of the LDL-bound, and 86% of the HDL-bound chemical were not excreted over the 6-hr period. Several explanations can account for this anomaly. One possibility is that, although metabolic enzymes were induced in the Aroclor-treated rats, the lipoproteins interfered in some way with the metabolism or the excretion of benzo(a)pyrene. Alternatively, Aroclor treatment may have impaired the hepatic uptake of lipoprotein-bound benzo(a)pyrene, or the lipoprotein-bound benzo(a)pyrene may have been sequestered at sites inaccessible to the metabolic enzymes in both the control and induced animals. Specific studies designed to address these possibilities are required to distinguish among them.

While we cannot at present evaluate the likelihood of these possibilities, our excretion data are consistent with those obtained by other investigators. When the amount of carcinogen injected was several times the saturation for biliary excretion in the rat, biliary excretion was enhanced and inhibited by agents that induced and inhibited, respectively, the metabolism of 3-methylcholanthrene (4, 6). When a lower level of benzo(a)pyrene was injected, drug pretreatment stimulated the rate of benzo(a)pyrene excretion for 30 min after benzo(a)pyrene injection (11). Total benzo(a)pyrene excretion in the pretreated animals was initially higher than that in control animals. However, by 10 hr, when the rate of carcinogen excretion approached zero in both pretreated and control animals, 42 to 47% of the carcinogen still remained in both the control and induced animals (11). Our results are qualitatively similar to these. Total carcinogen excretion was comparable for the control and the induced animals and amounted to 14 to 40% of the injected dose, leaving 60 to 86% still in the animal. Our results and those of Schlede et al. (11) suggest that a substantial part of the injected benzo(a)pyrene in the control and the microsomal induced rat is not excreted during the first few hr and may constitute a carcinogen pool that is slowly excreted.

ACKNOWLEDGMENTS

The authors thank Drs. J. Bartholomew and A. Nichols for helpful discussions during preparation of the manuscript and the National Cancer Institute for supplying benzo(a)pyrene metabolites.

REFERENCES

Systemic Excretion of Benzo(a)pyrene in the Control and Microsomally Induced Rat: The Influence of Plasma Lipoproteins and Albumin as Carrier Molecules

Helen P. Shu and Edwin N. Bymun


Updated version

Access the most recent version of this article at: [http://cancerres.aacrjournals.org/content/43/2/485](http://cancerres.aacrjournals.org/content/43/2/485)

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.