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Total Body Clearance of Circulating Benzo(a)Pyrene in Conscious Rats: Effect of Pretreatment with 3-Methylcholanthrene and the Role of Liver and Lung[1]

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ABSTRACT

The metabolic clearance of benzo(*a*)pyrene [B(*a*)P] was studied in conscious control and 3-methylcholanthrene (3-MC)-pretreated rats. The roles of liver and lung in total body B(*a*)P clearance were estimated *in vivo* in 3-MC-treated rats. During a 5-hr period, blood B(*a*)P concentration decreased rapidly in a biphasic manner. 3-MC pretreatment did not significantly affect the rate constants of B(*a*)P elimination, but the apparent volume of distribution increased, resulting in an increase in total body B(*a*)P clearance from 15 ± 1 to 48 ± 6 ml/min. Five hours after injection, B(*a*)P concentrations in most organs were lower in 3-MC-pretreated rats; however, lung B(*a*)P concentration was greater. In the 3-MC-pretreated rats, first-pass extraction of B(*a*)P by lung and liver was determined from comparison of areas under arterial blood B(*a*)P concentration *vs.* time curves after i.a., i.v. and intrahepatic portal venous administration. Liver had a first-pass extraction of 20% whereas that by lung was 33%. Organ B(*a*)P concentrations were correlated with the route of B(*a*)P administration. The results of this study suggest that 1) 3-MC prereatment increases the apparent volume of B(*a*)P distribution *in vivo*, 2) liver and lung of 3-MC-pretreated rats are about equal in their ability to extract circulating B(*a*)P *in vivo* and 3) 3-MC pretreatment enhances total body B(*a*)P clearance by increasing *extrahepatic* B(*a*)P elimination.

B(*a*)P and many other lipophilic xenobiotic compounds are metabolized by the mixed-function oxidase system to metabolites which are more easily excreted from the body (Levine, 1970; Chipman *et al.*, 1981a,b). This enzymic activity occurs in many organs (Prough *et al.*, 1979) and may be stimulated by exposure of animals to certain environmental contaminants (DePierre and Ernster, 1978) such as 3-MC.

The disposition of circulating B(*a*)P has been investigated in rats *in vivo* (Kotin *et al.*, 1959; Iqbal *et al.*, 1979; Vauhkonen *et al.*, 1980; Reeve and Gallagher, 1981; Chipman *et al.*, 1981a,b) as well as in isolated, perfused rat organs (Levine, 1970; Vainio *et al.*, 1976; Cohen *et al.*, 1977; Vahakangas *et al.*, 1977, 1979; Vahakangas, 1979; Wiersma and Roth, 1983). However, in none of these studies was a detailed pharmacokinetic analysis performed or the role of extrahepatic organs in the elimination process defined. In this study we examined the pharmacokinetics of B(*a*)P elimination in conscious control and 3-MC-pretreated rats. Additionally, we examined the role of liver and lung in B(*a*)P disposition *in vivo* after administration of B(*a*)P to 3-MC-pretreated rats.

Methods

Animals. Male, Sprague-Dawley rats (Spartan Research Animals, Inc., Haslett, MI) weighing approximately 200 to 300 g were used in these studies. The animals were housed in plastic cages on corn cob bedding in 12-hr light cycled, temperature controlled quarters. Food (Wayne Lab Blox, Allied Mills, Chicago, IL) and tap water were allowed *ad libitum*.

Treatment. 3-MC (Pfaltz and Bauer, Inc., Stamford, CT) was suspended in corn oil (Mazola, Best Foods, Englewood Cliffs, NJ) to a final concentration of 10 mg/ml. Each rat pretreated with 3-MC received an injection (20 mg/kg i.p.) 24 and 48 hr before use. Control animals received an equal volume of corn oil.

Preparation of B(*a*)P for injection. [G-^{3}H]B(*a*)P (specific activity, 17.4 Ci/mmole; Amersham, Inc., Arlington Heights, IL) was purified by the procedure of Van Cantfort *et al.* (1977) and 0.6 mCi was placed in a glass test tube along with 12 nmol of unlabeled B(*a*)P (99+%, Aldrich Chemical Co., Milwaukee, WI). The acetone solvent was evaporated at room temperature under a stream of nitrogen. The residue was stored under nitrogen in the dark at −20°C until time of use.

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ABBREVIATIONS: B(*a*)P, benzo(*a*)pyrene; 3-MC, 3-methylcholanthrene; h.p.v., intra-hepatic portal venous; DMSO, dimethyl sulfoxide; α, slope of initial disappearance phase; β, slope of final disappearance phase; V_d, volume of distribution; Cl_{TB}, total body clearance of B(*a*)P; AUC, area under the concentration curve from time zero to infinity.

On the day of use the B(a)P was suspended in plasma prepared from a donor rat. The resulting injection solution was analyzed for ^3H content.

Preparation of cannulas. Cannulas for the removal of blood from the aorta were prepared from microbore Tygon tubing of two sizes (Cole-Parmer, Inc., Chicago, IL). Tubing of 0.01 inch inside diameter × 0.03 inch outside diameter was soaked in 1:1 petroleum ether-toluene and stretched over 14-gauge stainless-steel wire to maintain internal diameter and reduce wall thickness. After curing in a steam oven overnight the wire was removed, the tubing trimmed to about 4 cm and inserted into a 9-inch segment of 0.02 inch inside diameter × 0.06 inch outside diameter tubing which had been looped at one end. The junction was sealed with an acrylic glue. Cannulas for the administration of the B(a)P solution were also constructed of 0.02 inch × 0.06 inch Tygon tubing into which was inserted a piece of tubing which was to be placed within the blood vessel. (For the i.a. and i.v. cannulas this second piece was a short length of PE-20 tubing; for the h.p.v. cannula a piece of stretched 0.01 inch × 0.03 inch microbore tubing was used.) As before, the junctions were sealed with acrylic glue.

Cannula implantation. Under pentobarbital anesthesia (50 mg/kg i.p.), cannulas for the administration of B(a)P and removal of blood samples were placed in appropriate blood vessels (schematically shown in fig. 1). Cannulas for i.a. administration were placed into the left carotid artery. The tip of the cannula just entered the aorta. The cannula for i.v. administration was placed in the left jugular vein with the cannula tip in the vena cava. The h.p.v. cannulas were placed into the ileocaecocolic vein. Cannulas for the removal of blood samples were placed in the left femoral artery. The tip of the cannula resided in the aorta at about the level of the lumbar artery.

All cannulas were filled with isotonic saline (0.9% NaCl) containing heparin (10 U/ml; Sigma Chemical Co., St. Louis, MO), tunnelled underneath the skin to an incision in the skin of the back between the scapulae and exteriorized. Incisions were closed with sutures painted with antiseptic solution (Acu-dyne, Acme United Corp., Medical Products Division, Bridgeport, CT) to retard infection. The rats were allowed 48 hr of recovery before initiation of the 3-MC pretreatment regimen.

Administration of B(a)P and collection of blood samples. On the day after the last corn oil or 3-MC injection, the pharmacokinetics of B(a)P were determined in conscious rats. All cannulas were flushed with a small volume of saline containing heparin (10 U/ml) and 300 to 400 U of heparin was administered. After the collection of a blood sample, 1 ml/kg of the [^3H]B(a)P solution was administered [117 nmol (2 mCi)/kg] and the cannula flushed with an equal volume of heparinized saline. Samples of blood (approximately 150 μl) were removed from the aorta 5 min before and 2, 5, 10, 20, 30, 45, 60, 105, 150, 195, 240 and 300 min after the administration of the B(a)P . Residual blood in the cannula was flushed into the aorta with heparinized saline. Blood (100 μl) was analyzed for the presence of B(a)P and B(a)P metabolites as described below. Packed cell volumes were determined at −5, 60 and 300 min.

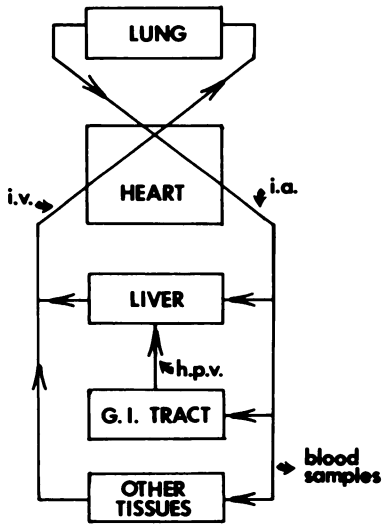

Fig. 1. Sites of cannula placement and circulatory relationships of organs involved in B(a)P disposition. Cannulas for B(a)P administration and blood sampling were placed in vessels at the indicated sites as described under "Methods."

After the final blood sample was removed, sodium pentobarbital (300 mg) was administered. Liver, lungs, epididymal fat, kidneys, spleen and muscle (diaphragm) were removed and the position of the cannulas verified. The organs were immediately placed on ice, weighed, frozen at −70°C and analyzed for B(a)P and metabolites.

Analysis for B(a)P and B(a)P metabolites. The content of B(a)P and B(a)P metabolite in blood and tissue samples was determined using a modification of the extraction procedure of Van Cantfort *et al.* (1977). Samples (0.10 ml) of blood diluted with 0.40 ml of saline or 0.50-ml samples of tissue homogenates were added to 1.0 ml of 0.15 N KOH in 85% DMSO (15 ml of 1 N KOH diluted to 100 ml with DMSO). This mixture was extracted with 5 ml of hexane, the layers allowed to separate and the hexane removed. The hexane phase was extracted again with fresh saline-KOH-DMSO. The aqueous phase was extracted again with another 5 ml of hexane and the two hexane layers evaporated to dryness in a Scintillation vial. Ten milliliters of ACS (Aqueous Counting Scintillant, Amersham, Inc.) was added and the amount of radioactivity in the vial determined. This procedure resulted in minimal contamination of the hexane layer with metabolites and accounted for 90 to 95% of the B(a)P in blood samples spiked with known amounts of [^3H]B(a)P . The KOH-DMSO layers for each sample were combined and 0.30 ml added to a scintillation vial. After neutralization with 0.3 ml of 0.15 N HCl and bleaching with 0.05 ml of 30% hydrogen peroxide, 15 ml of ACS was added and the radioactivity in the vial determined. This radioactivity represents B(a)P metabolites.

After a period of dark adaptation to reduce chemiluminescence, the vials were placed in a liquid scintillation counter (Beckman model LS-3150P, Beckman Instruments, Inc., Scientific instruments Division, Irvine, CA) and the radioactivity quantified. External standard quench correction was used to determine counting efficiency. Results of such analyses were expressed as the percent of the dose of [^3H]B(a)P administered per milliliter of blood or per gram of tissue.

Pharmacokinetic analysis. Blood B(a)P concentration *vs.* time data were subjected to pharmacokinetic analysis using a two-compartment open model and the program of Nielsen-Kudsk (1980) for minicalculators. The values of the first-order rate constants, α and β, for the initial and terminal components of the blood B(a)P concentration *vs.* time curves, respectively, were estimated by linear regression. The β was estimated by linear regression of the B(a)P concentrations after 3 hr postinjection. After subtraction of the contribution of the terminal phase, the α was estimated by linear regression of the residual data. V_d Cl_{TB} and AUC were calculated as follows:

$$V_d = \frac{\text{dose of B(a)P}}{B} \quad (1)$$

$$Cl_{TB} = V_d \cdot \beta \quad (2)$$

$$AUC = (A/\alpha) + (B/\beta) \quad (3)$$

A and B, respectively, are the B(a)P concentrations at zero times for the initial and terminal phases determined from extrapolations of the linear regression lines for these two phases of B(a)P concentration decline.

Calculation of extraction by organs *in vivo*. The fractions of the [^3H]B(a)P extracted by the lung and liver *in vivo* was calculated from the fraction of the dose available after first-pass through the organ (Cassidy and Houston, 1980), A dose of B(a)P administered i.a. is 100% available to the arterial circulation (see fig. 1). This is reflected in the arterial AUC after i.a. administration. Conversely, a dose given

i.v. must pass through the pulmonary circulation before entering the arterial circulation. Therefore, the arterial AUC will be reduced in proportion to the B(a)P removed during the first-pass through the pulmonary circulation. The fraction of the dose of B(a)P available to the arterial circulation after passage through the lung (f_P) is given by the following equation:

$$f_P = \frac{AUC_{i.v.}}{AUC_{i.a.}} \quad (4)$$

where $AUC_{i.v.}$ and $AUC_{i.a.}$ are the areas under the blood B(a)P concentration, vs. time curve from zero to infinity for i.v. and i.a. administration, respectively. Similarly, the fraction of the dose escaping removal on single passage through the liver (f_H) is given by the following equation:

$$f_H = \frac{AUC_{h.p.v.}}{AUC_{i.v.}} \quad (5)$$

where $AUC_{h.p.v.}$ is the area under the arterial blood B(a)P concentration *vs.* time curve after a h.p.v. administration.

The fraction of the dose extracted by the organ is that fraction which is not available. Thus, the fraction of the dose extracted by the lung (E_P) and that extracted by the liver (E_H), respectively, are given by the following equations:

$$E_P = 1 - f_P \quad (6)$$

$$E_H = 1 - f_H \quad (7)$$

Statistical analysis. Results are expressed as means ± S.E.M. Student's *t* test for unpaired observations was used to compare means between control and treated groups given B(a)P by the same route. One-way analysis of variance, completely random design, was used to detect differences resulting from varied routes of administration. $P < .05$ was chosen as the level of significance. The least significant difference test was used to compare the means (Steel and Torrie, 1960). Where unequal variances were noted, appropriate tests were performed on transformed data (logarithmic transformation).

Results

Body weight remained constant or was slightly reduced by the cannula implantation and pretreatment regimens. At the time of B(a)P administration, however, body weights among the four groups of animals were not significantly different. During the B(a)P pharmacokinetic determinations the animals moved freely about their cages and consumed both food and water. Hematocrit decreased during the course of blood sampling from 45 ± 1% at the beginning of the experiment to 41 ± 1% at 60 min and to 34 ± 1% by 300 min after B(a)P administration. This pattern was observed in all rats and was affected by neither 3-MC pretreatment nor route of B(a)P administration.

Figure 2 depicts the disappearance of B(a)P from the blood of rats. The same general pattern was observed for all animals irrespective of preteatment or the route of B(a)P administration. From 2 to 60 min after B(a)P administration there was a sharp decline in blood B(a)P concentration which slowed to a more gradual decline between 1 and 5 hr. These blood B(a)P concentration data were subjected to pharmacokinetic analysis as described under "Methods."

Effects of 3-MC pretreatment. As demonstrated in figure 2, 3-MC pretreatment reduced the B(a)P concentration in the blood during the terminal phase of the concentration decline. This was reflected as a significant increase in the pharmacokinetic parameters, V_d and clearance (table 1, compare i.a. corn oil with i.a. 3-MC). V_d increased 4 times, whereas Cl_{TB} increased

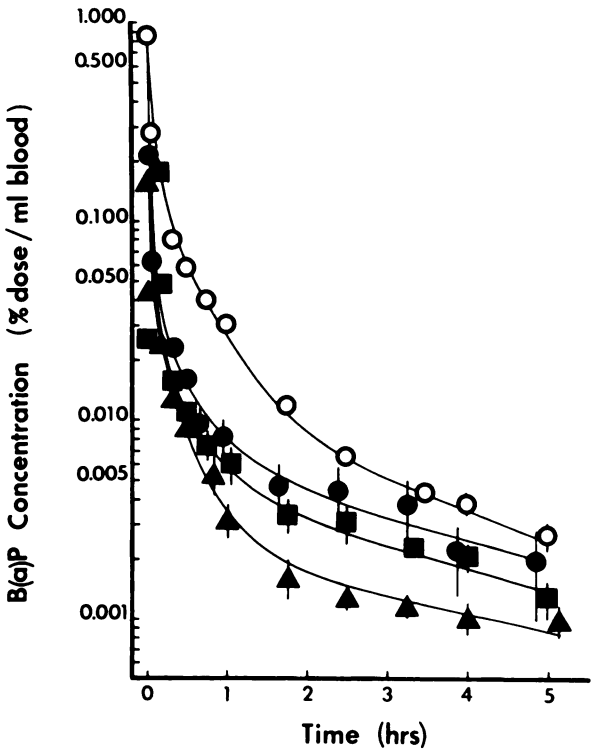

Fig. 2. Elimination of [^{3}H]B(a)P from conscious rats. Corn oil (control) and 3-MC-pretreated rats were given [^{3}H]B(a)P (117 nmol, 2 mCi/kg) in rat plasma by one of three routes. Periodically, blood samples were removed and B(a)P concentration determined as described under "Methods." Data points represent mean B(a)P concentrations ± S.E.M. of four to eight experiments. If no S.E.M. is apparent, it was less than the symbol size. The number of animals in each group is presented in table 1. Open symbols represent corn oil-pretreated rats; closed symbols represent 3-MC-pretreated rats. Circles, i.a. administration; squares, i.v. administration; triangles, h.p.v. administration.

from 15 to 48 ml/min. AUC was decreased to 37% of that in control rats whereas the first-order rate constants were not significantly affected.

Five hours after B(a)P administration, liver and fat B(a)P concentration was significantly reduced in 3-MC-pretreated rats as compared with controls (fig. 3), whereas B(a)P concentration in lung doubled. B(a)P concentrations in kidney, spleen and muscle were not significantly affected by 3-MC pretreatment.

3-MC pretreatment altered the appearance of B(a)P metabolites in the blood (fig. 4). The peak metabolite concentration was not increased by 3-MC pretreatment. However, the time to peak concentration was reduced. Despite this change, no statistically significant differences were observed in the AUC of these metabolites (table 2). No significant alterations in organ B(a)P metabolite concentration 5 hr postinjection resulted from 3-MC pretreatment (fig. 5).

Effects of varied route of B(a)P administration. The effect of different routes of B(a)P administration on organ extraction was examined in 3-MC-pretreated rats to evaluate the role of liver and lung in the total body disposition of B(a)P. As shown in figure 2, varying the route of B(a)P administration altered the blood B(a)P concentration at which the terminal phase of concentration decline began. Pharmacokinetic analysis of these data (table 1) demonstrated that there were no significant differences in the first-order rate constants for either the initial or terminal phase of B(a)P concentration

TABLE 1

Pharmacokinetic parameters of B(*a*)P disposition in control and 3-MC-pretreated rats given [^{3}H]B(*a*)P by various routes of administration

Conscious rats were given [^{3}H]B(*a*)P (117 nmol, 2 mCi/kg) dissolved in plasma by the indicated route. Blood samples were withdrawn periodically between 2 and 300 min after B(*a*)P injection and B(*a*)P concentration determined. Pharmacokinetic parameters were determined using a two-compartment open model. Results are mean ± S.E.M. of number of determinations. Asterisks denote control (corn oil) values significantly different than values from 3-MC-pretreated rats given B(*a*)P by the same route of administration (Student's *t* test, unpaired design). In the 3-MC groups, any two means in a row with different superscript letters are significantly different (one-way analysis of variance, completely random design; mean comparison by L.S.D. test), $P < .05$. V_d, Cl_{TB} and AUC calculated according to equations 1, 2 and 3, respectively.

Pretreatment	Corn Oil	3-MC		
Route of B(*a*)P Administration	i.a.	i.a.	i.v.	h.p.v.
α (min^{-1})	0.037 ± 0.002	0.064 ± 0.012	0.064 ± 0.007	0.061 ± 0.004
β (min^{-1})	0.0063 ± 0.0007	0.0058 ± 0.0009	0.0048 ± 0.0008	0.0035 ± 0.0004
V_d(l)	2.52 ± 0.17*	10.1 ± 2.8^a	17.9 ± 4.5^{a,b}	23.7 ± 4.7^b
Cl_{TB} (ml/min)	15.3 ± 1.1*	48.3 ± 6.1^a	63.5 ± 8.0^{a,b}	82.5 ± 20.3^b
AUC (% dose/min/ml blood)	6.73 ± 0.16*	2.51 ± 0.61^a	1.75 ± 0.20^a	1.38 ± 0.23^a
N	6	7	8	4

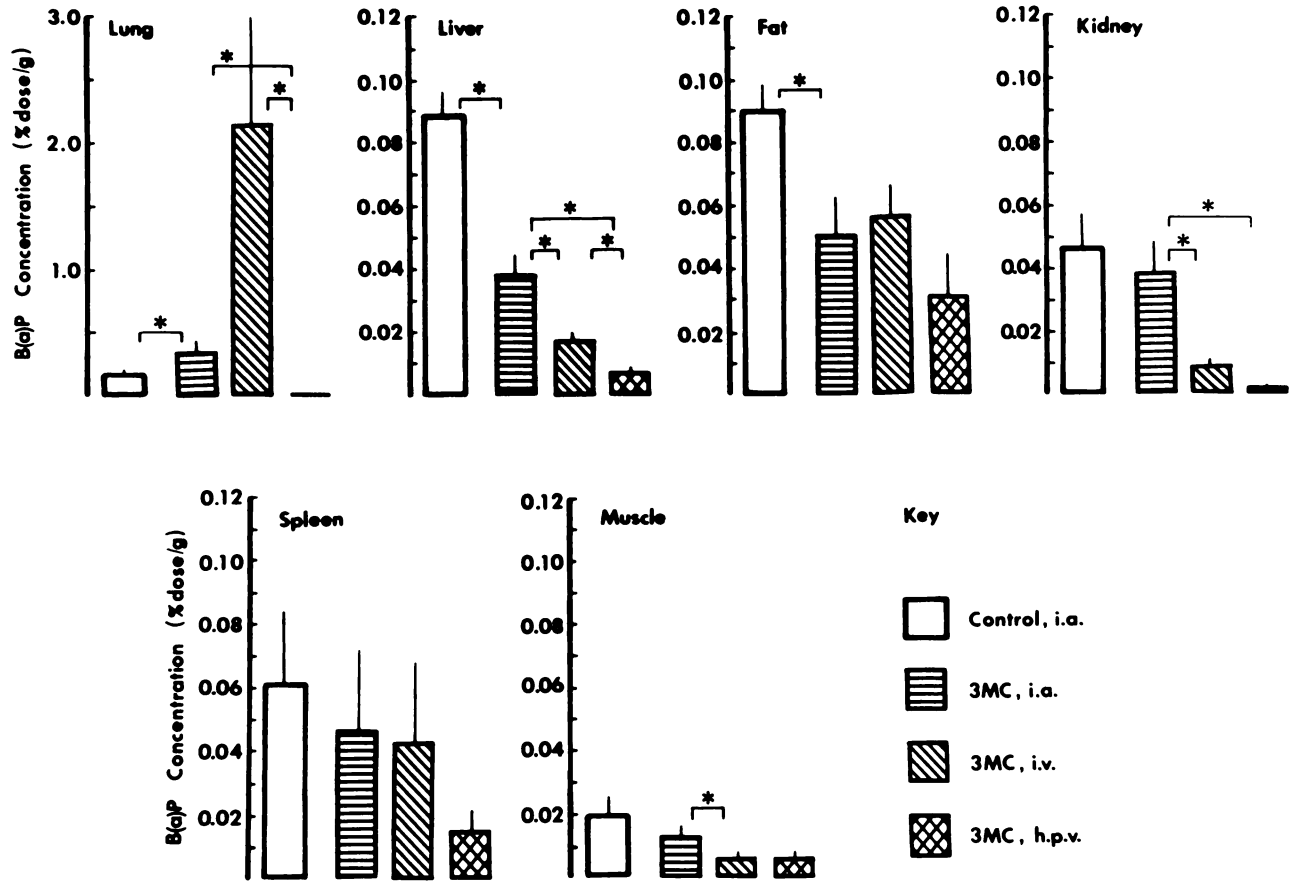

Fig. 3. B(*a*)P concentration in organs of control and 3-MC-pretreated rats given [^{3}H]B(*a*)P by various routes of administration. [^{3}H]B(*a*)P (117 nmol, 2 mCi/kg) dissolved in rat plasma was injected into conscious rats by the indicated routes. At 300 min post administration the rats were sacrificed, organs removed and B(*a*)P concentrations determined. Results are mean concentrations ± S.E.M. Asterisks denote significantly different mean concentrations ($P < .05$). Note that the ordinal scale for lung differs from that of the others.

decline. However, differences were observed in V_d and clearance. Both parameters doubled when B(*a*)P administration was varied between the i.a. and h.p.v. routes. Values after i.v. administration were intermediate to those of the other two routes. Although a trend toward a decreasing AUC for B(*a*)P after i.a., i.v., and h.p.v. routes of administration was apparent, no statistically significant differences in this parameter were detected.

In 3-MC-pretreated rats, the fraction of the dose escaping first-pass removal by lung and liver was calculated from AUC values (table 3). Seventy-nine % of the B(*a*)P dose escaped extraction on the first pass through the liver. Lung allowed 70% of the dose to pass. Thus, the first-pass extraction of B(*a*)P by these two organs *in vivo* was about equal at 21% for liver and 30% for lung.

Whereas no significant changes occurred in blood B(*a*)P metabolite concentration with varying the route of B(*a*)P administration (fig. 4; table 2), altered B(*a*)P and B(*a*)P metabolite concentrations in organs were observed (fig. 3 and 5). After i.a. and i.v. B(*a*)P administration to 3-MC-pretreated rats, the highest organ B(*a*)P concentration 5 hr after B(*a*)P administration occurred in the lung. However, after h.p.v. ad-

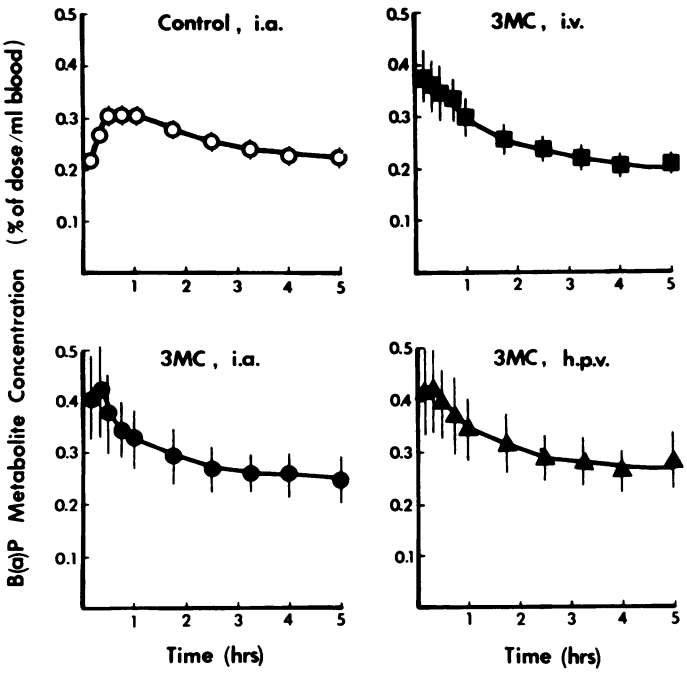

Fig. 4. Appearance of [^{3}H]B(*a*)P metabolites in the blood of rats. Conscious control and 3-MC-pretreated rats were given [^{3}H]B(*a*)P (117 nmol, 2 mCi/kg) in rat plasma by the indicated route. Periodically, blood samples were withdrawn and the concentration of total B(*a*)P metabolites determined as [^{3}H]B(*a*)P equivalents as described under "Methods." Data points represent mean total metabolite concentration ± S.E.M. Lack of S.E.M. indicates S.E.M. less than symbol size.

TABLE 2

Area under the arterial blood B(*a*)P metabolite concentration *vs.* time curves (AUC) for conscious rats given [^{3}H]B(*a*)P

Control (corn oil) and 3-MC-pretreated rats were given [^{3}H]B(*a*)P (117 nmol, 2 mCi/kg) in plasma by the indicated routes. B(*a*)P metabolite concentration of blood samples drawn between 2 and 300 min after B(*a*)P administration was determined and the AUC from zero to infinite time determined by pharmacokinetic analysis. Results are mean ± S.E.M.

Pretreatment	Route of Administration	AUC
		% dose/min/ml blood
Control	i.a.	225 ± 21
3-MC	i.a.	364 ± 63
3-MC	i.v.	240 ± 25
3-MC	h.p.v.	344 ± 77

ministration the highest B(*a*)P concentration was detected in fat. For most tissues in which significant changes in B(*a*)P concentration were observed due to varying the route of administration (liver, kidney and muscle), the tissue concentration after h.p.v. administration was less than that after i.v. administration which was, in turn, less than that after i.a. administration. Such differences were not seen in each tissue. For example, lung B(*a*)P concentration was highest after i.v. administration. No significant differences in B(*a*)P concentration due to route of B(*a*)P administration were detected in fat or spleen 5 hr after administration.

Few differences occurred in organ B(*a*)P metabolite concentration due to varied routes of B(*a*)P administration. The metabolite concentration in lung of the h.p.v. group was lower than in the i.v. group, whereas in liver metabolite concentration was lower after i.v. administration than when B(*a*)P was given i.a. Highest B(*a*)P metabolite concentrations occurred in the kidney irrespective of the route of B(*a*)P administration.

Discussion

B(*a*)P was rapidly removed from the blood after intravascular administration (fig. 2). Such rapid distribution into tissues is probably a consequence of the high lipid solubility of B(*a*)P. This disappearance of B(*a*)P from the blood occurred in two phases and is similar to that observed by Iqbal *et al.* (1979) and Vauhkonen *et al.* (1980). To assess the pharmacokinetics of the B(*a*)P elimination, blood B(*a*)P concentrations were observed until 5 hr post-B(*a*)P administration.

Concurrent with the initial fall in blood B(*a*)P concentration, a rapid increase in blood-borne B(*a*)P metabolites was observed, followed by a slow decline (fig. 4). In fact, 20 min after B(*a*)P administration, B(*a*)P metabolite concentration in the blood exceeded that of B(*a*)P itself. Inasmuch as the major route of B(*a*)P metabolite elimination is by way of biliary excretion (Kotin *et al.*, 1959; Levine, 1970), this time course of blood B(*a*)P metabolite concentration suggests that these more water soluble compounds initially are produced at a rate greater than their biliary elimination. As production decreases with lower B(*a*)P concentrations, these metabolites may be removed from the blood by reuptake into the liver and excreted.

Pretreatment of the rats with 3-MC enhanced the CL_{TB}. However, the rate constant of elimination (β), and its pharmacokinetic counterpart, the biological half-life ($T_{1/2} = 0.693/\beta$), were unaltered despite the ability of the 3-MC treatment regimen to increase the activity of microsomal enzymes which hydroxylate B(*a*)P in both liver and extrahepatic organs (Lake *et al.*, 1973; Wiersma and Roth, 1983). This result demonstrates the usefulness of expressing xenobiotic elimination as a clearance term, as a marked change in the ability of an animal to clear circulating agents may occur without an alteration in the biological half-life (Wilkinson, 1975).

The pharmacokinetic parameter altered by 3-MC pretreatment was V_d. This suggests that 3-MC pretreatment produces changes in the body which result in a greater distribution of B(*a*)P into the tissues. This may arise from either enhanced sequestration of B(*a*)P in tissues or decreased association of B(*a*)P with blood components. Exposure to 3-MC increases the amount of hepatic smooth endoplasmic reticulum (Fouts and Rogers, 1965) and ligandin, an intracellular xenobiotic-binding protein (Reyes *et al.*, 1972; Clifton and Kaplowitz, 1978). Thus, an increased V_d after 3-MC pretreatment may be due to an enhanced intracellular binding of B(*a*)P. The effect of 3-MC pretreatment on blood components or on the distribution of B(*a*)P between them is not known. Although our study did not examine this possibility, it may be that 3-MC decreases B(*a*)P binding to blood components, enhancing its partitioning into tissues.

The increased Cl_{TB} after 3-MC pretreatment may be due to increased participation in the clearance process by extrahepatic organs. In a recent study (Wiersma and Roth, 1983) utilizing isolated organs, the clearance of perfused B(*a*)P by liver was not altered by 3-MC pretreatment. However, pulmonary B(*a*)P clearance was increased approximately 8-fold. Thus, the altered Cl_{TB} may result from greater participation by extrahepatic organs in the B(*a*)P elimination process.

Apparent Cl_{TB} was also altered by varying the route of B(*a*)P administration to 3-MC-pretreated animals. Clearance after hepatic portal administration was greater than that after i.v. administration, which in turn was greater than that after i.a. administration (table 1). These differences in B(*a*)P clearance

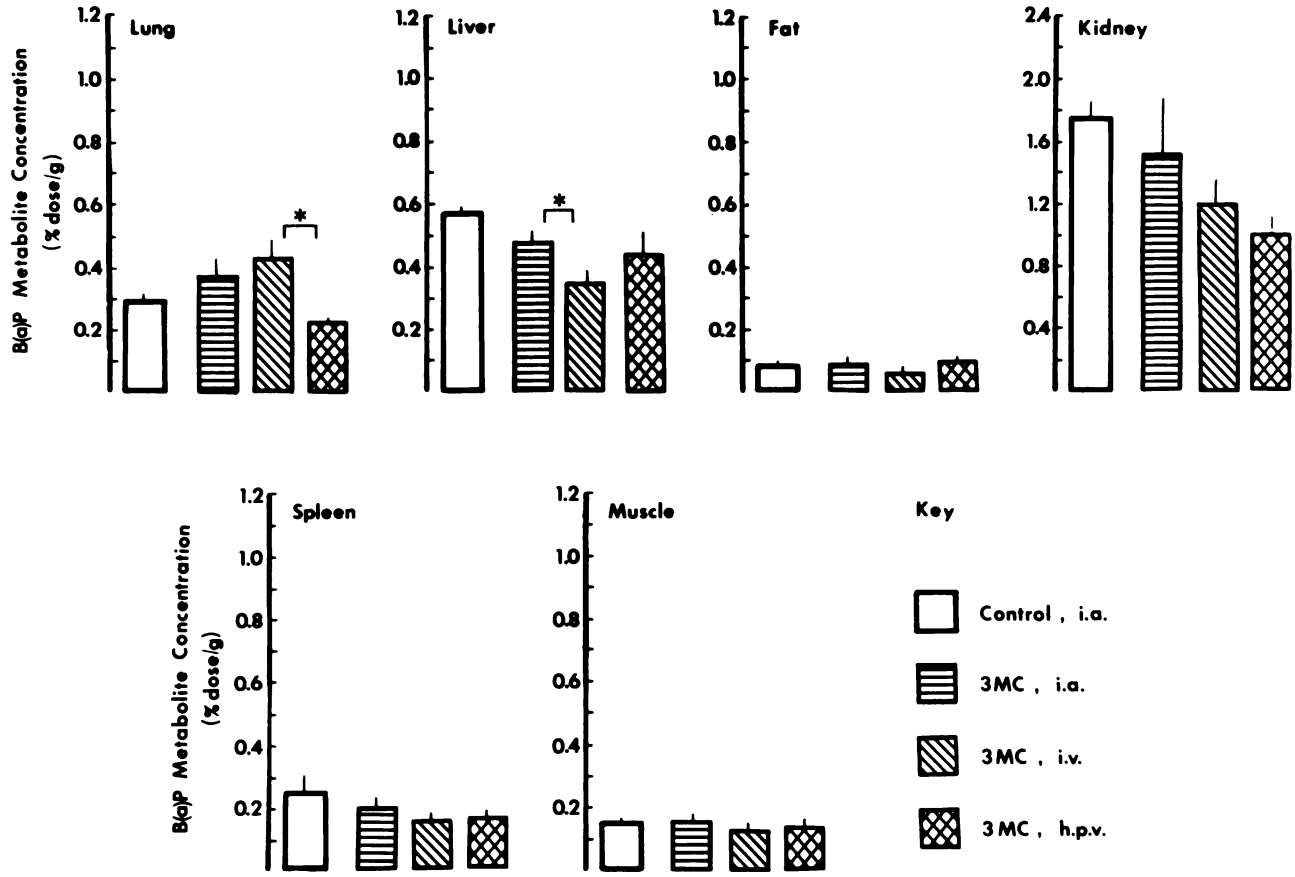

Fig. 5. [^{3}H]B(*a*)P metabolite concentration in organs of control and 3-MC-pretreated rats given [^{3}H]B(*a*)P by various routes of administration. [^{3}H]B(*a*)P (117 nmol, 2 mCi/kg) dissolved in rat plasma was injected into conscious rats by the indicated routes. At 300 min postadministration the rats were sacrificed, organs removed and B(*a*)P concentrations determined. Results are mean concentration ± S.E.M. Asterisks denote significantly different mean concentrations ($P < .05$). Note that the ordinal scale for kidney differs from that of the others.

TABLE 3

The fraction of the dose available (f) and the fraction of the dose extracted (E) by liver and lung of 3-MC-pretreated rats

Organ	f[a]	E[b]
Liver	0.79 (0.49–1.2)	0.21 (−0.20–0.51)
Lung	0.70 (0.41–1.6)	0.30 (−0.6–0.59)

[a] Calculated as the ratios of the AUC as described under "Methods." Values in parentheses are the 95% confidence limits for the ratio (Goldstein, 1967). Actual values of f cannot exceed 1, whereas values of E cannot be less than zero.

[b] Extraction = 1 − f.

were associated with different V_d rather than a change in the rate constants of elimination. However, our calculations of V_d did not take into account first-pass effects by the organs between the injection site and the sampling site. Thus, these differences in V_d are a function of the fraction of the dose of B(*a*)P removed by these organs before entry into the arterial circulation (Shand *et al.*, 1975). By using the data from table 3, the fraction of the dose escaping first-pass removal can be estimated. Accordingly, correction of the dosage value in equation 1 to reflect the effective dose reaching the arterial circulation resulted in equal Cl_{TB} values for the three routes of B(*a*)P administration (i.a., 48 ml/min; i.v., 45 ml/min; h.p.v., 45 ml/min).

Collins and Dedrick (1982) and Gillette (1982) have recently suggested that the lung may in certain situations play a larger role in Cl_{TB} than has previously been thought due to its unique anatomical position between the venous and arterial circulations. Thus, lung has the opportunity for a substantial first-pass effect on removal of xenobiotic compounds from the blood. The first-pass effect is greatest when the sequential first-pass components of both the hepatic and pulmonary capillary beds are combined as would be the case after oral or h.p.v. administration. In the 3-MC-pretreated rats, Cl_{TB} was 48 ml/min when given i.a. Adding the first-pass effect of lung by administration i.v. increased Cl_{TB} by almost a third. This increase was the same as that due to the first-pass effect by liver (compare i.v. with h.p.v. data, table 1). Thus, it appears that in 3-MC-pretreated rats lung and liver function equally in removing B(*a*)P from the circulation. This conclusion is supported by isolated organ studies (Wiersma and Roth, 1983) in which the ability of liver and lung to clear B(*a*)P was measured directly.

In summary, these experiments demonstrated that B(*a*)P is rapidly removed from the blood and metabolized. Pretreatment with 3-MC resulted in a more rapid Cl_{TB}. An examination of Cl_{TB}s after administration at several sites allowed a determination of first-pass effects of liver and lung. The results suggested that in 3-MC-pretreated rats lung and liver each contribute substantially to the elimination process. Thus, an increased elimination *in vivo* of certain compounds such as B(*a*)P after pretreatment with inducers of microsomal enzymes may be due to enhanced contributions by extrahepatic organs rather than increased hepatic disposition alone.

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