Biochemical Pharmacology, Vol. 33, No. 21, pp. 3433–3438, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00
© 1984 Pergamon Press Ltd.

INFLUENCE OF RED BLOOD CELLS, SERUM ALBUMIN, AND SERUM LIPOPROTEINS ON THE CLEARANCE OF BENZO[*a*]PYRENE BY ISOLATED LIVERS OF 3-METHYLCHOLANTHRENE-TREATED RATS

DAVID A. WIERSMA,* PAUL M. STEMMER and ROBERT A. ROTH†
Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824, U.S.A.

(*Received* 23 *May* 1983; *accepted* 6 *April* 1984)

Abstract—Red blood cells, serum albumin, and serum lipoproteins transport benzo[*a*]pyrene and other xenobiotic compounds in the circulation. The distribution of benzo[*a*]pyrene and its metabolites among these blood components was examined, and the effect of their presence in the perfusion medium on the ability of isolated livers from 3-methylcholanthrene-pretreated rats to clear circulating benzo[*a*]pyrene was determined. A large fraction (45%) of the benzo[*a*]pyrene in rat blood was associated with the serum lipoproteins. However, only 8% of the benzo[*a*]pyrene metabolites was associated with this component. Forty to forty-five percent of each was associated with red blood cells. Benzo[*a*]pyrene clearance by isolated rat livers was 1.8 ± 0.2 ml/min when the medium contained only red blood cells and buffer. Addition of serum lipoproteins or serum albumin increased benzo[*a*]pyrene clearance to 5.1 ± 0.5 or 8.5 ± 0.9 ml/min respectively. Appearance of benzo[*a*]pyrene metabolites in perfusion medium and bile was similarly altered by the changes in medium composition. These results indicate that the clearance of benzo[*a*]pyrene by rat liver depends on the composition of the medium perfusing the organ and suggest that alterations in blood components *in vivo* may influence the metabolic disposition of this carcinogen.

Benzo[*a*]pyrene (B[*a*]P)‡ is a ubiquitous environmental contaminant [1]. Its elimination from the body occurs by its metabolism to more water-soluble and excretable compounds [2, 3]. This metabolic activity is present in many tissues, including liver, and may be stimulated in animals by pretreatment with 3-methylcholanthrene (3-MC) [4].

Highly lipid-soluble xenobiotic compounds, such as B[*a*]P, are not usually transported in the blood in the free form, but are associated with blood components. Albumin and lipoproteins are among the serum components capable of carrying these hydrophobic compounds in the blood [5–7]. Recent studies have demonstrated that lipoproteins carry B[*a*]P *in vitro* and *in vivo*, while albumin seems to function as a carrier for B[*a*]P metabolites [8–10]. Erythrocytes are also capable of transporting lipophilic, xenobiotic agents [11]; however, their role has received scant attention. The purpose of this study was to examine the role of red blood cells, albumin and serum lipoproteins in hepatic B[*a*]P clearance by altering the composition of the medium perfusing isolated livers of 3-MC-pretreated rats.

EXPERIMENTAL PROCEDURES

Animals. Male, Sprague–Dawley rats (Spartan Research Animals, Inc., Haslett, MI) weighing 260–310 g were used in these studies. They were housed in plastic cages on corn cob bedding in 12 hr light/dark cycled quarters. Food (Wayne Lab-Blox, Allied Mills, Chicago, IL) and tap water were allowed *ad lib.*

Pretreatment with 3-methylcholanthrene. 3-Methylcholanthrene (Pfaltz & Bauer, Stamford, CT) was suspended in corn oil (Mazola, Best Foods, Englewood Cliffs, NJ) to a concentration of 10 mg/ml. Each rat received an injection (20 mg/kg, i.p.) 24 and 48 hr prior to the experiment.

Preparation of [^{3}H]B[a]P solution for injection. [G-^{3}H]Benzo[*a*]pyrene (Amersham Inc., Arlington Heights, IL) of specific activity 17.4 Ci/mmole was purified by the procedure of Van Cantfort *et al.* [12], and a solution of [^{3}H]B[*a*]P for injection was prepared in serum from a donor rat. [^{3}H]B[*a*]P (0.6 mCi, 105 nmoles) and unlabeled B[*a*]P (12 nmoles) (99+%, Aldrich Chemical Co., Milwaukee, WI) in acetone were placed in a glass tube. The acetone was evaporated under nitrogen at room temperature and the residue was stored under nitrogen at −20°. On the day of the experiment the residue was resuspended in 0.6 ml of serum prepared from a donor rat, and an aliquot was analyzed for radioactivity.

Treatment with B[a]P. Rats to be given B[*a*]P were placed in a restrainer, and a 25 gauge needle attached to PE-20 tubing was inserted into a tail vein. After ensuring that the needle was in the vein by drawing a small amount of blood, 1.0 ml/kg of the [^{3}H]B[*a*]P solution was injected. Residual solution was flushed from the tubing with an equal volume of 0.9% NaCl, and the rat was then removed from the restrainer.

* Present address: Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ 85721, U.S.A.
† Author to whom all correspondence should be sent.
‡ Abbreviations: B[*a*]P, benzo[*a*]pyrene; 3-MC, 3-methylcholanthrene; DMSO, dimethyl sulfoxide; and RBC, red blood cell.

Approximately 17 min after administration of the B[*a*]P, the rats were anesthetized with ether, the abdomen was opened, and blood was withdrawn from the descending aorta through a 20 gauge hypodermic needle. The sample was placed in a glass tube and allowed to coagulate.

Isolation of lipoproteins. The total lipoprotein fraction was isolated from rat serum using a modification of the method of Nervi and Dietschy [13]. Samples of clotted blood were centrifuged at 2000 *g* for 20 min to prepare serum. The density of the serum was adjusted to 1.215 g/ml by the addition 0.302 g KBr/ml serum. The adjusted serum was centrifuged at 43,000 rpm in either a 50Ti rotor for 40 hr or a 50.2 Ti rotor for 35.5 hr at 5° in a preparative ultracentrifuge (Beckman Instruments, Inc., Spinco Division, Palo Alto, CA, model L8-55). The resultant layer containing the lipoproteins was removed from the top of the tube and dialyzed (Spectropor 2, Spectrum Medical Industries, Inc., Los Angeles, CA) against three changes of 4 liters of 0.9% NaCl, containing 0.1% EDTA for 24 hr at 4°. Lipid content of the resulting lipoprotein preparation was determined by gravimetric analysis following extraction of the lipids [14]. Protein content of the preparation was assessed by the method of Lowry *et al.* [15]. Human serum protein standard (Sigma Chemical Co., St. Louis, MO) served as reference protein.

Isolated, perfused organs. The procedures for isolation and perfusion of the livers have been described [16, 17]. Isolated livers from 3-MC-pretreated rats were perfused at 37° with recirculating medium at 10 ml/min. Following an equilibration period in which the perfusion pressure was allowed to stabilize, 20 nmoles of [^{3}H]B[*a*]P (sp. act. 2 Ci/mmole) was added to the perfusion medium reservoir. At various times following the addition of the B[*a*]P, samples (0.50 ml) of medium were withdrawn from the reservoir, and the content of B[*a*]P and metabolites was determined as described below.

Perfusion media. Three different perfusion media were prepared. All contained washed human erythrocytes (American Red Cross, Lansing, MI) at a final packed cell volume of 20%. The remaining 80% of the 100 ml of medium for each liver consisted of one of the following: (1) Kreb's bicarbonate buffer (pH 7.4) alone, (2) buffer with 4% bovine serum albumin (Fraction V, Pentex, Miles Laboratories, Elkhart, IN) or (3) buffer with the serum lipoproteins prepared from 100 ml of rat blood obtained from untreated rats. The buffer was equilibrated with 95% O_2–5% CO_2 prior to the addition of the albumin or serum lipoproteins.

Analysis of samples for B[a]P and B[a]P metabolites. B[*a*]P and B[*a*]P metabolites in samples of blood, serum, bile, lipoprotein fractions or perfusion medium were separated by the method of Van Cantfort *et al.* [12]. Prior to analysis, samples of blood, serum, or bile were diluted to 0.50 ml with 0.9% NaCl. Samples of perfusion medium were analyzed without dilution. The entire lipoprotein layer was diluted to 10 ml with 0.9% NaCl; 0.5 ml of these mixtures was added to 1.0 ml of 0.15 N KOH in 85% dimethyl sulfoxide (DMSO) and extracted with 2 × 5 ml hexane. Each hexane layer was back extracted into a fresh 0.5 ml 0.9% NaCl in 1.0 ml KOH/DMSO mixture to remove contaminating metabolites. This KOH/DMSO layer was combined with the initial layer, and an aliquot was placed in a scintillation vial. Hexane layers for each sample were combined in plastic scintillation vials and the hexane was allowed to evaporate. Ten milliliters of ACS (Amersham, Inc.) was added to each hexane vial and 15 ml to each of the vials containing a KOH/DMSO layer sample. The radioactivity in each vial was determined by liquid scintillation spectrometry. Quenching was corrected by external standardization.

Calculation of B[a]P clearance. Preliminary experiments demonstrated that B[*a*]P was removed from the perfusion medium in a biphasic manner. From semilogarithmic plots of the disappearance of B[*a*]P from the perfusion medium reservoir with time during the terminal phase, the clearance (Cl) of B[*a*]P was calculated using the following equations:

$$Cl = V_d \cdot k_e \quad (1)$$

$$V_d = \text{dose}/C_0 \quad (2)$$

where k_e, the first-order elimination rate constant, is the slope of the disappearance curve determined by linear regression analysis and V_d is the apparent volume of distribution, determined as the dose of B[*a*]P divided by the perfusion medium concentration of B[*a*]P at zero time, C_0. C_0 was determined by extrapolation of the disappearance curve to the ordinate. Values reported for B[*a*]P clearance were not corrected for clearance by the perfusion apparatus, which was 0.39 ± 0.16 ml/min.

Statistical analysis. Results are presented as means ± S.E.M. Comparisons between groups were made by one-way analysis of variance, completely random design. Tukey's ω-procedure was used to compare the means [18]. $P < 0.05$ was chosen as the level of significance.

RESULTS

B[*a*]P in rat blood was not uniformly distributed between the various blood components. In arterial blood drawn 20 min after i.v. administration of [^{3}H]B[*a*]P (117 nmoles/kg) to rats, the concentration of B[*a*]P was 11.2 ± 2.2 pmoles/ml blood (Table 1). Thus, by this time less than 1% of the administered B[*a*]P remained in the blood. Of this B[*a*]P, 60% was associated with the serum (the serum recovered accounted for 46% of the blood volume), indicating an approximately equal distribution of B[*a*]P between red blood cells and serum. Seventy-five percent of the B[*a*]P in the serum was associated with the lipoprotein fraction. Thus, 45% of the B[*a*]P in rat blood was associated with the serum lipoproteins.

After 20 min of circulation, the blood concentration of B[*a*]P metabolites (25.6 ± 2.6 pmoles of B[*a*]P equivalents/ml) was more than twice that of B[*a*]P. Seventy percent of these metabolites was recovered along with the serum, indicating that the B[*a*]P metabolites were preferentially distributed into serum. In contrast to B[*a*]P, however, only 8% of the metabolites in the blood was associated with the lipoprotein fraction. Thus, B[*a*]P metabolites do

Table 1. Distribution of B[a]P and B[a]P metabolites in rat blood*

Blood component	Fraction associated (% of total)†	
	B[a]P	B[a]P metabolites
Serum	59.1 ± 10.6	69.3 ± 5.7
Serum lipoproteins	44.8 ± 8.9	7.8 ± 0.9

* [^{3}H]B[a]P (117 nmoles, 2 mCi/kg) dissolved in rat serum was injected into the tail vein of conscious rats. Under ether anesthesia, blood was drawn from the abdominal aorta 19.5 min after administration. Serum was prepared and the serum lipoprotein fraction was isolated as described in Experimental Procedures. B[a]P was separated from metabolites by hexane extraction. Results are expressed as the percent of the total amount in the blood ± S.E. for five animals.

† Concentrations of B[a]P and B[a]P metabolites in the blood were 11.2 ± 2.2 and 25.6 ± 2.6 pmoles/ml respectively.

not concentrate within the serum lipoprotein fraction.

The influence of alterations in perfusion medium composition on the disposition of B[a]P was examined in isolated, perfused livers from 3-MC-treated rats. All perfusion media contained red blood cells at a packed cell volume one-half that of normal rat blood. Variations in medium composition were made in the additions to the buffer component. One group of livers was perfused with medium containing red blood cells and buffer alone. A second group of livers was perfused with medium which included serum lipoproteins isolated from an equivalent volume of blood, resulting in a final protein concentration of 43.4 mg/100 ml and a final lipid concentration of 126 mg/100 ml. The third group was perfused with medium containing added serum albumin at a final concentration of 3.2 g protein/100 ml. Several parameters were measured in these experiments to assess the liver preparations. As shown in Table 2, the post-perfusion weight of the isolated livers was not influenced by the composition of the medium. The liver to body weight ratios of these perfused livers were not significantly different than

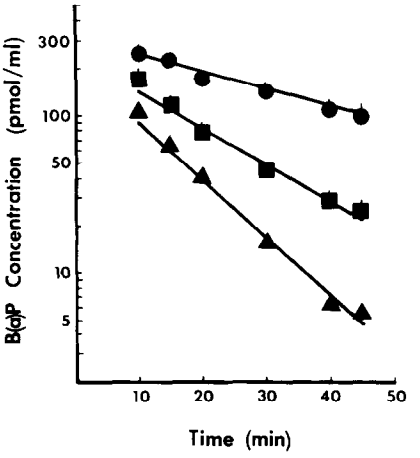

Fig. 1. Disappearance of B[a]P from the perfusion medium reservoir with time for isolated livers of 3-MC-pretreated rats perfused with various media. Isolated livers of 3-MC-pretreated rats were perfused at 37° in a recirculating manner at a constant flow of 10 ml/min. At 0 min, 20 nmoles of [^{3}H]B[a]P was added to the reservoir. Samples were taken at various times thereafter and analyzed for B[a]P content. Results are the mean concentration ± S.E. of four or five liver preparations. Key: (●) data from livers perfused with red blood cells (RBCs) and buffer; (■) livers perfused with RBCs and buffer containing serum lipoprotein fraction; and (▲) livers perfused with RBCs and buffer containing serum albumin. Media were prepared as described in Experimental Procedures.

those of livers from 3-MC-pretreated rats that were not perfused (data not presented). Addition of albumin to the perfusion medium significantly reduced the inflow pressure. The increase in perfusion pressure that occurred during the experiment was not affected by the medium composition. No significant difference in bile flow was observed between the livers perfused with medium containing serum albumin or lipoproteins. In the group of livers perfused with medium containing only red blood cells and buffer, bile flow was 30% less than that of the other groups.

Figure 1 depicts the disappearance of B[a]P from the perfusion medium reservoir with time during the

Table 2. Isolated perfused rat liver parameters*

Perfusion medium composition	Liver wt / Body wt (%)	P_{IN} (mm Hg)	Δ P (mm Hg)	Bile flow (μl/min)
RBCs, buffer	5.3 ± 0.1	4.4 ± 0.3	3.6 ± 1.0	4.8 ± 0.6†
RBCs, buffer with albumin	4.9 ± 0.1	2.5 ± 0.7†	4.0 ± 1.2	7.2 ± 0.3
RBCs, buffer with lipoproteins	5.1 ± 0.2	3.9 ± 0.3	4.2 ± 0.8	6.9 ± 0.5

* Isolated livers from 3-MC-pretreated rats were perfused at 10 ml/min in a recirculating manner at 37° with [^{3}H]B[a]P for 45 min as described in Experimental Procedures. Each perfusion medium contained washed human red blood cells (RBCs) at a hematocrit of 20%. Results are mean ± S.E. of four or five livers. Abbreviations: P_{IN}, initial inflow perfusion pressure; and ΔP, change in perfusion pressure during the experiment.

† Mean is significantly different ($P < 0.05$) from the other means in the same column.

Table 3. Pharmacokinetic parameters*

Perfusion medium composition	k_e (min^{-1})	V_d (ml)	Cl (ml/min)
RBCs, buffer	0.027 ± 0.007†	83.6 ± 22.1	1.82 ± 0.20†
RBCs, buffer with albumin	0.087 ± 0.002‡	97.8 ± 10.8	8.45 ± 0.87‡
RBCs, buffer with lipoproteins	0.055 ± 0.004§	94.1 ± 8.8	5.07 ± 0.48§

* Isolated livers of 3-MC-pretreated rats were perfused with [^{3}H]B[*a*]P at 10 ml/min in a recirculating manner with perfusion medium of the indicated composition. Pharmacokinetic parameters were determined from individual B[*a*]P disappearance curves as described in Experimental Procedures. Results are means ± S.E. Abbreviations: k_e, first-order elimination rate constant; V_d, apparent volume of distribution; Cl, clearance; and RBCs, red blood cells.

†–§ Any two means in the same column with different superscripts are significantly different ($P < 0.05$) by one-way analysis of variance.

terminal phase. B[*a*]P removal appeared to be first-order irrespective of the type of perfusion medium used. Pharmacokinetic parameters determined from such plots for each liver are presented in Table 3. No differences were observed in the apparent volume of distribution of B[*a*]P. Differences among the three groups occurred, however, in the first-order rate constant of elimination and in B[*a*]P clearance. Clearance by isolated livers perfused with medium containing only red blood cells and buffer was low. The highest B[*a*]P clearance was by the group of livers with albumin in the medium (8.5 ± 0.9 ml/min), while livers perfused with medium containing added serum lipoproteins had an intermediate clearance (5.1 ± 0.5 ml/min).

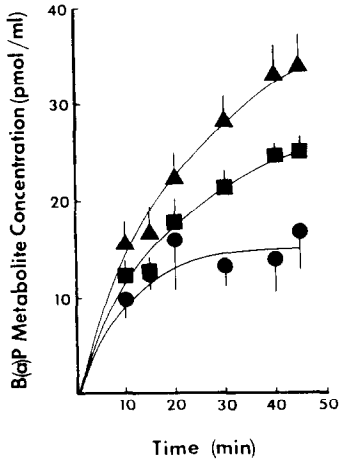

Fig. 2. Appearance of B[*a*]P metabolites in the perfusion medium reservoir with time for isolated livers of 3-MC-pretreated rats perfused with various media. Isolated livers of 3-MC-pretreated rats were perfused at 37° in a recirculating manner at a constant flow of 10 ml/min. At 0 min, 20 nmoles of [^{3}H]B[*a*]P was added to the reservoir. Samples were taken at various times thereafter and analyzed for total B[*a*]P metabolite content. Results are the mean concentration ± S.E. of four or five liver preparations. Key: (●) data from livers perfused with red blood cells (RBCs) and buffer; (■) livers perfused with RBCs and buffer containing serum lipoproteins; and (▲) livers perfused with RBCs and buffer containing serum albumin. Media were prepared as described in Experimental Procedures.

Appearance of B[*a*]P metabolites in the perfusion medium paralleled the differences in clearance. As demonstrated in Fig. 2, livers perfused with red blood cells and buffer released a minimal amount of metabolites into the medium, whereas those perfused with albumin-containing medium had the greatest metabolite production. Livers perfused with medium containing serum lipoproteins were intermediate in metabolite appearance.

Biliary excretion of B[*a*]P was low by all three groups of livers (Table 4), amounting to only about 0.006% of the amount of B[*a*]P administered. In contrast, significant amounts of B[*a*]P metabolites appeared in the bile. In livers perfused with albumin, about 2% of the B[*a*]P was excreted into the bile as metabolite during the 45-min perfusion period. This was significantly greater than the amount of metabolite excreted by the group of livers perfused with medium containing serum lipoprotein. Perfusion of livers with only red blood cells and buffer resulted in the lowest biliary excretion of B[*a*]P metabolites.

Table 4. Amount of B[*a*]P and B[*a*]P metabolite appearing in the bile of isolated, perfused livers*

Perfusion medium composition	B[*a*]P (pmoles)	B[*a*]P metabolites (pmoles)
RBCs, buffer	0.8 ± 0.2	92 ± 29†
RBCs, buffer with albumin	1.3 ± 0.1	451 ± 27‡
RBCs, buffer with lipoproteins	1.3 ± 0.4	303 ± 40§

* Isolated livers of 3-MC-pretreated rats were perfused in a recirculating manner for 45 min at 10 ml/min with the indicated medium containing 20 nmoles [^{3}H]B[*a*]P. Bile produced was analyzed for B[*a*]P and metabolite content as described in Experimental Procedures. Results are mean amount ± S.E.

†–§ Means with different superscripts are significantly different ($P < 0.05$) from the other means in the same column.

DISCUSSION

Previous studies in this laboratory suggested that the isolated liver clears B[a]P at a higher rate than the liver *in vivo* [19, 20]. The major difference between the two preparations used in these experiments was that blood perfused the liver *in vivo*, whereas the isolated liver was perfused with a medium containing erythrocytes and albumin. This suggested that the difference in clearance was possibly due to different carriers for the B[a]P.

Analysis of the distribution of B[a]P in rat blood (Table 1) indicated that, of the 60% of the B[a]P that was found in the serum, 75% was associated with the serum lipoproteins. The remainder was either free in the serum or probably associated with plasma protein. Forty percent of the B[a]P remained with the red blood cells. These results are similar to those obtained *in vitro* by Shu and Nichols [8] using human blood. These data suggest that serum lipoproteins may be the more important carrier for such highly lipophilic compounds.

In the present study, we examined the influence of red blood cells, albumin and serum lipoproteins on B[a]P clearance by isolated, perfused livers from 3-MC-pretreated rats. Alteration of the perfusion medium composition produced differences in some of the parameters used to assess the perfused liver preparation (Table 2). Initial inflow pressure of livers perfused at constant flow with medium containing albumin was less than that of the other groups. The reason for this may be related to the oncotic pressure exerted by the albumin in the medium. Since the other two media did not contain such an osmotic substance, the relative tissue pressure may have been greater, requiring greater perfusion pressure to open capillaries. Bile flow was reduced significantly in livers perfused with medium composed of erythrocytes and buffer compared to those livers perfused with medium containing lipoproteins or albumin. Since bile flow in the rat is related to the rate of bile acid secretion [21, 22], this flow reduction may be due to a decreased bile acid availability in the livers perfused with red blood cells and buffer alone. Although we did not measure bile acids in this study, albumin and lipoprotein preparations may have provided a source of these compounds.

In addition, the composition of the perfusion medium affected the disposition of B[a]P by the perfused livers (Table 3). Livers perfused with buffered medium containing erythrocytes alone were least able to clear circulating B[a]P. Inclusion of serum lipoproteins in the medium enhanced B[a]P clearance 2.5 times, while the presence of serum albumin resulted in a clearance about 4 times greater than when buffer alone was present. These alterations in clearance were associated with changes in the elimination rate constant and not the apparent volume of distribution.

Changes in clearance can occur by an alteration in the free concentration of the compound in the blood [23]. For example, in isolated livers in which the albumin concentration of the perfusion medium was decreased, phenytoin clearance was increased along with the concomitant increase in the free fraction of phenytoin observed [24]. Opposite results were obtained in our study, however, since inclusion in the perfusion medium of blood components capable of binding B[a]P, and thus decreasing its free concentration, *increased* the ability of the livers to extract the circulating B[a]P. Thus, addition of either albumin or lipoproteins to medium containing red blood cells and buffer enhanced the ability of the livers to extract circulating B[a]P.

One explanation for this carrier-mediated enhancement of B[a]P clearance may be that hepatocytes possess receptors for these compounds. In fact, Forker and Luxon [25] and Weisiger *et al.* [26] have shown that albumin receptors play a role in the hepatic uptake of taurocholate and oleate respectively. Thus, it may be that B[a]P bound to albumin or lipoprotein is extracted by an interaction of the carrier with hepatocyte surface receptors through either an internalization of the complex or an efficient presentation of the B[a]P to the membrane surface. However, Remsen and Shireman [27] discovered that, although they possess lipoprotein receptors, cultured lung fibroblasts do not take up lipoprotein bound B[a]P at a rate greater than free B[a]P. Thus, an involvement of receptors may not explain the effect of added lipoprotein in our study.

Another model of carrier-enhanced hepatic extraction suggests that the increased removal of B[a]P observed when albumin or lipoproteins are added to the medium may be due to a short dissociation time between these carriers and the free B[a]P in the medium. Only a very small fraction of B[a]P exists free in the medium due to its low water solubility. Thus, since hepatic extraction of B[a]P is high, a large portion of the bound B[a]P must dissociate from its carriers during the relatively short transit time through the liver. Lipid-containing carriers such as red blood cells and lipoproteins might be expected to have longer dissociation times and, therefore, lower B[a]P clearances, while less lipophilic carriers, such as albumin, might display shorter dissociation times and higher clearances. While the dissociation times of B[a]P from these carriers have not been measured, the B[a]P clearances we obtained with these three media suggest that clearance of lipophilic, carrier-bound substances may be inversely related to the lipophillicity of the carrier.

A third explanation for the observed difference in B[a]P clearances is suggested by the anatomy of the liver. The circulation of the liver is separated from the hepatocytes by a layer of fenestrated endothelial cells and the space of Disse. Thus, bulky carriers such as red blood cells are unable to penetrate this endothelial barrier and present B[a]P to the hepatocyte efficiently. This may result in a lower extraction. Lipoproteins and albumin, however, may easily diffuse through the fenestrated cell layer and present the B[a]P to the hepatocytes, resulting in a greater extraction. Accordingly, B[a]P clearance is enhanced by those carrier molecules which penetrate the space of Disse.

These three possible explanations of our results are not mutually exclusive. Further experimentation will be required to determine the role each plays in the B[a]P clearance process.

Alteration in B[a]P clearance by changes in per-

fusion medium composition was also reflected in the metabolism of B[*a*]P by the perfused livers. The B[*a*]P clearance and the amounts of metabolite in the bile and in the perfusion medium (Fig. 2; Table 4) were correlated. Thus, the metabolism of B[*a*]P by these perfused livers was limited by the amount of B[*a*]P which was taken up into the liver.

In summary, B[*a*]P in rat blood was associated primarily with red blood cells and serum lipoproteins. When red blood cells alone served as a carrier for B[*a*]P in the isolated liver, clearance of B[*a*]P was low. However, when either serum lipoproteins or albumin were included in the perfusion medium, B[*a*]P clearance was greater. Clearance was greatest when albumin acted as the carrier. Thus, those blood components capable of binding such lipophilic foreign compounds as B[*a*]P may increase the ability of the liver to clear these compounds from the circulation. Since lipoproteins serve as the major carrier of B[*a*]P *in vivo*, these results suggest that clearance of highly extracted, lipophilic compounds *in vivo* may be less than that in isolated livers in which albumin is the major carrier.

Acknowledgements—We thank the American Red Cross, Great Lakes Regional Center, for their supply of outdated red blood cells, Miss Michelle M. Imlay for her technical assistance, and Miss Diane Hummel for her help with manuscript preparation. This work was supported by NIH Grant HL25962. D. A. Wiersma was supported by an advanced predoctoral fellowship from the Pharmaceutical Manufacturer's Association Foundation, and P. M. Stemmer by National Institutes of Health Training Grant GM07392.

REFERENCES

1. J. W. DePierre, M. S. Moron, K. A. M. Johannesen and L. Ernster, *Analyt. Biochem.* **63**, 470 (1975).
2. P. Kotin, H. L. Falk and R. Busser, *J. natn. Cancer Inst.* **23**, 541 (1959).
3. W. G. Levine, *J. Pharmac. exp. Ther.* **175**, 301 (1970).
4. P. M. Warren and G. D. Bellward, *Biochem. Pharmac.* **27**, 2537 (1978).
5. J. Avigan, *Cancer Res.* **19**, 831 (1959).
6. R. Ranke, *Biochim. biophys. Acta* **160**, 378 (1968).
7. E. Tipping, B. P. Moore, C. A. Jones, G. M. Cohen, B. Ketterer and J. W. Bridges, *Chem. Biol. Interact.* **32**, 291 (1980).
8. H. P. Shu and A. V. Nichols, *Cancer Res.* **39**, 1224 (1979).
9. H. P. Shu and A. V. Nichols, *Biochim. biophys. Acta* **665**, 376 (1981).
10. H. P. Shu and E. N. Bymun, *Cancer Res.* **43**, 485 (1983).
11. M. LeMaire and J. P. Tillement, *J. Pharm. Pharmac.* **34**, 715 (1982).
12. J. Van Cantfort, J. DeGraeve and J. E. Gielen, *Biochem. biophys. Res. Commun.* **79**, 505 (1977).
13. F. O. Nervi and J. M. Dietschy, *J. biol. Chem.* **250**, 8704 (1975).
14. W. M. Sperry and F. C. Brand, *J. biol. Chem.* **213**, 69 (1955).
15. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
16. D. A. Wiersma and R. A. Roth, *J. Pharmac. exp. Ther.* **212**, 97 (1980).
17. R. A. Roth and R. J. Rubin, *J. Pharmac. exp. Ther.* **199**, 61 (1976).
18. R. G. D. Steel and J. H. Torrie, *Principles and Procedures of Statistics*. McGraw-Hill, New York (1960).
19. D. A. Wiersma and R. A. Roth, *J. Pharmac. exp. Ther.* **225**, 121 (1983).
20. D. A. Wiersma and R. A. Roth, *J. Pharmac. exp. Ther.* **226**, 661 (1983).
21. C. Balabaud, K. Kron and J. J. Gumucio, *J. Lab. clin. Med.* **89**, 393 (1977).
22. B. L. Blitzer and J. L. Boyer, *Gastroenterology* **82**, 346 (1982).
23. G. R. Wilkinson and D. G. Shand, *Clin. Pharmac. Ther.* **18**, 377 (1975).
24. D. G. Shand, R. H. Cotham and G. R. Wilkinson, *Life Sci.* **19**, 125 (1976).
25. E. L. Forker and B. A. Luxon, *J. clin. Invest.* **67**, 1517 (1981).
26. R. Weisiger, J. Gollan and R. Ockner, *Science* **211**, 1048 (1981).
27. J. F. Remsen and R. B. Shireman, *Cancer Res.* **41**, 3179 (1981).