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Movements of Benzo[a]pyrene across the Hemochorial Placenta of the Guinea Pig[1,2] (41307)

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Abstract. In an effort to determine fetal exposure resulting from maternally administered benzo(a)pyrene (BaP), the clearance of radiolabeled BaP from mother to fetus was measured across the hemochorial placenta of the guinea pig at 60 days of gestation. Using techniques previously reported for other toxic materials, the fetal circulation of the placenta was isolated, BaP injected into the maternal circulation, and the concentration of BaP determined in the perfusate. The clearance of BaP from mother to fetus was high following intravenous injection. Clearances appeared to be a function of umbilical blood flow, and ranged from 0.59 to 2.40 ml/min at an umbilical flow of 2.5 ml/min. Since clearances of BaP approximated those obtained for tritiated water, it is apparent that circulating BaP gains easy access to the fetus.

Maternal exposure to carcinogenic agents can lead to malignancies in offspring, a phenomenon termed "transplacental carcinogenesis." Benzo[a]pyrene (BaP), a polycyclic aromatic hydrocarbon, has been shown to be a transplacental carcinogen in sensitive rodent strains. For example, Nikonova (1) reported incidences of pulmonary adenomas as high as 89% in male offspring of mice dosed subcutaneously with 6 mg of BaP on day 18 of gestation (dg 18). Bulay (2) found an incidence of pulmonary adenomas as high as 62% in offspring of mice dosed subcutaneously with 4 mg of BaP on dg 11, 13, or 15.

Benzo[a]pyrene has also been shown to be embryotoxic and teratogenic (3). When administered intraperitoneally at a dose of 150 mg/kg to BALB/c female mice on dg 8.5, BaP reduced the live birth rate to 52%. All survivors showed a shift in the number of presacral vertebrae, from 26 in controls to 27 in pups from treated mothers. The incidence of frank skeletal malformations also increased in offspring from treated females as compared to control females.

The morphologic and carcinogenic consequences of prenatal BaP administration suggest that the parent compound (or its metabolites) has either a direct effect on the developing organism or an indirect effect, mediated through disturbances in placental function. There seems to be little doubt that either BaP or its metabolites are capable of crossing the placenta and directly affecting the developing fetus. For example, large amounts of either BaP or its metabolites were evident in fetal rats 3 hr after their dams were dosed intragastrically with 200 mg/kg on dg 21 (4). Napalkov (5) has interpreted such data to indicate that BaP (or its metabolites) reach the fetus primarily by simple diffusion across the placenta; that the concentration of BaP-related material in the fetus is most likely determined by maternal metabolic rates; and that transplacental carcinogenic responses are likely due to maternal production of carcinogenic metabolites.

It is not possible, from data in the literature, to define the relative roles of placental transport and of placental, maternal, and fetal metabolism in the production of BaP effects in the developing organism. The purpose of this study was to measure the clearance of BaP from maternal to fetal circulation of the hemochorial placenta, independent of the presence of the fetus.

Methods. Outbred Harley guinea pigs were bred immediately after farrowing so

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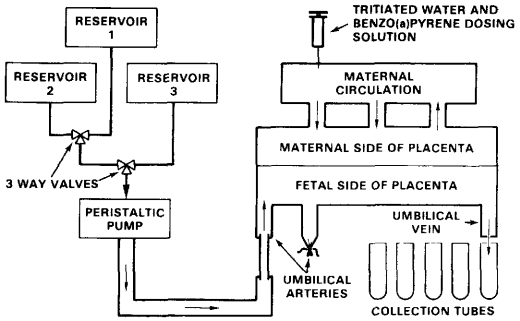

FIG. 1. Schematic diagram of the perfusing system.

that gestational age was known (within 24 hr). The animals used in this study were approximately 60 (±1) dg.

Surgical procedures. The perfusion technique used in these experiments has been extensively described (6–9). A schematic diagram of the perfusing system is shown in Fig. 1. The dam was anesthetized with a combination of sodium pentobarbital injected ip and Innovar-Vet (Pitman-Moore) injected not more than 30 min before surgery. The dam, in a supine position, was placed in a bath of mammalian Ringer's solution which covered the lower legs and part of the posterior abdomen. Fetuses were exposed, and the uterus and products of conception immersed in the bath. An umbilical artery was cannulated with flexible tubing which passed through a peristaltic pump and then to a reservoir. After cannulation of the umbilical vein, perfusion fluid was passed through the placenta and collected in a series of tubes. Following establishment of a satisfactory outflow from the venous cannula, 10 μCi of tritiated water and the dosing solution was injected into the maternal jugular vein. After the first experiment using [^{14}C]BaP, maternal blood samples were collected from the carotid artery at 2- to 10-min intervals, depending on the rapidity of change in plasma BaP concentrations observed in the initial [^{14}C]BaP experiment.

Since preliminary experiments indicated that BaP was retained on plastic surfaces, most of the cannula material and tubing exposed to BaP was either glass or metal. The dosing solution was administered from a glass syringe. The maternal jugular cannula consisted of a metal, 23-ga needle, with the tip covered by approximately 1 mm of polyethylene tubing to protect the jugular wall. The maternal carotid cannula consisted of approximately 7 cm of polyethylene tubing connected to a metal needle. Perfusion pressure, maternal cardiac rate, ECG, blood pressure, and respiratory rate were monitored continously during each perfusion. Umbilical flow rates were varied between 0.7 and 2.3 ml/min. As previously described these rates overlap the physiological rate of umbilical blood flows in the guinea pig at 60 dg (6).

Solution composition and analysis. The fetal perfusate consisted of sterile, filtered plasma (pH 7.35) from adult guinea pigs. Preparation of this perfusing solution was identical to that previously described (6, 8, 9). The perfusate was collected and analyzed for BaP content after a single passage.

Maternal dosing solutions consisted of unlabeled BaP (Aldrich Chemical Co., Gold Label, 99+%) or 7, 10-[^{14}C]BaP (Amersham Corporation) in dimethylsulfoxide (DMSO; Eastman Kodak Co., Spectro Grade). One animal was administered 27 mg of unlabeled BaP in 800 μl DMSO without radiolabeled water. Four additional animals were administered 20 μCi of [^{14}C]BaP (21.7 mCi/mmole) in 100 μl DMSO in addition to radiolabeled water.

A Hewlett–Packard 5840A gas chromatograph with a flame ionization detector and auto sampler was used to analyze BaP in samples from the animal dosed with unlabeled BaP (10). Our limit of detection was approximately 5 ng BaP. The [^{14}C]BaP was analyzed using standard liquid scintillation techniques. Since less than 1 μmole of [^{14}C]BaP was administered to each dam receiving radiolabeled BaP, we did not attempt to analyze these samples by gas chromatography.

Calculations. The clearance of tritiated water from the maternal circulation was calculated as previously described (6–9) by the following formula:

$$\text{tritium clearance} = (P/M)R,$$

where *tritium clearance* is equivalent to the milliliters of maternal plasma which would

contain an amount of tritium equal to that entering the perfusate per minute (ml/min). P is the tritium concentration (μCi/ml) measured in the perfusate, and M is the average maternal tritium concentration (μCi/ml), determined from a series of linear approximations of a graph of maternal tritium concentration versus time. R is the flow rate of the perfusate (ml/min), and is equivalent to the umbilical flow rate.

The clearance of BaP from maternal to fetal circulation of the placenta was calculated in an analogous manner by the equation:

$$\text{BaP clearance} = (P/M)R,$$

where *BaP clearance* is equivalent to the milliliters of maternal plasma which would contain an amount of either unlabeled or labeled BaP equal to that entering the perfusate per minute (ml/min). P is either the concentration of unlabeled BaP or [^{14}C]BaP (μg/ml or μCi/ml) measured in the perfusate, and M is the average maternal unlabeled BaP or [^{14}C]BaP concentration (μg/ml or μCi/ml), determined from a series of linear approximations of a graph of maternal BaP concentration versus time. Again, R is the rate of flow of the perfusing fluid.

Results. During *in situ* perfusions, measurements of changes in maternal blood flow to the placenta are necessary to avoid confounding influences when determining the rate at which substances move across the placenta. We have used the characteristics of the clearance of tritiated water from mother to fetus to indicate changes in maternal blood flow to the placenta. This use of tritiated water is based on the premise that it diffuses freely across the placenta and equilibrates rapidly between maternal and fetal circulations. Because of the rapid equilibration, tritium movements across the placenta are a function of flow rates on both sides. Tritiated water clearance has been shown to be a linear function of perfusion rate in experiments in which it was established that minimal changes occur in maternal blood flow to the placenta. Changes in maternal blood flow to the placenta are the most likely source of variation from the linear relationship between perfusion rate and tritiated water clearance. Complete descriptions of the use of tritium clearance as an indicator of changes in maternal blood flow have been published (6–8).

Data obtained from eight placentas from seven dams are summarized in Table I. The clearance of labeled water from mother to fetal circulation of the placenta (T_{MF}) showed the same characteristics and fell within the same range as in the studies previously cited in this manuscript. In most perfusions, T_{MF} was a linear function of umbilical flow rate and, as just described,

TABLE I. BENZO[A]PYRENE CLEARANCE FROM MOTHER TO FETUS

		Clearance measurements (ml/min)					
		Labeled water		Benzo[a]pyrene			
						Correlation with T_{MF}	
Dam No.	Placenta No.	at 2.5 ml/min[a] (±SE)	Range	At 2.5 ml/min[a] (±SE)	Range	r^b	P
1	1	—	—	0.60 ± <0.01	0.26–0.71	—	—
2	1	3.25	0.33–0.71	2.40	0.71–1.10	0.99	<0.001
3	1	1.72 ± 0.10	0.39–1.74	1.62 ± 0.11	0.31–1.64	0.98	<0.001
4	1	1.14 ± 0.09	0.54–1.92	1.09 ± 0.07	0.72–1.53	0.99	<0.001
4	2	1.16 ± 0.05	0.65–1.15	1.03 ± 0.06	0.44–1.00	0.97	<0.01
5	1	2.08 ± 0.10	1.52–2.61	2.62 ± 0.41	2.08–3.32	0.67	<0.05
6	1	2.51 ± 0.06	1.69–2.15	2.67 ± 0.25	0.70–4.31	0.95	<0.001
7	1	0.58 ± 0.02	0.28–0.43	0.30 ± 0.02	0.16–0.26	0.98	<0.001

[a] As predicted from the regression of T_{MF} or BaP_{MF} on umbilical flow.
[b] Correlation coefficient for the regression of BaP_{MF} on T_{MF}.

that relationship was used as a defining characteristic for determining when maternal blood flows to the placenta were changing.

The clearance of BaP from mother to fetal circulation of the placenta (BaP_{MF}) was determined in two types of experiments. In the first, one dam was administered 27 mg of unlabeled BaP; T_{MF} was not measured in this experiment. BaP_{MF} was linearly related to umbilical flow, which was predicted to be 0.60 ml/min at an umbilical flow rate of 2.5 ml/min, and ranged in magnitude from 0.26 to 0.71 ml/min. Although concentrations of unlabeled BaP were low in both maternal plasma and perfusate, we believe that there was mostly parent compound present in these fluid compartments, since not more than 30 min elapsed between intravenous administration of the parent compound and the last sampling period.

The remaining animals were administered 230 μg of [^{14}C]BaP, and T_{MF} was measured. In nearly all cases, movements of BaP from mother to fetal circulation of the placenta were similar to those of water. The BaP_{MF} predicted at an umbilical flow rate of 2.5 ml/min (from the regression of BaP_{MF} on umbilical flow rate) was 91 ± 9% (mean ± SE) of the T_{MF} predicted at an umbilical flow rate of 2.5 ml/min (from the regression of T_{MF} on umbilical flow rate). Furthermore, BaP_{MF} was highly correlated with T_{MF} in six of the seven perfusions in which data on maternal blood flow to the placenta were obtained.

Discussion. Our studies indicate that BaP introduced directly into the maternal circulation gains easy access to the fetus. The characteristics of BaP_{MF} were sufficiently like those of T_{MF} that one would expect circulating BaP to cross the placenta rapidly, and by simple diffusion, just as water does.

A plethora of data exists showing that homogenates or tissue cultures of human and nonhuman fetal and placental tissues are capable of forming potentially carcinogenic materials from BaP (11–13). Recently, Namkung and Juchau (14) have shown that many of the qualitative differences which have been reported are due to a technical artifact resulting from the use of substrate concentrations high enough to assure zero-order kinetics since large substrate concentrations produce an inhibitory effect on the formation of dihydrodiols. While it appears from these studies that nearly all placental/fetal tissues have the capacity to metabolize BaP to highly carcinogenic diols at various times during gestation, data obtained from tissue homogenates is difficult to extrapolate to the intact pregnant animal where many processes not present in homogenates determine which materials will reach the fetus. The data reported in our study suggest that a large fraction of the radiolabeled material entering the fetal circulation of the placenta was unmetabolized BaP. Because plasma BaP concentrations were close to our detection limits, there is a possibility that appreciable amounts of metabolites may have been present in plasma samples, although this appears unlikely due to the short time interval between injection of BaP and sampling from the fetal circulation of the placenta. It therefore appears evident that the metabolic capabilities of fetal tissue itself may play an important part following exposure to BaP.

While it is apparent from our data that circulating BaP reaches the fetus, additional data are necessary before the hazard associated with fetal exposure to BaP can be determined. In particular, BaP concentrations in maternal plasma must be determined following exposure by means more appropriate to intact animals, such as oral or dermal routes. Determination of maternal plasma concentrations, coupled with data such as those described in this report, should then allow quantitative estimation of the amount of BaP to which fetal tissues are exposed.

1. Nikonova TV. Bull Exp Biol Med **84**:1025, 1977.
2. Bulay OM, Wattenberg LW. Proc Soc Exp Biol Med **135**:84, 1970.
3. Russell LB, McKinley TW Jr. In: Mahlum DD, Sikov MR, Hackett PL, Andrew FD, eds. Developmental Toxicology of Energy-Related Pollutants. Springfield, Va., CONF-771017, NTIS, p175, 1978.
4. Alexandrov VA. Neoplasma **23**:285, 1976.

5. Napalkov NP. Special Problems with Carcinogenicity Protocols. Toxicology Forum, Washington D.C., February 19–22, 1978.
6. Kelman BJ. Toxicol Appl Pharmacol **41**:659, 1977.
7. Kelman BJ. Fed Proc **38**:2247, 1979.
8. Kelman BJ, Walter BK. Proc Soc Exp Biol Med **156**:68, 1977.
9. Kelman BJ, Walter BK. Proc Soc Exp Biol Med **163**:278, 1980.
10. Beernaert HJ. Chromatography **173**:109, 1979.
11. Selkirk JK, Wiebel FJ. Prog Exp Tumor Res **24**:61, 1979.
12. Berry DL, Zahariah PK, Slaga TJ, Juchau MR. Eur J Cancer **13**:667, 1977.
13. Wang IY, Rasmussen RE, Creasey R, Crocker TT. Life Sci **20**:1265, 1977.
14. Namkung MJ, Juchau MR. Toxicol Appl Pharmacol **55**:253, 1980.

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