Estimation of Transplacental and Nonplacental Diphenhydramine Clearances in the Fetal Lamb: The Impact of Fetal First-Pass Hepatic Drug Uptake

SANJEEV KUMAR, GEORGE R. TONN, EDDIE KWAN, CAROLINE HALL, K. WAYNE RIGGS, JAMES E. AXELSON and DAN W. RURAK

Division of Pharmaceutics and Biopharmaceutics, Faculty of Pharmaceutical Sciences (S.K., G.R.T., K.W.R., J.E.A.), and Department of Obstetrics and Gynecology, Faculty of Medicine (E.K., C.H., D.W.R.), University of British Columbia, Vancouver, B.C., Canada

Accepted for publication April 8, 1997

ABSTRACT

Previous estimates of maternal and fetal placental and nonplacental clearances in pregnant sheep using a two-compartment open model have revealed higher values of fetal placental clearance (CLfm) compared to the maternal placental clearance (CLmp) for most drugs. This includes the anti-histamine diphenhydramine (DPHM), which also has the highest weight-corrected fetal nonplacental clearance (CLfn) among the drugs studied. This study was designed to determine the reasons for this CLmf difference and to identify the sites of high CLfn for DPHM. DPHM and a stable isotope-labeled analog, [2H10]DPHM, were simultaneously infused to steady state to the mother and fetus, respectively, in five pregnant sheep. CLmp, CLfm, CLfn and CLfo averaged 50.3 ± 13.2, 214.4 ± 30.8, 36.6 ± 1.9 and 109.8 ± 22.3 ml/min⁻¹/kg⁻¹, respectively. By measuring diphenylmethoxyacetic acid and [2H10]diphenylmethoxyacetic acid levels in samples obtained from our previous study of fetal hepatic first-pass DPHM uptake, the hepatic first-pass extraction ratio of the drug from umbilical venous blood was estimated to be 0.44 ± 0.05. This can account for virtually all of CLfn. Fetal hepatic first-pass uptake of maternally derived DPHM in the paired infusion study reduces the fetal/maternal plasma DPHM concentration ratio and results in significant underestimation of CLmp. When the CLfn estimate is corrected for this factor and for maternal-fetal DPHM plasma protein binding differences, its value approaches CLmp. Fetal hepatic first-pass uptake may also be a factor in the underestimation of CLmp for most of the other drugs. Conversely, a lower value of CLmp compared with CLfn provides evidence for significant fetal hepatic uptake of these compounds.

The extent of fetal exposure to maternally administered drugs is determined by a number of factors, including physicochemical characteristics of the drug in question, permeability characteristics of the placenta and the length of time the drug is present in the maternal circulation (Reynolds and Knott, 1989; Rurak et al., 1991). During long-term administration with steady-state drug concentrations in the mother, two additional factors become important in determining fetal exposure: maternal and fetal plasma protein binding and fetal nonplacental clearance of the drug. Szeto et al. (1982a) have shown that under steady-state conditions, the fetal-to-maternal drug concentration ratio during maternal drug administration is determined by the maternal placental clearance divided by the sum of fetal placental and nonplacental clearances (i.e., $\frac{C_{fn}}{C_{max}} = \frac{CL_{mp}}{CL_{mp} + CL_{fn}}$). Thus, when the fetus is able to eliminate the drug via nonplacental routes, fetal drug exposure is reduced.

Maternal and fetal placental and nonplacental clearances

Received for publication July 22, 1996.

1 This project was supported by Medical Research Council of Canada Program Grant PG-11120.

2 S.K. is the recipient of a University of British Columbia Graduate Fellowship.

3 G.R.T. was supported by a Medical Research Council of Canada Studentship.

4 D.W.R. is the recipient of an Investigatorship award from the British Columbia Children’s Hospital Foundation.

ABBREVIATIONS: DPHM, diphenhydramine; [2H10]DPHM, deuterium-labeled diphenhydramine; DPMA, diphenylmethoxyacetic acid; [2H10]DPMA, deuterium-labeled diphenylmethoxyacetic acid; Clmp, maternal total body clearance; Cltn, fetal total body clearance; Clttn, maternal-to-fetal transplacental clearance; Cltnp, fetal-to-maternal transplacental clearance; Clpmp, maternal nonplacental clearance; Clfn, fetal nonplacental clearance; AUC, area under the plasma concentration-vs.-time curve; MA, maternal femoral artery; MV, maternal femoral vein; FA, fetal femoral artery; UV, umbilical vein; TV, fetal lateral tarsal vein; CA, fetal carotid artery; Cmax, maternal plasma steady-state DPHM concentration after maternal administration; Cmax, fetal plasma steady-state DPHM concentration after maternal administration; Cmax, maternal plasma steady-state [2H10]DPHM concentration after fetal administration; Cmax, fetal plasma steady-state [2H10]DPHM concentration after fetal administration; Qm, umbilical blood flow; ER, fetal hepatic extraction ratio for DPHM present in umbilical blood; F, fetal systemic availability for DPHM present in umbilical blood; GFR, glomerular filtration rate; GC-MS, gas chromatography-mass spectrometry; LOQ, limit of quantification.
have been determined for a number of drugs in pregnant sheep. The most commonly used method is the two-compartment open model proposed by Szeto et al. (1982a). This technique involves paired maternal and fetal intravenous infusions of the drug to steady state and collection of paired maternal and fetal plasma samples. From the infusion rates of the drug and the maternal and fetal steady-state drug concentrations, maternal and fetal placental (i.e., bidirectional) and nonplacental clearance values can be estimated. To date, this method has been used with morphine and methadone (Szeto et al., 1982b), acetaminophen (Wang et al., 1986), metoclopramide (Riggs et al., 1990) and DPHM (Yoo et al., 1993). With the exception of acetaminophen, all of the compounds studied thus far have exhibited a higher value for CL_{fm} compared to CL_{nf}. This has also been found with labetalol using a different method to estimate maternal and fetal transplacental clearances (Yeleswaram et al., 1993). Given that the placental transfer of all these drugs appears to occur by passive diffusion and that CL_{nf} should equal CL_{fm} (see Discussion for further comment on this point), these findings are surprising. Yoo et al. (1993) reported that the magnitude of the CL_{fm} − CL_{nf} difference is linearly related to the fetal placental clearance. Of drugs studied to date, the greatest difference between fetal and maternal placental clearance occurs with the histamine antagonist DPHM, with the fetal clearance value being 3.7 times that determined in the ewe (Yoo et al., 1993). However, no explanation for this phenomenon appears to have been provided in the literature. The weight-normalized DPHM nonplacental clearance in the fetal lamb is also higher than the corresponding maternal value, but the routes of fetal nonplacental elimination have not been totally elucidated. This is also the case for other drugs that have been studied in pregnant sheep.

We consider that there could be at least two possible explanations for the higher value of fetal placental clearance. One relates to a methodological issue. Ideally, the two-compartment model experimental protocol should use simultaneous maternal and fetal drug infusions. However, this requires a stable isotope-labeled analog of the drug and an analysis method to quantify both forms of the drug when present together in biological fluids. This was not possible in previous studies, and time-separated maternal and fetal drug administration was carried out. Although the order of drug administration was randomized, it seems possible that with the rapid growth and maturation of the fetus in late gestation, the time separation of the maternal and fetal infusions could have artifactually affected the clearance estimates. The other possibility involves fetal hepatic first-pass uptake of a portion of the drug transferred to the fetus via placenta. Drug administered to the mother reaches the fetus via the umbilical vein (UV), and ~50% of UV flow enters the fetal liver before reaching the fetal systemic circulation (Holzman, 1984). If fetal hepatic drug uptake was significant, the fetal systemic availability and plasma concentration of maternally administered drug reaching the fetal systemic circulation would be reduced, and this would result in an underestimation of maternal placental clearance. Recently, however, we reported that there is no detectable hepatic first-pass uptake of DPHM from the UV blood in the fetal lamb in a study involving simultaneous UV and tarsal venous (TV) administration of unlabeled and deuterium-labeled DPHM and measurement of the two forms of drug in fetal systemic circulation (Tonn et al., 1996).

The overall objective of the current study was to determine the reason or reasons for the difference in maternal and fetal placental clearances and to elucidate the components of high fetal nonplacental clearance for DPHM. Because this drug exhibits the greatest CL_{fm} − CL_{nf} difference of all drugs studied in pregnant sheep, an explanation for this difference could also be relevant to other compounds. To achieve this objective, we reassessed maternal and fetal clearances of DPHM using simultaneous maternal and fetal infusions of unlabeled and stable isotope-labeled drug. We also reexamined fetal hepatic first-pass uptake of DPHM from the UV blood by measuring the fetal plasma concentrations of the labeled and unlabeled forms of a DPHM metabolite, DPMA, which has a very low placental permeability in sheep compared to the parent drug. Using these data, the contribution of fetal hepatic clearance to overall CL_{fm} was assessed; in addition, the maternal and fetal renal clearances of DPHM and DPMA were measured. Finally, we assessed the impact of fetal hepatic first-pass uptake of maternally derived DPHM on the estimates of maternal and fetal clearance parameters calculated using the two-compartment open model.

**Methods**

**Animals and Surgical Preparation**

This study was approved by the University of British Columbia Animal Care Committee, and all procedures performed on the sheep conformed to the guidelines of the Canadian Council on Animal Care. Five pregnant Dorset Suffolk cross-bred ewes, with a maternal body weight of 70.5 ± 3.4 kg (mean ± S.E.M.), were surgically prepared at 119 to 127 days gestation (122 ± 1 day, term − 145 days). Surgery was performed aseptically under halothane (1–2%) and nitrous oxide (60%) anesthesia (balance O2), after induction of anesthesia with intravenous sodium pentothal (1 g) and intubation of the ewe. Silicon rubber catheters (Dow Corning, Midland, MI) were implanted in PA and CA, common UV and lateral tarsal veins, trachea, urinary bladder (via a suprapubic incision) and the amniotic cavity. Electrodes (Cooper Corporation, Chatsworth, CA) were implanted biaxially on the dura to record the fetal electrocorticogram. In four animals, a transit-time 4SB blood flow transducer (Transonic Systems, Ithaca, NY) was placed around the common umbilical artery to measure umbilical blood flow. Catheters were also implanted in a maternal femoral artery (MA) and maternal femoral vein (MV). The catheters, electrodes and flow cables were tunneled subcutaneously to a small incision on the flank of the ewe where they exited. They were stored in a denim pouch when not in use. Each vascular catheter was flushed daily with ~2 ml of sterile 0.9% sodium chloride containing 12 units of heparin/ml to maintain catheter patency. Intramuscular injections of 500 mg of ampicillin and 80 mg of gentamicin were administered to the ewe on the day of surgery and for 3 days after surgery. Ampicillin (500 mg) and gentamicin (40 mg) were administered via the amniotic cavity immediately after surgery and then daily thereafter. After surgery, animals were kept in holding pens with other sheep and were given free access to food and water. The sheep were allowed to recover for 4 to 8 days before experimentation. On the morning of the experiment, a Foley bladder catheter was inserted via the urethra of the ewe and attached to a sterile polyvinyl bag for cumulative urine collection.

**Experimental Protocol**

Experiments were conducted at 125 to 133 days (128.8 ± 1.4 days) (term 145 days gestation). Before each experiment, DPHM · HCl
(Sigma Chemical, St. Louis, MO) and [2H10]DPHM · HCl (synthesized and purified in our laboratory; Tonn et al., 1993) were weighed to obtain the correct dose for administration. The weighed doses were dissolved in sterile 0.9% sodium chloride for injection and then filtered through a 0.22-μm nylon syringe filter (MSI, Westboro, MA) into a capped sterile injection vial.

Two types of experiments (studies 1 and 2) were carried out on this group of animals, as described below.

**Study 1: Fetal isotope effect studies.** These studies were conducted on two animals to check for the presence of any isotope effects in the disposition of [2H10]DPHM and the metabolite [3H10]DPMA compared with DPHM and DPMA. Equimolar doses of DPHM and [2H10]DPHM were simultaneously administered via the TV catheter as a 2.0-mg loading dose followed immediately by a 90-min infusion (60 μg/min). Serial samples were collected at −5, 5, 15, 30, 45, 60, 75 and 90 min from the FA and CA (1.5 ml each) and MA (3.0 ml) catheters. Amniotic fluid and fetal urine samples (3.0 ml) were also collected at −5, 30, 60 and 90 min.

**Study 2: Paired maternal-fetal infusions.** Simultaneous infusions of DPHM and [2H10]DPHM to the ewe and fetus, respectively, were carried out on all five sheep. In the two animals (animals E2241 and E2181) used for the isotope effect study, the paired maternal-fetal infusions were carried out 72 hr later. DPHM was administered as a 20-mg intravenous loading dose over 1.0 min, followed immediately by an infusion of 670 μg/min via the TV. Simultaneously, a 5.0-mg intravenous loading dose of [2H10]DPHM was given via the TV over 1.0 min, followed by an infusion of the compound at 170 μg/min. Simultaneous blood samples were collected from the FA (1.5 ml) and MA (3.0 ml) catheters at −5, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360 min during the infusion and at 30, 60, 120, 180, 240 and 360 min and 8, 12, 18, 24, 30 and 40 hr after the infusion. FA samples (0.6 ml) were also collected at the same time intervals for blood gas analysis and measurement of glucose and lactate concentrations. CA and UV blood samples (1.5 ml) were collected at −5, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360 min during the infusion period. All fetal blood removed for sampling was replaced at intervals during the experiment by an equal volume of maternal blood obtained before the start of the experiment. Amniotic and tracheal fluid (3.0 ml) and maternal urine (10 ml) samples were obtained at −5, 30, 60, 120, 180, 240, 300 and 360 min during the infusion and at 60, 120, 180, 240 and 360 min and 8, 12, 18, 24, 30 and 40 hr postinfusion. Maternal and fetal blood samples collected for drug analysis were placed into heparinized 10-ml Vacutainer tubes (Becton-Dickinson, Rutherford, NJ) and gently mixed. These samples were then centrifuged at 2000 × g for 10 min. The plasma supernatant was removed and placed into clean borosilicate test tubes that were capped with polytetrafluoroethylene-lined caps. Amniotic fluid and urine samples were also placed into clean borosilicate test tubes. All samples were stored frozen at −20°C until the time of analysis (<3 months from sample collection).

**Study 3: Simultaneous fetal UV and tarsal venous administration.** In addition to the studies described above, data that we recently obtained using samples collected from eight animals used in our previous study of fetal DPHM hepatic first-pass uptake (Tonn et al., 1996) are reported here. As noted in the introduction, ~50% (range, 30–80%) of the UV flow traverses the fetal liver before reaching the fetal systemic circulation (Edestone et al., 1978). Moreover, UV provides a major vascular input into the fetal liver, supplying ~93% and ~60% of the total blood flow to the left and the right and caudate lobes, respectively (Edestone et al., 1978; Holzman, 1984). Thus, the drug present in UV could undergo a "partial" first-pass hepatic uptake before entering the fetal circulation if the fetal liver was sufficiently active in metabolizing the drug (fig. 1). The surgical preparation and experimental protocol used in these animals have been previously fully described (Tonn et al., 1996). The surgery was similar to that described above, with the exception that the fetus and maternal bladder catheters were not used. To assess the fetal first-pass hepatic uptake for DPHM, we used simultaneous but separate bolus injections of [2H10]DPHM and DPHM (5.0 mg each) via the common UV and TV (which drains directly into the inferior vena cava) or 90-min intravenous infusions of the compounds (60 μg/min each, preceded by a 2.0 mg intravenous bolus of each) via the same routes. This was coupled with collection of fetal arterial plasma for the measurement of [2H10]DPHM and DPHM concentrations. In the present study, fetal plasma samples remaining from four of these fetal bolus and four of the infusion studies were used for the measurement of [2H10]DPMA and DPMA concentrations.

**Physiological Recording and Monitoring Procedures**

From ≥24 hr before to ≥24 hr after the infusion period, fetal amniotic, tracheal and FA pressures, heart rate, electrocortical activity and urine production rate were continuously monitored. However, these data will be reported separately. In the animals with an implanted umbilical flow transducer, UV blood flow was measured with a Transonic model T201 transit-time flowmeter (Transonic Systems, Inc., Ithaca, NY). Fetal urine flow rate was estimated using a computer-controlled roller pump assembly developed in our laboratory. The fetal bladder catheter was allowed to drain by gravity into a sterile reservoir (10-ml syringe barrel) to which a disposable DTX transducer was connected. When the pressure in the reservoir increased above a preset level (usually 3 mm Hg) due to urine collection, the computer activated a roller pump (DIAS Ex154, DIAS Inc., Kalamazoo, MI), which pumped a calibrated volume of urine from the reservoir back to the amniotic cavity (via the amniotic catheter) during control periods. During the experimental period, the urine was collected into a sterile sample collection syringe, and at hourly intervals a 5-ml aliquot was taken, with the remainder of the urine returned to the amniotic cavity. The cumulative volume

![Fig. 1. Sketch of the fetal circulation showing the position of fetal liver and sites of drug administration for assessment of fetal first-pass hepatic drug uptake from UV (study 3).](image-url)
pumped/min, which equals fetal urine production/min, was stored on disk.

Blood pH, PO2, and Pco2 were measured using an IL 1306 pH/blood gas analyzer (Allied Instrumentation Laboratory, Milan, Italy). Blood O2 saturation and hemoglobin concentration were determined using a Hemoximeter (Radiometer, Copenhagen, Denmark). Blood glucose and lactate concentrations were determined with a 2300 STAT plus glucose/lactate analyzer (Y.S.I. Inc., Yellow Springs, OH).

**Plasma Protein Binding of DPMA in Fetal and Maternal Plasma**

The plasma protein binding of DPMA was measured in vitro in fetal and maternal plasma using equilibrium dialysis as described by Tonn et al. (1995). Maternal and fetal plasma for these measurements was obtained from two additional sheep in our laboratory set up for other experiments, and the drug-free plasma was obtained on nonexperimental days.

**Drug and Metabolite Analysis**

The concentrations of DPHM and [2H10]DPHM in all biological fluids collected were measured using a previously developed GC-MS assay capable of simultaneously measuring DPHM and [2H10]DPHM (LOQ = 2 ng/ml each; Tonn et al., 1993). The concentrations of DPMA and its stable isotope labeled analog, [2H10]DPMA, were measured simultaneously using another GC-MS method, which was also developed in our laboratory (LOQ = 2.5 ng/ml each; Tonn et al., 1995).

**Pharmacokinetic Analysis**

**Study 2: Paired maternal-fetal infusions.** The fetal and maternal plasma concentration-time data from this study were fit separately to a two-compartment open model with elimination occurring from the central compartment (Gibaldi and Perrier, 1982). The data were fit using ADAPT II pharmacokinetic modeling program and a maximum likelihood fitting algorithm (variance model for Cp: Var = δ(Cp) + γ, where δ and γ are estimated variance parameters) (D’Argenio and Schumitzky, 1992). Plasma concentration at time 0 (Cp0) was extrapolated from the fitted equation.

The maternal and fetal steady-state arterial plasma DPHM and [2H10]DPHM concentration data obtained from study 2 were treated according to a two-compartment open model to estimate the placental and nonplacental clearance parameters of DPHM and [2H10]DPHM in the ewe and fetus, respectively. This model assumes steady-state plasma concentrations and drug elimination from both the maternal and fetal compartments (Szeto et al., 1982a). Clearances were calculated from the equations 1 to 6 as previously described (Szeto et al., 1982a).

\[
\text{CL}_{\text{mm}} = \frac{k_e}{[C_{\text{mm}} - C_{\text{fo}}*(C_{\text{mm}}/C_{\text{fo}})]} 
\]

\[
\text{CL}_{\text{m}} = \frac{k_e}{[C_{\text{fo}} - C_{\text{mm}}*(C_{\text{fo}}/C_{\text{mm}})]} 
\]

\[
\text{CL}_{\text{mf}} = \text{CL}_{\text{mf}}^{*} \frac{C_{\text{fo}}}{C_{\text{mm}}} 
\]

\[
\text{CL}_{\text{fm}} = \text{CL}_{\text{fm}}^{*} \frac{C_{\text{mm}}}{C_{\text{fo}}} 
\]

\[
\text{CL}_{\text{mm}} = \text{CL}_{\text{mm}}^{*} - \text{CL}_{\text{mf}} 
\]

\[
\text{CL}_{\text{fm}} = \text{CL}_{\text{fm}}^{*} - \text{CL}_{\text{mf}} 
\]

The symbols \(k_e\) and \(k_e^{*}\) denote the drug infusion rates to the mother (DPHM) and fetus ([2H10]DPHM), respectively.

Fetal DPHM placental clearance were also calculated using the Fick method in two animals in which umbilical blood flow and the concentrations of DPHM and [2H10]DPHM were measured in paired samples of FA and fetal UV plasma. The purpose was to assess whether placental metabolism could contribute to the high fetal nonplacental clearance of the drug (Yoo et al., 1993) because with the two-compartment model, placental drug metabolism on the fetal side of placenta would appear as fetal nonplacental clearance (Szeto et al., 1982a). The equation for Fick estimation is given below:

\[
\text{CL}_{\text{mm}} = Q_{\text{um}} \frac{[\text{[2H}_{10}\text{]DPHM}]_{\text{FA}} - [\text{[2H}_{10}\text{]DPHM}]_{\text{UV}}}{[\text{[2H}_{10}\text{]DPHM}]_{\text{FA}}} 
\]

where \(Q_{\text{um}}\) is the umbilical blood flow, and \([\text{[2H}_{10}\text{]DPHM}]_{\text{FA}}\) and \([\text{[2H}_{10}\text{]DPHM}]_{\text{UV}}\) refer to [2H10]DPHM concentrations in FA and fetal UV.

The fetal first-pass hepatic extraction ratio for the maternally derived DPHM in UV was indirectly estimated (assuming sole hepatic formation of the DPMA and [2H10]DPMA in the fetus as well as no placent al transfer of the DPMA metabolite formed in the mother) as follows (see Appendix for theoretical basis and derivation):

\[
\text{ER} = \frac{(\text{AUC}_{\text{DPMA}}/\text{AUC}_{\text{DPHM}}) - (\text{AUC}_{\text{[2H10]DPMA}}/\text{AUC}_{\text{[2H10]DPHM}})}{(\text{AUC}_{\text{DPMA}}/\text{AUC}_{\text{DPHM}})} 
\]

where AUC_{DPMA}, AUC_{[2H10]DPMA}, AUC_{DPHM} and AUC_{[2H10]DPHM} are the FA AUCs of DPHM, [2H10]DPHM, DPMA and [2H10]DPMA, respectively. DPHM was infused to the mother and hence reaches the fetus via the UV, whereas [2H10]DPHM was directly infused to the fetus via the TV. In this study (study 2), all AUCs were estimated from time 0 to infinity.

The renal clearance values for DPHM, DPMA, [2H10]DPHM and [2H10]DPMA in the ewe and the fetus were calculated using the following equations (Gibaldi and Perrier, 1982):

\[
\text{CL}_{\text{ren}(\text{DPHM or [2H10]DPHM})} = \frac{X_u^{*}}{\text{AUC}_{\text{0-}\infty}(\text{DPHM or [2H10]DPHM})} 
\]

\[
\text{CL}_{\text{ren}(\text{DPMA or [2H10]DPMA})} = \frac{X_u^{*}}{\text{AUC}_{\text{0-}\infty}(\text{DPMA or [2H10]DPMA})} 
\]

where \(X_u^{*}\) is the total cumulative amount of DPHM or DPMA excreted in the urine, and AUCs refer to respective area under the plasma concentration-time curve of drug or metabolite in the ewe or the fetus.

The contribution of maternal DPHM and fetal [2H10]DPHM renal elimination to respective DPHM nonplacental clearance was calculated as the ratio of their renal clearance values to nonplacental clearance values (CL_{mm} and CL_{fa}, respectively). The percentage of administered maternal DPHM and fetal [2H10]DPHM dose excreted as DPMA and [2H10]DPMA in maternal and fetal urine, respectively, was calculated from the ratio of cumulative amount of unlabeled or labeled DPMA (corrected for mass difference between parent drug and metabolite) excreted in the urine at time infinity to the administered DPHM or [2H10]DPHM dose.

**Study 3: Simultaneous fetal UV and TV administration:** For these fetal hepatic first-pass experiments, fetal systemic availability of DPHM (or [2H10]DPHM) after UV administration was calculated from the parent drug data as previously described (Tonn et al., 1996):

\[
F = \frac{\text{AUC}_{\text{parent}(\text{UV})}}{\text{AUC}_{\text{parent}(\text{TV})}} 
\]

where respective AUC values refer to AUC of parent drug after UV or TV administration.

Fetal hepatic extraction ratio of DPHM (or [2H10]DPHM) after UV administration was calculated as:

\[
\text{ER} = 1 - F 
\]

In these experiments, the fetal hepatic extraction ratio of DPHM (or [2H10]DPHM) was also calculated from the metabolite (DPMA and [2H10]DPMA) data in an analogous fashion to equation 8 as...
Drug Clearance in the Fetal Lamb

follows:

\[
ER = \frac{(AUC_{\text{metabolite}}(UV)/AUC_{\text{parent}}(UV)) - (AUC_{\text{metabolite}}(TV)/AUC_{\text{parent}}(TV))}{(AUC_{\text{metabolite}}(UV)/AUC_{\text{parent}}(UV))}
\]

(13)

where \(AUC_{\text{metabolite}}(UV)\) and \(AUC_{\text{metabolite}}(TV)\) refer to the AUCs of the metabolite after UV and TV administration of the parent drug, respectively. In this study (study 3), all AUCs were calculated from time 0 to the time of the last sampling point.

Fetal systemic availability using this extraction ratio data was also calculated using equation 12.

The AUCs \((AUC_{0-\infty} \text{ or } AUC_{\text{total}})\) for parent drug or metabolite in all experiments were calculated using the linear trapezoidal method (Gibaldi and Perrier, 1982).

Statistical analysis. All values are reported as the mean ± S.E.M. The achievement of steady state was determined using three groups of mean concentration values (i.e., 150 and 240 and 270, and 330 and 360 min) with a repeated-measures analysis of variance (Zar, 1984). A paired \(t\) test was used to test for differences between fetal and maternal pharmacokinetic parameters. The significance level was \(P < 0.05\) in all cases. Fetal weight in utero at the time of experimentation was estimated from the weight at birth and the time interval between the experiment and birth (Koong et al., 1975).

Results

Study 1: Fetal Isotope Effect Studies

In two experiments, DPHM and \(^{[2H_{10}]}\)DPHM were simultaneously infused via the TV for 90 min to determine possible differences in the disposition of two forms of the drug. The overall mean steady-state plasma concentrations of DPHM and \(^{[2H_{10}]}\)DPHM in the two animals were 183.6 ± 30.9 and 182.4 ± 30.3 ng/ml, respectively. For DPMA and \(^{[2H_{10}]}\)DPMA, steady-state concentrations were not achieved during the infusion, but there were no apparent differences between the AUCs for DPMA (1598.2 ng.min/ml) and \(^{[2H_{10}]}\)DPMA (1429.5 ng.min/ml). In addition, no differences were observed in the concentrations of DPHM and \(^{[2H_{10}]}\)DPHM in maternal plasma (10.4 ± 0.5 vs. 11.0 ± 0.5 ng/ml), AUCs in amniotic fluid (1054.8 vs. 1101.0 ng.min/ml) and fetal urinary concentrations at the end (90 min) of the infusion (2944.0 vs. 2874.3 ng/ml). Overall, the data do not indicate any significant isotope effect in the disposition of labeled forms of DPHM and DPMA.

Study 2: Paired Maternal/Fetal Infusions

The five experiments involving the simultaneous 6-hr infusions of DPHM and \(^{[2H_{10}]}\)DPHM to ewe and fetus, respectively, were carried out at 125 to 133 days gestation (128.8 ± 1.4 days). Estimated fetal weight was 2.46 ± 0.09 kg. During the control period, the fetal femoral arterial values for pH, \(P_{O_2}\), \(P_{CO_2}\), \(O_2\) saturation, hemoglobin, glucose and lactate concentrations were 7.36 ± 0.02, 22.6 ± 1.65 mm Hg, 47.3 ± 0.5 mm Hg, 55.3 ± 23.8%, 10.0 ± 0.3 g/dl, 0.98 ± 0.09 mM and 0.70 ± 0.11 mM, respectively. There were no consistent changes in any of these variables during or after the infusion period. Likewise umbilical blood flow (281 ± 39 ml/min/kg, \(n = 3\)) was not consistently altered during the experiment.

Maternal and fetal plasma DPHM and \(^{[2H_{10}]}\)DPHM concentrations and placental and nonplacental clearance values. The average plasma concentrations of DPHM and \(^{[2H_{10}]}\)DPHM in MA and FA plasma are illustrated in figure 2. They reached a plateau at ~120 min, and there were no statistical differences between the plasma concentrations at 150 and 180, 240 and 270, and 330 and 360 min, suggesting the achievement of steady state by 150 min. Thus, mean steady-state concentration values used for subsequent calculations were taken from 150 to 360 min. The mean steady-state concentrations of DPHM were 260.8 ± 18.9 and 45.6 ± 17.4 ng/ml in MA and FA plasma, respectively, whereas the mean concentrations of \(^{[2H_{10}]}\)DPHM in the same vessels were 44.6 ± 5.8 and 244.0 ± 42.4 ng/ml, respectively. The total MA and FA steady-state concentrations of DPHM (i.e., labeled and unlabeled DPHM) were 305.4 ± 24.4 and 289.6 ± 57.6 ng/ml. In the four fetuses in which there was a functional CA catheter, the mean concentrations of \(^{[2H_{10}]}\)DPHM (infused via the tarsal vein) were 203.7 ± 29.9 and 186.1 ± 27.0 ng/ml in FA and CA plasma, respectively. The mean FA-CA concentration difference of 17.5 ± 6.1 ng/ml was significantly different from 0. In contrast, the concentrations of DPHM (infused to the ewe) in FA and CA plasma averaged 27.2 ± 4.4 and 26.6 ± 4.3 ng/ml in these four animals and were not significantly different. As in previous studies (Rurak et al., 1991), there was accumulation of DPHM (both labeled and unlabeled) in fetal lung and amniotic fluids. The average drug concentration ratio between lung fluid and FA.

![Fig. 2. Plasma concentrations of DPHM and \(^{[2H_{10}]}\)DPHM in MA and FA plasma during and after simultaneous intravenous infusions of DPHM (670 \(\mu g/min\)) to the ewe and of \(^{[2H_{10}]}\)DPHM (170 \(\mu g/min\)) to the fetus. O, Maternal DPHM; □, maternal \(^{[2H_{10}]}\)DPHM; ●, fetal DPHM; ■, fetal \(^{[2H_{10}]}\)DPHM.](image-url)
plasma was 4.0 ± 1.7 for DPHM and 4.5 ± 1.6 for \[^{12}H_{10}\]DPHM, whereas the corresponding ratios in amniotic fluid (i.e., amniotic fluid/FA) were 0.6 ± 0.2 and 0.8 ± 0.2 for labeled and unlabeled drug, respectively. After the infusion, concentrations of DPHM and \[^{12}H_{10}\]DPHM in all fluids declined rapidly with a terminal elimination half-life in plasma of 70.5 ± 6.9 and 51.8 ± 7.2 min in the ewe and fetus, respectively.

The nonplacental and transplacental plasma clearance parameters calculated in the mother and the fetus are shown in table 1. The weight-normalized estimates of CL\(_{fm}\) (214.4 ± 30.8 ml/min/kg), CL\(_{fo}\) (109.8 ± 22.3 ml/min/kg) and CL\(_{mf}\) (324.2 ± 46.6 ml/min/kg) were all significantly higher than the corresponding maternal values for CL\(_{mf}\) (50.3 ± 13.2 ml/min/kg), CL\(_{mm}\) (36.6 ± 1.9 ml/min/kg) and CL\(_{mo}\) (38.4 ± 2.3 ml/min/kg). The nonplacental contribution to total body clearance averaged 95.5 ± 0.9% and 33.4 ± 4.1% in ewe and fetus, respectively, and again these were significantly different. In ewes 122z and 2181, the presence of an umbilical arterial flow probe and functional UV catheter also allowed calculation of the fetal transplacental clearance value using the Fick principle (equation 7). The umbilical extraction ratio for \[^{12}H_{10}\]DPHM averaged 0.58 ± 0.02, which is consistent with uptake from fetus to placenta. Mean umbilical blood flow in these animals was 281 ± 39 ml/min/kg, and the Fick estimate of CL\(_{fm}\) averaged 140.0 ± 4.2 ml/min/kg. The two-compartment estimate of CL\(_{fm}\) in these two animals was 183.2 ± 8.6 ml/min/kg; however, because of the low n value, a statistical comparison of the estimates was not possible. The clearance parameters were also calculated in four animals using the CA reference drug concentrations rather than FA concentrations. The mean values for CL\(_{mf}\), CL\(_{mm}\), CL\(_{mo}\) and CL\(_{fo}\) thus obtained compared with those from FA drug concentrations in these four animals were 39.8 ± 8.2 vs. 38.4 ± 7.5, 253.2 ± 24.3 vs. 234.6 ± 30.0, 35.6 ± 2.1 vs. 35.6 ± 2.1 and 140.7 ± 32.8 vs. 118.4 ± 26.5 ml/min/kg, respectively. Although the estimates of CL\(_{mm}\) and CL\(_{fo}\) using CA concentrations are higher than those calculated using the FA drug concentrations, the differences are not statistically significant.

Fetal and maternal DPMA and \[^{12}H_{10}\]DPMA plasma concentrations. A mean plasma concentration-vs.-time plot of DPMA and \[^{12}H_{10}\]DPMA in MA and FA plasma is shown in figure 3. Although concentrations of DPHM and \[^{12}H_{10}\]DPHM reached steady state at ~120 min from the start of the infusion (fig. 2), the plasma levels of DPMA and \[^{12}H_{10}\]DPMA did not reach steady state during the entire duration of infusion and continued to increase for 30 to 120 min after infusion. At all time points during the infusion period, the concentration of \[^{12}H_{10}\]DPMA was higher in the fetus than in the mother, whereas for the unlabeled metabolite the situation was reversed. The peak concentrations of DPMA in maternal and fetal plasma averaged 137.4 ± 18.5 and 92.8 ± 16.8 ng/ml, respectively, whereas the peak maternal and fetal plasma concentrations of \[^{12}H_{10}\]DPMA were 28.7 ± 4.3 and 135.0 ± 20.1 ng/ml, respectively. The maternal-fetal concentration differences for both forms of the metabolite were significantly different from 0. The time at which the peak levels occurred postinfusion was 18.0 ± 7.3 min for both labeled and unlabeled DPMA in the ewe, whereas in the fetus the value was 87.0 ± 14.5 min. After the peak, the fetal metabolite levels declined much more slowly than in the ewe.
The elimination half-life of the metabolite in the fetus and ewe (determined by simultaneous fitting of the parent drug and metabolite data) was 15.2 ± 2.5 and 3.0 ± 0.2 hr, respectively. These values are significantly different. In the two animals with functional UV catheters, the extraction ratio of $[^{2}H_{10}]$DPMA across the fetal side of the placenta averaged 0.06 ± 0.01. This value is not significantly different from 0 but is different from the umbilical extraction ratio for $[^{2}H_{10}]$DPHM given above. Finally, DPMA or $[^{2}H_{10}]$DPMA metabolites were never detected in amniotic or fetal lung fluid.

Table 2 gives the AUC values for DPHM, $[^{2}H_{10}]$DPHM, DPMA and $[^{2}H_{10}]$DPMA in MA and FA plasma. The fetal AUC$_{DPMA}$/AUC$_{DPHM}$ ratio (8.20 ± 1.62) was significantly higher than the corresponding AUC ratio for $[^{2}H_{10}]$DPMA/$[^{2}H_{10}]$DPHM (2.24 ± 0.53). In the ewe, however, there was the opposite situation. The AUC$_{DPMA}$/AUC$_{DPHM}$ ratio (0.62 ± 0.07) was significantly less than the corresponding ratio for $[^{2}H_{10}]$DPMA/$[^{2}H_{10}]$DPHM (0.96 ± 0.12), although the magnitude of the difference is much smaller than that in the fetus. Table 2 also gives the estimates of fetal hepatic first-pass extraction ratio for maternally derived DPHM calculated using equation 8, with the mean value being 0.71 ± 0.07.

**Fetal and maternal renal elimination of DPHM, $[^{2}H_{10}]$DPHM, DPMA and $[^{2}H_{10}]$DPMA.** The renal clearances of DPHM, $[^{2}H_{10}]$DPHM, DPMA and $[^{2}H_{10}]$DPMA in the ewe and fetus are given in table 3. DPMA and $[^{2}H_{10}]$DPMA were both present in adult urine during and after the infusion. In contrast, only very small quantities were detected in fetal urine. The cumulative excretion plot for DPHM and $[^{2}H_{10}]$DPHM in maternal and fetal urine clearly shows a plateau after the infusion (fig. 4A), whereas the metabolite appears to be near a plateau only at the end of the experimental protocol (fig. 4B). Thus, cumulative excretion of the metabolite may have been somewhat underestimated. The weight-corrected renal clearance of DPHM was ~200-fold less in adult sheep compared with fetal lambs, whereas the opposite situation existed for the metabolite (relative renal clearance ~50-fold greater in mother than in fetus). The contribution of renal DPHM clearance to maternal nonplacental clearance was 0.025 ± 0.011%, whereas in the fetus the contribution was 2.22 ± 0.42% (table 3). These values are significantly different. The percentage of total maternal DPHM and fetal $[^{2}H_{10}]$DPHM dose excreted as DPMA and $[^{2}H_{10}]$DPMA, respectively, averaged 0.95 ± 0.07% in the ewe and 0.014 ± 0.011% in the fetus. These values are also significantly different from each other.

**Study 3: Simultaneous Fetal UV and TV Administration**

Figure 5 illustrates the concentration-vs.-time plots for unlabeled and labeled forms of DPHM and DPMA measured from samples obtained in our previous study of fetal hepatic first-pass DPHM uptake after UV administration (Tonn et al., 1996). Figure 5A shows the data from a bolus experiment in which $[^{2}H_{10}]$DPHM was administered via the UV, whereas figure 5B shows the data from an infusion study in which unlabeled DPHM was infused via the umbilical route. In both experiments, there were no consistent differences in the fetal arterial concentrations of DPHM and $[^{2}H_{10}]$DPHM. In contrast, the plasma concentration of the form of DPMA derived from the drug administered via the UV was consistently higher than that derived from drug given via the TV. Table 4 gives the FA plasma AUC values for labeled and unlabeled DPHM and DPMA. The estimates of fetal first-pass hepatic extraction ratio based on intact drug concentrations were obtained using equations 11 and 12, and the mean value of $0.066 ± 0.006$ is not significantly different from 0, as reported previously (Tonn et al., 1996). In contrast, the estimates of fetal hepatic extraction ratio obtained using the AUC values for DPMA and $[^{2}H_{10}]$DPMA and equation 13 indicated significant drug uptake by the fetal liver. The mean extraction ratio was 0.44 ± 0.05, and this was significantly lower than the value of 0.71 ± 0.07 obtained above in the paired maternal-fetal infusion protocol (table 2).

**Fetal and Maternal Plasma Protein Binding of DPMA**

The time required to reach equilibrium for the determination of the binding of DPMA was 8 hr. No significant volume shifts were associated with this equilibrium time, and no nonspecific binding of DPMA to the equilibrium dialysis cell and the membrane could be detected. The metabolite was highly bound in both maternal and fetal plasma, with the percent bound averaging 99.4 ± 0.01% and 98.9 ± 0.07%, respectively.
TABLE 2
MA and FA AUC values for unlabeled and labeled forms of DPHM and DPMA, metabolite/parent drug AUC ratios and estimates of fetal hepatic extraction of the maternally derived DPHM in five maternal-fetal paired infusion experiments

<table>
<thead>
<tr>
<th>Ewe No.</th>
<th>Site</th>
<th>DPHM</th>
<th>[(^{2}\text{H}_0)]DPHM</th>
<th>DPMA</th>
<th>[(^{2}\text{H}_0)]DPMA</th>
<th>DPMA/DPHM</th>
<th>[(^{2}\text{H}_0)]DPMA/[(^{2}\text{H}_0)]DPHM</th>
<th>Fetal hepatic first-pass extraction ratio for DPHM</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2101</td>
<td>MA</td>
<td>87,278.0</td>
<td>12,645.7</td>
<td>62,067.8</td>
<td>14,522.5</td>
<td>0.71</td>
<td>1.15</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>FA</td>
<td>7,270.7</td>
<td>87,988.8</td>
<td>95,138.8</td>
<td>140,002.3</td>
<td>13.08</td>
<td>1.59</td>
<td></td>
</tr>
<tr>
<td>E122Z</td>
<td>MA</td>
<td>95,396.9</td>
<td>14,089.4</td>
<td>40,862.4</td>
<td>12,114.4</td>
<td>0.43</td>
<td>0.86</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>FA</td>
<td>16,805.3</td>
<td>83,072.2</td>
<td>110,297.6</td>
<td>279,327.6</td>
<td>6.56</td>
<td>3.36</td>
<td>0.49</td>
</tr>
<tr>
<td>E2177</td>
<td>MA</td>
<td>88,534.1</td>
<td>17,516.2</td>
<td>44,028.7</td>
<td>14,709.4</td>
<td>0.50</td>
<td>0.84</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>FA</td>
<td>45,697.4</td>
<td>299,601.8</td>
<td>332,695.2</td>
<td>10.71</td>
<td>3.40</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>E2241</td>
<td>MA</td>
<td>128,464.1</td>
<td>26,465.9</td>
<td>84,048.4</td>
<td>17,131.6</td>
<td>0.81</td>
<td>1.29</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>FA</td>
<td>14,847.2</td>
<td>60,600.8</td>
<td>68,176.8</td>
<td>4.08</td>
<td>0.62</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td></td>
<td>98,876.7 ± 7,570.9</td>
<td>17,340.8 ± 2,426.4</td>
<td>61,577.4 ± 8,594.9</td>
<td>15,828.4 ± 1,444.6</td>
<td>0.62 ± 0.07</td>
<td>0.96 ± 0.12</td>
<td>0.71 ± 0.07</td>
</tr>
</tbody>
</table>

TABLE 3
Renal clearance values for DPHM, DPMA, \([\(^{2}\text{H}_0\)]\)DPHM and \([\(^{2}\text{H}_0\)]\)DPMA in the ewe and fetus, percent contribution of DPHM and \([\(^{2}\text{H}_0\)]\)DPHM renal clearances to maternal (\(\%\text{CL}_{\text{mo}}\)) and fetal (\(\%\text{CL}_{\text{fo}}\)) nonplacental clearance of the intact drug, respectively, and percent of administered dose (\(\%\text{dose}\)) excreted as DPMA or \([\(^{2}\text{H}_0\)]\)DPMA in maternal or fetal urine, respectively

<table>
<thead>
<tr>
<th>Ewe No.</th>
<th>Maternal(^a)</th>
<th>Fetal(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPHM</td>
<td>(%\text{CL}_{\text{mo}})</td>
</tr>
<tr>
<td>2101</td>
<td>N.D.</td>
<td>1.98</td>
</tr>
<tr>
<td>1222</td>
<td>0.009</td>
<td>1.00</td>
</tr>
<tr>
<td>2177</td>
<td>0.023</td>
<td>0.52</td>
</tr>
<tr>
<td>2181</td>
<td>0.014</td>
<td>0.54</td>
</tr>
<tr>
<td>2241</td>
<td>0.001</td>
<td>0.42</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>0.012 ± 0.005</td>
<td>0.026 ± 0.011</td>
</tr>
</tbody>
</table>

\(^a\) All maternal renal clearance values (ml/min) are normalized to maternal weight.

\(^b\) All fetal renal clearance values (ml/min) are normalized to fetal weight.

\(^c\) Significantly different from the corresponding maternal value.

N.D., not detectable.
respectively. The free fraction in adult plasma (0.006 ± 0.002) was significantly less than that in fetal plasma (0.010 ± 0.001).

Discussion

Use of Stable Isotope-Labeled Compounds to Study Maternal-Fetal Drug Disposition

The current study appears to be the first in which a stable isotope-labeled drug analog has been used to study maternal-fetal drug disposition in pregnant sheep. The use of stable isotope-labeled compounds provides several advantages in studies of drug disposition during pregnancy, particularly in situations in which the simultaneous administration of the drug *via* two routes is to be used. As noted in the introduction, our primary reason for using this methodology in the current study was to eliminate the potential confounding effects of fetal growth and maturation that occur over the period between time-separated maternal and fetal drug infusions. This approach also reduces the overall duration of the experiment. This is an important factor in studies involving chronically instrumented pregnant animals in which there is a finite time window available for each preparation and, hence, shorter experiments allow for additional studies to be conducted on the same animal. However, it is important that the labeled and unlabeled forms of the drug be biologically equivalent (Baillie, 1981). If this is not so, the labeled drug could display different dispositional characteristics compared with the unlabeled drug and thus be of limited use in pharmacokinetic studies (Baillie, 1981). Consequently, it was first necessary to determine whether such an "isotope effect" existed for [2H10]DPHM in fetal sheep (study 1). The data obtained on the concentrations of DPHM, [2H10]DPHM, DPMA and [2H10]DPMA during and after simultaneous intravenous infusions of DPHM (670 μg/min) to the ewe and of [2H10]DPHM (170 μg/min) to the fetus.
TABLE 4

FA AUC values of labeled and unlabeled DPHM and DPMA after simultaneous but separate UV and TV administration of DPHM and [2H10]DPHM and estimates of fetal hepatic extraction ratio of DPHM in umbilical venous first-pass experiments

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>E889</td>
<td>Bolus</td>
<td>4,688.0</td>
<td>5,787.2</td>
<td>3,334.5</td>
<td>2,718.7</td>
<td>0.08</td>
<td>0.43</td>
<td>0.04</td>
<td>0.36</td>
</tr>
<tr>
<td>E2088</td>
<td>Bolus</td>
<td>4,272.0</td>
<td>5,802.0</td>
<td>6,952.5</td>
<td>2,820.8</td>
<td>0.23</td>
<td>0.50</td>
<td>0.23</td>
<td>0.50</td>
</tr>
<tr>
<td>E1142</td>
<td>Infusion</td>
<td>8,462.1</td>
<td>794.7</td>
<td>8,298.1</td>
<td>6,526.9</td>
<td>0.22</td>
<td>0.70</td>
<td>0.10</td>
<td>0.70</td>
</tr>
<tr>
<td>E1242</td>
<td>Infusion</td>
<td>3,240.8</td>
<td>521.1</td>
<td>3,826.8</td>
<td>1,468.8</td>
<td>0.01</td>
<td>0.37</td>
<td>0.10</td>
<td>0.51</td>
</tr>
</tbody>
</table>

In these animals, [2H10]DPHM was given via the UV, and DPHM was given via the TV.

In two of the animals, we also measured the fetal transplacental clearance of [2H10]DPHM via the Fick principle by using umbilical blood flow and the umbilical arteriovenous difference in drug concentrations (equation 7). This was done to assess one potential limitation of the two-compartment open model: the failure to take into account placental metabolism of the drug in question. If drug metabolism did occur in...
the fetal component of the placenta, the loss of drug by this route would be included in the calculation of fetal nonplacental clearance, thus resulting in an overestimation of the latter value. However, placental drug metabolism is considered to be of minor importance in the maternal-fetal unit (Juchau, 1982). In a previous study in pregnant sheep, the fetal placental clearance of acetaminophen estimated with the Fick method was not different from the clearance value calculated using the two-compartment open model (Wang et al., 1986). However, both $\text{CL}_{\text{fm}}$ (31 ml/min/kg) and $\text{CL}_{\text{fo}}$ (11 ml/min/kg) for acetaminophen are much lower than those for DPHM (table 1), so it was of interest to see whether the Fick and two-compartment placental clearance rates for the latter compound would be similar. Although the Fick estimates are lower than those obtained using the two-compartment model, the difference was not great. Moreover, if there was placental metabolism of DPHM, the Fick estimate of $\text{CL}_{\text{fm}}$ should have been higher than the two-compartment value, whereas the opposite situation was the case. Thus, for these two drugs with very different physicochemical properties and clearance values, placental drug metabolism does not appear to be of significance in terms of fetal drug elimination in pregnant sheep.

Maternal and fetal plasma concentrations of DPMA and $[\text{H}^{10}]\text{DPMA}$. In humans, monkeys and dogs, DPHM is thought to be metabolized via two sequential N-demethylation steps followed by deamination to DPMA. This DPMA metabolite and its conjugates are the major urinary metabolites of DPHM in these species (Chang et al., 1974; Drach et al., 1970; Drach and Howell, 1968; Glazko et al., 1974). DPMA is also present in the urine and plasma of nonpregnant ewes after DPHM administration (Tonn et al., 1995). In the present study, DPMA and $[\text{H}^{10}]\text{DPMA}$ were detected in both maternal and fetal plasma during and after the simultaneous infusions of DPHM and $[\text{H}^{10}]\text{DPHM}$ to the ewe and fetus, respectively. The consistently higher concentrations of the labeled metabolite in fetal plasma compared with those in the mother during the infusion period provide strong evidence for its formation in the fetus. The presence of DPMA in the fetus and $[\text{H}^{10}]\text{DPMA}$ in the ewe could be the result of two processes: (1) placental transfer of DPHM to the fetus and $[\text{H}^{10}]\text{DPHM}$ to the ewe, with subsequent formation of the unlabeled and labeled metabolites in fetal and maternal compartments, respectively; and (2) fetal-to-maternal transfer of $[\text{H}^{10}]\text{DPMA}$ and maternal-to-fetal transfer of DPMA. However, it seems unlikely that the latter process could be of much importance because the minimal umbilical extraction ratio of $[\text{H}^{10}]\text{DPMA}$ (−0.03) demonstrates that although organic cation tubular secretion is developed in utero during late gestation, renal elimination pathways for organic anions must develop some time after birth. The low fetal renal clearance of DPMA may explain the lack of measurable quantities in the amniotic fluid because fetal urine is a major source of amniotic fluid during late gestation (Brace, 1994). However, compounds in fetal blood may also reach amniotic fluid via the intramembranous pathway (Gilbert et al., 1995). The reason for the apparent lack of this transport

---

mechanism for DPMA may be its high degree of plasma protein binding in fetal plasma.

Studies 2 and 3: Fetal Hepatic Uptake and Metabolism of DPHM

Evidence of fetal hepatic first-pass DPHM uptake from UV. In a previous study (Tonn et al., 1996), we examined the fetal hepatic first-pass uptake of DPHM after UV drug administration after both bolus and constant-rate intravenous infusions and found no evidence for a first-pass effect. In contrast, a substantial (>90%) hepatic presystemic elimination of the drug was observed with mesenteric venous administration in adult sheep. However, the results from the fetal experiments did not completely rule out the involvement of fetal liver in DPHM metabolism/DPMA formation, and we recently demonstrated formation of the DPMA metabolite when fetal hepatic microsomal preparations are incubated with DPHM. The data from study 2 on DPHM/DPH (8.20 ± 1.62) and [3H10]DPMA/[3H10]DPHM AUC (2.24 ± 0.53) ratios in the fetus (table 2) indicate that more of the maternally derived form of the drug (reaching the fetus via the UV and hence undergoing a “partial” fetal hepatic first-pass) is converted to the metabolite than is the form administered directly to the fetus. These AUC ratios clearly demonstrate that the fetal liver is involved in the metabolism and nonplacental clearance of the drug. With equation 8 and the AUC ratios from the current study, fetal hepatic first-pass extraction of DPHM present in UV blood averaged 0.71 ± 0.07. However, with the DPHM, [3H10]DPHM, DPMA and [3H10]DPMA concentrations measured in samples from our previous umbilical hepatic first-pass experiments (study 3 and equation 13 (analogous to equation 8), a mean value of 0.44 ± 0.05 was obtained. We believe that the higher estimate of this parameter obtained in the paired infusion study (study 2) is due to maternal-to-fetal transfer of a portion of the maternally formed DPMA to artifactualy increase the fetal AUCDPMA/AUCDPHM ratio. In the fetal hepatic first-pass study (study 3), both labeled and unlabeled DPHM were administered to the fetus, so that maternal-to-fetal transfer of intact drug or metabolite was unlikely. Thus, the mean value of 0.44 for fetal hepatic DPHM extraction estimated from direct fetal UV administration is probably more accurate.

The failure to detect a fetal hepatic first-pass effect in our previous study, which measured only the concentrations of DPHM and [3H10]DPHM, may have resulted from the geometry and hemodynamics of the fetal circulation as well as the high placental permeability for the intact drug. In terms of the former factors, a greater portion of UV and fetal hepatic venous return is preferentially distributed to the upper body, with only ~20% reaching the placenta in one circulation time (Edelstone and Rudolph, 1979; Reuss and Rudolph, 1980). In contrast, ~50% of inferior vena cava blood reaches the placenta (Edelstone and Rudolph, 1979; Reuss and Rudolph, 1980). In addition, as was demonstrated in study 2 above, ~60% of the drug delivered to the placenta is extracted at this site. Thus, after one pass through the fetal circulation, the average systemic availability of the drug (DPHM or [3H10]DPHM) injected via the UV will be ~50%. This is because on average, ~50% of the drug injected via the UV is extracted in a single pass through the circulation, calculated as the fraction removed via hepatic first-pass extraction (~44%), plus the fraction extracted by the placenta (~6%), for a total of ~50%. Similarly, after a single pass through the fetal circulation, the systemic availability of the drug administered at the inferior vena cava (TV) will be ~70% because ~30% of the drug will be extracted at the placenta. In addition to this factor, fetal circulatory transit times from UV to placenta (~5.1 sec) and from inferior vena cava to placenta (~3.7 sec) are different (Power and Longo, 1975), whereas the transit time to the placenta for the ~50% of the umbilical blood flow that passes through the fetal liver is even longer (~9.8 sec; Power and Longo, 1975). Due to the above described factors, the form of drug administered at the UV site likely experiences a “fetal hepatic first-pass effect,” whereas that administered at the TV site experiences a “placental first-pass effect” (fig. 1). Thus, even though a fetal hepatic first-pass DPHM uptake from UV blood was present, the high placental extraction of drug administered via the TV may act to nullify the difference between systemic concentrations (AUCs or Cmax) of the two forms of the parent drug. This would result in minimal and inconsistent differences in the systemic arterial levels and AUCs of the two forms of drug and thus to an apparent lack of fetal hepatic DPHM elimination, as was concluded in our previous study (Tonn et al., 1996). However, if we assume that fetal hepatic DPHM elimination occurs via its metabolism in the fetal liver and placental elimination involves simple drug transfer to the maternal circulation, there should be differences in the fetal plasma concentrations of the DPHM metabolite (e.g., DPMA and [3H10]DPMA) formed from the two forms of the drug. Also, these differences are more likely to be maintained over time because of the limited placental permeability of more polar and highly protein bound DPMA (see above) in contrast to the parent drug, which readily crosses placenta. In agreement with this, a consistent concentration difference between the labeled and unlabeled forms of DPMA in fetal arterial plasma was observed (study 3) that results from the hepatic first-pass uptake of umbilically administered drug and subsequent formation of higher amounts of metabolite from this form of the drug (fig. 5).

The data on fetal plasma DPMA and [3H10]DPH concentrations allowed an indirect estimation of the fetal hepatic first-pass extraction of the parent drug after UV administration with the use of equation 13. The estimate of 0.44 for fetal hepatic DPHM extraction is less than that found in adult sheep (0.93; Tonn et al., 1996). However, ~50% of UV return bypasses the fetal liver via the ductus venosus (Holzman, 1984), and thus drug present in this blood is not available for hepatic first-pass uptake. When the fetal hepatic extraction estimate is corrected for this, it is similar to the adult value (0.88 vs. 0.92). Consequently, it appears that the liver of the fetal lamb in late gestation is almost as effective as the adult liver in metabolizing DPHM. Moreover, when the “true” hepatic extraction estimate of 0.88 is multiplied by a reported value for total hepatic blood flow in the fetal lamb (137 ml/min/kg fetal weight; Edelstone et al., 1978), the resulting estimate of fetal hepatic clearance is ~120 ml/min/kg. This comprises ~100% of CLfo (table 1), suggesting that the fetal liver is the major organ responsible for fetal nonplacental clearance of DPHM. Renal clearance of DPHM contributes to...
only ∼2% to \( CL_{mf} \); previously, we found that fetal pulmonary extraction of DPHM contributes another 8% (Rurak et al., 1991). Thus, combined average clearances of the liver, kidney and lung are ∼10% greater than our current estimate of \( CL_{mf} \), but given that fetal hepatic extraction is estimated by an indirect method and that the other data come from different studies, this difference is not too great.

The metabolic fate of the DPHM taken up by the fetal liver remains to be determined. As noted above, DPMA appears to play only a minor role in the overall nonplacental elimination of the parent drug in both fetal and adult sheep. We recently obtained evidence for the presence of large amounts of an N-oxide metabolite of DPHM in adult and fetal sheep plasma but further studies are required to determine its quantitative importance in the metabolism of drug.

**Impact of fetal hepatic drug uptake on two-compartment model estimates of maternal and fetal clearances.** The placental clearance values calculated using the two-compartment model are the “fundamental” clearances of the maternal-placental-fetal system and can be used to estimate the maximum possible rate of drug transfer across the placenta (under given conditions of blood flow and protein binding) assuming sink conditions on the other side. These clearance parameters are thus reflective of the true placental permeability of the drug in question. This is in contrast to the net rate of placent al drug flux, which depends only on the rate of nonplacental drug elimination on the other side of the placenta.

It is important at this point to realize that the proposed two-compartment system incorporates both maternal and fetal drug elimination. Hence, after both maternal and fetal drug administration, this system never reaches a state of equilibrium (defined as equal bidirectional drug fluxes and no net transfer of drug across the placenta). It does, however, reach a steady state in which the rate of drug flux across the placenta becomes equal to the rate of drug elimination from the fetus via nonplacental pathways, as given by the following equation:

\[
CL_{mf} \times C_{mass} - CL_{fm} \times C_{fss} = CL_{mo} \times C_{fss}
\]

(14)

During steady-state maternal drug administration, there is net maternal-to-fetal drug transfer, and rate of this transfer is equal to the rate of drug elimination from the fetus via nonplacental pathways, as given by the following equation:

\[
CL_{mf} \times C_{fss} = CL_{mo} \times C_{fss}
\]

(15)

Hence, after maternal steady-state drug administration, the maternal-to-fetal drug flux always exceeds that in the opposite direction. The situation is reversed after fetal drug administration, and fetal-to-maternal flux exceeds that in the opposite direction.

Consider a hypothetical situation where equal steady-state drug concentrations are achieved in the mother and fetus after separate drug administration. In this case, the rate of maternal-to-fetal drug flux after maternal administration should be equal to the rate of fetal-to-maternal drug flux after maternal administration. This is because of the presence of the same drug diffusion barrier in both directions (i.e., placenta) and a similar rate of drug delivery to the placenta (due to relative equality between maternal and fetal placental blood flows and equal drug concentrations). Placental drug flux is a product of placental clearance and steady-state drug concentration (maternal-to-fetal flux, \( CL_{mf} \times C_{mass} \), fetal-to-maternal flux, \( CL_{fm} \times C_{fss} \)). Because we are assuming equal drug concentrations (\( C_{mass} \) and \( C_{fss} \) after separate maternal and fetal drug administration, respectively), the placental clearance (\( CL_{mf} \) and \( CL_{fm} \)) in both directions should be equal. Also, in linear pharmacokinetic systems, clearance is constant at all drug concentrations. Thus, \( CL_{mf} \) and \( CL_{fm} \) should be equal at all rates of drug infusion as long as the assumption of linearity holds. However, as noted in the introduction, with the exception of acetaminophen, all drugs studied in pregnant sheep have values of \( CL_{fm} \) that are greater than values of \( CL_{mf} \).

In the case of DPHM, we believe that the lower value of \( CL_{mf} \) compared to \( CL_{fm} \) is in part due to fetal hepatic first-pass uptake of the maternally administered form of the drug that reaches the fetus via the UV. As noted above, we believe that the value of 0.44 is a more accurate estimate of this fetal hepatic extraction of DPHM. Because this means that ∼44% of the maternally derived drug present in UV blood will not reach the fetal systemic arterial circulation, its steady-state concentration and AUC in fetal arterial plasma will be reduced by the same magnitude. Thus, the clearance estimates obtained by equations 1 to 6 will be affected by this reduction in \( C_{fss} \). When these estimates are corrected for fetal hepatic first-pass extraction (using a systemic availability of 0.56 for maternally derived DPHM), \( CL_{mf} \) (39.4 ml/min/kg, corrected vs. uncorrected), \( CL_{ff} \) (332.1 vs. 324.2 ml/min/kg), \( CL_{fm} \) (219.7 vs. 214.4 ml/min/kg), \( CL_{mo} \) (36.1 vs. 36.6 ml/min/kg) and \( CL_{f} \) (112.4 vs. 109.8 ml/min/kg) are only minimally altered. However, the corrected estimate of \( CL_{mf} \) (92.6 ml/min/kg) is substantially higher than the uncorrected value (50.2 ml/min/kg). It is still lower than the \( CL_{fm} \) value (214.4 ml/min/kg). However, it is assumed that only the free unbound drug can diffuse across the placenta, and we previously reported that the free fraction of DPHM in fetal plasma (0.277) is significantly higher than that in maternal plasma (0.141; Yoo et al., 1993). When \( CL_{mf} \) is corrected for the difference in maternal and fetal free fractions of DPHM (i.e., by a factor of 1.96), the estimate of \( CL_{mf} \) (181.9 ml/min/kg) approaches \( CL_{fm} \). Thus, the apparent difference between \( CL_{mf} \) and \( CL_{fm} \) for DPHM, when the two-compartment open model is used to determine the clearance values, appears to be due to two factors: a difference in maternal and fetal plasma protein binding and a significant first-pass uptake of maternally derived drug by the fetal liver.

As noted in the introduction, the estimates of \( CL_{mf} \) are lower than those for \( CL_{fm} \) for all drugs studied using the two-compartment open model, with the exception of acetaminophen. For some of these drugs, there are no differences between maternal and fetal plasma protein binding, so the clearance difference could largely be due to fetal hepatic first-pass uptake of maternally derived drug. The calculated clearance values could thus be in error, but this could only be determined by obtaining an estimate of fetal hepatic extrac-

---

tion for each drug. However, from another viewpoint, the difference in maternal and fetal placental clearances of these drugs may provide evidence for significant fetal hepatic clearance of these compounds. In figure 6, estimates of CLfo for the drugs studied in pregnant sheep are plotted against their CLfm - CLmf difference. There is a highly significant, linear relationship between the two variables, suggesting that with the exception of acetaminophen, fetal hepatic first-pass uptake is a significant factor in fetal nonplacental elimination of these compounds. From this relationship, methadone and DPHM will be predicted to exhibit the most pronounced fetal hepatic first-pass effect; in agreement with this, these drugs also have the highest values for CLfo. In the case of acetaminophen, the lack of evidence for any fetal first-pass effect is consistent with the low value of CLfo for this drug.

In summary, the use of simultaneous maternal and fetal infusions of unlabeled and labeled DPHM coupled with measurements of DPHM, [2H10]DPHM, DPMA and [2H10]DPMA in maternal and fetal plasma has allowed determination of transplacental and nonplacental clearances and an estimate of fetal hepatic first-pass uptake of the maternally derived drug. In addition, we obtained strong evidence for the formation of the deaminated metabolite of DPHM, DPMA, in both the mother and fetus. DPMA has a substantially longer half-life in the fetus compared to the adult, perhaps due to limited placental transfer and lack of fetal elimination capability for the metabolite. The data indicate that DPHM uptake and metabolism by the fetal liver play a major role in fetal nonplacental elimination of the drug. Moreover, the fetal first-pass uptake of the form of drug infused to the mother also appears to be in part responsible for an underestimation of maternal-to-fetal placental clearance. This may also be the case for other drugs studied in pregnant sheep using the two-compartment open model that demonstrate higher values of CLfm compared with CLmf. Conversely, the difference between fetal and maternal placental clearance for these drugs may provide evidence for fetal hepatic metabolism of these compounds. Renal excretion of DPHM and DPMA is markedly different in the ewe and fetus in that although the adult kidney appears to reabsorb DPHM and secrete DPMA, neither process is evident in the fetus. Together, the fetal kidney and lung appear to be responsible for ~10% of total nonplacental clearance in the fetus, with the fetal liver accounting for the remainder. However, the precise hepatic metabolic pathways involved remain to be determined.

**Appendix**

Equation 8 is derived from principles of the well-stirred model of hepatic drug elimination (Pang and Rowland, 1977; Wilkinson and Shand, 1975). In the current study, the fetal UV input of the drug (form of drug given to mother, i.e., DPHM) is considered equivalent to portal venous administration after birth because ~50% of the UV blood flow passes through the fetal liver before reaching the fetal circulation (Edelstone et al., 1978). The bypass of the remaining ~50% of umbilical blood flow from the fetal liver does not jeopardize the validity of this assumption because we were in fact interested in the fetal hepatic drug extraction ratio after umbilical input only. TV administration (form of drug given directly to the fetus, i.e., [2H10]DPHM) is equivalent to the usual intravenous input.

In the following section, the symbols D and M denote parent drug (DPHM or [2H10]DPHM) and metabolite (DPMA or [2H10]DPMA), respectively. The subscripts u and l represent unlabeled and labeled drug or metabolite, respectively.

The fraction of DPHM (or [2H10]DPHM) metabolized to DPMA (or [2H10]DPMA) (fn) can be described in terms of intrinsic clearances of the liver as the following

$$fn = \frac{(CL_{f,int})_{Du \text{ or } Dl} \cdot (CL_{f,int})_{Mu \text{ or } Ml} \cdot (CL_{int})_{other}}{(CL_{f,int})_{Du \text{ or } Dl} \cdot (CL_{int})_{Mu \text{ or } Ml} + (CL_{int})_{other}} \quad(16)$$

where \((CL_{f,int})_{Mu \text{ or } Ml}\) is the intrinsic formation clearance of DPMA or \([2H10]DPMA\) from DPHM or \([2H10]DPHM\), respectively. The symbol \((CL_{int})_{Du \text{ or } Dl}\) represents the total intrinsic clearance of DPHM or \([2H10]DPHM\), and \((CL_{int})_{other}\) is the intrinsic clearance of DPHM or \([2H10]DPHM\) via other pathways other than the formation of DPMA or \([2H10]DPMA\).

Also, according to the well-stirred model of hepatic drug elimination (Pang and Rowland, 1977; Wilkinson and Shand, 1975):

$$Q_H^{-1}(CL_{int})_{Du \text{ or } Dl} = \frac{Q_H^{-1}(CL_{int})_{Du \text{ or } Dl}}{Q_H^{-1}(CL_{int})_{Du \text{ or } Dl}}$$

$$Q_H^{-1}(CL_{int})_{Mu \text{ or } Ml} = \frac{Q_H^{-1}(CL_{int})_{Mu \text{ or } Ml} + (CL_{int})_{other}}{Q_H^{-1}(CL_{int})_{Mu \text{ or } Ml} + (CL_{int})_{other}} \quad(17)$$

where \(Q_H\) is the total hepatic blood flow. The formation clearance of DPMA (or \([2H10]DPMA\)) can be expressed as:

$$(CL_{f})_{Mu \text{ or } Ml} = fn \cdot (CL_{Du \text{ or } Dl})$$

$$(CL_{Du \text{ or } Dl})_{Mu \text{ or } Ml} = \frac{Q_H^{-1}(CL_{int})_{Mu \text{ or } Ml}}{Q_H^{-1}(CL_{int})_{Mu \text{ or } Ml} + (CL_{int})_{other}} \quad(18)$$

With equations 16 and 17, equation 18 becomes:

$$(CL_{Du \text{ or } Dl})_{Mu \text{ or } Ml} = \frac{Q_H^{-1}(CL_{int})_{Mu \text{ or } Ml}}{Q_H^{-1}(CL_{int})_{Mu \text{ or } Ml} + (CL_{int})_{other}} \quad(19)$$

As discussed above, \([2H10]DPHM\) administration is equivalent to intravenous input, and the total amount of labeled
metabolite \( ([^{2}H_{10}]\text{DPMA}) \) formed in the fetus from \( ([^{2}H_{10}]\text{DPHM}) \) during the experimental period is equal to the total amount of metabolite eliminated (all of the formed metabolite is ultimately eliminated). This can be described by the following relationship (assuming a one-compartment fetus):

\[
k_{f}^{*}\text{Vd}_{D}^{*}\text{AUC}_{D} = k_{m}^{*}\text{Vd}_{M}^{*}\text{AUC}_{M}
\]

where \( k_{f} \) and \( k_{m} \) are the formation and elimination rate constants of the \( ([^{2}H_{10}]\text{DPMA}) \) metabolite, respectively; \( \text{Vd}_{D} \) and \( \text{Vd}_{M} \) are the volumes of distribution of \( ([^{2}H_{10}]\text{DPMA}) \) and \( ([^{2}H_{10}]\text{DPMA}) \), respectively; and \( \text{AUC}_{D} \) and \( \text{AUC}_{M} \) are the systemic arterial UCs of \( ([^{2}H_{10}]\text{DPMA}) \) and \( ([^{2}H_{10}]\text{DPMA}) \), respectively.

On rearranging, equation 20 becomes:

\[
\frac{\text{AUC}_{M}}{\text{AUC}_{D}}_{\text{i.v.\ input}} = \left( \frac{k_{f} \text{Vd}_{D}}{k_{m} \text{Vd}_{M}} \right)_{\text{i.v.\ input}} \left( \frac{\text{CL}_{d}^{\text{M}}}{\text{CL}_{d}^{\text{M}}} \right)
\]

where \( \text{CL}_{d}^{\text{M}} \) and \( \text{CL}_{d}^{\text{M}} \) are the systemic formation and elimination clearances of the \( ([^{2}H_{10}]\text{DPMA}) \) metabolite, respectively.

Substituting the right-hand side of equation 19 for \( \text{CL}_{d}^{\text{M}} \) in equation 21, we obtain:

\[
\frac{\text{AUC}_{M}}{\text{AUC}_{D}}_{\text{i.v.\ input}} = \left( \frac{\text{CL}_{d}^{\text{int}}\text{b}_{d}}{\text{CL}_{d}^{\text{int}}\text{b}_{d}} \right)_{\text{i.v.\ input}} \left( \frac{Q_{H}^{*}(\text{CL}_{d}^{\text{int}}\text{b}_{d})}{\text{CL}_{d}^{\text{int}}\text{b}_{d}} \right)_{\text{i.v.\ input}} + \left( \frac{\text{CL}_{d}^{\text{int}}\text{b}_{M} + \text{CL}_{d}^{\text{int}}\text{other}}{\text{CL}_{d}^{\text{int}}\text{b}_{M}} \right)
\]

After UV input (equivalent to portal or oral route in postnatal circulation), systemic formation clearance of the metabolite is in fact equivalent to its intrinsic formation clearance (Pang and Rowland, 1977; Wilkinson and Shand, 1975).

Hence, for umbilical input of the drug (unlabeled DPHM in the case of study 2), an expression equivalent to equation 21 can be written as:

\[
\frac{\text{AUC}_{M}}{\text{AUC}_{D}}_{\text{umbilical\ input}} = \left( \frac{\text{CL}_{d}^{\text{int}}b_{d}}{\text{CL}_{d}^{\text{int}}b_{d}} \right)_{\text{umbilical\ input}} \left( \frac{Q_{H}}{\text{CL}_{d}^{\text{int}}b_{M}} + (\text{CL}_{d}^{\text{int}}b_{M} + \text{CL}_{d}^{\text{int}}\text{other})_{\text{umbilical\ input}} \right)
\]

Using equation 23, equation 22 becomes:

\[
\frac{\text{AUC}_{M}}{\text{AUC}_{D}}_{\text{i.v.\ input}} = \left( \frac{\text{AUC}_{M}}{\text{AUC}_{D}}_{\text{umbilical\ input}} \right)_{\text{i.v.\ input}} \left( \frac{Q_{H}}{\text{CL}_{d}^{\text{int}}b_{M}} + (\text{CL}_{d}^{\text{int}}b_{M} + \text{CL}_{d}^{\text{int}}\text{other})_{\text{umbilical\ input}} \right)
\]

Again, according to the well-stirred model of hepatic drug elimination, the hepatic extraction ratio is given by the following equation (Pang and Rowland, 1977; Wilkinson and Shand, 1975).

\[
\text{ER} = \frac{(\text{CL}_{d}^{\text{int}}b_{M} + \text{CL}_{d}^{\text{int}}\text{other})_{\text{i.v.\ input}}}{Q_{H} + (\text{CL}_{d}^{\text{int}}b_{M} + \text{CL}_{d}^{\text{int}}\text{other})_{\text{i.v.\ input}}}
\]

And, hence, systemic availability (\( F = 1 - \text{ER} \)) will be:

\[
F = 1 - \text{ER} = \frac{Q_{H}}{Q_{H} + (\text{CL}_{d}^{\text{int}}b_{M} + \text{CL}_{d}^{\text{int}}\text{other})_{\text{i.v.\ input}}}
\]


Send reprint requests to: Dr. Dan W. Rurak, B.C. Institute for Child and Family Health, 950 West 28th Avenue, Vancouver, B.C., Canada V5Z 4H4.