DIPHENHYDRAMINE DISPOSITION IN THE SHEEP MATERNAL-PLACENTAL-FETAL UNIT:
GESTATIONAL AGE, PLASMA DRUG PROTEIN BINDING, AND UMBILICAL BLOOD FLOW
EFFECTS ON CLEARANCE

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ABSTRACT:
The objective of this study was to examine the interrelationships
between maternal and fetal plasma drug protein binding, umbilical
blood flow (Qum), gestational age (GA), and maternal-fetal diphen-
hydramine (DPHM) clearances in chronically instrumented preg-
nant sheep. Maternal and fetal DPHM placental (CLmf and CL fm ,
respectively) and nonplacental (CLmo and CLfo, respectively) clear-
ances and steady-state plasma protein binding were determined in
18 pregnant sheep at 124 to 140 days’ gestation (term, ~145 days).
The data demonstrated a highly significant fall of ~66% in CLfm
and a decreasing trend in CLfo (~47%) over the GA range studied.
However, no such relationships existed between GA and CLmf or
CLmo. Concomitant with this was a decrease in fetal DPHM plasma
unbound fraction with GA, with no such change being evident in
the mother. Both CLmo and CLfo were related to the respective
DPHM plasma unbound fraction. A strong relationship also existed
between fetal plasma unbound fraction and CLfm. Thus, the de-
crease in fetal unbound fraction of DPHM during gestation could
contribute to the fall in CLfm, and possibly CLfo. However, over the
GA range studied, fetal DPHM free fraction decreased by ~47%,
whereas CLfm fell by ~66%. Because fetal unbound fraction and
CLfm are linearly related, the GA-associated fall in unbound frac-
tion appears to be insufficient to account for the entire decline in
CLfm. In separate studies in pregnant sheep, we observed a ~40% fall
in weight-normalized Qum between 125 and 137 days’ gesta-
tion. Because CLfm for DPHM is similar to that of flow-limited
compounds (e.g., ethanol, antipyrine), this decrease in Qum may
also contribute to the GA-related fall in CLfm.

The final trimester of gestation is a very dynamic period in terms of
fetal development and is associated with profound changes in numer-
ous physiological variables that may alter different aspects of mater-
nal-fetal drug disposition. These include changes in fetal plasma
protein concentrations, and the extent of drug binding (Szeto et al.,
1982a; e.g., propranolol and methadone, Czuba et al., 1988); changes
in the drug metabolism capacity of the fetal liver (Wang et al., 1986);
development of fetal renal function and drug excretion; and alterations
in fetal circulatory and hemodynamic processes (Battaglia and Me-
schia, 1988). Little has been done experimentally to eluci-
date the quantitative influence of these gestational age (GA)1-related
factors on drug disposition in the maternal-placental-fetal unit and the
resulting alterations in fetal drug exposure.

A two-compartment open model is most commonly used to de-
scribe the pharmacokinetics of drugs in the maternal-fetal unit (Fig. 1; 
Szeto et al., 1982b,c; Wang et al., 1986; Riggs et al., 1990; Yoo et al.,
1993; Kumar et al., 1997, 1999a). This model has four clearance
parameters: the maternal (CLmf) and fetal (CLfm) placental clearances
are related to the efficiency of bidirectional placental transfer of the
drug, whereas the corresponding nonplacental clearances (CLmo and

1 Abbreviations used are: GA, gestational age; CL mf, maternal-to-fetal placental
clearance; DPHM, diphenhydramine; [H32]DPHM, deuterium-labeled diphenhydra-
mine; CLt b, maternal total body clearance; CL f t, fetal total body clearance; CL f t-m f ,
fetal-to-maternal placental clearance; CL f t-n m, maternal nonplacental clearance; CL n m ,
fetal nonplacental clearance; C m, maternal plasma steady-state diphenhydramine
concentration after maternal administration; C f, fetal plasma steady-state diphenhydra-
mine concentration after maternal administration; C f t-m f , maternal plasma steady-
state diphenhydramine (or deuterium-labeled diphenhydramine) concentration after
fetal administration; M-UF, maternal hepatic blood flow; Qum, umbilical blood flow.
CL_{eo}, CL_{mo}, CL_{fm}, CL_{ft}
\begin{align*}
\text{MATERNAL COMPARTMENT} & \quad \text{CL}_{mo} \quad \text{CL}_{ft} \\
\text{CL}_{en} & \quad \text{CL}_{ef} \\
\text{Placenta} & \quad \text{CL}_{fm}
\end{align*}
\begin{align*}
\text{FETAL COMPARTMENT} & \quad k_{o} \quad k_{o'}
\end{align*}

Fig. 1. A representation of various placental and nonplacental drug clearances in the two-compartment pharmacokinetic model of the maternal-fetal unit.

\text{CL}_{eo}\) describe the extent of maternal and fetal drug elimination via all other routes (e.g., drug metabolism, renal excretion, pulmonary elimination, and so on).

Diphenhydramine, or 2-(diphenylmethoxy)-N,N-dimethylamine (DPHM), is a potent histamine H\textsubscript{1}-receptor antagonist. It has been widely used during human pregnancy for the treatment of nausea and vomiting, insomnia, allergic rhinitis, and common coughs and colds. Previous studies in our laboratory, with the use of chronically instrumented pregnant sheep, demonstrated that DPHM readily crosses the ovine placenta and is eliminated from the fetus via both placental and nonplacental routes (Yoo et al., 1986a). In continuation of these studies, we used DPHM as a model high-clearance drug that undergoes rapid and extensive placental transfer to examine the factors affecting different aspects of maternal-fetal drug disposition of this class of compounds. This includes the study of comparative maternal-fetal drug clearance (Yoo et al., 1993), in utero fetal hepatic drug uptake and its relation to fetal drug clearance (Kumar et al., 1997), and in utero functional capacity of fetal drug metabolism pathways compared with the mother (Kumar et al., 1999a). As part of these studies, we determined DPHM placental and nonplacental clearances in 18 pregnant sheep during the final 2 weeks of their gestation. In the present study, we retrospectively examined the overall interrelationships between maternal and fetal plasma protein binding, umbilical blood flow, GA, and DPHM maternal and fetal clearances using pooled data from these three studies (Yoo et al., 1993; Kumar et al., 1997, 1999a). Our aim was to better understand the qualitative and quantitative importance of these factors in determining the GA-related alterations in maternal-fetal drug clearance.

Materials and Methods

Animals and Surgical Preparation. A total of 18 pregnant sheep were used in these studies. All studies were approved by the University of British Columbia Animal Care Committee, and the procedures performed on sheep conformed to the guidelines of the Canadian Council on Animal Care. The detailed surgical procedures have been described previously (Yoo et al., 1993; Kumar et al., 1997, 1999a). Briefly, 18 pregnant Dorset Suffolk cross-bred ewes, with a maternal body weight of 76.9 ± 12.6 kg (mean ± S.D.), were surgically prepared at 115 to 129 days’ gestation (term, ~145 days). Surgery was performed aseptically under halothane (1–2%) and nitrous oxide (60%) anesthesia (balance O\textsubscript{2}), after induction with i.v. sodium pentothal (1 g) and amnesia (balance O\textsubscript{2}, after induction with i.v. sodium pentothal (1 g) and nitrous oxide (60%). Intermuscular injections of 500 mg ampicillin were administered to the ewe on the day of surgery and for 3 days postoperatively. Ampicillin (500 mg) was also injected into the amniotic cavity immediately after surgery and daily thereafter. After surgery, animals were kept in holding pens with other sheep and were allowed free access to food and water. The sheep were allowed to recover for 4 to 8 days before experimentation.

Experimental Protocols. All experiments were conducted between 124 and 140 days’ gestation. A total of 31 experiments were conducted in 18 pregnant sheep. Each animal received one of the following: 1) a 90-min separate maternal and fetal steady-state DPHM (DPHM hydrochloride; Sigma Chemical Co., St. Louis, MO) infusion with an appropriate washout period (n = 8, experiments from Yoo et al., 1993); 2) a 6-h separate maternal and fetal steady-state DPHM infusion with an appropriate washout period (n = 3, experiments from Kumar et al., 1999a); 3) a 6-h separate maternal and fetal steady-state [\textsuperscript{2}H\textsubscript{10}]DPHM (a deuterium-labeled analog of DPHM synthesized in our laboratory; Tonn et al., 1993) infusion with an appropriate washout period (n = 2, experiments from Kumar et al., 1999a); or 4) a 6-h simultaneous steady-state infusion of DPHM to the mother and [\textsuperscript{2}H\textsubscript{10}]DPHM to the fetus (n = 5, experiments from Kumar et al., 1997). The doses were prepared in sterile water for injection and were sterilized by filtering through a 0.22-μm nylon syringe filter (MSI, Westboro, MA) into a capped empty sterile injection vial.

Drug (DPHM or [\textsuperscript{2}H\textsubscript{10}]DPHM) was administered to the mother in each experiment as a 20-μg i.v. loading dose over 1.0 min, followed immediately by an infusion at 670 μg/min via the maternal femoral vein. In fetal experiments, a 5.0-μg i.v. loading dose of DPHM or [\textsuperscript{2}H\textsubscript{10}]DPHM was administered via the fetal lateral tarsal vein over 1.0 min, followed by an infusion of the same compound at a rate of 170 μg/min. Simultaneous serial blood samples were collected from the fetal (1.5 ml) and maternal (3.0 ml) femoral arterial catheters. Fetal femoral arterial samples (0.5 ml) were also collected at the same time intervals for blood gas analysis and measurement of glucose and lactate concentrations. 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 (2.0 ml) and fetal (5.0 ml) and maternal urine (10.0 ml) samples were also collected to examine the excretion of DPHM into these fluids; these data have been reported previously (Yoo et al., 1993; Kumar et al., 1997, 1999a).

Maternal and fetal blood samples collected for drug analysis were placed into heparinized Vacutainer tubes (Becton-Dickinson, Rutherford, NJ), gently mixed, and then centrifuged at 2000g for 10 min. The plasma supernatant was removed and placed into clean borosilicate test tubes 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.

Physiological Recording and Monitoring Procedures. From at least 24 h before and at least 24 h after each infusion period, amniotic pressure, fetal tracheal and femoral arterial pressures, and fetal heart rate were continuously monitored. In some animals with implanted cortical electrodes and fetal bladder catheters, fetal electrocorticogram, and urine flow rate were also measured. Some of these data have been reported separately (Rurak et al., 1988).

Fetal blood pH, pO\textsubscript{2}, and pCO\textsubscript{2} were measured using an IL 1306 pH/blood gas analyzer (Allied Instrumentation Laboratory, Milan, Italy). Blood O\textsubscript{2} saturation and hemoglobin concentration were determined using an 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). All of these fetal blood gases and metabolite concentrations have been reported in our previous publications and were within the normal range observed in our and other laboratories at this stage of gestation in fetal sheep (Yoo et al., 1993; Kumar et al., 1997, 1999a).

Protein Binding of DPHM and [\textsuperscript{2}H\textsubscript{10}]DPHM in Fetal and Maternal Plasma. The protein plasma binding/unbound fraction of DPHM (or [\textsuperscript{2}H\textsubscript{10}]DPHM) was measured ex vivo in pooled fetal and maternal steady-state plasma samples collected from the above DPHM infusion studies using an equilibrium dialysis
procedure described by Yoo et al. (1993). Maternal plasma protein binding was measured in plasma samples obtained during maternal drug infusion, whereas fetal plasma protein binding was measured in plasma samples obtained during fetal drug infusion.

**Drug Analysis.** The concentrations of DPHM in all biological fluids collected were measured using either a gas chromatographic-nitrogen phosphorus detection method (Yoo et al., 1986b; studies in Yoo et al., 1993) or a gas chromatographic-mass spectometric assay (studies in Kumar et al., 1997, 1999a) capable of simultaneously measuring DPHM and [3H10]DPHM (Tonn et al., 1993). These assays have been shown to be comparable to each other with a similar limit of quantification (2.0 ng/ml; Tonn et al., 1993).

**Pharmacokinetic Analysis.** The maternal and fetal steady-state arterial plasma DPHM and [3H10]DPHM concentration data were treated according to a two-compartment open model to estimate the placental and nonplacental clearance parameters of DPHM (or [3H10]DPHM when present) in the ewe and fetus. This model assumes steady-state plasma concentrations and drug elimination from both the maternal and fetal compartiments. The equations to estimate placental and nonplacental clearance parameters have been previously described (Szeto et al., 1982b). Pharmacokinetic modeling of the data, wherever necessary, was carried out using the nonlinear least-squares fitting program ADAPT II (D’Argenio and Schumitzky, 1997).

**Statistical Analysis.** All values are reported as mean ± S.D. All linear correlational analyses were performed by computing Pearson’s correlation coefficient (r). The significance level was P < .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**

The average maternal body weight was 76.9 ± 12.6 kg, and the estimated fetal body weights on the day of maternal and fetal DPHM (or [3H10]DPHM) infusion were 2.61 ± 0.61 and 2.56 ± 0.54 kg, respectively.

The mean GA on the day of maternal and fetal steady-state DPHM infusion experiments was 130.9 ± 4.1 (range, 124–140) and 130.4 ± 3.7 (range, 125–136) days, respectively, and these were not statistically different (paired t test, P > .05). The average washout period between maternal and fetal DPHM infusion experiments was 2.4 ± 2.2 days. The average maternal and fetal steady-state plasma drug concentrations in these animals after maternal administration (Cm and Cm', respectively) were 228.0 ± 56.1 (range, 140.3–360.3) and 43.1 ± 31.2 (range, 3.5–124.1) ng/ml, respectively, whereas those after fetal drug infusion were 35.3 ± 11.9 (Cf'; range, 17.9–66.3) and 331.1 ± 172.4 (Cf'; range, 132.5–697.9) ng/ml, respectively. The steady-state maternal and fetal plasma unbound fractions of the drug were 0.120 ± 0.069 (range, 0.032–0.293) and 0.301 ± 0.094 (range, 0.165–0.527), respectively. The average maternal plasma unbound fraction (M-UF) was significantly lower compared with the average fetal plasma unbound fraction (F-UF; unpaired t test, P < .0001). Maternal and fetal steady-state unbound plasma drug concentrations were calculated by multiplying the appropriate total plasma concentration with the corresponding plasma unbound fraction. The mean steady-state unbound plasma concentrations thus obtained were Cm = 25.1 ± 11.4 (range, 8.6–45.6) ng/ml, Cf = 12.0 ± 8.6 (range, 1.9–40.4) ng/ml, Cm' = 3.9 ± 1.8 (range, 0.9–7.2) ng/ml, and Cf' = 89.3 ± 32.0 (range, 46.1–166.2) ng/ml.

Mean values for maternal total (CLmm), nonplacental (CLmo), and placental (CLmf) clearances were 41.7 ± 9.6, 40.2 ± 9.4, and 45.0 ± 30.3 ml/min/kg, respectively. Similarly, fetal total (CLff), nonplacental (CLfo), and placental (CLfm) clearances were 263.0 ± 111.8, 102.3 ± 46.4, and 160.7 ± 80.7 ml/min/kg, respectively. All clearances, except CLmm and CLmo, are normalized to the estimated fetal body weight on the day of experiment. All fetal clearances were significantly higher compared with the corresponding maternal clearance parameters (unpaired t test, P < .0001 in all cases), as reported previously (Yoo et al., 1993; Kumar et al., 1997, 1999a). However, the contribution of CLfo to CLff (39.5 ± 10.7%) was significantly lower compared with that of CLmm to CLmm (96.3 ± 2.8%; unpaired t test, P < .0001).

**Relationships of Maternal and Fetal DPHM Clearances with GA.** Figure 2 depicts the alterations in maternal and fetal clearances with advancing gestation for the 2-week period during which our experiments were conducted. CLff and CLfm exhibit a highly significant negative linear relationship with GA (Fig. 2A and B). The calculated regression equations predict a fall of ~59% (from 374.3 to 153.2 ml/min/kg) and ~66% (from 247.0 to 83.1 ml/min/kg) for CLff and CLfm, respectively, from 125 to 136 days’ gestation. Although the CLfo parameter also exhibits a decreasing trend (~47%) with gestation, this relationship is only near statistical significance (Fig. 2C).

Also, the percent contribution of CLfo to CLff did not change as a function of GA (% [CLfo/CLff] versus GA, r = 0.2892, P > .2, data not shown). In contrast to fetal clearances, none of the maternal clearance parameters show any relationship with GA (CLmm versus...
GA, \( r = 0.0113, P > .9 \); CL\textsuperscript{mF} versus GA, \( r = -0.0021, P > .9 \); CL\textsuperscript{mm} versus GA, \( r = -0.0041, P > .9 \); data not shown).

**Plasma Protein Binding Effects on Maternal and Fetal DPHM Clearances.** Figure 3 depicts the underlying relationships between maternal and fetal steady-state plasma unbound fraction and the corresponding clearance parameters. CL\textsubscript{ff} and CL\textsubscript{fm} are highly correlated with F-UF (Fig. 3A and B). In contrast to CL\textsubscript{fm}, there was no relationship between CL\textsubscript{mf} and M-UF (Fig. 3E). Also, CL\textsubscript{fm} and CL\textsubscript{mf} were not significantly related to the extent of drug protein binding on the other side of the placenta (i.e., maternal and fetal plasma protein binding, respectively; data not shown).

Although the linear relationships between CL\textsubscript{mo} and M-UF and between CL\textsubscript{fo} and F-UF were statistically significant (CL\textsubscript{mo} versus M-UF: CL\textsubscript{mo} = 107.7 \times M-UF + 28.8, \( r = 0.7649, P < .0005 \); CL\textsubscript{fo} versus F-UF: CL\textsubscript{fo} = 234.7 \times F-UF + 31.6, \( r = 0.4749, P < .05 \), a better fit of the data (Fig. 3C and F) was obtained using an equation of the form (see Discussion for further details):

\[
y = \frac{P1(x)}{P2 + P1(x)}
\]

where \( P1 \) and \( P2 \) are the parameters required to describe the relationship.

This equation is exactly analogous to the following relationship describing hepatic uptake of compounds based on the well-stirred model of the liver (Wilkinson and Shand, 1975):

\[
CL_{H} = \frac{Q_{H}(f_{un})(CL_{un})}{(Q_{H} + f_{un})(CL_{un})}
\]

where \( Q_{H} \), CL\textsubscript{H}, CL\textsubscript{un}, and \( f_{un} \) are the total hepatic blood flow, total hepatic clearance, hepatic intrinsic clearance of the unbound drug, and the unbound fraction of the drug, respectively.

In our previous studies, we have shown that the liver is the major organ of nonplacental clearance in the fetus (Kumar et al., 1997), whereas both the liver and gut contribute significantly to DPHM clearance in adult sheep (Kumar et al., 1999b). Thus, in the fetus, the terms P1, P2, and P1/P2 of eq. 1 correspond to \( Q_{H} \), CL\textsubscript{H}, and CL\textsubscript{un}, respectively. In the mother, these parameters describe the combined blood flow and intrinsic clearance of the liver and gut. This analysis produced fits that were statistically at least as good as (CL\textsubscript{fo} versus F-UF; F test on sum of squared residuals, \( P > .05 \)) or significantly better than (CL\textsubscript{mo} versus M-UF; F test on sum of squared residuals, \( P < .05 \)) the corresponding linear model fits. From the fitting of CL\textsubscript{mo} versus maternal plasma unbound fraction data to eq. 1, DPHM CL\textsubscript{un} and \( Q_{H} \) in pregnant adult sheep were estimated to be 1242.6 \( \pm \) 176.8 and 60.2 \( \pm \) 5.7 ml/min/kg (mean \( \pm \) S.E.), respectively (Fig. 3F). A similar hyperbolic relationship was apparent between CL\textsubscript{mm} and M-UF as well because CL\textsubscript{mo} is the major contributor to CL\textsubscript{mm} (Fig. 3D). Similarly, the treatment of CL\textsubscript{fo} and F-UF data according to eq. 1 produced the fetal CL\textsubscript{un} and \( Q_{H} \) estimates of 517.6 \( \pm \) 251.2 and 318.7 \( \pm \) 306.6 ml/min/kg (mean \( \pm \) S.E.), respectively (Fig. 3C).

**Changes in Maternal and Fetal Plasma Protein Binding with GA.** Figure 4 shows the changes in fetal and maternal steady-state plasma unbound fractions with increasing GA during the period of gestation under study. F-UF falls significantly from 125 to 136 days' gestation by \( \sim 47\% \) (from 0.383 to 0.203 as predicted by the regression equation; Fig. 4A). In contrast, M-UF does not change significantly during this period of gestation (Fig. 4B).
Discussion

A lower-than-unity \( C_f/C_m \) ratio of the unbound as well as total DPHM indicates that fetus can eliminate this drug via routes other than the placenta (Szeto et al., 1982b). However, a lower contribution of \( CL_{fo} \) to \( CL_{ff} \) (39.5 ± 10.7%) compared with that of \( CL_{mm} \) to \( CL_{num} \) (96.3 ± 2.8%) emphasizes greater importance of the placenta in overall fetal drug elimination.

One of the first observations we made from our DPHM clearance data in 18 pregnant sheep was a significant fall (~66%) in \( CL_{fm} \) and a decreasing trend in \( CL_{fo} \) (~47%) with increasing GA over the period of our study (Fig. 2, B and C). This resulted in a ~59% decrease in \( CL_{ff} \) during the 12-day gestation period of 125 to 136 days (Fig. 2A). The decreasing trend in \( CL_{fo} \) with GA was also consistent with the fact that despite the large decrease in \( CL_{fm} \) with advancing gestation, the percent contribution of \( CL_{fo} \) to \( CL_{ff} \) did not increase with increasing GA. Our subsequent analysis has focused on evaluating the mechanisms of this GA-associated fall in fetal DPHM clearances.

It is widely accepted that only the free or unbound (not bound to plasma proteins) form of the drug is able to cross biological membranes. Hence, we examined the steady-state unbound fractions of DPHM in maternal and fetal plasma and their relationship with maternal and fetal drug clearances. DPHM is moderately to extensively bound in both maternal (range, 71–97%) and fetal (range, 47–84%) plasma, with the average F-UF being significantly higher. This is similar to the fetal plasma protein binding of a number of other basic drugs in sheep as well as in humans (e.g., propranolol, methadone, and lidocaine) and may be related to lower fetal plasma \( \alpha_1 \)-acid glycoprotein concentrations (Szeto et al., 1982a; Hill et al., 1986; Czuba et al., 1988; Hill and Abramson, 1988). The data in Fig. 3, A and D, show that plasma protein binding of the drug is an important determinant of its total clearance in both the mother and the fetus. The relationship of F-UF with \( CL_{fr} \) appears to be linear, whereas that of M-UF with \( CL_{rn} \) is closer to a hyperbola (see later). The F-UF also correlates closely with \( CL_{fr} \) (Fig. 3B), indicating that fetal-to-maternal placental transfer of the drug is highly dependent on its binding in fetal plasma. In contrast to \( CL_{rn} \), \( CL_{mf} \) was not significantly related to M-UF (Fig. 3E). At first, this may seem somewhat difficult to understand, but a very plausible explanation can be made for this phenomenon. We demonstrated earlier that after maternal DPHM administration, the fetal liver (due to its unique anatomical position) can metabolize a significant but variable and unquantifiable proportion of DPHM transferred across the placenta in a first-pass manner before it reaches the fetal circulation (Kumar et al., 1997). This leads to a corresponding variable underestimation of \( CL_{mf} \) if the data are treated according to the two-compartment model (Kumar et al., 1997). Thus, in this study, \( CL_{mf} \) cannot be estimated accurately, and we believe that this underlies the lack of any obvious relationship between M-UF and \( CL_{mf} \).

Because F-UF is an important factor determining the magnitude of \( CL_{rn} \), an obvious question is whether F-UF changes significantly during 125 to 136 days’ gestation and whether this change can explain the observed fall of ~66% in \( CL_{rn} \). The data in Fig. 4A demonstrate a highly significant fall of ~47% in F-UF during these 12 days of gestation. Although we did not measure total or specific plasma protein concentrations during the course of the study, at least total plasma protein concentrations in fetal sheep are known to gradually increase over this period of gestation (Kwan et al., 1995), and this might be related to the above fall in fetal DPHM plasma unbound fraction with advancing gestation. The observed fall in \( CL_{rn} \) of ~66% over the same gestational period is somewhat greater than the ~47% decrease in F-UF. Because F-UF is linearly and tightly related to \( CL_{fm} \) (Fig. 3B), the changes in F-UF do not appear to account for the entire observed fall in \( CL_{rn} \) with GA. This, combined with two additional observations, strongly suggests the involvement of another factor in this \( CL_{rn} \)-GA relationship. First, Fig. 5 shows the relationship between \( CL_{rn} \) clearance of the unbound DPHM (calculated using the unbound drug concentrations) with GA; this parameter also falls significantly by ~43% from 125 to 136 days. Because, as expected, \( CL_{rn} \) (unbound drug) bears no correlation with F-UF (\( r = 0.1852, P = 0.5 \); data not shown), there must be an additional factor involved in this \( CL_{rn} \)-GA relationship. Second, in Fig. 6, we plotted a relationship between GA (from 109 to 134 days) and acetaminophen \( CL_{rn} \) data in pregnant sheep obtained by Wang et al. (1986). Similar to DPHM (although much less pronounced), a significant inverse relationship between acetaminophen \( CL_{rn} \) and GA is also evident, suggesting that a GA-related fall in \( CL_{rn} \) may be a more general phenomenon. However, plasma protein binding of acetaminophen is low in both the ewe and the fetus (~10%; Wang et al., 1986) and would not be a limiting factor for the placental clearance of the drug. Hence, it appears that protein binding is not the only factor involved in DPHM and acetaminophen \( CL_{rn} \) alterations with GA. We believe

\[
F-UF = -0.0163(GA) + 2.42
r = -0.6427
p < 0.005
\]

**Fig. 4.** Alterations in fetal (A) and maternal (B) steady-state plasma free fraction of DPHM with increasing GA.

Actual experimental data (scatterpoints), regression line (solid), and 95% confidence interval (dotted) are depicted.
that the other possible factor affecting $CL_{fm}$ is umbilical blood flow ($Q_{um}$). It has been shown that weight-normalized $Q_{um}$ in sheep gradually decreases from 103 to 141 days’ gestation (Hedriana et al., 1995). We have also observed a $\sim 37\%$ fall in $Q_{um}$ over 125 to 136 days’ gestation in pregnant sheep in our laboratory (S.K., K.W.R., and D.W.R., unpublished data). This may represent an inability of the fetal cardiovascular system to sustain both fetal regional perfusion and $Q_{um}$ during the rapid phase of fetal growth during late gestation. In contrast to $Q_{um}$, there is no evidence for a fall in either uterine or maternal placental blood flow per kilogram of fetal weight during late gestation in sheep (Rosenfeld et al., 1974). The relationship between fetal placental clearance of flow-limited compounds (ethanol and antipyrine) and uterine ($Q_{ut}$) and umbilical ($Q_{um}$) blood flow in sheep is described by the equation:

$$CL_{fm} = 1/(1/0.91Q_{ut} + 1/0.83Q_{um})$$

(Wilkningen et al., 1982). This equation predicts that a fall in either $Q_{ut}$ or $Q_{um}$ will reduce the $CL_{fm}$ of these compounds. At a constant $Q_{ut}$, $Q_{um}$ will lead to a less-than-proportional decrease in $CL_{fm}$. Because $CL_{fm}$ for DPHM is similar to the values for ethanol and antipyrine (98.6 ± 11.9 and 113.4 ± 13.5 ml/min/kg, respectively; Wilkening et al., 1982), the above relationship likely holds for DPHM and other high placental clearance drugs as well. The combined fall in F-UF and $Q_{um}$ appears sufficient to explain the observed relationship between DPHM $CL_{fm}$ and GA. The $CL_{fm}$ of acetaminophen is much lower compared with that of DPHM, ethanol, and antipyrine. However, in vitro studies with perfused human placenta demonstrated that the placental transfer of a low placental permeability compound, cimetidine, was also significantly affected by alterations in the rate of umbilical perfusion (Bassily et al., 1995). Thus, GA-related fall in $Q_{um}$ may be responsible for a decrease in acetaminophen $CL_{fm}$ with advancing gestation. Also, in contrast to DPHM, $Q_{um}$ is likely the only variable involved in the acetaminophen $CL_{fm}$-GA relationship because the plasma protein binding for this drug is not a limiting factor.

The linear regression of both $CL_{fo}$ versus F-UF and $CL_{fo}$ versus M-UF was statistically significant. However, the y-axis zero intercepts of both these relationships were positive, being 31 and 72% of the average $CL_{fo}$ and $CL_{fm}$ estimates, respectively. This may be explained by the fact that the relationship of plasma unbound fraction with drug clearance, as described by the most common models of organ clearance (e.g., well-stirred, parallel tube, and dispersion models) is in fact curvilinear (Wilkinson, 1987). Thus, it would be conceptually inaccurate to fit the nonplacental clearance versus unbound fraction data to a simple linear model, and perhaps that underlies the high positive y-intercepts obtained earlier. Hence, these relationships were analyzed according to the most commonly studied and accepted well-stirred model of organ drug clearance (eqs. 1 and 2; Fig. 3, C and F) (Wilkinson and Shand, 1975; Wilkinson, 1987). The $CL_{in}$ and $Q_{H}$ parameters in the mother were estimated to be 1242.6 ± 176.8 and 60.2 ± 5.7 ml/min/kg, respectively. The estimated $Q_{H}$ is very similar to that reported for pregnant sheep (65 ml/min/kg; Katz and Bergman, 1969), supporting the validity of our analysis. From the fit of $CL_{fo}$ versus F-UF data, $CL_{in}$ and $Q_{F}$ in the fetus were estimated to be 517.6 ± 251.2 and 318.7 ± 306.6 ml/min/kg, respectively. The estimated mean value of $Q_{H}$ is higher compared with the average reported for fetal sheep at this stage of gestation ($\sim 137$ ml/min/kg; Edelstone et al., 1978). This and the high standard error in the $CL_{in}$ and $Q_{F}$ estimates in the fetus may be related to a relatively larger variability in the $CL_{in}$ versus F-UF relationship (Fig. 3C). The latter in turn may be due to the fact that F-UF was generally higher compared with M-UF and may not be a strongly limiting factor for $CL_{fo}$. Overall, this analysis indicates that plasma protein binding is an important factor in the hepatic (hepatic and gut in case of the mother) uptake of DPHM in both the mother and the fetus. Because F-UF falls with GA, it may explain the decreasing trend in $CL_{fo}$ with GA (Fig. 2C). Also, $\sim 75\%$ of the fetal hepatic blood supply comes from $Q_{um}$ (Edelstone et al., 1978), and the GA-related fall in $Q_{um}$ may also contribute to the observed negative trend in $CL_{fo}$ with GA via reductions in fetal $Q_{H}$.

In summary, our data demonstrate that plasma protein binding of the drug is an important determinant of placental and nonplacental DPHM clearances in the mother as well as in the fetus. DPHM fetal total, placental, and possibly nonplacental clearances decrease during the final period of gestation in sheep, with the fetal placental clearance demonstrating the most pronounced change. An increase in fetal protein binding of the drug and a decrease in weight-normalized umbilical blood flow with advancing gestation appear to be responsible for these clearance changes. A similar phenomenon is
DRUG CLEARANCE IN THE MATERNAL-FETAL UNIT

apparent in the literature data for acetaminophen, a drug with much lower placental clearance compared with DPHM, indicating that it is not limited to high placental clearance drugs.

References


