

FETAL MORPHINE METABOLISM AND CLEARANCE ARE CONSTANT DURING LATE GESTATION

Marianne Garland, Kirsten M. Abildskov, Samantha Taylor, Kenza Benzeroual, Casper S. Caspersen, Sylvia E. Arroyo, Tung-wah Kiu, Boris Reznik, Piper Weldy, Salha S. Daniel, and Raymond I. Stark

Perinatal Research Laboratory, Division of Neonatology, Columbia University, New York, New York

Received October 4, 2005; accepted January 25, 2006

ABSTRACT:

Fetal metabolism significantly contributes to the clearance of drugs from the fetus. To understand how the changes in fetal metabolism expected in late gestation alter fetal drug clearance, serial measurements of morphine metabolism were made in the fetal baboon over the latter third of gestation. Clearance and metabolism were evaluated in the context of fetal growth, onset of labor, and the administration of classical enzyme induction agents. Morphine, a probe substrate for the enzyme uridine diphosphate glucuronosyltransferase 2B7 (UGT2B7), was continuously infused to chronically catheterized fetal baboons while measuring morphine, morphine-3- β -glucuronide, and morphine-6- β -glucuronide concentrations. In some animals, intermittent infusions of the metabolites provided estimates of metabolite clearance and, hence, the rate of formation of metabolites and metabolic clearance.

Overall, metabolic clearance of morphine from the fetus was $27 \pm 9.0 \text{ ml} \cdot \text{min}^{-1}$ or 32% of total clearance. This is similar to the overall clearance in the adult baboon when standardized to weight. No change in any measure of metabolism or clearance of morphine or its glucuronide metabolites was found with gestational age, the presence of labor, or administration of UGT enzyme induction agents. Interpreting these findings using a physiologically based approach suggests that the intrinsic clearance of the fetal liver toward morphine is of sufficient magnitude that fetal hepatic clearance is flow-limited. The implication of a high intrinsic clearance is for significant placento-hepatic first-pass metabolism when drugs are administered to the mother. The previously held view of the "inadequacy of perinatal glucuronidation" needs to be reconsidered.

More than 90% of women take some form of prescribed medication during pregnancy (Glover et al., 2003). This high prevalence of drug use by pregnant women underscores the importance of understanding drug disposition in the fetus. The general consensus is that almost all drugs cross the placenta to some extent. Several factors such as protein binding, ionization, fetal metabolism, and active placental transport are known to influence fetal drug exposure (Garland, 1998). However, no comprehensive pharmacokinetic model exists that predicts fetal drug concentrations, let alone drug metabolite concentrations. For the most part, both toxic and direct beneficial effects of an agent occur in the fetus in a dose-dependent manner. Thus, models are critical to assess the risk or efficacy of an agent and to optimize the choice of agent for use in pregnancy. The chronically catheterized pregnant baboon provides an animal model with close parallels to human pregnancy (Stark et al., 1989, 1993, 1999; Daniel et al., 1992; Grieve et al., 1994; Garland et al., 1996, 1998a,b). The model has considerable utility

and direct application to understanding the processes contributing to fetal drug disposition. The studies reported here are part of a larger project to develop a comprehensive pharmacokinetic model of pregnancy that incorporates metabolite disposition (Garland et al., 2005b). Morphine is used as a model drug.

The physiology of pregnancy is highly dynamic. These physiologic processes contribute to pharmacokinetic parameters, and, as such, a pharmacokinetic model must take into account the likely changes in model parameters over gestation. A major influence on fetal drug concentration is direct clearance of drug from the fetus (Szeto et al., 1982). Fetal metabolism was found to be a significant contributor to nonplacental fetal clearance (Garland et al., 2005b). Approximately one third of the elimination of morphine from the fetal compartment is accounted for by fetal metabolism. Changes in the expression and activity of drug-metabolizing enzymes that can occur with development, and increase in the absolute amount of enzyme that results from growth are expected to alter fetal metabolism (Ring et al., 1999). Morphine is a probe substrate for UGT2B7, a member of the 2B family of uridine diphosphate glucuronosyltransferases (UGTs). Furthermore, both the glucuronide metabolites of morphine that are found in humans, that is, morphine-3- β -glucuronide (M3G) and morphine-6- β -glucuronide (M6G), are formed by the fetal baboon. The glucuronosyltransferase enzyme system is known to undergo considerable up-regulation in the perinatal period (Coffman et al., 1997). However,

This research was supported by the National Institute on Drug Abuse and was initially presented at the Society for Pediatric Research, 2003 [Garland M, Benzeroual K, Taylor S, Kiu T, Abildskov K, and Stark R (2003) *Pediatr Res* 53 (4 Part 2):558A].

Article, publication date, and citation information can be found at <http://dmd.aspetjournals.org>.

doi:10.1124/dmd.105.007567.

ABBREVIATIONS: UGT, UDP-glucuronosyltransferase; HPLC, high-performance liquid chromatography; M3G, morphine-3- β -glucuronide; M6G, morphine-6- β -glucuronide; CL, clearance.

the timing of induction before or after delivery is not well delineated for most UGT isoforms (Ring et al., 1999).

The objectives of these studies were to delineate the changes in fetal metabolism of morphine across late gestation in the nonhuman primate. The hypotheses were that there are gradual increases in fetal metabolism commensurate with fetal growth and that a more marked increase is initiated with the onset of labor. Furthermore, exogenous UGT enzyme-inducing agents lead to a premature enhancement of metabolism. To examine these changes in fetal metabolism of morphine with gestational age, fetal morphine metabolism was quantified during the latter third of gestation from studies infusing morphine and its glucuronide metabolites.

Materials and Methods

Study Population. A breeding colony of baboons (*Papio* species), housed in the Institute of Comparative Medicine, Columbia University, New York, was available for study. Gestational age was determined using the midpoint of timed matings as the estimated day of conception (± 3 days, term ~ 175 days). Ultrasound was performed at 70 to 110 days to confirm singleton pregnancy, fetal size, and placental location. Lights were cycled (7:00 AM on, 7:00 PM off) and feeding times (8:00 AM and 4:00 PM) were constant. Animals were maintained in accordance with all National Institutes of Health, U.S. Department of Agriculture, and American Association for the Accreditation of Laboratory Animal Science regulations for the care and use of laboratory animals. Research protocols were approved by the Institutional Animal Care and Use Committee at Columbia University.

Surgical Procedure and Tethering System. The animals were studied using a system of individualized backpacks and tether. This system, along with the methods for maintenance, breeding, preconditioning, anesthesia, surgery, and postoperative care, were described in detail in a previous report and are only summarized here (Stark et al., 1989). Surgery was scheduled between 120 and 145 days. Approximately 2 weeks before surgery, the animal was fitted to a backpack and sham tethered. This allowed proper adjustment of the backpack straps and assessment of acceptance of the animal to the tether system. Only those animals who readily adapted to the system were studied.

Surgery was performed under general anesthesia (isoflurane, nitrous oxide) using sterile surgical techniques. Vascular catheters were placed in the maternal femoral artery and vein and in the fetal carotid artery and jugular vein. An amniotic fluid catheter was attached on the neck. Electroencephalogram, electro-oculogram, and electrocardiogram electrodes and tracheal catheter were also placed to monitor fetal well being and drug effects. The uterus was closed in two layers, taking care to appose amniotic membranes. After closure of the rectus sheath and before skin closure, the catheters and electrodes were tunneled under the skin to the mid-scapular region of the mother, where they exited to the backpack.

The backpack housed the pressure transducers and the catheter and electrical connectors. It was attached to a stainless steel tether cable that connected to a freely rotating pole on the top of the cage. Peristaltic infusion pumps and solutions were fixed to the pole (P720; Instech Laboratories, Inc., Plymouth Meeting, PA). The maternal and fetal vascular catheters were continuously infused with normal saline containing heparin ($2 \text{ U} \cdot \text{ml}^{-1}$) at rates of 5 and $2 \text{ ml} \cdot \text{h}^{-1}$, respectively, to keep catheters patent. Catheters were accessible at the top of the cage to obtain blood and amniotic fluid samples. An electrical swivel at the top of the pole allowed the whole apparatus to turn with the activity of the animal while providing electrical power for pumps and signal transmission of physiological data.

Postoperative analgesia was supplied with a continuous infusion of morphine sulfate ($50\text{--}150 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) to the mother. This was reduced stepwise as she resumed normal activity in the 2 to 4 days after surgery. No tocolytic agents were used. Cefazolin was administered daily for 3 days to the mother and into the amniotic fluid, and then as indicated. The mothers were maintained in their home cages near other familiar animals. A minimum of 6 days postsurgery and 48 h after cessation of postoperative morphine was allowed for stabilization before the start of infusion protocols.

Mothers were monitored for signs of labor assessed by maternal behavior and the pressure recordings from the amniotic fluid catheter (Cobe low-level pressure transducers; Cobe, Boulder CO; Hewlett Packard 8805-B Amplifier;

Hewlett Packard, San Diego, CA). Physiologic stability of the fetus was assessed by fetal heart rate, blood pressure, arterial acid base status, and blood gas values (Hewlett Packard 3040A heart rate monitor; Cobe low-level pressure transducers; Hewlett Packard 8805-B Amplifiers; Radiometer ABL720, Radiometer America, Westlake, OH). This model allowed the pharmacokinetic studies to be performed during the last trimester of a stable pregnancy without the need of anesthesia or undue restraint.

Drug Preparation. M3G and M6G were obtained from the National Institute on Drug Abuse, Division of Neuroscience and Behavioral Research, Bethesda, MD. A morphine sulfate injection solution, confirmed by HPLC in this laboratory to be a concentration of 15 mg/ml morphine, was purchased from Elkins Sinn (Cherry Hill, NJ). Other injection solutions included dexamethasone sodium phosphate injection, USP, 4 mg/ml (American Regent Laboratories, Shirley, NY) and heparin, sodium injection, USP, 5000 U/ml (American Pharmaceutical Partners, Inc., Schaumburg, IL).

All M3G solutions were prepared from a 2 mg/ml stock solution in normal saline and stored at -70°C until use. Solutions of morphine, a combination of M6G and morphine, and dexamethasone were prepared directly from the chemicals listed above. All infusion solutions were prepared in normal saline so that when infused at $2 \text{ ml} \cdot \text{h}^{-1}$, they would deliver the doses given below. The infusions contained 0.5 U/ml heparin. They were filtered through 0.22- μm , 25-mm MCE filters (Fisher Scientific, Pittsburgh, PA) into sterile Viaflex infusion bags (Baxter-Clinitech Division, Deerfield, IL). The infusion bags were wrapped in aluminum foil and stored at -20°C until they were ready to be used on the animal.

Infusion and Sampling Protocols. Results from several related infusion protocols have been grouped together for this report because they were all directed toward examining the changes in fetal metabolism across the latter stages of gestation. This is done in part because of the small number of animals available for research, but more because the implications drawn from the results are similar across studies and lend strength to the conclusions. For some studies, only relative changes in metabolism are evaluated, whereas in others, metabolism was quantified by intermittent infusion of the morphine metabolite(s). Metabolite infusions were used to define the clearance of the metabolite from the fetus so that the rate of formation of the metabolite can be calculated from the concentration of morphine metabolites during fetal morphine infusion (see below). The 12 pregnancies were distributed among four protocols described below (Table 1).

Group 1 ($n = 1$). Morphine was infused to the fetus at a continuous rate (0.4 mg/h) for 12 days until delivery. The morphine dose selected was in the therapeutic range and known to produce plasma levels of morphine, M3G, and M6G that were well above the limit of quantification (Garland et al., 2005b). This animal (240) had completed a previous protocol in which morphine had been infused to the fetus for 48 h and, a week later, to the mother for 48 h to determine placental clearances. The fetus remained stable and thus was recruited to examine the changes in metabolism near term and with the onset of labor. The animal had labor for 3 days before delivery, at which time the infusion ended. Samples were collected each day where possible.

Group 2 ($n = 5$). Three doses of M3G (0.125, 0.25, and 0.5 mg/h) were infused to the fetal baboon to determine the metabolite clearance. For practical reasons, two timed samples were obtained between 8 and 16 h after the start of the infusion: the first dose was started at 5:00 PM with samples obtained at 8:00 AM and 9:00 AM the following morning; the second dose was then started with samples taken at 5:00 PM and 9:00 PM that evening; the third dose was then started with samples obtained at 8:00 AM and 9:00 AM the next day. Start times of infusions and sample times were precisely recorded for these shorter infusions. The doses used spanned the concentration of M3G found during morphine infusion and provided a calibration curve for M3G formation. They were selected from doses used and concentrations obtained in previous dose-kinetic studies (Garland et al., 2005b).

The M3G infusions were followed by a continuous infusion of morphine to the fetus at 0.5 mg/h for 7 to 10 days. During morphine infusion, two fetal blood samples were collected on alternate days. This sampling schedule was selected to provide information on within- and between-day variability. The morphine infusion was then stopped for 24 h to allow clearance of drug and metabolite. The fetus was again infused with M3G at the same three doses to assess changes in M3G clearance. After reassessment of M3G clearance, morphine infusion was restarted. M3G clearance was repeated in this manner

TABLE 1
Demographic data on study animals

Study Group	Animal No.	Maternal Weight	Gestational Age			Birth Weight	Sex	Induction Agent
			Surgery	Start	Delivery			
		kg		days		g		
1	240	17	123	146	158	676	M	None
2	278	16.5	126	135	156	760	F	None
2	290	17.5	136	142	158	778	M	None
2	292	19	133	141	164			None
2	294	18	135	142	151	720	M	None
2	297	12	129	137	148 ^a			None
3	248	16.5	129	146	177	680	M	PB/BT
3	319	12.5	137	145	172	668	F	DX/PB
3	322	15.5	134	141	151	680	F	DX/PB
4	327	14.5	129	138	153	671	M	SAL
4	331	14.5	139	146	156	469	F	DX
4	342	15.75	131	139	153	570	M	DX

BT, betamethasone; DX, dexamethasone; PB, phenobarbital; SAL, saline.

^a Gestational age at fetal demise.

every 7 to 10 days, followed by resumption of the morphine infusion. Infusions were continued for the duration of the pregnancy.

Group 3 ($n = 3$). Classical UGT enzyme-inducing agents were evaluated to assess which one would be appropriate for further study. The UGT enzyme-inducing agents were selected based on their wide use in research for induction of UGTs and for their clinical relevance. Phenobarbital is perhaps the most widely used UGT enzyme inducer. It was administered directly to the fetus at a dose of $1 \text{ mg} \cdot \text{h}^{-1}$ for 72 h. This is a relatively high dose, based on estimated fetal weight, but does not exceed that based on maternal weight for clinical use. Glucocorticoids are widely used for induction of drug-metabolizing enzymes including UGTs. Betamethasone was administered as two 6-mg doses given intramuscularly to the mother, 24 h apart. This treatment regimen is similar to that used in human pregnancy for lung maturation in threatening preterm delivery (Liggins and Howie, 1972). Dexamethasone is an alternative glucocorticoid that is more potent but has a shorter half-life. As such, this was administered directly to the fetus as a continuous infusion for 72 h. The dose used, $0.002 \text{ mg} \cdot \text{h}^{-1}$, has been shown to cause physiological effects in fetal lambs (Fletcher et al., 2000). The UGT enzyme-inducing agents that individual animals received are indicated in Table 1.

Morphine was infused continuously at a dose of 0.5 mg/h . After a minimum of 72 h of infusion, three samples at least 1 h apart were obtained to define steady-state values. After the third sample, the UGT enzyme-inducing agent was administered; 72 h later, three more samples were obtained. The timing of the second set of samples was chosen to reflect anticipated maximal induction. After these samples were obtained, the morphine infusion continued, whereas, for 7 days, no further UGT enzyme-inducing agent was administered. The effects of phenobarbital and prenatal steroid administration in human pregnancy diminish after this time period. Plasma samples were obtained throughout the week to evaluate whether decrease in effect was evident. After the recovery period, three samples were again obtained and a second UGT enzyme-inducing agent was administered, with three additional samples obtained 72 h later. Thereafter, daily sampling was continued where possible until the animal delivered.

One animal assigned to this group (248) had been in a previous protocol in which M3G was infused to the fetus for 8 h at five doses in random order, on alternate days, to examine the dose kinetics (Garland et al., 2005b). The results from that study were used to provide a value for M3G clearance for this animal; thus, an estimate of fetal metabolism was available for this animal.

Group 4 ($n = 3$). The effects of dexamethasone were studied in a blinded fashion. Eight animals were to be studied with interim review after two animals had received dexamethasone. This was because no obvious effect was seen in the results from group 3, but good data were only available for one animal with dexamethasone. The absence of inducibility was thought to be of enough importance to warrant confirming this finding. The design used each animal as its own control and included a saline group to control for gestational age effects.

The infusion protocol was based on the protocols from groups 2 and 3 above, but modified to obtain measures of metabolite clearance without dis-

continuing morphine. It had appeared that stopping morphine to infuse M3G may have precipitated delivery in protocol 2. After an initial M3G infusion series (see doses in group 2), animals in group 4 received a combined infusion of morphine ($0.5 \text{ mg} \cdot \text{h}^{-1}$) and M6G ($0.25 \text{ mg} \cdot \text{h}^{-1}$) for 24 h before continuation of morphine infusion alone. This allowed for an initial determination of M3G clearance, a comparison with M6G clearance, and subsequent further evaluations of metabolite clearance without discontinuing morphine by repeating the infusion of M6G and morphine combined at appropriate intervals. M6G clearance is similar to that of M3G and both are linear (Garland et al., 2005b). The rate of formation of M6G from morphine infusion was expected to account for $<5\%$ of that measured when M6G was infused.

As in the protocol for group 3 above, at least 72 h of morphine infusion was allowed to ensure that plasma concentrations were at steady state before the initiation of dexamethasone. The same dose of dexamethasone was used as above, except that the animals were randomized to receive dexamethasone or saline. Investigators who conducted the study were blinded to this assignment. Again, three samples were obtained on the day before the start of the dexamethasone infusion and three samples 72 h later. In addition, a single blood sample was obtained on all other days where possible. After the final set of three samples was obtained during dexamethasone infusion, a final M6G/morphine combination was infused overnight while dexamethasone or saline continued. A final pair of samples was obtained and the fetuses were delivered by cesarean section for collection of tissues.

For all protocols, morphine and morphine metabolite infusions were administered through the fetal venous catheter replacing the normal saline infusion that maintained catheter patency. Samples were obtained from the fetal arterial catheter. If samples were consistently not available through the arterial catheter yet could be obtained through the venous catheter, a decision was made to switch sites for the remainder of the study. UGT enzyme-inducing agents that were administered directly to the fetus were given through the sampling catheter to avoid disturbing the rate of infusion of morphine to the fetus. Although all pumps were precalibrated to deliver $2 \text{ ml} \cdot \text{h}^{-1}$, some differences in rates had been noted once attached to the animal, presumably due to increased resistance. Hence, infusion bags were weighed before and after the infusions to determine the actual drug delivery rate. The pump rate during infusion remained stable and the same pump was used throughout. The mean rate of infusion within each animal was used to determine actual doses delivered.

Blood samples were obtained by drawing back the dead space of the catheter plus 0.5 to 1 cc of blood for the fetus and a minimum of 3.0 cc of blood for the mother. The sample volume of 0.6 cc was then obtained for drug analyses. The extra blood withdrawn was returned and the line flushed with 3 cc of normal saline, which was sufficient to clear the line. Blood was placed in heparinized Microtainer tubes (BD, Franklin Lakes, NJ), gently mixed, and then separated by centrifugation. Plasma was transferred to plastic vials and stored at -20°C until analysis.

Fetal and Hepatic Weights. Animals from the same colony in different protocols over the same time period, and those in group 4, underwent cesarean

section in time mated pregnancies. The fetus was euthanized while still under anesthesia, and then weighed. Fetal organs were dissected and weighed individually.

HPLC Method for Analysis of Morphine, M3G, and M6G in Plasma Samples. Morphine, M3G, and M6G were measured by HPLC using an automated Waters Alliance 2695 HPLC system (Waters, Milford, MA) with a combination of fluorometric and coulometric detection, as described previously (Garland et al., 2005).

Extraction Procedure for Plasma Samples. Sample preparation was performed using solid phase extraction columns (1-ml Bond Elute C18 50-mg columns; Varian Inc., Sugarland, TX) on a Visiprep-DL Solid Phase Vacuum Manifold (model 5-7250), followed by sample evaporation under nitrogen with a Visidry drying attachment (model 5-7124; Supelco, Bellefonte, PA). After conditioning twice with 1 ml of methanol (HPLC grade, Mallinkrodt; Fisher Scientific), 1 ml of purified water (Milli-Q NanoPure Water Filtration System; Millipore, Bedford, MA), and 1 ml of 0.01 M ammonium bicarbonate buffer (pH 9.3, HPLC grade, Mallinkrodt; Fisher Scientific) under vacuum, the solid phase extraction columns were loaded by gravity with 200 μ l of ammonium bicarbonate buffer (pH 9.3), 100 μ l of sample plasma or, for standards, 100 μ l of blank pooled plasma with 20 μ l of 5 \times standard, 100 μ l of the internal standard nalorphine hydrochloride (800 ng \cdot ml⁻¹; Sigma, St. Louis, MO), and then an additional 200 μ l of ammonium bicarbonate buffer (pH 9.3). After equilibration, the column was washed with 1 ml of 0.01 M ammonium bicarbonate (pH 9.3) and dried under vacuum. The sample was eluted twice with 150 μ l of methanol (HPLC grade; Fisher Scientific), dried under nitrogen, and reconstituted in 200 μ l of mobile phase. All samples were extracted in duplicate.

Chromatographic Conditions. The separation was performed isocratically on a Waters Alliance 2695 HPLC system using a Spherisorb C18 column (ODS2, 3 μ m, 4.6 \times 100 mm i.d.) at ambient temperature, with a flow rate of 1.5 ml \cdot min⁻¹. The mobile phase was 10 mM sodium phosphate monobasic (Fisher Scientific), 1.5 mM sodium dodecyl sulfate (ultraPURE; Invitrogen, Carlsbad, CA), and 24% acetonitrile (Fisher Scientific) adjusted to pH 2.1 with *o*-phosphoric acid (HPLC grade; Fisher Scientific). After injection of 50 μ l of sample, the eluting compounds were measured by fluorescence detection (excitation 210 nm and emission 340 nm) on a Linear model LC305 detector (Linear Instruments; ESA, Bedford, MA), followed by coulometric detection (electrode 1, +225 mV, and electrode 2, +350 mV) with an ESA model 5200A Coulochem II detector.

The three compounds (morphine, M3G, and M6G), as well as the internal standard nalorphine, were detectable by fluorescence, whereas M3G was the only compound not electrochemically active. Values determined by the two detection systems were not different, although electrochemical detection gave greater sensitivity and was used when available. The lower limit of detection was taken as the lowest point on the standard curve, which was 1.0, 1.0, and 5.0 ng/ml for morphine, M6G, and M3G, respectively. Values for a control sample included in each extraction had mean (coefficient of variation) values of 18.5 (8.8%), 5.6 (12.1%), and 260 (12.3%) ng \cdot ml⁻¹ in 16 extractions over a period of 2 years.

Pharmacokinetic and Statistical Analyses. *Morphine Infusions.* Morphine, M3G, and M6G concentrations during morphine infusion were examined for effects of route of infusion and effects of uterine activity or labor. The mean of appropriate samples was then used to define steady-state concentrations. Clearance of morphine was determined by dividing dose (corrected to morphine base) by concentration of morphine at steady state.

Metabolite Infusions. M3G clearance was determined similarly during M3G infusions, using the mean of the two concentration measurements obtained to represent steady state. Assessment of approximation to steady state was done by evaluation of the paired values. To calculate M6G clearance, M6G concentration was first corrected by subtracting the mean M6G concentration during morphine infusion from the mean M6G concentration during the combined infusion of morphine and M6G. The effect of gestation on metabolite clearance was determined for animals with multiple measures to allow correction for metabolite clearance in calculating the rate of formation of metabolite.

Fetal Metabolism. Rate of formation of metabolite during morphine infusion was calculated by multiplying metabolite steady-state concentration during morphine infusion by the metabolite clearance (corrected if necessary)

calculated from metabolite infusion. Measures of morphine metabolism evaluated during long-term infusion were fetal morphine, M3G, and M6G concentrations, total fetal morphine clearance, fetal metabolite to drug ratios, and calculated rates of formation of metabolite.

Placental Clearance. The difference between total fetal clearance of morphine and fetal metabolic clearance was taken as an indirect measure of placental clearance. In addition to clearances, fetal to maternal drug and metabolite ratios were used to evaluate changes in placental clearance.

Statistical Analyses. Dose kinetics of M3G were assessed by linear regression and by comparison of clearances with repeated measures analysis. M6G and M3G clearances in the same animal were compared with a paired *t* test. The effect of UGT enzyme-inducing agents was examined with a paired *t* test comparing the mean steady-state concentrations before and after administration of the agent. Changes with gestational age were evaluated using linear regression analysis. Mean and standard deviation (S.D.) are reported unless otherwise stated. Alpha of 0.05 was considered significant. All statistical analyses were done using the Systat statistical package (Systat Software, Inc., Point Richmond, CA).

Results

Animal Demographics. Twelve animals were studied in the various infusion protocols (Table 1). Fetal surgery was performed between 123 and 139 days of gestation. Animals 240 and 248 began these protocols 23 and 17 days after fetal surgery, respectively, because they had prior assignment to other research protocols, as described under *Materials and Methods*. Protocols continued until delivery or catheter failure. Mean age of delivery was 158 \pm 9 days, with average birth weight of 667 \pm 90 g. Eleven of the 12 fetuses were alive through the onset of labor. One fetus died as the result of a catheter disconnection. Fetal heart rate and blood pressure were stable on all animals, and all acid base and blood gas values were within previously documented fetal norms (Daniel et al., 1992).

Effect of Infusion/Sampling Site. In three animals, the site of infusion was changed from the venous to the arterial catheter because of problems obtaining samples through a patent arterial catheter. During arterial infusion of morphine, plasma concentrations of morphine were almost twice those measured during venous infusion (Table 2). There was no demonstrable effect on metabolite concentrations during morphine infusion. A similar effect should be seen with infusion of the metabolite. In animal 278, results during both venous and arterial M3G infusions were also available. Clearance measurements were compared since different doses were used. The mean clearance during venous infusion (1.74 \pm 0.096 ml \cdot min⁻¹; *n* = 6) was not different from that during arterial infusion (1.69 \pm 0.473 ml \cdot min⁻¹; *n* = 6); however, the variability among venous samples was considerably greater. There were more confounding variables (dose, gestational age, and labor, for example) to account for this. Therefore, in the following comparisons between animals, only values from venous infusions with arterial samples are used. In longitudinal analyses, the route of infusion is indicated and evaluated separately if necessary.

Metabolite Clearances. In all, 31 M3G infusions were conducted in eight animals. In four infusions, only one sample was obtained, and in another, one value was excluded because it was an order of magnitude below that expected. To evaluate whether values from the two samples are a reasonable approximation of steady state, the difference between paired values was examined. Of the 26 paired values obtained, 18 lay within 5% of the mean of the pair, with an even distribution above and below the mean with respect to time. Dividing the paired values into three groups based on the timing of samples showed no relationship between the concentrations standardized to a 0.25 mg \cdot h⁻¹ dose and the duration of infusion or the time separating the measurements (Fig. 1). The only pair >15% different

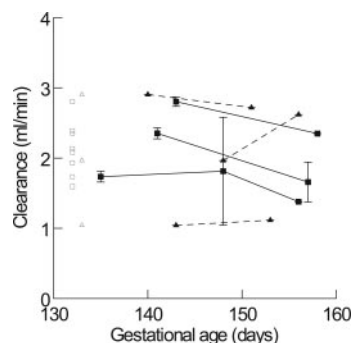


FIG. 2. Morphine metabolite clearances at different gestational ages. M3G (squares) and M6G (triangles) clearances at different gestational ages in individual animals (connecting lines) are shown. Where values from more than one infusion were available, mean \pm S.E.M. was plotted. Values from all animals in Table 2 are plotted at the left-hand edge for comparison (open symbols).

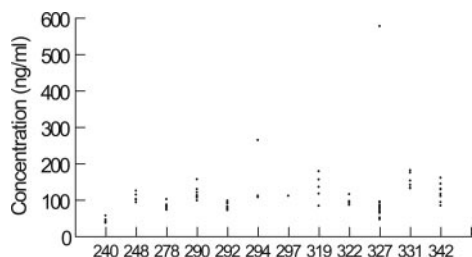


FIG. 3. Variability in morphine concentrations. Morphine concentrations from venous infusions and before the initiation of any induction agent are plotted for each animal. This demonstrates the consistency of values within animals, apart from two outlying values, and the considerable variability between animals. The outlying values were excluded from subsequent analyses.

3). The contribution of metabolism to M6G was minimal and accounted for less than 1% of total morphine clearance (Table 3).

Effect of Classical UGT Enzyme-Inducing Agents on Fetal Morphine Metabolism. Mean morphine, M3G, and M6G concentrations, and the metabolite to drug ratios before and after exposure to phenobarbital, betamethasone, or dexamethasone are presented in Table 4. Before and after values were compared using paired t test and no significant effect was found (p , N.S.). The individual values across gestational age (discussed below) are shown in the right panel of Fig. 4.

Changes in Morphine Disposition with Gestational Age. The individual morphine and metabolite concentrations for all animals

with a minimum of 7 days of data are shown in Fig. 4. Periods in which infusions were switched from venous to arterial route and where UGT enzyme-inducing agents were administered are indicated. There were no consistent trends across gestation, although, in some individual animals, significant changes (mostly decreases) in concentrations could be discerned. Overall, morphine and metabolite concentrations remained remarkably constant in all animals. This is exemplified in Fig. 5, where data from all animals are combined. The molar ratio of metabolite to drug is presented as a more sensitive measure of metabolism because this ratio controls for placental clearance as well as dose. Morphine clearance is also presented to illustrate the similarity in total clearance over this latter part of gestation. The statistical parameters are provided in Table 5.

Fetal Growth over the Latter Part of Gestation. The weights of a contemporary group of fetal baboons undergoing cesarean delivery at gestational ages comparable to those of the fetuses in the present study are shown in Fig. 6. Both fetal body weight and liver weight increased with gestational age (slope, $7.45 \text{ g} \cdot \text{day}^{-1}$, $r = 0.68$, $p < 0.0001$; and slope, $0.3 \text{ g} \cdot \text{day}^{-1}$, $r = 0.21$, $p < 0.06$, respectively). Over a 30-day period of gestation, the fetal weight increases by $\sim 220 \text{ g}$ and the liver weight by $\sim 9 \text{ g}$, an increase of approximately 30 to 50%. The birth weights of the current group of animals and the liver weights from the two animals from which tissues were collected are superimposed on Fig. 6. These demonstrate that the fetuses in the study are appropriate for gestational age and that there were no overt effects on fetal growth as a result of administration of UGT enzyme inducers.

Effect of Uterine Contractions or Labor on Disposition. Labor was diagnosed retrospectively by the presence of uterine activity with progression to delivery. All of the animals in the left panel of Fig. 4 exhibited uterine activity during the last one to four sample collections before delivery. Animal 319 had significant uterine activity for 2 days at 160 days of gestation. No effect of uterine activity is seen. One of the outlier points omitted above (animal 294) was during active labor. Although a marked decrease in placental clearance might have explained this high morphine concentration, the metabolite concentrations should also have increased, and this was not the case. There was no evidence for either an increase in metabolism with the onset of parturition, nor was there evidence for a decrease in placental permeability.

Placental Clearance. Estimated placental clearance for each animal is provided in Table 3. Changes in total morphine clearance with gestational age that cannot be attributed to changes in metabolism may reflect changes in placental clearance. Close inspection of the data in Fig. 4 may

TABLE 4

Effect of classical induction agents on morphine clearance ($\text{ml} \cdot \text{min}^{-1}$), and M3G and M6G to morphine molar ratios

Mean and S.D. are reported for the day before initiation of UGT enzyme-inducing agent and 72 h after the agent.

Animal	Agent	Clearance	M3G/morphine	M6G/morphine
248	Saline	84 ± 12	13 ± 2.0	0.52 ± 0.096
	Phenobarbital	75 ± 11	12 ± 2.2	0.48 ± 0.13
	Saline	92 ± 8.8	10 ± 1.4	0.40 ± 0.069
319	Betamethasone	95 ± 6.6	9.6 ± 0.86	0.48 ± 0.048
	Saline	67 ± 25	11 ± 2.5	0.38 ± 0.11
	Dexamethasone			
322	Saline	48 ± 5.4	8.4 ± 0.56	0.26 ± 0.014
	Phenobarbital	45 ± 1.8	10 ± 0.62	0.33 ± 0.024
	Saline	79 ± 12	15 ± 0.67	0.30 ± 0.039
331	Dexamethasone	77 ± 5.8	13 ± 1.0	0.33 ± 0.013
	Saline	82 ± 11	13 ± 2.3	0.28 ± 0.048
	Phenobarbital	85 ± 8.5	8.7 ± 2.7	0.21 ± 0.091
342	Saline	54 ± 7.4	6.9 ± 1.6	0.23 ± 0.044
	Dexamethasone	49 ± 7.1	6.0 ± 1.2	0.19 ± 0.047
	Saline	80 ± 11	13 ± 1.6	0.51 ± 0.085
	Dexamethasone	71 ± 7.3	9.8 ± 0.90	0.49 ± 0.031

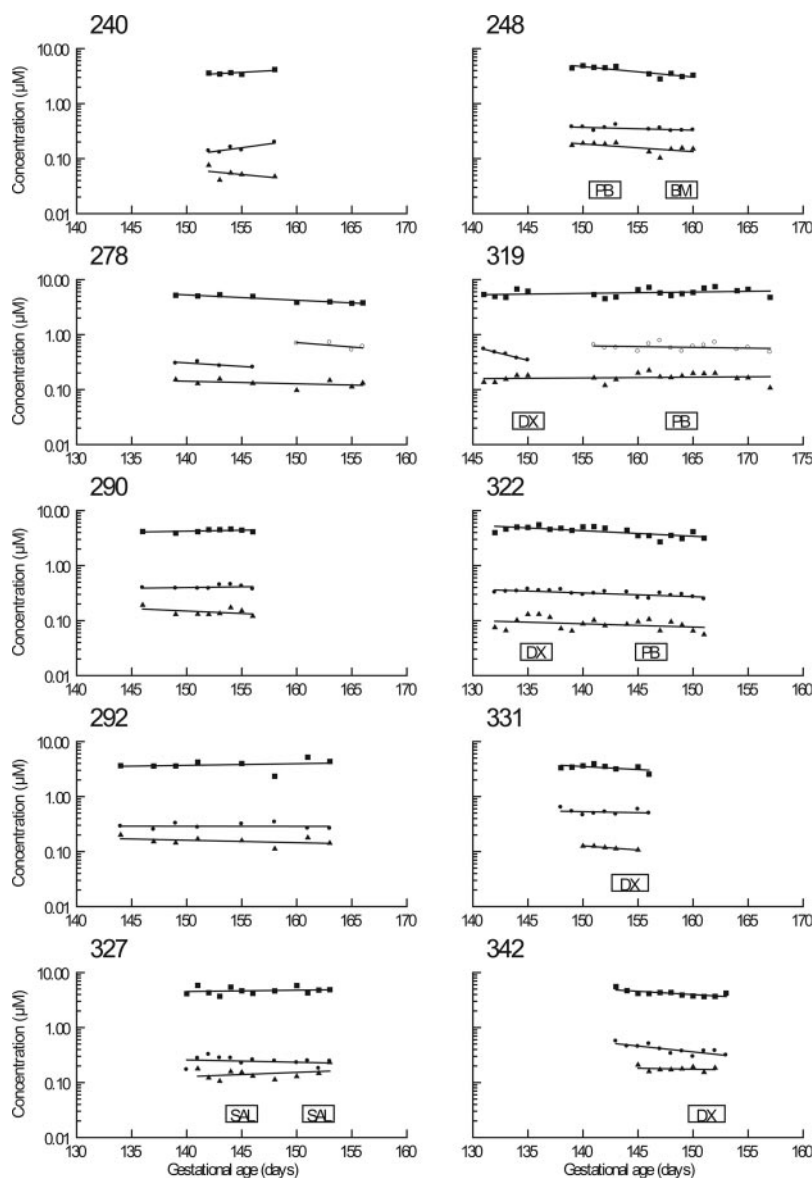


FIG. 4. Effect of gestational age on morphine, M3G, and M6G concentrations. Morphine (circles), M3G (squares), and M6G (triangles) concentrations for individual animals are plotted against gestational age to examine changes in those animals in which more than 7 days of infusion occurred. Open circles represent morphine concentrations when the infusion was switched to the arterial catheter. To maintain scale, a 30-day period is shown in each figure. The lines superimposed are least-squares regression lines. Animals in the left panel delivered vaginally, with all experiencing between 1 and 3 days of uterine activity preceding actual delivery. Animals in the right panel were administered UGT enzyme-inducing agents as indicated in the boxes (BM, betamethasone; DX, dexamethasone; PB, phenobarbital; SAL, saline; see *Materials and Methods* for doses).

suggest that in some animals there are slight decreases in all three analytes. This would support the conclusion that in these animals, there was a slight increase in placental clearance with advancing gestation. Clearly, this was not a consistent feature. Maternal concentrations of morphine and M3G during fetal morphine infusions were evaluated as a surrogate marker of placental clearance in protocol 2. As expected, they were much lower than fetal concentrations, with mean maternal morphine, M3G, and M6G concentrations across animals of 0.040 ± 0.014 , 0.24 ± 0.039 , and $0.012 \pm 0.007 \mu\text{M}$, respectively. Although the data are limited, the concentrations remained relatively constant through the infusion period. This is in keeping with the absence of any change in fetal or placental parameters.

Discussion

Morphine is metabolized by the fetal baboon during the third trimester of pregnancy. Surprisingly little change in the extent of

metabolism or placental clearance is apparent over this latter part of gestation, despite evidence of considerable fetal growth. Furthermore, the use of classical exogenous UGT enzyme inducers does not enhance fetal drug metabolism. Clearly, these results do not support the original hypotheses.

It was proposed that an increase in morphine metabolism would be expected with advancing gestational age, based on an increase in the size of the fetal liver. Instead, no change in fetal metabolism was apparent during late gestation, even though fetal weight increases by 30 to 50% over the period studied. Only one other study has examined fetal clearances over a similar period of gestation (Wang et al., 1986a). In that sheep study, fetal growth resulted in an increase in metabolic clearance of acetaminophen by the fetus. This increase in metabolism disappeared when the data were corrected for estimated fetal weight, implying that the increase in metabolism was due to growth and not increased expression of the UGTs that metabolize

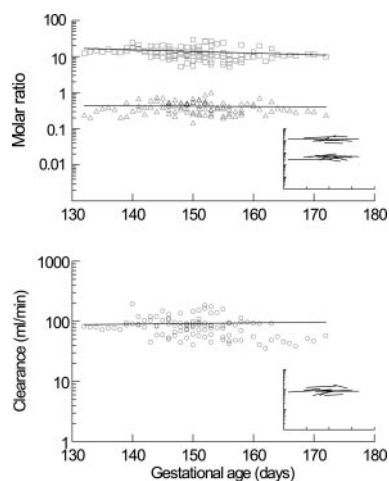


FIG. 5. Effect of gestational age on metabolite to drug ratio and morphine clearance. The upper panel shows the M3G (squares) and M6G (triangles) to morphine ratios for all animals across the latter part of gestation. The lower panel shows morphine clearance (circles). The superimposed lines are least-squares regression lines calculated from the mean value and gestational age for each animal excluding data obtained during arterial infusion. The insets show least-squares regression lines for the individual animals (see Table 5 for values).

acetaminophen. From a physiological standpoint, growth in the liver represents an absolute increase in both the amount of enzyme available, which is reflected in the intrinsic hepatic clearance ($CL_{\text{intrinsic}}$), and the total hepatic blood flow (Q_H). These are the two parameters that contribute to hepatic clearance (Gibaldi and Perrier, 1982).

$$CL_{\text{hepatic}} = Q_H \cdot CL_{\text{intrinsic}} / (Q_H + CL_{\text{intrinsic}}) \quad (1)$$

Hence, independent of the intrinsic hepatic clearance of morphine by the fetal liver, growth should result in greater metabolism.

Several other factors can contribute to the disposition of drug and metabolite, but they are not likely explanations for the lack of increase in the present study (Garland, 1998). In animals exposed to glucocorticoids, lack of growth may explain the lack of increase, but this is not supported by weights at delivery and the similar findings in fetuses not exposed to glucocorticoids. Change in the extent of drug ionization in the fetus is an unlikely explanation since this group of fetuses was documented to have stable pH values. Protein binding of morphine is in the order of 15 to 20% and is proportional to serum albumin concentration, the main binding protein (Tocque et al., 1980).

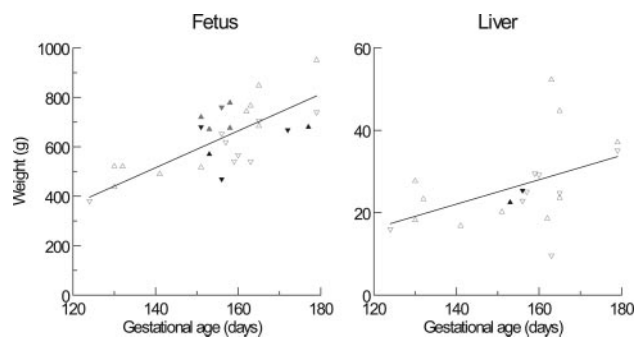


FIG. 6. Changes in fetal hepatic weight and body weight with gestational age. Weights are from a contemporaneous group of fetal baboons. The fetuses from the present study are superimposed. Least-squares regression lines are for the nonstudy animals only. Slope, r^2 , and p values for fetal weight are 7.45, 0.68, and <0.0001 , and for fetal liver, 0.3, 0.21, and <0.06 . Open symbols, contemporaneous group of fetuses; solid symbols, study group; gray, not exposed to glucocorticoid; black exposed to glucocorticoid; inverted triangle, female; regular triangle, male.

Whereas the fetal albumin concentration increases during pregnancy and maternal concentration decreases, the concentrations are fairly similar during the third trimester (Krauer et al., 1984). In addition, protein binding of morphine reported in human infants is similar to that in adults (McRorie et al., 1992; Milne et al., 1996). Thus, although increases in clearance could be masked by increases in protein binding, the magnitude of the increase in protein binding would be insufficient to mask a 30 to 50% increase in clearance that could be expected based on the increase in size of the liver.

An alternative explanation for the lack of increase in metabolism is the method used to quantify fetal metabolism in the present study. This method may have underestimated the capacity of the entire fetal liver (Wang et al., 1986a; Kumar et al., 1999; Garland et al., 2005b). Direct infusion of drug to the fetus will deliver drug to that part of the liver supplied by the hepatic artery and the portal vein. The concentration of drug reaching the liver via the umbilical vein will be low because of transfer to the mother across the placenta. Over this period in gestation, the proportion of the liver supplied from the umbilical vein increases as the percentage of blood shunted through the ductus venosus decreases from almost 50% at mid-gestation to around 10% at term (Bellotti et al., 2000). Hence, the part of the liver supplied by the fetal hepatic artery and portal vein does not increase proportionally. Thus, despite an increase in overall size of the fetal liver, the proportion of the liver that is perfused by the fetal hepatic artery and

TABLE 5

Statistical parameters for changes in metabolism and total clearance with gestational age

For comparative purposes, change is presented as percent change per day, determined by dividing the slope of the individual regression lines by the mean parameter value for individual animals $\times 100$. Overall changes are determined from the regression lines of the mean parameter values and mean gestational age during venous infusion for each animal as drawn in Fig. 5.

Animal	Infusion Route	Clearance		M3G Ratio		M6G Ratio	
		% change \cdot day $^{-1}$	r^2	% change \cdot day $^{-1}$	r^2	% change \cdot day $^{-1}$	r^2
240	Venous	-5.9	0.74	-3.8	0.76	-11	0.60
248	Venous	1.1	0.30	-3.2	0.58*	-2.0	0.22
278	Venous	2.9	0.75	2.5	0.52	1.6	0.10
	Arterial	-7.7	0.56	-13	0.55	-11	0.78
290	Venous	-0.53	0.07	0.27	0.03	-3.0	0.45
292	Venous	0.06	0.00	1.2	0.08	-0.9	0.07
319	Venous	11	0.99*	18	0.84*	20	0.97*
	Arterial	0.69	0.06	1.6	0.22	0.09	0.00
322	Venous	1.6	0.57*	-0.66	0.08	0.20	0.00
327	Venous	1.0	0.06	2.0	0.14	5.5	0.52*
331	Venous	1.2	0.09	-1.4	0.06	-6.7	0.89*
342	Venous	4.9	0.65*	2.2	0.23	3.4	0.16
Overall	Venous	0.25	0.00	-1.0	0.02	-0.20	0.00

* $p < 0.05$.

portal vein may not increase. This provides an explanation for the lack of increase in metabolism despite increase in liver size. Total fetal hepatic clearance may, in fact, be much higher when drug is delivered to the fetus across the placenta, that is, administered to the mother rather than by fetal infusion.

A striking finding is the confirmation of the high metabolic clearance of morphine by the fetus (Garland et al., 2005b). The estimate for mean metabolic clearance from the present study, $27 \pm 9.0 \text{ ml} \cdot \text{min}^{-1}$, when corrected for estimated fetal weight ($\sim 500 \text{ g}$), is of the same order of magnitude as total morphine clearance in the pregnant baboon ($\sim 43 \pm 15 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) (Garland et al., 2005a). As noted above, this may not even capture the full capacity of the fetal liver to metabolize morphine. Errors in the estimation of metabolism could arise from calculations made from samples obtained before steady state. Whereas steady state was well defined for morphine and the metabolites during morphine infusion, this was not the case for metabolite concentrations during M3G infusions. Underestimation of steady-state concentrations of M3G during M3G infusion would overestimate M3G clearance and, hence, morphine metabolism. Any overestimate in clearance would be proportional to the extent that steady state is underestimated. The lack of change between the samples spaced at 8 and 12 h and the similarity in concentrations, whether infused for 8, 12, or 16 h, limit any underestimation in steady-state concentration to 5% and, thus, make the estimate for fetal metabolic clearance reliable (Fig. 1).

The parallel circuitries in the fetal vasculature can lead to systematic biases in measurements. This was well demonstrated in the present study when infusion and sampling routes were switched. Based on the flow characteristics in the fetal heart, drug infused retrograde into the right carotid artery of the fetus preferentially flows to the left carotid artery and returns to the right atrium. Samples are then obtained from the right jugular venous catheter. Thus, the higher morphine concentrations during arterial morphine infusion most likely reflect an enrichment of the sampling site by the relative proximity of the infusion site. Alternatively, it is possible that even under steady-state conditions, the concentration of drug in the carotid artery may be less than that in the descending aorta and lead to an overestimation of clearance. This alternative seems unlikely because no effect was seen on the metabolite concentrations. In addition, during maternal infusions, there is no difference between concentrations in fetal carotid arterial or jugular venous samples (unpublished observation). Another potential explanation for the high metabolism is that all the fetuses have undergone a "stressful" surgery and been exposed to morphine for analgesic purposes before study infusions and measurements. Comparison of enzyme activity in age-matched fetuses that have not been exposed to the stress of surgery or to previous exposure to morphine would address this possibility.

The high level of metabolism in the baboon fetus seems, at first, to be in conflict with human data. Although no measures of metabolism are available from the human fetus, total morphine clearance by the human neonate is markedly less than that in the adult (Gerdin et al., 1990b; Chay et al., 1992; Hartley et al., 1993; Milne et al., 1993; Scott et al., 1999). Morphine clearance in newborns ranges from 1 to $10 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$, depending on the degree of maturity and postnatal age, whereas, in pregnant women, total clearance is $\sim 42 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$. This developmental difference between human infants and adults is supported by reduced UGT activity toward morphine in microsomes prepared from human fetal liver compared with adult microsomes (Pacifci et al., 1982). Measures of enzyme activity in fetal liver from *Macaca* species also show similarly reduced activity when compared with adults (Dvorchik et al., 1979; Rane et al., 1984). These findings might imply that baboons are different from other

primates; however, the disposition of zidovudine, another UGT2B7 substrate, is very similar in the baboon and macaque (Garland et al., 1998b; Tuntland et al., 1998). Furthermore, the picture in sheep is similar to that in the baboon, with in vivo measures of metabolism in the fetus similar to those in the adult; yet enzyme activity toward morphine is also reduced in the fetus (Dvorchik et al., 1986; Olsen et al., 1988; Milne et al., 1993). No measures of morphine metabolism are available in newborn sheep or baboon to ascertain whether morphine metabolic clearance is reduced after birth when a marked reduction in hepatic blood flow is anticipated (Townsend et al., 1989).

Data are available for acetaminophen clearance in all three developmental periods of sheep; however, acetaminophen is metabolized primarily by a different UGT isoform (UGT1A6). In this case, clearance of acetaminophen in both the ovine fetus and lamb is approximately 15 to 20% of the adult values, similar to the difference between fetal and adult microsomes (Wang et al., 1986b, 1990). The data currently available do not show any clearly distinguishing differences between enzyme kinetics for morphine and acetaminophen that could explain the difference across development except, perhaps, a slightly lower affinity toward acetaminophen. These discrepancies between enzyme activity data and in vivo metabolic clearance data suggest that the level of intrinsic hepatic clearance of morphine in the fetus is sufficiently high that total hepatic clearance is not primarily dependent on the metabolic capacity of the tissues. Rather, fetal hepatic clearance is more dependent on hepatic blood flow.

It is noteworthy that the values for metabolic clearance in the fetus (sheep and baboon), and total clearances in the adult (sheep, baboon, and human), in which total clearance is predominantly metabolism, are all very similar (Wang et al., 1986a; Olsen et al., 1988; Milne et al., 1993, 1996; Garland et al., 2005a,b). These clearances actually exceed hepatic blood flow for the respective species, a phenomenon that suggests a high intrinsic hepatic clearance, and/or extensive extrahepatic clearance. Evaluation of the extraction ratios across the placenta and fetal liver comparing acetaminophen, which the above discussion would suggest has a lower intrinsic clearance, with morphine would be useful to confirm this hypothesis. That glucuronidation by the fetuses of the respective species fall into this classification of high intrinsic clearance belies the previous position, at least for morphine, on the "inadequacy of perinatal glucuronidation" (Coughtrie et al., 1988).

In light of the high level of fetal metabolism, it is no longer as surprising that exposure to UGT enzyme-inducing agents did not lead to enhanced metabolism. The lack of response to these agents supports the concept that the intrinsic clearance of the fetal liver is sufficiently high that induction would have little effect on overall hepatic clearance. The same applies to natural induction expected to occur around the time of birth, although natural induction may very well be a postnatal event (Dvorchik et al., 1979). Alternatively, the lack of response to the UGT enzyme-inducing agent may indicate that UGT2B7 does not respond to that agent, that the dose used was insufficient, or that the stress of surgery and/or the previous exposure to morphine may have already maximally turned on the fetal enzyme as noted above. Examination of enzyme activity in developmentally timed tissues with and without exposure to UGT enzyme-inducing agents is necessary to determine the effect of inducers, either endogenous or exogenous, on enzyme expression. Another explanation for a lack of response to a UGT enzyme inducer is that a more fundamental inhibitory process is at work in the fetus, preventing induction. At least one UGT isoform responds to phenobarbital through the constitutive androstane receptor, a nuclear receptor that is reported to be reduced in the neonatal period (Sugatani et al., 2001; Huang et al., 2003). Answers to the question of fetal induction await greater un-

derstanding of the specific processes that regulate enzyme activity in utero.

Total fetal morphine clearance represents fetal placental clearance in addition to fetal metabolic clearance and other elimination processes. Although not a stated hypothesis, it was anticipated that the placental component would increase with gestational age. This has previously been shown in fetal sheep for acetaminophen, although the increase was not directly proportional to fetal weight (Wang et al., 1986b). In the present study, an increase in placental clearance would manifest as an increase in total morphine clearance and clearance of the metabolites. In the longitudinal data, morphine, M3G, and M6G concentrations should decrease in parallel in the absence of any increase in the metabolite to drug ratio, that is, in metabolic clearance. This may have occurred to a small degree in some of the animals but was not a consistent finding, nor was it apparent in the pooled data when controlled for route of infusion and dose (Figs. 4 and 5). Most drugs cross the placenta by passive diffusion with the expectation that diffusibility would continue to increase during the latter stages of pregnancy. The surface area of the placenta increases with branching of villi, and the diffusional distance decreases with thinning of the syncytiotrophoblast (Fox, 1995). Efflux transporters in the placenta can also enhance clearance from the fetus (Young et al., 2003). Hence, the lack of any change in either total morphine clearance or metabolite clearances needs further consideration.

In addition to placental attributes, the clearance of morphine by the placenta is determined by the permeability characteristics engendered by the physicochemical properties of the drug and to the activity of any placental transporters. Assuming that placental clearance accounts for all fetal clearance not attributable to fetal metabolism, placental clearance would be $\sim 60 \text{ ml} \cdot \text{min}^{-1}$ or $\sim 120 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$. This approaches reported values for umbilical venous blood flow in human fetuses, but is only two-thirds that reported in fetal sheep (Berman et al., 1975; Galan et al., 1999; Bellotti et al., 2000). The intrinsic placental clearance of morphine may be sufficient such that the changes in the capacity of the placenta to clear drugs would not lead to an increase in placental clearance.

This is clearly not the case for the metabolites of morphine, for which total clearance is only $\sim 2 \text{ ml} \cdot \text{min}^{-1}$. It has been assumed, so far (in this discussion), that the elimination pathway of the glucuronide metabolites is primarily placental; however, there is only little corroborative evidence for this assumption. In primates, glucuronide metabolites have been shown to cross the placenta, whereas, in sheep, glucuronide metabolites have virtually no placental clearance (Wang et al., 1986a; Olsen et al., 1988; Gerdin et al., 1990a; Garland et al., 1998c). The extent to which renal excretion to the amniotic fluid, with subsequent fetal swallowing and sequestration in the fetal intestinal tract, or transfer back to the mother directly across fetal membranes, might contribute to overall clearance is not known (Tuntland et al., 1998; Adams et al., 2005). These pathways might also be expected to mature, leading to enhanced clearance with gestational age.

Perhaps the most important concept to emphasize is that fetal metabolic activity must be considered in the context of the physiological environment to predict fetal exposure to drug and metabolites. One implication of a high intrinsic clearance of morphine by the fetal liver would be a significant fetal first-pass effect when drug is administered to the mother. This first-pass effect would lead to reduced exposure to drug for the fetus but also increased exposure to metabolites. The current findings would lend support to a significant first-pass clearance. This can be estimated by equating the fetal to maternal ratio (c_f/c_m) during maternal infusion (MI ; 0.32) to drug clearance to and from the fetus:

$$\frac{c_f}{c_m}(MI) = \frac{CL_p}{CL_p + CL_{F(\text{hepatic})} + CL_{(\text{placento-hepatic})}} \quad (2)$$

The experimentally derived numbers for placental clearance (CL_p ; $56 \text{ ml} \cdot \text{min}^{-1}$) and fetal metabolism during fetal infusion ($CL_{F(\text{hepatic})}$; $27 \text{ ml} \cdot \text{min}^{-1}$) can be substituted. Thus, to account for a fetal to maternal concentration ratio of 0.32, found previously during maternal morphine infusion, a first-pass fetal hepatic clearance ($CL_{FFP(\text{placento-hepatic})}$) of $\sim 92 \text{ ml} \cdot \text{min}^{-1}$ is needed (Garland et al., 2005b). This value, when corrected for fetal weight, is in the range of umbilical blood flow for fetal sheep (Berman et al., 1975; Galan et al., 1999).

Results from this study contribute to the understanding of fetal drug disposition, in particular, the role of fetal metabolism. Clarification of the extent of first-pass metabolism of drugs across the placenta and fetal liver, and the pathways of metabolite clearance from the fetus, is still needed for prediction of fetal drug concentrations. Prediction is critically important when trying to achieve therapeutic effects in the fetus, for instance, in the treatment of fetal arrhythmias or prevention of perinatal HIV transmission (Connor and Mofenson, 1995; Simpson and Sharland, 1998). Prediction of fetal drug levels is equally important for devising strategies to minimize fetal exposure to maternally indicated drugs. In addition to morphine and acetaminophen, many drugs undergo glucuronidation as part of the clearance process. These drugs include zidovudine, fluoxetine, and labetalol as examples of agents that are commonly used in pregnancy. Classification of drugs by the degree of fetal intrinsic hepatic clearance is likely to lead to the development of general principles for prediction of fetal concentrations of drug and metabolite (Wu and Benet, 2005). Furthermore, the principles garnered with respect to glucuronidation should be applicable to other conjugating systems and to metabolism by cytochrome P450 enzymes.

In summary, the major findings are that the baboon fetus exhibits a high level of metabolism of morphine that rivals that in the adult. No change in metabolism occurs with advancing gestational age, onset of labor, or in response to classical inducers of UGT activity. These findings suggest that, despite reports of diminished enzyme activity in fetal hepatic tissue, the intrinsic hepatic clearance of morphine in the fetus is sufficient for metabolic clearance to be dependent on hepatic blood flow and not the absolute metabolic capacity of the fetal liver. This differs from the previously held view that the fetus has limited capacity for glucuronidation—a view based on in vitro enzyme research. Although many questions are raised by these findings, they provide a framework for further research to understand fetal drug disposition and prediction of fetal drug concentrations.

Acknowledgments. We acknowledge Dr. Hazel H. Szeto, Cornell University, New York, for her support and mentorship of Dr. Garland.

References

- Adams EA, Choi HM, Cheung CY, and Brace RA (2005) Comparison of amniotic and intramembranous unidirectional permeabilities in late-gestation sheep. *Am J Obstet Gynecol* **193**:247–255.
- Bellotti M, Pennati G, De Gasperi C, Battaglia FC, and Ferrazzi E (2000) Role of ductus venosus in distribution of umbilical blood flow in human fetuses during second half of pregnancy. *Am J Physiol* **279**:H1256–H1263.
- Berman W Jr, Goodlin RC, Heymann MA, and Rudolph AM (1975) Measurement of umbilical blood flow in fetal lambs in utero. *J Appl Physiol* **39**:1056–1059.
- Chay PC, Duffy BJ, and Walker JS (1992) Pharmacokinetic-pharmacodynamic relationships of morphine in neonates. *Clin Pharmacol Ther* **51**:334–342.
- Coffman BL, Rios GR, King CD, and Tephly TR (1997) Human UGT2B7 catalyzes morphine glucuronidation. *Drug Metab Dispos* **25**:1–4.
- Connor EM and Mofenson LM (1995) Zidovudine for the reduction of perinatal human immunodeficiency virus transmission: pediatric AIDS Clinical Trials Group Protocol 076—results and treatment recommendations. *Pediatr Infect Dis J* **14**:536–541.
- Coughtrie MW, Burchell B, Leakey JE, and Hume R (1988) The inadequacy of perinatal glucuronidation: immunoblot analysis of the developmental expression of individual UDP-

- glucuronosyltransferase isoenzymes in rat and human liver microsomes. *Mol Pharmacol* **34**:729–735.
- Daniel SS, James LS, MacCarter G, Morishima HO, and Stark RI (1992) Long-term acid-base measurements in the fetal and maternal baboon. *Am J Obstet Gynecol* **166**:707–712.
- Dvorchik BH, Stenger VG, and Hartman RD (1979) Drug metabolism by the fetal stump-tailed monkey (*Macaca arctoides*). Hepatic microsomal N-demethylation and glucuronidation as measured by radiometric assays. *Pharmacology* **18**:241–250.
- Dvorchik BH, Woodward G, Sitar DS, and Tweed WA (1986) Hydroxylation and glucuronidation of various xenobiotics by hepatic microsomes from the fetal lamb, pregnant ewe and human fetus. *Dev Pharmacol Ther* **9**:282–289.
- Fletcher AJ, Goodfellow MR, Forhead AJ, Gardner DS, McGarrigle HH, Fowden AL, and Augment the glycemic response to acute hypoxemia in fetal sheep during late gestation. *Pediatr Res* **47**:684–691.
- Fox H (1995) Placental structure, in *Scientific Basis of Obstetrics and Gynaecology* (Macdonald RR ed), pp 1–28. Churchill Livingstone, Edinburgh.
- Galan HL, Jozwik M, Rigano S, Regnault TR, Hobbins JC, Battaglia FC, and Ferrazzi E (1999) Umbilical vein blood flow determination in the ovine fetus: comparison of Doppler ultrasonographic and steady-state diffusion techniques. *Am J Obstet Gynecol* **181**:1149–1153.
- Garland M (1998) Pharmacology of drug transfer across the placenta. *Obstet Gynecol Clin North Am* **25**:21–42.
- Garland M, Abildskov KM, Altincatal A, and Stark RI (2005a) Models of morphine disposition in the fetal baboon. *Pediatric Academic Societies PAS* **57**:1944.
- Garland M, Abildskov KM, Kiu TW, Daniel SS, and Stark RI (2005b) The contribution of fetal metabolism to the disposition of morphine. *Drug Metab Dispos* **33**:68–76.
- Garland M, Szeto HH, Daniel SS, Tropper PJ, Myers MM, and Stark RI (1996) Zidovudine kinetics in the pregnant baboon. *J Acquir Immune Defic Syndr Hum Retrovirol* **11**:117–127.
- Garland M, Szeto HH, Daniel SS, Tropper PJ, Myers MM, and Stark RI (1998a) Implications of the kinetics of zidovudine in the pregnant baboon following oral administration. *J Acquir Immune Defic Syndr Hum Retrovirol* **19**:433–440.
- Garland M, Szeto HH, Daniel SS, Tropper PJ, Myers MM, and Stark RI (1998b) Placental transfer and fetal metabolism of zidovudine in the baboon. *Pediatr Res* **44**:47–53.
- Garland M, Szeto HH, and Stark RI (1998c) The primate placenta is permeable to glucuronide metabolites. *Pediatr Res* **43**:59A.
- Gerdin E, Gabrielson J, Lindberg B, and Rane A (1990a) Disposition of morphine-3-glucuronide in the pregnant rhesus monkey. *Pharmacol Toxicol* **66**:815–819.
- Gerdin E, Salmonson T, Lindberg B, and Rane A (1990b) Maternal kinetics of morphine during labour. *J Perinat Med* **18**:479–487.
- Gibaldi M and Perrier D (1982) *Pharmacokinetics*. Marcel Dekker, Inc., New York.
- Glover DD, Amonkar M, Rybeck BF, and Tracy TS (2003) Prescription, over-the-counter and herbal medicine use in a rural, obstetric population. *Am J Obstet Gynecol* **188**:1039–1045.
- Grieve PG, Myers MM, and Stark RI (1994) Behavioral states in the fetal baboon. *Early Hum Dev* **39**:159–175.
- Hartley R, Quinn M, Green M, and Levene MI (1993) Morphine glucuronidation in premature neonates. *Br J Clin Pharmacol* **35**:314–317.
- Huang W, Zhang J, Chua SS, Qatanani M, Han Y, Granata R, and Moore DD (2003) Induction of bilirubin clearance by the constitutive androstane receptor (CAR). *Proc Natl Acad Sci USA* **100**:4156–4161.
- Krauer B, Dayer P, and Anner R (1984) Changes in serum albumin and alpha 1-acid glycoprotein concentrations during pregnancy: an analysis of fetal-maternal pairs. *Br J Obstet Gynaecol* **91**:875–881.
- Kumar S, Riggs KW, and Rurak DW (1999) Role of the liver and gut in systemic diphenhydramine clearance in adult nonpregnant sheep. *Drug Metab Dispos* **27**:297–302.
- Liggins GC and Howie RN (1972) A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. *Pediatrics* **50**:515–525.
- McRorie TI, Lynn AM, Nespeca MK, Opheim KE, and Slattery JT (1992) The maturation of morphine clearance and metabolism. *Am J Dis Child* **146**:972–976.
- Milne RW, Nation RL, and Somogyi AA (1996) The disposition of morphine and its 3- and 6-glucuronide metabolites in humans and animals and the importance of the metabolites to the pharmacological effects of morphine. *Drug Metab Rev* **28**:345–472.
- Milne RW, Sloan PA, McLean CF, Mather LE, Nation RL, Runciman WB, Rutten AJ, and Somogyi AA (1993) Disposition of morphine and its 3- and 6-glucuronide metabolites during morphine infusion in the sheep. *Drug Metab Dispos* **21**:1151–1156.
- Olsen GD, Sommer KM, Wheeler PL, Boyea SR, Michelson SP, and Cheek DB (1988) Accumulation and clearance of morphine-3-beta-D-glucuronide in fetal lambs. *J Pharmacol Exp Ther* **247**:576–584.
- Pacifici GM, Sawe J, Kager L, and Rane A (1982) Morphine glucuronidation in human fetal and adult liver. *Eur J Clin Pharmacol* **22**:553–558.
- Rane A, Sawe J, Lindberg B, Svensson JO, Garle M, Erwald R, and Jorulf H (1984) Morphine glucuronidation in the rhesus monkey: a comparative in vivo and in vitro study. *J Pharmacol Exp Ther* **229**:571–576.
- Ring JA, Ghabrial H, Ching MS, Smallwood RA, and Morgan DJ (1999) Fetal hepatic drug elimination. *Pharmacol Ther* **84**:429–445.
- Scott CS, Riggs KW, Ling EW, Fitzgerald CE, Hill ML, Grunau RV, Solimano A, and Craig KD (1999) Morphine pharmacokinetics and pain assessment in premature newborns. *J Pediatr* **135**:423–429.
- Simpson JM and Sharland GK (1998) Fetal tachycardias: management and outcome of 127 consecutive cases. *Heart* **79**:576–581.
- Stark RI, Daniel SS, James LS, MacCarter G, Morishima HO, Niemann WH, Rey H, Tropper PJ, and Yeh MN (1989) Chronic instrumentation and longterm investigation in the fetal and maternal baboon: tether system, conditioning procedures and surgical techniques. *Lab Anim Sci* **39**:25–32.
- Stark RI, Daniel SS, Kim YI, Leung K, Rey HR, and Tropper PJ (1993) Patterns of development in fetal breathing activity in the latter third of gestation of the baboon. *Early Hum Dev* **32**:31–47.
- Stark RI, Myers MM, Daniel SS, Garland M, and Kim YI (1999) Gestational age related changes in cardiac dynamics of the fetal baboon. *Early Hum Dev* **53**:219–237.
- Sugatani J, Kojima H, Ueda A, Kakizaki S, Yoshinari K, Gong QH, Owens IS, Negishi M, and Sueyoshi T (2001) The phenobarbital response enhancer module in the human bilirubin UDP-glucuronosyltransferase UGT1A1 gene and regulation by the nuclear receptor CAR. *Hepatology* **33**:1232–1238.
- Szeto HH, Umans JG, and Rubinow SI (1982) The contribution of transplacental clearances and fetal clearance to drug disposition in the ovine maternal-fetal unit. *Drug Metab Dispos* **10**:382–386.
- Tocque B, Pontikis R, Nam NH, Hoellinger H, and Leroux Y (1980) Morphine and methadone binding to human serum proteins. *J Pharm Pharmacol* **32**:729–731.
- Townsend SF, Rudolph CD, and Rudolph AM (1989) Changes in ovine hepatic circulation and oxygen consumption at birth. *Pediatr Res* **25**:300–304.
- Tuntland T, Odinecs A, Nosbisch C, and Unadkat JD (1998) In vivo maternal-fetal-amniotic fluid pharmacokinetics of zidovudine in the pigtailed macaque: comparison of steady-state and single-dose regimens. *J Pharmacol Exp Ther* **285**:54–62.
- Wang LH, Rudolph AM, and Benet LZ (1986a) Pharmacokinetic studies of the disposition of acetaminophen in the sheep maternal-placental-fetal unit. *J Pharmacol Exp Ther* **238**:198–205.
- Wang LH, Rudolph AM, and Benet LZ (1990) Comparative study of acetaminophen disposition in sheep at three developmental stages: the fetal, neonatal and adult periods. *Dev Pharmacol Ther* **14**:161–179.
- Wang LH, Zakim D, Rudolph AM, and Benet LZ (1986b) Developmental alterations in hepatic UDP-glucuronosyltransferase. A comparison of the kinetic properties of enzymes from adult sheep and fetal lambs. *Biochem Pharmacol* **35**:3065–3070.
- Wu CY and Benet LZ (2005) Predicting drug disposition via application of BCS: transport/absorption/elimination interplay and development of a biopharmaceutics drug disposition classification system. *Pharm Res (NY)* **22**:11–23.
- Young AM, Allen CE, and Audus KL (2003) Efflux transporters of the human placenta. *Adv Drug Deliv Rev* **55**:125–132.

Address correspondence to: Marianne Garland, Columbia University, College of Physicians and Surgeons, 622 West 168th Street, PH4W-465, New York, NY 10032. E-mail: mg71@columbia.edu
