THE CONTRIBUTION OF FETAL METABOLISM TO THE DISPOSITION OF MORPHINE

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ABSTRACT:

The contribution of fetal metabolism to drug disposition in pregnancy is poorly understood. With maternal administration of morphine, like many drugs, steady-state concentrations in fetal plasma are less than in maternal plasma. The contribution of fetal metabolism to this difference is unknown. Morphine was used as a model drug to test the hypothesis that fetal metabolism contributes significantly to drug clearance by the fetus. Infusions of morphine, morphine-3-β-glucuronide (M3G), and morphine-6-β-glucuronide (M6G) were administered to the fetal baboon. Plasma concentrations of drug and metabolite obtained near steady state were measured by high-performance liquid chromatography. During morphine infusion, morphine, M3G, and M6G concentrations rose linearly with dose. M3G concentrations exceeded M6G by 20-fold. Mean ± S.D. clearances of morphine, M3G, and M6G from the fetus were 69 ± 17, 23 ± 0.80, and 1.6 ± 0.24 ml⋅min⁻¹, respectively. Clearances seemed to be dose-independent. The mean ± S.D. fraction of morphine dose metabolized was 32 ± 5.5%. This converts to a fetal metabolic clearance of 22 ± 6.5 ml⋅min⁻¹. In conclusion, one third of the elimination of morphine from the fetal baboon is attributable to metabolism, one third to passive placental transfer, and one third undefined. Furthermore, there is no evidence for saturation of metabolism. Fetal metabolism is surprisingly high compared with in vitro estimates of metabolism and morphine clearance in human infants. For morphine, fetal drug metabolism accounts for half the difference between fetal and maternal plasma concentrations.

The safe and effective use of drugs during pregnancy requires detailed knowledge of drug disposition and action in both the mother and the fetus. Changes in distribution, clearance, and effect in the mother can be studied in a clinical population during therapeutic use of a drug (Gerdin et al., 1990c; O’Sullivan et al., 1993); however, mothers and clinicians are primarily concerned about any adverse effects of pharmacological agents on the developing fetus. This makes it equally if not more important to develop an in-depth understanding of the bioavailability of drugs to the fetus. It is obviously not possible to perform detailed pharmacological studies in the human fetus (Gerdin et al., 1990b). A nonhuman primate model provides an opportunity to delineate disposition pathways of drug and metabolite from which relevant parallels can be drawn to human pregnancy. Similarities between human and nonhuman primate pregnancy include endocrinology of pregnancy, structure and function of the placenta, metabolism of drugs, and fetal cardiorespiratory and neurobehavioral development (Dvorochik et al., 1979; Goland et al., 1990; Daniel et al., 1992; Stark et al., 1993, 1999; Grieve et al., 1994; Garland et al., 1996, 1998a; Enders et al., 1997). The principles uncovered in this research provide the foundations for developing pharmacokinetic/pharmacodynamic models that are appropriate for human pregnancy.

Glucuronidation is a major drug detoxification and elimination pathway in humans (Owens and Ritter, 1995; Meech and Mackenzie, 1997). To date, 15 isoforms of UDP-glucuronosyltransferase (UGT) enzymes have been identified in humans, with many parallel homologs found among primates (Mackenzie et al., 1997). Although a certain degree of specificity exists, individual isoforms bind a diverse range of endogenous and exogenous compounds. Furthermore, each UGT is uniquely regulated in response to developmental, hormonal, or environmental cues. For most isoforms characterized using probe substrates, metabolic activity in fetal hepatic tissues is markedly reduced compared with that in adult liver (Ring et al., 1999). UGTs catalyze the transfer of a sugar molecule from UDP-glucuronic acid to the drug or an intermediary metabolite. The enhanced water solubility of the metabolite facilitates renal and/or biliary excretion. From the fetal perspective, enhanced water solubility leads to decreased placental transfer and so-called “trapping” within the fetal compartment (Szeto et al., 1982b; Olsen et al., 1988).

It had been reasoned that fetal enzymes are down-regulated to prevent accumulation of metabolites during intrauterine life. Zidovudine is metabolized by the isoform UGT2B7 (Barbier et al., 2000). Despite limited activity toward probe substrates for this isoform in fetal hepatic tissue, there is evidence for significant metabolism of zidovudine by the primate fetus (Pacifici et al., 1982; Garland et al., 1998b). This metabolism was not accompanied by an accumulation of zidovudine glucuronide in either fetal plasma or amniotic fluid (Gar-
These findings differ from the suppositions previously made toward fetal disposition of the drug and its glucuronide metabolite. The capacity of the fetus to metabolize drugs is a critical component in any pharmacokinetic model that defines the underlying relationships of maternal and fetal bioavailability. Moreover, pharmacokinetic modeling points out that the relationship between fetal and maternal free drug concentrations largely depends on placental permeability or transport and fetal nonplacental clearance pathways (Szetó et al., 1982b; Garland et al., 1998b). Hence, determining the contribution of metabolism to fetal nonplacental clearance pathways is paramount in understanding fetal drug disposition.

Morphine is a probe substrate for UGT2B7 and, as such, is an appropriate drug for investigating the determinants of fetal drug disposition in pregnancy (Coffman et al., 1997). An added dimension to research with morphine is the measurement of the pharmacodynamic consequences of disposition in the fetus, because the glucuronide metabolites of morphine [morphine-3-β-glucuronide (M3G) and morphine-6-β-glucuronide (M6G)] have pharmacological activity (Labella et al., 1979; Pasternak et al., 1987). In this study, the dose kinetics of morphine and its glucuronide metabolites in the fetal baboon were examined using a series of steady-state infusions. The results were used to quantify drug and metabolite clearance from the fetal baboon and to determine the rate of formation of metabolites by the fetus. Several hypotheses were tested, including 1) the fetal baboon makes both M3G and M6G; 2) fetal metabolism becomes saturated in the pharmacological dose range; 3) metabolism by the fetus makes a significant contribution to overall morphine clearance; and 4) deconjugation of morphine glucuronide does not account for metabolite clearance from the fetus.

### Materials and Methods

#### Study Population

Pregnant baboons (papio species) from onsite (Columbia University) and offsite (Biological Research Laboratory, University of Illinois, Chicago, IL) breeding colonies were available for study. Gestational age (GA) was determined by using the midpoint of timed matings as the estimated day of conception (+3 days, term = 175 days). An 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. Offsite animals were transported to Columbia University around 110 days of gestation, allowing a minimum of 2 weeks of acclimatization to the new facility prior to surgery. 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 Committees at both Columbia University and the University of Illinois.

#### Surgical Procedure and Tethering System

The animals were studied using a system of individualized backpacks and tether systems. This system, along with the methods for maintenance, breeding, preconditioning, anesthesia, surgery, and postoperative care, were described in detail in a previous report (Stark et al., 1989) and only summarized here. Surgery was scheduled between 125 and 140 days. Approximately 2 weeks prior to 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. Animals that showed suppressed appetite, weight loss, agitation, or repeatedly removed the backpack were not considered as candidates for surgery. With a careful selection of animals, this was a rare occurrence. Most animals adapted readily to the backpack within 1 to 2 days. Surgery was done 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 anesthetic fluid catheter was attached on the neck. An 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. All 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, catheter, and electrical connectors and 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. The maternal and fetal vascular catheters were continuously infused with normal saline containing 2 U · ml⁻¹ h⁻¹ heparin at rates of 5 and 2 ml · h⁻¹, respectively, to maintain patency. 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 (50–150 µg · kg⁻¹ · 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 (500 mg) 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 following cessation of postoperative morphine, sulfate was allowed for stabilization prior to 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. Physiologic stability of the fetus was assessed by fetal heart rate, blood pressure, arterial acid base status, and blood gas values. This model allowed the pharmacokinetic and pharmacodynamic studies to be performed over the last trimester of a stable pregnancy without needing anesthesia or undue restraint.

#### Infusion Protocol

Morphine sulfate, M6G, and M3G infusions were studied sequentially. Where possible, studies were conducted at similar gestational ages. The doses of agents used were selected based on animal and human literature and were those expected to span the concentrations measured in clinical studies. The initial study design assigned four animals to each drug. An additional animal was assigned to morphine sulfate study because of an incomplete data set caused by the displacement of a fetal venous catheter that had gone undetected until sample analysis. Additional animals were assigned to low-dose morphine infusions to span the concentrations measured during morphine infusion. Morphine sulfate doses were 0.1, 0.2, 0.5, 1, and 2 mg · h⁻¹, and M3G and M6G doses were 0.025, 0.05, 0.1, 0.25, and 0.5 mg · h⁻¹. The additional M6G doses were 0.004, 0.01, and 0.025 mg · h⁻¹. Morphine saline infusion solutions were prepared from morphine sulfate pentahydrate (National Institute of Drug Abuse, Rockville, MD) for the initial four animals or morphine sulfate solution (Elkins-Sinn, Cherry Hill, NJ) for the additional study. Metabolite infusion solutions were prepared from the M6G and M3G compound (National Institute of Drug Abuse). Solutions were passed through a 0.22-µm filter into sterile infusion bags. Solutions were stored at −20°C until required and then thawed at room temperature. Samples of each solution were obtained at the time of infusion to confirm dose delivered and to check for degradation. The order of administration of the various doses was randomized to control for gestational age and the possibility of incomplete studies from premature delivery or catheter blockage. When conducting the animal studies, investigators were blinded to dose. The investigator responsible for sample analysis was aware of dosages because this information was necessary to set instrumentation gains.

On the first study day, an initial sample was obtained from the fetal arterial catheter. Following this, the dead space of the fetal venous line (2.5 ml) was primed with the drug solution and then infused at a rate of 2 ml · h⁻¹ using a calibrated P720 Intech peristaltic infusion pump (Intech Laboratories, Inc., Plymouth Meeting, PA). The infusion rate ranged from 1.8 to 2.5 ml · h⁻¹ between animals but was only ± 0.1 ml · h⁻¹ within the studies of an individual animal. Sites of fetal infusion and sampling were only reversed if samples could not be obtained through the fetal arterial line. Reversal of the infusion and sampling site variably increases drug plasma concentrations, sometimes by as much as 2-fold, but does not affect metabolite concentrations (unpublished observation). This can be explained by circulatory patterns in the fetus. To validate drug delivery, the infusion bags were weighed at the beginning and end of the infusion. The duration of each infusion was 8 h. This duration of infusion was selected for pharmacological and practical reasons. Ideally, to approximate steady state, a minimum duration of infusion of four elimination half-lives is needed. Although the elimination half-life of morphine...
is almost certainly less than 2 h, the only elimination data from a fetus for M3G are from the sheep, where, in keeping with an epitheliochorial placenta, the elimination half-life is ~15 h (Olsen et al., 1988). The elimination half-life of a molecule with similar size and structure to the glucuronide metabolites, the glucuronide metabolite of zidovudine, is only 2 h in the fetal baboon. It seems likely that the elimination half-life of the glucuronide metabolites of morphine would be of a similar order of magnitude. Although 8 h may not be sufficient to achieve steady state, it is sufficient to address the aims of this study. Furthermore, to complete the series of infusions in the time frame available for studies, to have drug-free periods between infusions, and to ensure the research staff was available to collect samples, an 8-h infusion was selected. Blood samples for drug analysis (0.6 ml) were obtained at 0, 7, and 8 h of infusion, with care being taken that timing was consistent in each infusion. Following the final sample, the infusion was stopped, the line was cleared of drug, and saline infusion was reinstituted. Blood was collected in microtainer tubes containing heparin and the plasma separator (BD Biosciences, San Jose, CA). After centrifugation of the sample, the plasma was transferred to 1.5-ml plastic vials and stored at ~20°C within 30 min of collection. Fetal well being was assessed throughout the study period by evaluating fetal heart rate and heart rate variability and stability of blood pressure. In addition, samples for acid base and blood gas analysis (0.2 ml) were obtained at 0, 2, 4, and 8 h and injected directly into a Radiometer ABL30 blood gas analyzer (Radiometer America, Westlake, OH). During the morphine infusion, blood was also collected for glucose analysis (0.2 ml) at 0, 2, 4, and 8 h. Hematocrit was checked twice weekly. The extent of labor was recorded from the preceding and following night.

High-Performance Liquid Chromatography. Morphine, M3G, and M6G were measured in plasma samples using a modified high-performance liquid chromatography (HPLC) method (Stensson et al., 1982; Hartley et al., 1993a). This method uses a combination of fluorometric and coulochemical detection to increase sensitivity.

**HPLC equipment.** Two HPLC systems were used. The first was an automated system that consisted of a Gilson model 307 isocratic pump equipped with a model 10 WTI titanium pump head, a Gilson model 231-401 autosampling injector (Gilson Medical Electronics, Middleton, WI). The second was an automated Waters Alliance 2695 HPLC system (Waters, Milford, MA). Detection on both systems was made by a Linear model LC305 fluorescence spectrophotometer with a Pulsed Xenon lamp (Linear Instruments; Thermo Separation Products, Inc., West Palm Beach, FL) and an ESA model 5200A Coulochem II detector with an ESA model 5011 high-sensitivity analytical cell (ESA, Inc., Chelmsford, MA). The systems were controlled by a Gilson 506C System Interface with UniPoint software v. 1.71 (Gilson Medical Electronics) or busSAT/IN module with Millenium32 software v. 4.0 (Waters), with a pentium PC for system control, data acquisition, and data processing.

**Chromatographic conditions.** The separation was performed on a Waters Spherisorb ODS2 C18 (3 µm, 4.6 × 100 mm i.d.) column. The mobile phase was 0.01 M ammonium phosphate monobasic, 1.5 mM SDS, and 24% acetonitrile at pH 2.1. Sodium phosphate monobasic, α-phosphoric acid to adjust pH, and acetonitrile were HPLC grade (Fisher Scientific Co., Pittsburgh, PA). SDS was ultrapure (Invitrogen, Carlsbad, CA). The chromatography was performed at ambient temperature with a flow rate of 1.5 ml · min⁻¹, with the eluting compounds measured by fluorescence detection (excitation, 210 nm; emission, 340 nm) followed by coulochemical detection (electrode 1, +225 mV; electrode 2, +350 mV).

**Extraction procedure.** Sample preparation was performed using solid-phase extraction columns (1 ml of Bond Elute C18 50-µg columns; Varian Inc., Palo Alto, CA) on a model 5-7250 Visisprep DL Solid Phase Vacuum Manifold, followed by sample evaporation with a model 5-7124 Visidyris drying attachment (Supelco, Bellefonte, PA).

**Results**

Thirteen baboon pregnancies were studied (Table 1). The number of infusions in each pregnancy ranged from 2 to 10. The reasons for an incomplete series of infusions were premature delivery (n = 2) and catheter-related problems (n = 5). A total of 69 infusions were done, with samples collected in 65. In all except one pregnancy, infusions were made through the fetal venous catheter, with sampling from the fetal arterial catheter. The exception was pregnancy no. 244, where, for the last four infusions, the drug was administered through the fetal...
arterial catheter, and samples were obtained from the venous catheter because of arterial sampling difficulties. In three pregnancies, there was significant uterine activity. One (no. 183) delivered prematurely 13 days after surgery, having had moderate uterine activity since surgery. Another (no. 181) delivered vaginally 29 days after the fetal surgery on the evening of the final infusion, having had no previous uterine activity. A third animal (no. 197) had significant contractions during the first infusion that settled to mild nocturnal contractions by the third infusion. Approximately half the pregnancies had mild contractions each evening that lasted from 3 to 6 h. Overall, the fetal study period averaged 33 days (range, 13–49 days), whereas the period over which individual infusion sets were administered did not exceed 9 days. All but four fetuses were alive until delivery and were normally grown. All animals displayed normal physiologic variables prior to and following infusion. During morphine infusions, fetal breathing activity was reduced. No changes in heart rate or blood pressure occurred to indicate fetal distress. Acid-base status and glucose measurements did not change during the infusions and were not different from previously published norms (Daniel et al., 1992). Data from these physiologic recordings will be presented in a separate pharmacodynamic report.

Initial studies began at a minimum of 6 days after surgery and at least 60 h after the cessation of postoperative morphine. Plasma concentrations prior to initial infusion were <1.0, 3.0, and 15.0 ng·ml⁻¹ for morphine, M6G, and M3G, respectively, indicating almost complete drug clearance prior to the initiation of the study. After the initial dose, baseline concentrations prior to infusion were only analyzed for the infusion following the high dose or, if not available, the second highest dose. The maximum levels recorded were 8.6, 7.7, and 20 ng·ml⁻¹ for morphine, M6G, and M3G, respectively. These levels are below those measured during infusion even for the lowest dose in the initial series of infusions and would not affect subsequent levels. Of a total of 65 infusions, all but two had two samples available. In one infusion, samples were obtained at 8 and 9 h. One paired value from each of three sample pairs (all from different animals) were excluded from analysis because they lie more than two standard deviations below the values expected. In two animals, clearance values abruptly increased to more than 4-fold average values. It seemed unlikely that this represented physiologic changes, so a presumptive assessment was made that the venous catheter had dislodged such that the drug was delivered to the amniotic fluid. Displacement of the catheter had been detected in one animal with the subsequent infusion. Data from one further infusion were excluded because samples were contaminated with morphine. Visual inspection of the 7- and 8-h samples and statistical analysis (paired t test) showed little difference between the paired values. During morphine infusion, morphine concentrations in the 8-h samples were on average 10% less than the 7-h samples, and M3G and M6G concentrations were 3% more than the 7-h samples. Although the 10% difference reached statistical significance (p < 0.5), this difference is in the opposite direction to that expected if steady state were not approximated. During M6G and M3G infusion, M6G and M3G concentrations, respectively, were on average 4% higher in 8-h samples. All these values are consistent with assay and sampling variability. Differences in dose kinetics between concentrations in pregnancy no. 244 prompted a comparison between analyses of morphine infusion solutions. This showed that the commercially prepared solution was 15% higher than that expected and was corrected for in subsequent calculations and analyses.

During morphine infusion, morphine, M3G, and M6G concentrations rose linearly as a function of dose (Fig. 1). M3G was the major metabolite, with concentrations averaging more than 20 times that of M6G. No M6G was present in the morphine solution to be metabolized. Metabolite concentrations were also dose-independent (Table 2). During M3G and M6G infusion, M6G and M3G concentrations, respectively, were on average 4% higher in 8-h samples. All these values are consistent with assay and sampling variability. Differences in dose kinetics between concentrations in pregnancy no. 244 prompted a comparison between analyses of morphine infusion solutions. This showed that the commercially prepared solution was 15% higher than that expected and was corrected for in subsequent calculations and analyses.

TABLE 1
Demographic data

<table>
<thead>
<tr>
<th>Pregnancy No.</th>
<th>Agent</th>
<th>Maternal Weight</th>
<th>GA at Surgery</th>
<th>Infusions</th>
<th>Monitor</th>
<th>Sex</th>
<th>Birth Weight</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>180</td>
<td>MOR</td>
<td>16</td>
<td>132</td>
<td>5</td>
<td>24</td>
<td></td>
<td>660</td>
<td>Uncomplicated</td>
</tr>
<tr>
<td>181</td>
<td>MOR</td>
<td>15½/3</td>
<td>127</td>
<td>5</td>
<td>29</td>
<td>F</td>
<td>525</td>
<td>Delivered prematurely, labor during infusion 3</td>
</tr>
<tr>
<td>183</td>
<td>MOR</td>
<td>16½/3</td>
<td>133</td>
<td>3</td>
<td>13</td>
<td>F</td>
<td>840</td>
<td>Infusion catheter dislodged for all but first infusion</td>
</tr>
<tr>
<td>184</td>
<td>M6G</td>
<td></td>
<td>136</td>
<td>5</td>
<td>34</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>188</td>
<td>M6G</td>
<td>18½/3</td>
<td>132</td>
<td>5</td>
<td>37</td>
<td>F</td>
<td>938</td>
<td>Uncomplicated</td>
</tr>
<tr>
<td>192</td>
<td>M6G</td>
<td>16½/3</td>
<td>127</td>
<td>3</td>
<td>44</td>
<td>F</td>
<td>620</td>
<td>Catheter occlusion after infusion 3</td>
</tr>
<tr>
<td>197</td>
<td>M3G</td>
<td>16½/3</td>
<td>126</td>
<td>5</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>244</td>
<td>M3G</td>
<td>16½/3</td>
<td>134</td>
<td>5</td>
<td>28</td>
<td>M</td>
<td>770</td>
<td>Switch of infusion/sampling site for last four infusions, labor during final infusion</td>
</tr>
<tr>
<td>246</td>
<td>MOR</td>
<td></td>
<td>127</td>
<td>5</td>
<td>35</td>
<td>M</td>
<td>745</td>
<td>Catheter occlusion, physiology last two doses</td>
</tr>
<tr>
<td>248</td>
<td>M3G</td>
<td>16½/3</td>
<td>129</td>
<td>5</td>
<td>49</td>
<td>M</td>
<td>680</td>
<td>Uncomplicated</td>
</tr>
<tr>
<td>251</td>
<td>M3G</td>
<td>16½/3</td>
<td>128</td>
<td>5</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>255</td>
<td>M6G</td>
<td>18½/3</td>
<td>129</td>
<td>2</td>
<td>27</td>
<td>M</td>
<td>902</td>
<td>Near-term delivery prior to final scheduled infusion</td>
</tr>
<tr>
<td>265</td>
<td>M6G</td>
<td>19</td>
<td>125</td>
<td>3</td>
<td>36</td>
<td></td>
<td></td>
<td>Catheter occlusion, physiology only last dose</td>
</tr>
</tbody>
</table>

MOR, morphine.
had no effect on clearance within animals over the 10 days to complete each series of infusions. Uterine activity was expected to decrease placental clearance. It was a relatively rare occurrence but had no discernible effect on fetal clearance.

During infusion of M6G, both morphine and M3G were detectable in fetal plasma. At the highest M6G dose, morphine concentration was 3.6 ng · ml⁻¹ (range, 2.1–5.0 ng · ml⁻¹), and M3G concentration was 17 ng · ml⁻¹ (range, 13–21 ng · ml⁻¹). Analysis of the infusion solution showed that M6G solutions contained approximately 1% morphine, which would account for the concentrations of both morphine and M3G. M3G concentrations in fetal plasma were perhaps lower than that for M6G. The fetal-to-maternal metabolite ratios were similar during morphine infusion compared with infusion of the M6G solution. During infusion of M6G, both morphine and M3G were detectable, although there was more variability. The M3G infusion solution contained ~0.5% morphine and 0.005% M6G, which would account for their presence in fetal plasma. Maternal samples collected during the highest infusion rates of each drug ranged from 10 to 50 times less than those in the fetus, confirming fetal origin of the metabolites (Fig. 3). The mean fetal-to-maternal concentration ratio of M3G was lower than that for M6G. The fetal-to-maternal metabolite ratios were similar during morphine infusion compared with infusion of the metabolite alone.

Discussion

The major finding from this study is that, on average, one third of the morphine delivered to the fetus is eliminated through metabolism by the fetus. These results demonstrate that fetal metabolism is a significant contributor to fetal drug disposition of morphine. Furthermore, in the clinical dose range, metabolic clearance by the fetus is not saturated. Both of the glucuronide metabolites of morphine, M3G and M6G, are made by the fetus. Although M3G is formed at a substantially greater rate, the metabolites otherwise behave in a similar manner. Finally, deconjugation, suggested as a mechanism for facilitating the elimination of glucuronide metabolites from the fetal compartment, seems to have an insignificant contribution to overall disposition.

Until now, fetal nonplacental clearance of drugs in primate had been poorly defined. In contrast, the sheep fetus has been shown to metabolize several drugs (Wang et al., 1985; Olsen et al., 1988; Kumar et al., 2000). In addition, studies of drug metabolism in fetal microsomes from both human and nonhuman primates support some, albeit reduced, metabolism of drugs by the fetus (Dvorchik et al., 1979; Pacifici et al., 1982). Zidovudine, and now morphine, are the only drugs where fetal metabolism has been evaluated and confirmed in vivo in nonhuman primates (Garland et al., 1998). Fetal metabolism represents a fetal nonplacental clearance pathway and is thought to be at least partly responsible for the reduced steady-state concentrations of drugs in fetal plasma compared with maternal plasma following maternal administration. This has been found in essentially all studies in sheep and primates (Szeto et al., 1982a; Wang et al., 1986; Kumar et al., 1997; Garland et al., 1998b; Tunland et al., 1998). Quantitative analysis of fetal metabolism and total fetal clearance of morphine from this study allow an evaluation of the contribution of metabolism to this difference in concentrations between the mother and fetus.

The simplest pharmacokinetic representation of pregnancy, the two-compartment model, suggests that both fetal nonplacental clearance (CL_{Fnp}), and placental permeability (maternal-to-fetal and fetal-to-maternal placental clearances (CL_{FMp} and CL_{Fmp}, respectively) are primary contributors to active drug concentrations in the fetus (eq. 1: c, concentration; ss, steady state; F, fetal; M, maternal) (Szeto et al., 1982b; Garland, 1998).

\[
\frac{c_{Fss}}{c_{Mss}} = \frac{CL_{Fnp}}{CL_{FMp} + CL_{Fmp}}
\]

Using the assumptions that morphine crosses the placenta by passive diffusion (CL_{FMp} = CL_{Fmp} = CL_{Fm}) and that placental clearance and fetal metabolism (CL_{Fm}) account for all fetal drug clearance, a fetal-to-maternal concentration ratio of 0.68 would be expected. The fetal-to-maternal ratio in the baboon is 0.32 during steady-state infusion of morphine to the mother (Garland et al., 2001). This implies that not all clearance from the fetus was accounted for. By adding an unknown clearance (CL_{Fp}) to the sum of fetal clearances, this unaccounted clearance can be estimated (eq. 2).

\[
\frac{c_{Fss}}{c_{Mss}} = \frac{CL_{Fp}}{CL_{Fp} + CL_{Fnp} + CL_{FMp}}
\]

CL_{Fp} is the only contributor to the numerator, and the sum of the clearances in the denominator equals 1, so the fraction of total clearance that placental clearance contributes equals the fetal-to-maternal concentration ratio (0.32). From this study, fetal metabolism is 0.32 of total fetal clearance. This leaves 0.36 of total clearance from the fetus unaccounted for in the denominator. Several possible explanations exist for this unaccounted component of fetal clearance. In adult sheep, when M3G is formed, a substantial fraction is never seen in plasma (Milne et al., 1995). Glucuronide metabolites can be excreted in the bile; however, these are likely to reenter the circulation through intestinal reabsorption. Metabolism by the kidney with excretion directly into the urine is more likely (Milne et al., 1995). In support of this is the presence of enzyme activity toward morphine in human fetal kidney (Pacifici and Rane, 1982); however, in the fetus, metabolites excreted in urine are likely to eventually enter the circulation through swallowing of amniotic fluid. Moreover, clearance of morphine and M3G in the human neonate is quantitatively very low, making it unlikely that renal clearance is a major contributor in utero (Choonara et al., 1992; Hartley et al., 1993b). Another consideration would be the formation of metabolites other than monoglucuronides. These were not measured in this study. In adults, normorphine and morphine-3-sulfate can account for up to 10% of metabolites formed (Milne et al., 1996). Morphine-3-sulfate conjugates, although not detected in neonatal plasma, are present in higher concentrations than...
glucuronides in newborn urine (Choonara et al., 1990). A first-pass metabolism has been demonstrated in fetal sheep for drugs delivered across the placenta (Kumar et al., 1997). Unless the metabolites were excreted into bile and not seen in plasma, this first-pass metabolism would be measured during fetal infusions. An alternative, although not mutually exclusive, explanation is the active transport of morphine across the placenta from the fetus to the mother. P-glycoprotein is present in the placenta and has been shown to decrease fetal exposure across the placenta (Kumar et al., 1997). Unless the metabolites were in steady state, the conclusions from this study remain sound, even if steady state was not achieved for the metabolites. The amniotic fluid compartment is probably not at steady state by 8 h; however, exchange with the amniotic fluid compartment is likely to make only a small contribution to overall clearance. Another potential problem is the possible induction of the enzymes responsible for the metabolism of morphine by exposure to morphine. In pregnant guinea pigs, chronic morphine exposure increased enzyme activity in vitro but not in vivo. Morphine exposure did not affect fetal or newborn metabolic activity (Smith et al., 1999). In the present study, all animals were exposed to morphine postoperatively for several days to provide analgesia. The time course of the induction effect is not well defined; thus, if induction did occur, it is not clear to what extent the effects would persist or what effect the repeated experimental infusions would have. To partially control for this possibility, the order of doses was randomized. Ideally, for quantifying drug metabolism, the metabolite infusion would be done in the same animals. The major goal of this study was to establish the effect of dose on disposition and effects; thus, controlling for gestational age rather than intra-animal differences took priority.

Comparative studies of morphine metabolism in human fetal and adult liver tissues show markedly less activity in the fetal tissues (Pacifici et al., 1982). In addition, there are marked differences in morphine clearance between infant and adult humans (Gerdin et al., 1990c; Choonara et al., 1992; Hartley et al., 1993b; Milne et al., 1996). In pregnant women, morphine clearance is 42 ml min⁻¹ kg⁻¹, whereas in premature and term newborn infants, total morphine clearance ranges from 1 to 10 ml min⁻¹ kg⁻¹. In both sheep and primates, the fetal metabolic clearance of morphine seems higher than might be expected. Standardized to body weight, fetal metabolic clearance may even exceed that in the adult (Olsen et al., 1988; Milne et al., 1996). Clearance in the fetal baboon is also similar to that in the adult baboon. In the pregnant baboon, total clearance is 38 ± 11 ml min⁻¹ kg⁻¹, whereas, standardized to weight (~0.5 kg at 135–140 days of gestation), fetal metabolic clearance in the baboon would be ~40 ml min⁻¹ kg⁻¹ (Garland et al., 2001). This high clearance in the fetus may suggest that the fetal baboon and sheep are more precocious than humans. An alternative explanation may be the effect of greater hepatic blood flow in the fetus than in the newborn. In
sheep, hepatic blood flow in the fetus is 3-fold higher than in the immediate newborn period (Townsend et al., 1989). In human fetuses, ultrasound studies show that 60% of the umbilical blood flow is directed to the liver at mid-gestation, and this increases to 90% near term (Bellotti et al., 2000). Shunting of blood from the portal system through the ductus venous after birth may also contribute to the decreased metabolism in the newborn period.

The initial hypothesis that fetal metabolism would become saturated at higher concentrations was based on previous studies in which there were inconsistencies in placental and fetal clearances calculated from paired infusions of zidovudine to the mother and fetus (Garland et al., 1998b). Several independent methods had reported no evidence for active transport of zidovudine across the placenta that would explain higher fetal-to-maternal than maternal-to-fetal clearances (Liebes et al., 1990; Schenker et al., 1990; Bawdon et al., 1992). This bias in placental clearances is a common finding in studies that have used the paired infusion method in sheep and primates (Garland, 1998). Explanations put forth include saturation of fetal metabolism, high first-pass hepatic clearance in the fetus during maternal infusion, active placental transport, and paraplacental routes of elimination (Kumar et al., 1997; Garland et al., 1998b; Tuntland et al., 1998; Smit et al., 1999). The linearity in M3G and M6G concentrations during morphine infusion indicate that morphine metabolism by the fetal baboon exhibits first-order kinetics over the dose range studied. The calculated morphine clearances might suggest that clearance at the highest dose was lower; however, the confounding effect of switching the infusion site in one animal and the incomplete data sets in others make this difficult to confirm. If indeed the clearances do decrease with dose, this cannot be explained by saturation of fetal metabolism; however, it could reflect saturation of a placental transport mechanism. The therapeutic concentrations of morphine reported in new-

### Table 3

<table>
<thead>
<tr>
<th>Pregnancy No.</th>
<th>Mean GA</th>
<th>0.025</th>
<th>0.05</th>
<th>0.125</th>
<th>0.25</th>
<th>0.5</th>
<th>Mean ± S.D. by Animal</th>
</tr>
</thead>
<tbody>
<tr>
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<td>145</td>
<td>0.96</td>
<td>1.6</td>
<td>1.9</td>
<td>1.7</td>
<td>1.7</td>
<td>1.6 ± 0.35</td>
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<tr>
<td>246</td>
<td>136</td>
<td>2.1</td>
<td>2.7</td>
<td>2.9</td>
<td></td>
<td></td>
<td>2.6 ± 0.38</td>
</tr>
<tr>
<td>248</td>
<td>139</td>
<td>1.9</td>
<td>1.9</td>
<td>2.1</td>
<td>1.9</td>
<td>2.4</td>
<td>2.0 ± 0.22</td>
</tr>
<tr>
<td>251</td>
<td>144</td>
<td>2.8</td>
<td>3.1</td>
<td>3.2</td>
<td>2.6</td>
<td></td>
<td>3.0 ± 0.26</td>
</tr>
<tr>
<td>Mean ± S.D. by Dose</td>
<td></td>
<td>1.7 ± 0.63</td>
<td>2.3 ± 0.59</td>
<td>2.4 ± 0.66</td>
<td>2.4 ± 0.74</td>
<td>2.2 ± 0.51</td>
<td>2.3 ± 0.60</td>
</tr>
</tbody>
</table>

### Table 4

<table>
<thead>
<tr>
<th>Pregnancy No.</th>
<th>Mean GA</th>
<th>0.004</th>
<th>0.01</th>
<th>0.025</th>
<th>0.05</th>
<th>0.125</th>
<th>0.25</th>
<th>0.5</th>
<th>Mean ± S.D. by Animal</th>
</tr>
</thead>
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<td>188</td>
<td>142</td>
<td>1.3</td>
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<td>1.3</td>
<td>1.3</td>
<td>1.4</td>
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<td>1.4 ± 0.06</td>
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<tr>
<td>192</td>
<td>136</td>
<td>1.5†</td>
<td>2.0</td>
<td>1.3</td>
<td>1.5</td>
<td>1.5</td>
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<td>1.5 ± 0.04</td>
<td></td>
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<tr>
<td>217</td>
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<td>1.5</td>
<td>1.5</td>
<td>1.6</td>
<td>1.5</td>
<td></td>
<td>1.5 ± 0.04</td>
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</tr>
<tr>
<td>255</td>
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<td>1.2</td>
<td>1.2</td>
<td>1.6</td>
<td></td>
<td>1.9</td>
<td></td>
<td>1.8 ± 0.21</td>
<td></td>
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<tr>
<td>265</td>
<td>146</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td></td>
<td></td>
<td></td>
<td>1.3 ± 0.003</td>
<td></td>
</tr>
<tr>
<td>Mean ± S.D. by Dose</td>
<td></td>
<td>1.4 ± 0.15</td>
<td>1.6 ± 0.40</td>
<td>1.7 ± 0.37</td>
<td>1.6 ± 0.31</td>
<td>1.4 ± 0.10</td>
<td>1.4 ± 0.16</td>
<td>1.5 ± 0.04</td>
<td>1.6 ± 0.24</td>
</tr>
</tbody>
</table>

† Moderate to severe uterine activity.

### Table 5

**Fetal metabolism of morphine**

Estimated percentage (mean ± S.D.) of morphine dose converted to metabolites was calculated by dividing the metabolite formation rate (moles per minute) by the morphine dose (moles per minute) × 100. Metabolite formation was determined from M3G and M6G concentrations at each dose of morphine and average metabolite clearances from separate groups of animals. For pregnancy no. 244, M3G clearance was determined in this animal and used to calculate the percentage of morphine dose converted to metabolite instead of mean clearance. Metabolic clearance (mean ± S.D.) was calculated from total morphine clearance at each dose × the fraction of dose converted to M3G and M6G.

<table>
<thead>
<tr>
<th>Pregnancy No.</th>
<th>Morphine Dose</th>
<th>Metabolic %</th>
<th>Metabolic Clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M3G</td>
<td>M6G</td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>41 ± 17</td>
<td>0.88 ± 0.30</td>
<td>30 ± 13</td>
</tr>
<tr>
<td>181</td>
<td>31 ± 8.5</td>
<td>1.0 ± 0.20</td>
<td>20 ± 8.5</td>
</tr>
<tr>
<td>183</td>
<td>28 ± 3.6</td>
<td>0.84 ± 0.16</td>
<td>25 ± 11</td>
</tr>
<tr>
<td>184</td>
<td>26</td>
<td>1.2</td>
<td>23</td>
</tr>
<tr>
<td>244</td>
<td>29 ± 14</td>
<td>1.4 ± 0.48</td>
<td>13 ± 5.5</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>31 ± 5.6</td>
<td>1.1 ± 0.24</td>
<td>22 ± 6.5</td>
</tr>
</tbody>
</table>

![Fig. 3](https://example.com/f3.jpg)
born infants are usually between 100 to 150 ng \cdot ml^{-1}, although levels in excess of 400 ng \cdot ml^{-1} have been reported (Chay et al., 1992; Choonara et al., 1992; Hartley et al., 1993b). Mechanisms other than saturation of metabolism will need to be evaluated if results from paired infusion studies of morphine reveal the same discrepancies in placental clearances, since these studies are conducted in the therapeutic range. Even though morphine and zidovudine are both metabolized by UGT2B7, these results may not be applicable to zidovudine clearance. In humans, morphine is metabolized by at least three isoforms of UGT, whereas zidovudine metabolism seems to be limited to a single isoform (Court et al., 2003). Furthermore, molar concentrations of zidovudine during fetal infusion were 2- to 3-fold higher in the previous study (Garland et al., 1998b). Finally, as noted above, there is evidence that morphine but not zidovudine is transported by \(\text{p-glycoprotein (Leung and Bendayan, 1999; Xie et al., 1999). Although results of this study question the likelihood that fetal metabolism of zidovudine had become saturated, it does not exclude the possibility.}

It does, however, underscore the complexity of the interactions between the mechanisms that govern fetal drug disposition.

Morphine is metabolized to two glucuronide metabolites in several species (Milne et al., 1995; Lotsch et al., 1998; Garland et al., 2001). M3G is found in human neonates but was not found in the sheep fetus (Olsen et al., 1988; Choonara et al., 1992; Hartley et al., 1993b). In the fetal baboon, the concentration of M3G is 22 times that of M6G, whereas, in the adult baboon, the ratio is in the order of 40 times (Garland et al., 2001). This difference may arise from differences in formation and/or clearance of the metabolites. UGT2B7, UGT1A3, and UGT1A8 are the major human UGT isoforms responsible for morphine metabolism (Court et al., 2003). UGT2B7 forms both M3G and M6G, whereas other UGTs form only M3G. Little is known about the developmental regulation of UGT1A3 and UGT1A8, but if activity were low in utero compared with UGT2B7, a greater proportion of M6G would be expected. The calculated rates of formation of M3G at all doses of morphine were 31 times those of M6G formation. The difference between concentration ratios and the ratio of formation rates reflects the trend toward higher fetal clearance of M3G compared with M6G. This trend is supported by the lower fetal-to-maternal ratio of M3G than M6G. Early reports had suggested that M6G was also a substrate for \(\text{p-glycoprotein, but this has not been substantiated (Huwyler et al., 1996; Bourasset et al., 2003). It seems that glucose transporter 1, a glucose transporter in the placenta, is also a transporter for M6G (Bourasset et al., 2003). This transporter activity would presumably facilitate transfer in the direction of the concentration gradient, which, in this case, was from the fetus to the mother. This would not explain the higher M6G-to-M3G ratio in the fetus. M3G transport across the blood-brain barriers is inhibited in the presence of probenecid (Xie et al., 2000). There may be a similar transporter in the placenta that increases M3G efflux from the fetus that would explain the greater clearance of M3G from the fetus. The developmental differences in metabolite disposition may have more clinical relevance to human infants. In humans, M6G formation accounts for a greater proportion of metabolism—around 10% (Milne et al., 1996; Lotsch et al., 1998). In two human infant studies, M6G was actually one third of the M3G concentration (Choonara et al., 1992; Hartley et al., 1993b). M6G is reported to have potent opiate activity; however, physiological data recorded during these studies showed little effect of M6G at the levels expected from a pharmacological dose of morphine (Pasternak et al., 1987).

Deconjugation was investigated as a possible clearance mechanism of metabolites because of the presence of \(\beta\text{-glucuronidase in the placenta and fetal tissues (Lucier et al., 1977; Kushari and Mukherjea, 1980). This aspect of the study was confounded to some extent by impurities in M6G and M3G infusion solutions by small quantities of morphine and, in the case of M3G, minimally detectable amounts of M6G. The concentrations found in fetal plasma can be accounted for by the impurities alone; thus, the extent of deconjugation is minimal.}

Placental clearance of glucuronide metabolites is expected to be limited, although two reports in primates indicate transfer from the maternal circulation to the fetal circulation (Gerardin et al., 1990a; Garland et al., 1998c); thus, placental transfer seems likely to be the major clearance route for metabolites. The only other species in which fetal clearance of M3G has been quantified is the sheep. A major difference between the placenta of sheep (epitheliochorial) and primates (hemomonochorial) is the number of membrane layers that separate the fetal and maternal circulations (Enders and Carter, 2004). Both species have adapted to ensure adequate nutrient and oxygen supply to the fetus and removal of waste from the fetus. The sheep placenta probably affords greater isolation for the fetus but markedly restricts the transfer of large hydrophilic molecules such as glucuronide metabolites (Olsen et al., 1988). M3G clearance from fetal sheep is of the same order of magnitude as seen in the fetal baboon (Olsen et al., 1988). Despite this similar overall clearance, the smaller volume of distribution expected in the fetal baboon compared with the fetal sheep leads to a shorter elimination half-life in the baboon fetus. Neither the study in fetal sheep nor this study distinguished between placental transfer and renal excretion into the amniotic cavity. Urinary excretion is often considered as an elimination pathway; however, in the fetus, subsequent reuptake by swallowing of amniotic fluid is likely to occur, although some drugs and metabolites may be sequestered in the fetal intestinal tract (Garland, 1998). In addition, substances have been demonstrated to directly cross the placental base-plate, the fetal umbilical cord, and even fetal skin (Gilbert et al., 1996). Direct transfer back to the mother from the amniotic fluid may also occur. Ongoing studies are expected to address these additional clearance pathways.

Recognition of the many practical and ethical concerns in studying human pregnancy—particularly gaining access to the fetus—underscores the need for relevant animal models. Data are available from one study that examines morphine transfer to the human fetus when administered for sedation prior to percutaneous umbilical blood sampling (Gerardin et al., 1990b). Both morphine and M3G were found in fetal plasma. Most data from human pregnancy are collected at birth, when there are tremendous physiological changes taking place (Gerardin et al., 1990a; O’Sullivan et al., 1993). Even when samples are obtained during elective cesarian section, the effects of anesthesia and the ability to obtain data at only a single time point make interpretation difficult. This difficulty is compounded because drugs are usually administered as a bolus dose to the mother. The sheep model continues to be the mainstay for perinatal pharmacological research; however, the differences between human and sheep noted above lead to some difficulty in directly transferring results in ovine to human pregnancy. The similarities in key attributes of baboon and human pregnancy allow relevant parallels to be drawn with respect to the disposition and effects of drugs in the fetus. Findings with regard to the importance of fetal drug metabolism support the notion that this model is extremely useful, although studies are more difficult, more limited in scope, and more expensive to conduct.

In summary, drug concentration in the fetus depends on drug delivery across the placenta and the clearance of drug from the fetus. The fetal baboon metabolized morphine to both M3G and M6G and showed no evidence for saturation of fetal metabolism. One third of the clearance of morphine from the fetal baboon can be attributed to metabolism and one third to passive placental transfer, whereas the remaining third is yet to be
explained. The glucuronide metabolites are eliminated from the fetus, albeit at much lower rates than the parent drug. The pathways of metabolite clearance remain to be defined.

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References