Title: Fetal morphine metabolism and clearance are constant during late gestation

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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-ß-glucuronide (M3G), and morphine-6-ß-glucuronide (M6G) 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 ± 9.0 ml.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.
Introduction

More than 90 percent 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; Daniel et al., 1992; Stark et al., 1993; Grieve et al., 1994; Garland et al., 1996; Garland et al., 1998a; Garland et al., 1998b; Stark et al., 1999). 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 uridinediphosphate-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 glucuronyltransferase enzyme system is known to undergo considerable upregulation in the perinatal period (Coffman et al., 1997). However, the timing of induction prior to or following 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.
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 70-110 days to confirm singleton pregnancy, fetal size, and placental location. Lights were cycled (0700 h on, 1900 h off) and feeding times (0800 and 1600 h) 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 only summarized here (Stark et al., 1989). Surgery was scheduled between 120 and 145 days. Approximately two 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. 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 prior to 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 U.ml⁻¹) at rates of 5 and 2 mls.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.

Post-operative 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 2-4 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 post-surgery and 48 h following cessation of post-operative morphine 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 (Cobe low-level pressure transducers, Cobe, Boulder CO; Hewlett-Packard 8805-B Amplifier, 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, San Diego, CA; 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.** Morphine-3-β-D-glucuronide (M3G), and morphine 6-β-D-glucuronide (M6G) were obtained from the National Institute of Drug Abuse (NIDA), Division of Neuroscience and Behavioral Research, Bethesda, MD, USA. 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, USA). Other injection solutions included dexamethasone sodium phosphate injection, USP, 4 mg/ml (American Regent Laboratories, Shirley, NY, USA) and heparin, sodium injection, USP, 5000 U/ml (American Pharmaceutical Partners, Inc., Schaumburg, IL, USA.)
All M3G solutions were prepared from a 2mg/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 ml.h⁻¹, they would deliver the doses given below. The infusions contained 0.5 U/ml heparin. They were filtered through 0.22 µm, 25mm MCE filters (Fisher Scientific, Pittsburg, PA, USA) into sterile Viaflex infusion bags (Baxter-Clinitech Division, Dearfield, 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 as 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 4 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 where 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 so was recruited to examine the changes in metabolism near term and with the onset of labor. The animal had labor for 3 days prior to 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 1700 h with samples obtained at 0800 h and 0900 h the following morning; the second dose was then started with samples taken at 1700 and 2100 h that evening; the third dose was then started with samples obtained at 0800 and 0900 h 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 – 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 3 doses to assess changes in M3G clearance. Following reassessment of M3G clearance, morphine infusion was restarted. M3G
clearance was repeated in this manner every 7-10 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 mg.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 mg.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 infusion, 3 samples at least one hour apart were obtained to define steady state values. After the third sample, the UGT enzyme inducing agent was administered. 72 h later, 3 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 while, 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, 3 samples were again obtained and a second UGT enzyme inducing agent was administered with 3 further 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 where M3G was infused to the fetus for 8 h at 5 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 so 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 2 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 their 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 discontinuing 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 mg.h⁻¹) and M6G (0.25 mg.h⁻¹) for 24 h prior to 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 assure plasma concentrations were at steady-state prior to 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, 3 samples were obtained on the day prior to the start of the dexamethasone infusion and 3 samples 72 h later. In addition, a single blood sample was obtained on all other days where possible. After the final set of 3 samples were obtained during dexamethasone infusion, a final M6G/morphine combination was infused overnight while dexamethasone or saline continued. A final pair of samples were 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 
ml.h⁻¹, some difference 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 – 1 cc 
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, gently mixed, and then separated by centrifugation. Plasma was 
transferred to plastic vials and stored at −20º 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 coulochemical detection, as previously described (Garland et al., 2005).

**Extraction procedure for plasma samples.** Sample preparation was performed using Solid Phase Extraction (SPE) 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 Inc., Bellefonte, PA). After conditioning with 2 x 1 ml methanol (HPLC grade, Mallinkrodt, Fisher Scientific, Pittsburgh, PA), 1 ml purified water (Milli-Q NanoPure Water Filtration System, Millipore, Bedford, MA), and 1 ml 0.01M ammonium bicarbonate buffer (pH 9.3, HPLC grade, Mallinkrodt, Fisher Scientific) under vacuum, the SPE columns were loaded by gravity with 200 µl of ammonium bicarbonate buffer (pH 9.3), 100 µl of sample plasma or, for standards, 100 µl of blank pooled plasma with 20 µl of 5X standard, 100 µl of the internal standard nalorphine HCl (800 ng.ml⁻¹, Sigma, St. Louis, MO), then an additional 200 µl of ammonium bicarbonate buffer (pH 9.3). After equilibration, the column was washed with 1 ml of 0.01M ammonium bicarbonate (pH 9.3) and dried under vacuum. The sample was eluted with 2 x 150 µl methanol (HPLC grade, Fisher Scientific), dried under nitrogen, and reconstituted in 200 µl of mobile phase. All samples were extracted in duplicate.

**Chromatographic conditions.** The separation was performed isocratically on Waters Alliance 2695 HPLC system using a Spherisorb C18 column (ODS2, 3µm, 4.6 x 100 mm I.D., Waters, Milford, MA) at ambient temperature with a flow rate of 1.5 ml.min⁻¹. The mobile phase was 10 mM sodium phosphate monobasic (Fisher Scientific), 1.5 mM sodium dodecyl sulphate...
(ultraPURE, Gibco BRL, Grand Island, NY), and 24% acetonitrile (Fisher Scientific) adjusted to pH 2.1 with o-phosphoric acid (HPLC grade, Fisher Scientific). After injection of 50 µl 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 coulochemical detection (electrode 1: +225 mV and electrode 2: +350 mV) with a ESA Model 5200A Coulochem II detector (ESA, Bedford, MA).

The three compounds (morphine, M3G, M6G) as well as the internal standard nalorphine, were detectable by fluorescence while M3G was the only compound not electrochemically active. Values determined by the two detection systems were not different though 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.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 (SD) are reported unless otherwise stated. Alpha of .05 was considered significant. All statistical analyses were done using Systat statistical package (Systat Software, Inc., Point Richmond, CA).
Results

**Animal demographics.** 12 animals were studied in the various infusion protocols (Table 1). Fetal surgery was performed between 123 and 139 days 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 in the methods. Protocols continued until delivery or catheter failure. Mean age of delivery was 158 ± 9 days with average birth weight of 667 ± 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 3 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 ± 0.096 ml.min⁻¹; n=6) was not different from that during arterial infusion (1.69 ± 0.473 ml.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 8 animals. In four infusions only one sample was obtained, and in another, one value was excluded as it was an order of magnitude below that expected. To evaluate if values from the 2 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.h\(^{-1}\) dose and the duration of infusion or the time separating the measurements (Figure 1). The only pair > 15% different from the mean was found when the preceding morphine infusion was not discontinued until the start of the M3G infusion. This difference may reflect the presence of M3G that remained from the morphine infusion.

A clearance value was determined for each infusion then a mean for each animal. For comparative purposes among animals, only the initial series of M3G infusions was used to determine M3G clearance in Table 3. The mean M3G clearance was 2.13 ± 0.398 ml.min\(^{-1}\). There was no decrease in clearance with increasing dose (not shown). A corrected M6G clearance was determined in 3 animals (Table 3). No difference is apparent between M3G clearance and M6G clearance in these animals. Three animals had serial determinations of M3G clearance with an interval ranging from 15 to 21 days and 3 had serial M6G clearances at 8 – 11
day intervals (Figure 2). No change in either M3G or M6G clearance was apparent in this period of gestational age.

**Morphine clearance, rate of formation of metabolite, and metabolic clearance.** The arterial morphine concentrations during infusions taken prior to any UGT enzyme inducing agent are shown in Figure 3. The two obvious outliers were excluded from subsequent calculations (animals 294 and 327). While some variation in dose occurred across animals (0.38 – 0.60 mg.h\(^{-1}\)), this contributed very little to the between animal variability (r = 0.25; p, ns). From these samples, morphine clearances for each animal had an overall mean of 87 ± 29 ml.min\(^{-1}\) (Table 3). The formation of M3G and M6G was calculated from their respective concentrations during morphine infusion and the metabolite clearances for each individual. In animal 248, the prior study protocol had involved fetal M3G infusion so the previously established M3G clearance for this animal was used for determining the rate of formation of M3G (Garland et al., 2005b). The extent to which formation of M3G contributed to total morphine clearance was 32 ± 3.5 % with an overall estimated metabolic clearance of 27 ± 9.0 ml.min\(^{-1}\) (Table 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, ns).
The individual values across gestational age (discussed below) are shown in the right hand panel of figure 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 figure 4. Periods where 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 Figure 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 comparable gestational ages to the fetuses in the present study are shown in Figure 6. Both fetal body weight and liver weight increased with gestational age (slope, 7.45 g.d\(^{-1}\), \(r = 0.68, p < 0.0001\) and slope, 0.3 g.d\(^{-1}\) \(r = 0.21, p < 0.06\) respectively). Over a 30 day period of gestation the fetal weight increases by ~220 g and the liver weight by ~9 g, an increase of approximately 30 – 50 percent. The birth weights of the current group of animals and the liver weights from the two animals where tissues were collected are superimposed on figure 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 presence of uterine activity with progression to delivery. All of the animals in the left hand panel of Figure 4 exhibited uterine activity during the last 1 – 4 sample collections prior to delivery. Animal 319 had significant uterine activity for 2 days at 160 days gestation. No effect of uterine activity is seen. One of the outlier points omitted above (animal 294) was during active labor. While 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 figure 4 may suggest that in some animals there are slight decreases in all three analytes. This would support 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±0.007 µM respectively. Though 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 – 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 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 ($CL_{intrinsic}$), and the total hepatic blood flow ($Q_H$). These are the two parameters that contribute to hepatic clearance ($Gibaldi$ and $Perrier$, 1982).

$$CL_{hepatic} = \frac{Q_H \cdot CL_{intrinsic}}{(Q_H + CL_{intrinsic})}$$

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 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 as this group of fetuses was documented to have stable pH values. Protein binding of morphine is in the order of 15-20% and is proportional to serum albumin concentration, the main binding protein (Tocque et al., 1980). While 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, while 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 30–50% 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 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 do 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 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, $27 \pm 9.0 \text{ ml.min}^{-1}$, when corrected for estimated fetal weight (~500 g), is of the same order of magnitude as total morphine clearance in the pregnant baboon (~43 $\pm$ 15 ml.min$^{-1}$.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 prior to steady-state. While 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 (Figure 1).
The parallel circuitries in the fetal vasculature can lead to systematic biases in measurements. This is 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 prior to 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. While no measures of metabolism are available from the human fetus, total morphine clearance by the human neonate is markedly less that 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 – 10 ml.min\(^{-1}\).kg\(^{-1}\) depending on degree of maturity and postnatal age; whereas, in pregnant women total clearance is ~ 42 ml.min\(^{-1}\).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 (Pacifici 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 if 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 – 20% of the adult values, similar to the difference between fetal and adult microsomes (Wang et al., 1986b; Wang et al., 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 such 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.

Notedly, the values for metabolic clearance in the fetus (sheep and baboon), and total clearances in the adult, (sheep, baboon, and human) where total clearance is predominantly metabolism, are all very similar (Wang et al., 1986a; Olsen et al., 1988; Milne et al., 1993; Milne et al., 1996; Garland et al., 2005a; Garland et al., 2005b). 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 post-natal event (Dvorchik et al., 1979). Alternatively, the lack of response to UGT enzyme inducing agent may indicate that UGT2B7 does not respond to that agent, that the dose used was insufficient, or 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 active receptor (CAR), 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 understanding 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. While 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 (Figures 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 ~ 60 ml.min⁻¹ or ~ 120 ml.min⁻¹.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 where total clearance is only ~ 2 ml.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:

$$\frac{c_f}{c_m} (MI) = \frac{CL_P}{CL_P + CL_F(hepatic) + CL_{FFP}(placento-hepatic)}$$

The experimentally derived numbers for placental clearance ($CL_P$; 56 ml.min$^{-1}$), and fetal metabolism during fetal infusion ($CL_F(hepatic)$; 27 ml.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 ($CL_{FFP}(placento-hepatic)$) of ~92 ml.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. While 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.
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Footnotes

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Figure Legends

**Figure 1. Effect of infusion duration on M3G concentrations.** M3G concentrations were standardized to a 0.25 mg.h\(^{-1}\) dose for comparison. The infusions were assigned to one of 3 groups depending on the time at which samples were obtained. In the left panel, the two samples were obtained around 8 and 12 h after the start of the infusion, in the center panel, samples were obtained around 11 and 12 h after the start of the infusion, and in the last panel, samples were obtained around 15 and 16 h or more after the start of the infusion. The lack of change in concentration with separation of samples and duration of infusion support that M3G is close to steady-state by 8 – 12 h if not at steady-state.

**Figure 2. Morphine metabolite clearances at different gestational ages.** M3G (square) and M6G (triangle) clearances at different gestational ages in individual animals (connecting lines). Where values from more than one infusion available, mean±SEM plotted. Values from all animals in Table 2 are plotted at the left hand edge for comparison (open symbols).

**Figure 3. Variability in morphine concentrations.** Morphine concentrations from venous infusions and prior to the initiation of any induction agent are plotted for each animal. This demonstrates the consistency of values within animals apart from 2 outlying values and the considerable variability between animals. The outlying values were excluded from subsequent analyses.
Figure 4. Effect of gestational age on morphine, M3G, and M6G concentrations. Morphine (circle), M3G (square) and M6G (triangle) concentrations for individual animals are plotted against gestational age to examine changes in those animals where 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 d period is shown in each figure. The lines superimposed are least squares regression lines. Animals in the left hand panel delivered vaginally with all experiencing between 1 and 3 days of uterine activity preceding actual delivery. Animals in the right hand panel were administered UGT enzyme inducing agents as indicated in the boxes. (BM, betamethasone; DX, dexamethasone; PB, phenobarbital; SAL, saline; see methods for doses.)

Figure 5. Effect of gestational age on metabolite to drug ratio and morphine clearance. The upper panel shows the M3G (square) and M6G (triangle) to morphine ratios for all animals across the latter part of gestation. The lower panel shows morphine clearance (circle). 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 inset figures show least squares regression lines for the individual animals (see Table 5 for values).

Figure 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 square regression lines are for the non-study animals only. Slope, r², p values for fetal weight are 7.45, .68, < .0001, and for fetal liver 0.3, 0.21, and p < 0.6. (Open symbols, contemporaneous group of fetuses; filled symbols study group; grey, not exposed to
glucocorticoid; black exposed to glucocorticoid; down pointing triangle, female; up pointing triangle, male.)
Table 1. Demographic data on study animals

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Animal #</th>
<th>Maternal Weight kg</th>
<th>Surgery GA days</th>
<th>Start GA days</th>
<th>Delivery GA days</th>
<th>Birth Weight g</th>
<th>Sex</th>
<th>Induction agent</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>240</td>
<td>17</td>
<td>123</td>
<td>146</td>
<td>158</td>
<td>676</td>
<td>M</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>278</td>
<td>16.5</td>
<td>126</td>
<td>135</td>
<td>156</td>
<td>760</td>
<td>F</td>
<td>None</td>
</tr>
<tr>
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<td>290</td>
<td>17.5</td>
<td>136</td>
<td>142</td>
<td>158</td>
<td>778</td>
<td>M</td>
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</tr>
<tr>
<td>2</td>
<td>292</td>
<td>19</td>
<td>133</td>
<td>141</td>
<td>164</td>
<td></td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>294</td>
<td>18</td>
<td>135</td>
<td>142</td>
<td>151</td>
<td>720</td>
<td>M</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>297</td>
<td>12</td>
<td>129</td>
<td>137</td>
<td>148</td>
<td></td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>248</td>
<td>16.5</td>
<td>129</td>
<td>146</td>
<td>177</td>
<td>680</td>
<td>M</td>
<td>PB/BT</td>
</tr>
<tr>
<td>3</td>
<td>319</td>
<td>12.5</td>
<td>137</td>
<td>145</td>
<td>172</td>
<td>668</td>
<td>F</td>
<td>DX/PB</td>
</tr>
<tr>
<td>3</td>
<td>322</td>
<td>15.5</td>
<td>134</td>
<td>141</td>
<td>151</td>
<td>680</td>
<td>F</td>
<td>DX/PB</td>
</tr>
<tr>
<td>4</td>
<td>327</td>
<td>14.5</td>
<td>129</td>
<td>138</td>
<td>153</td>
<td>671</td>
<td>M</td>
<td>SAL</td>
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<tr>
<td>4</td>
<td>331</td>
<td>14.5</td>
<td>139</td>
<td>146</td>
<td>156</td>
<td>469</td>
<td>F</td>
<td>DX</td>
</tr>
<tr>
<td>4</td>
<td>342</td>
<td>15.75</td>
<td>131</td>
<td>139</td>
<td>153</td>
<td>570</td>
<td>M</td>
<td>DX</td>
</tr>
</tbody>
</table>

Abbreviation: BT, betamethasone; GA, gestational age (days); DX, dexamethasone; PB, phenobarbital; SAL, saline

1 GA at fetal demise
Table 2. Effect of infusion and sampling site on concentration (mean±SD\(^1\)) and ratio of mean values for samples obtained during venous and arterial infusions.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Infusion</th>
<th>Samples</th>
<th>Morphine</th>
<th>M3G</th>
<th>M6G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>#  ng.ml(^{-1})</td>
<td>ratio</td>
<td>ng.ml(^{-1})</td>
<td>ratio</td>
</tr>
<tr>
<td>278</td>
<td>Venous</td>
<td>7  83.2±9.63</td>
<td>0.46</td>
<td>2370±81.2</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Arterial</td>
<td>6  180±52.7</td>
<td>2170±129</td>
<td>1.3</td>
<td>57.5±14.2</td>
</tr>
<tr>
<td>297</td>
<td>Venous</td>
<td>1  111</td>
<td>0.36</td>
<td>2885</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>Arterial</td>
<td>2  122, 189</td>
<td>2390, 2020</td>
<td>0.65</td>
<td>61.4, 50.9</td>
</tr>
<tr>
<td>319</td>
<td>Venous</td>
<td>7  125±33.6</td>
<td>0.54</td>
<td>2470±415</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Arterial</td>
<td>16</td>
<td>170±23.5</td>
<td>2720±437</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Mean±SD 0.45±0.09 1.1±0.39 1.0±0.34

\(^1\) Where less than 3 samples available, the actual values are given.
Table 3. Summary of fetal clearances and morphine metabolism (mean±SD).

<table>
<thead>
<tr>
<th>Animal</th>
<th>Clearances (ml.min⁻¹)</th>
<th>Morphine metabolized (%)</th>
<th>Metabolic Clearance (ml.min⁻¹)</th>
<th>Placental Clearance (ml.min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>240</td>
<td></td>
<td>150±24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>248</td>
<td>2.0±0.22⁴</td>
<td>83±9.6</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>278</td>
<td>1.7±0.10</td>
<td>97±10</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>290</td>
<td>2.8±0.09</td>
<td>87±11</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>292</td>
<td>2.4±0.10</td>
<td>83±9.3</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>294</td>
<td>2.1±0.06</td>
<td>73±1.8</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>297</td>
<td>1.6±0.28</td>
<td>72</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>319</td>
<td></td>
<td>62±19</td>
<td></td>
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<td>322</td>
<td></td>
<td>79±8.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>327</td>
<td>2.4±0.10</td>
<td>2.8</td>
<td>140±33</td>
<td>33</td>
</tr>
<tr>
<td>331</td>
<td>2.1±0.30</td>
<td>2.0</td>
<td>54±6.6</td>
<td>27</td>
</tr>
<tr>
<td>342</td>
<td>1.9±0.17</td>
<td>1.0</td>
<td>65±15</td>
<td>33</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>2.1±0.39</td>
<td>1.9±0.91</td>
<td>87±29</td>
<td>32±3.5</td>
</tr>
</tbody>
</table>

¹ M3G clearances from initial series of M3G infusions
² M6G clearances corrected for simultaneous infusion of morphine (see methods)
³ For morphine clearances, from samples obtained during venous infusion and prior to administration of any induction agent.
⁴ M3G clearance from a previous study was not included in overall mean but used for calculation of metabolic clearance.
Table 4. Effect of classical induction agents on morphine clearance (ml.min⁻¹), and M3G and M6G to morphine molar ratios. Mean and SD reported for the day prior to initiation of UGT enzyme inducing agent and 72 h after the agent.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Agent</th>
<th>Clearance</th>
<th>M3G/morphine</th>
<th>M6G/morphine</th>
</tr>
</thead>
<tbody>
<tr>
<td>248</td>
<td>Saline</td>
<td>84±12</td>
<td>13±2.0</td>
<td>0.52±0.096</td>
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<tr>
<td></td>
<td>Phenobarbital</td>
<td>75±11</td>
<td>12±2.2</td>
<td>0.48±0.13</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>92±8.8</td>
<td>10±1.4</td>
<td>0.40±0.069</td>
</tr>
<tr>
<td></td>
<td>Betamethasone</td>
<td>95±6.6</td>
<td>9.6±0.86</td>
<td>0.48±0.048</td>
</tr>
<tr>
<td>319</td>
<td>Saline</td>
<td>67±25</td>
<td>11±2.5</td>
<td>0.38±0.11</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>48±5.4</td>
<td>8.4±0.56</td>
<td>0.26±0.014</td>
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<td>Phenobarbital</td>
<td>45±1.8</td>
<td>10±0.62</td>
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<td>79±12</td>
<td>15±0.67</td>
<td>0.30±0.039</td>
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<td>13±1.0</td>
<td>0.33±0.013</td>
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<td>0.21±0.091</td>
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<td>6.9±1.6</td>
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<td>0.19±0.047</td>
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<td>342</td>
<td>Saline</td>
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<td>13±1.6</td>
<td>0.51±0.085</td>
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<td>9.8±0.90</td>
<td>0.49±0.031</td>
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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 (d) determined by dividing the slope of the individual regression lines by the mean parameter value for individual animals x 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 Figure 5.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Infusion route</th>
<th>Clearance % change·d⁻¹</th>
<th>$r^2$</th>
<th>M3G Ratio % change·d⁻¹</th>
<th>$r^2$</th>
<th>M6G Ratio % change·d⁻¹</th>
<th>$r^2$</th>
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<tr>
<td>240</td>
<td>V</td>
<td>-5.9</td>
<td>0.74</td>
<td>-3.8</td>
<td>0.76</td>
<td>-11</td>
<td>0.60</td>
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<td>248</td>
<td>V</td>
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<td>-3.2</td>
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<td>-2.0</td>
<td>0.22</td>
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<tr>
<td>278</td>
<td>V</td>
<td>2.9</td>
<td>0.74</td>
<td>2.5</td>
<td>0.52</td>
<td>1.6</td>
<td>0.10</td>
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<tr>
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<td>0.56</td>
<td>-13</td>
<td>0.55</td>
<td>-11</td>
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<td>0.03</td>
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<td>0.06</td>
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<td>0.57*</td>
<td>-0.66</td>
<td>0.08</td>
<td>0.20</td>
<td>0.00</td>
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<tr>
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<td>0.06</td>
<td>2.0</td>
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<td>5.5</td>
<td>0.52*</td>
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<tr>
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<td>V</td>
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<td>0.09</td>
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<td>-6.7</td>
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<tr>
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<td>V</td>
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<td>0.65*</td>
<td>2.2</td>
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<tr>
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<td>V</td>
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<td>0.00</td>
<td>-1.0</td>
<td>0.02</td>
<td>-0.20</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* $p < .05$
Figure 1
Figure 2
Figure 4
Figure 5
Figure 6