Pharmacokinetics and conjugative metabolism of labetalol stereoisomers in pregnant sheep: A chiral drug case study in pregnancy

By

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ABSTRACT

A chiral HPLC assay for the separation of the four stereoisomers of labetalol in biological fluids was developed. The limit of detection of the individual isomers was 0.15 ng (0.6 ng of injected racemic labetalol). In maternal plasma the concentration of the RR isomer was 25±12% higher than those of the SR, SS and RS isomers throughout the infusion and post infusion period. The RR isomer had the lowest steady-state clearance (50.93±4.64 mL/min/kg) and the longest half-life (5.60±0.86 h). Of the 280 mg of labetalol administered, 11.04±2.76 mg was excreted as unconjugated while 65.84±7.19 mg was excreted as glucuronide conjugate. Approximately 32% of the unconjugated labetalol was in the form of the RR isomer compared to 19% for the other isomers. In fetal plasma, the concentration of the RR isomer (24 ± 5 ng/mL at steady-state) was 50% higher than that of the SR (11.00±2.38 ng/mL), SS(13.02±3.04 ng/mL) and RS(12.05±2.85 ng/mL) isomers throughout the infusion and post-infusion period, while the concentration of the SR isomer was consistently lower over the same interval. The terminal elimination half-life (t_{1/2}) and the mean residence time (MRT) of the RR isomer were 13.07 ± 4.02 h, and 17.19 ± 5.63 h, respectively. Labetalol fetal infusion studies were performed in four chronically instrumented pregnant ewes. In fetal plasma, the concentration of the RR isomer at steady-state was 51.22 ± 4.55 ng/mL, which was significantly higher than the other three isomers ([SR]= 30.33 ± 3.41 ng/mL, [SS]= 33.70 ± 3.22 ng/mL, [RS]=34.93 ± 2.75). The terminal elimination half-life (t_{1/2}) of the RR isomer was 8.62 ± 2.85 h, which was significantly lower than that obtained following maternal infusion of labetalol. Of the 9,400 ug of labetalol administered, 73.79 ± 9.64 mg (0.80 ±
0.10% was excreted as unconjugated while $133.44 \pm 25.20$ mg (1.4%) was excreted as glucuronide.

Pharmacokinetics, pharmacodynamics and conjugative metabolism of dilevalol were compared to those obtained previously for dilevalol from racemic labetalol infusion experiments. The results suggest that virtually all of the previously observed pharmacological effects of labetalol in non-pregnant sheep were elicited by dilevalol.
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LIST OF ABBREVIATIONS

A: Exponential equation constant
α: Alpha, an exponential rate constant
AUC: Area under the concentration vs time curve
AUMC: Area under the first moment curve (i.e. concentration*time vs time)
AM: Amniotic fluid
A-V: Arterio-venous
3-APB: 3-amino-1-phenylbutane
B: Exponential equation constant
BE: Blood base excess
β: Beta, an exponential rate constant
bpm: Beats per minute
°C: Degree Celsius
C: Arterial plasma concentration of labetalol
Css: Arterial plasma concentration of labetalol at steady-state
C_t-last: Arterial plasma concentration of labetalol in the last sample obtained or the last sample in which labetalol could be quantitated
cAMP: Cyclic adenosine monophosphate
CL: Total body clearance
CO2: Carbon dioxide
C_t: Concentration of labetalol at time t
CV: Coefficient of variation
EM: Emission wavelength
EX: Excitation wavelength
FA: Fetal arterial
g: gram
GC: Gas chromatography
h: hour(s)
Hb: Hemoglobin
Hg: Mercury
HLA: Hind limb artery (pudendo-epigastric artery)
HLV: Hind limb vein (pudendo-epigastric vein)
HPLC: High performance liquid chromatography
Hz: Hertz
i.d.: Internal diameter
I.U.: International units
i.v.: Intravenous
JANA: Computer program for pharmacokinetic modeling
kq: Infusion rate
kg: kilogram
L: Liter
M: Molar (as a concentration term)
MA: Maternal arterial
MANOVA: Multivariate analysis of variance
meq: Milliequivalents
μg: Microgram
μL: Microliter
μm: Micrometer
mg: milligram
min: Minute(s)
mL: milliliter
mm: millimeters
mmol: millimole(s)
mol: Mole(s)
n: Number of samples
N: Normal (as a concentration term)
NAD: Nicotinamide adenine dinucleotide
NADH: Reduced form of nicotinamide adenine dinucleotide
ND: Not detectable
NE: Norepinephrine
ng: nanogram
nm: nanometer
NONLIN: Computer program for pharmacokinetic modeling
O2: Oxygen
p: Probability factor
pCO2: Partial pressure of carbon dioxide
pH: Negative logarithm (base 10) of the hydrogen ion concentration
pO2: Partial pressure of oxygen
r: Correlation coefficient
r2: Coefficient of determination
rpm: revolutions per minute
sec: seconds
SCH: Schering Corporation's compound, 5-{2-[4-(4-methylphenyl)-2-butylamino]-1-hydroxyethyl} salicylamide hydrochloride hemihydrate
SEM: Standard error of mean
\( \sigma^2 \): Sigma squared, notation for variance

ss: (as a suffix) steady-state

TBAP: Tetrabutylammonium phosphate

t-test: Student's t-test for statistical analysis

t_{1/2}: Half-life

t-last: Time at which the last sample was collected

t_{\text{max}}: Time corresponding to the maximum concentration

TR: Tracheal fluid

TRIS: Tris(hydroxymethyl)aminomethane

UV: Umbilical vein

VD: Apparent volume of distribution

*: Notation for product, \textit{i.e.}, multiplication
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Dedication

This work is dedicated to Shamsi Rahimi.
1. INTRODUCTION

Labetalol {2-hydroxy-5-[1-hydroxy-2-(1-methyl-3-phenylpropylamino)ethyl] benzamide7} hydrochloride (Trandate®) is a unique drug with complex pharmacological properties exhibiting combined $\alpha_1$ and $\beta$-adrenoceptor antagonist properties and partial $\beta_2$-agonist activity (Farmer et al., 1972; Kennedy and Levy, 1975; Carpenter, 1981). Labetalol, an antihypertensive, has been used in the management of pregnancy-induced hypertension or pre-eclampsia. It was introduced in 1984 for general clinical use in the US.

Labetalol has two asymmetrical centers resulting in four stereoisomers, designated RR, SS, SR and RS (Fig 1). The drug is supplied as an approximately equicomponent mixture of all four isomers. Labetalol is a weak base ($pK_a = 8.3$) and is administered as the hydrochloride salt.

The four stereoisomers of labetalol contribute to its overall pharmacological properties. Since it was shown that the $\alpha$-blocking action of labetalol can lead to orthostatic hypotension (Brodgen, R., et al., 1978), the search for a compound with less inherent orthostatic potential led to the synthesis and development of dilevalol, the RR isomer. Dilevalol has very little $\alpha$-blocking activity and was thought to be a suitable candidate to replace labetalol for the management of hypertension. This did not happen, however, as dilevalol was withdrawn from the North American market in 1991 because of suspected hepatotoxicity.

1.1 PHARMACOLOGY

Labetalol exerts reversible and competitive $\alpha_1$- and $\beta$-blocking activity (Brittain and Levy, 1976; Mehta and Cohen, 1977) and is approximately one-
Figure 1: Structure of Labetalol and proposed pharmacological activity of its 4 stereoisomers:

- $\beta$ - Non-selective beta-adrenoceptor blocking activity
- $\beta_2$ - Selective beta2-adrenoceptor agonist activity
- $\alpha_1$ - Selective beta1-adrenoceptor blocking activity

* Denotes Chiral Center
fourth as potent as propranolol in blocking β-receptors and one-tenth as potent as phentolamine in blocking α₁-receptors. The pharmacological profile of the four stereoisomers differs substantially from that of labetalol and from that of one another.

Of the four stereoisomers of labetalol, dilevalol (the RR stereoisomer) has the most potent β-blocking activity. In fact, virtually all of the β-receptor blockade and β₂-receptor-mediated vasodilation (partial β₂-agonist activity) attributed to labetalol is produced by dilevalol (Sybertz, 1981). The RR isomer is approximately 4 to 6 times as potent a β-blocker as the parent racemate (Baum, et al., 1981) but only 10% as potent an α₁-blocker (Sybertz, 1981).

Among the four isomers, the SR isomer is the most potent antagonist of α₁-receptors, whereas the RS isomer has α- and β-blocking activity which is intermediate between that of the RR and the SR isomer (Brittain, et al., 1982). Finally, the SS isomer has very little α- or β-blocking activity.

1.2 PHARMACOKINETICS

Although the pharmacokinetic behavior of racemic labetalol and dilevalol has been studied in humans and animals, there are no pharmacokinetic data for the other individual isomers; SR, SS and RS, due in part to the lack of availability of the individual isomers for scientific study. Thus far, the analytical techniques used in the study of the pharmacokinetics of labetalol have not been sensitive enough for stereoselective pharmacokinetic studies. Therefore, the current pharmacokinetic profile of labetalol is based on the result of simultaneous administration of four stereoisomers as a racemate, all with different
pharmacological properties, and most likely, different pharmacokinetic characteristics, as well.

In humans, labetalol and dilevalol are rapidly absorbed following oral administration with peak plasma concentration occurring within 1 to 3 hours (McNeil and Louis, 1984; Abernethy et al., 1986; Tenero, et al., 1989). The oral bioavailability of labetalol and dilevalol are in the range of 11 to 86% and 10 to 30% (Kramer, et al., 1988), respectively. In normotensive or hypertensive subjects, the elimination half-life of labetalol is between 3 to 8 h (Goa et al., 1989) whereas that of dilevalol is higher, ranging from 8 to 12 h (Kramer et al., 1988). The apparent volume of distribution of labetalol in humans (2.5-15.5 L/kg) is lower than the estimated 25 L/kg reported for dilevalol (Goa et al., 1989; Chrisp and Goa, 1990). The plasma protein binding of labetalol is about 50% while that of dilevalol is about 73-77%. The total body clearance of these two compounds is in the range of 10 - 25 mL/min/kg (Nyberg, et al., 1982; Kramer, et al., 1988).

The pharmacokinetics of labetalol have been studied in pregnant sheep following a 100 mg maternal iv bolus dose (Yeleswaram et al., 1992A). Labetalol exhibited a maternal total body clearance of 30.8 ± 3.8 mL/min/kg and a terminal elimination half-life of 2.79 ± 0.66 h and 3.71 ± 0.5 h in the mother and the fetus, respectively. The apparent volume of distribution of labetalol in sheep was reported to be 6.48 ± 0.72 L/kg (Yeleswaram et al.,1993). Labetalol was found in the amniotic and tracheal fluid at concentrations 2- to 4 times the fetal plasma concentration (33.7 ± 5.8 ng/mL) for up to 24 hours following drug administration.

In the fetal lamb, the pharmacokinetic parameters of labetalol following a 4 mg direct fetal i.v. bolus were found to be significantly higher than the corresponding values in the ewe (Yeleswaram, et al., 1993). The fetal total body
clearance and elimination half-life of labetalol were 50.4 ± 1.4 mL/min/kg and 4.4 ± 0.4 h, respectively.

1.3 METABOLISM

The metabolic fate of labetalol has been studied in rat, rabbit, dog, sheep and man (Martin, et al., 1976). The drug is excreted in urine, feces and bile, with urinary excretion appearing to be the predominant pathway. The major metabolic pathways of labetalol include oxidation, hydroxylation, N-dealkylation, sulfation and direct glucuronidation (Gal, et al., 1988; Gal and Zirrolli, 1991). Glucuronidation occurs at both the phenolic (o-phenyl glucuronide) and benzylic hydroxyl groups (Martin, et al., 1976; Niemeijer, et al., 1991).

The metabolites of labetalol identified, so far, in man, mouse, rat, rabbit and dog are o-phenyl glucuronide, aliphatic glucuronide, hydroxy labetalol, glucuronide conjugates of hydroxy labetalol, 3-amino-1-phenylbutane (3-APB) and the p-hydroxy derivative of 3-APB, 3-AHP (Martin, et al., 1976; Gal and Zirrolli, 1991). The 3-APB metabolite was also identified in pregnant ewes following bolus administration of labetalol (Yeleswaram, et al., 1992B). However, the metabolite was not detected in the fetal lamb following a 4 mg bolus dose.

In humans, less than 5% of labetalol and dilevalol were excreted as unchanged drug in the urine and about 20% of labetalol and 80% of dilevalol were recovered as the glucuronide conjugates (Martin, et al., 1976; Sugeno, et al., 1987a). In sheep, very little is excreted as unchanged drug (1%) or as sulfate (1.5%) while glucuronide excretion appears to be significant at 11.4% of the dose (Yeleswaram, et al., 1993a.).
1.4 CHIRAL ASPECTS OF DRUG PHARMACOKINETICS AND METABOLISM

1.4.1 Chirality

Chirality was discovered by Louis Pasteur in 1848. He observed that the two crystalline forms of tartaric acid had the same melting point, solubility and physicochemical properties, except in solution where one form would rotate the plane of polarized light to the right (dextro) and the other to the left (levo). Pasteur proposed that "The optical activity of organic solutions is determined by molecular asymmetry, which produces non-superimposable mirror image structures" (Drayer, 1988). The non-superimposable mirror images are called enantiomers. In 1904 the two enantiomers of atropine were found to have different pharmacological properties. It is now known that when a pair of enantiomers is placed in an asymmetric environment, each enantiomer interacts differently with that environment. This asymmetric environment could be pharmacological receptors, protein binding sites, enzymes and areas at the site of action where the dextro or levo drug enantiomer will interact. When enantiomers react specifically with a receptor, the nature or the magnitude of the pharmacological effect may be quite different for each enantiomer. Differences in the pharmacological activities of enantiomers have been widely reported for calcium channel blockers (Echizen et al., 1985), non-steroidal anti-inflammatory drugs (Jamali et al., 1988), alkaloids (White et al., 1983) and β blockers (Barret and Cullum, 1968, Francis et al., 1982). There is also a well established model for the variable fit of β blocker enantiomers to the β-adrenoceptor binding sites (Walle et al., 1988).
1.4.2 Pharmacokinetic Considerations

Individual enantiomers often behave as distinctly different chemicals with respect to their pharmacological and toxicological actions and with regard to their fate in the body. In some cases, the difference between the pharmacological activity of enantiomers may be of pharmacokinetic as well as pharmacodynamic origin. In most cases, the blood concentration and pharmacokinetics of racemates do not reflect those of the individual active enantiomers (Walle, 1985). Therefore, the importance of measuring the individual concentrations of enantiomers of racemic drugs is becoming more apparent. In numerous studies on the pharmacokinetics of the enantiomers of propranolol, it was found that the systemic and oral clearances of the S-enantiomer were lower than those of the R-enantiomer, resulting in the higher plasma concentration of the pharmacologically active form (Jackman, et al., 1981; Silber, et al., 1982; Olanoff, et al., 1984).

Until recently, the measurement of drugs in biological fluids was based largely on techniques unable to differentiate between individual enantiomers. Therefore, for some drugs the question of whether the enantioselectivity of processes such as absorption, distribution, metabolism and excretion is large enough to be of therapeutic significance remains unanswered. However, it is known that plasma protein binding and hepatic metabolizing enzymes show significant stereoselectivity for drugs (Williams and Lee, 1985).

To date there is little or no information in the literature about the pharmacokinetics and pharmacodynamics of labetalol stereoisomers either given as the pure isomers (except for dilevalol) or when given as a racemate.
1.4.3 Metabolic Considerations

The metabolism of a drug results in either bioactivation or bioinactivation of the molecule. The stereochemistry of the drug has direct (pharmacological) and indirect (metabolic) consequences for the type of action and activity of the drug. There are a number of enantioselective metabolic conversions of drugs which have been reported in the literature (Jenner, 1980; Vermeulen, 1983; Simonyi, 1984). For example, the enantioselective biochemical conversion may occur through (Ariëns, 1986):

1. **substrate stereoselectivity**, in which one isomer is preferentially converted by the enzyme.
2. **product stereoselectivity**, in which a non-chiral substrate is converted preferentially to one of the possible enantiomers of the product.
3. **enzymatic inversion**, in which one of the enantiomers is preferentially inverted to the other enantiomer.


As mentioned earlier, labetalol undergoes extensive direct glucuronidation. Glucuronidation involves glucuronyltransferase-catalysed SN2 coupling of a nucleophilic substrate (alcohol, acid, amine) with uridine diphosphate glucuronic acid, UDPGA (Fenselau and Johnson, 1980). The stereochemical aspects of the glucuronidation conjugation reaction have only been investigated within the last
decade. Evidence in the literature indicates that glucuronyltransferase (which can exist in many forms), in some instances, preferentially conjugates one enantiomer over another. Examples include the stereoselective conjugation process for propranolol (Yost, et al., 1981), oxazepam (Yost and Finely, 1985; Sisenwine, et al. 1987) and morphine (Rane, et al., 1985). The glucuronidation conjugation of morphine was shown to be both stereoselective (conjugation of 3-phenolic group is highly stereoselective for S-morphine) and regioselective (S-morphine was exclusively conjugated at the 3-phenolic group while R-morphine is preferentially conjugated at the 6-hydroxyl group).

In the rat and rabbit, labetalol undergoes direct glucuronide conjugation at the phenolic hydroxy group (Martin, 1976), whereas in dog and bovine liver, conjugation occurs at both the aliphatic and the phenolic hydroxy groups (Niemeijer, 1991). Whether the process of glucuronidation of labetalol is stereo- or regioselective has not been investigated to-date.

1.5 METHODS OF CHIRAL SEPARATION

1.5.1 Background

Several approaches for the chromatographic separation of enantiomeric mixtures have been reported in the literature. They all fall within 2 categories: direct and indirect methods.

Indirect methods are based on the reaction of the enantiomeric mixture with chiral reagents to form a pair of diastereomers. The diastereomers which have different physicochemical properties can then be separated on a non-chiral column. Indirect methods have several disadvantages:
i) They require expensive and optically pure derivatizing agents because enantiomeric contamination of the reagents could lead to false determination.

ii) They require further treatment to recover the starting enantiomers.

iii) Because the diastereomers have different physicochemical properties the rate of formation may not be the same for each member of the pair.

Direct methods do not require prior derivatization. These methods use chiral stationary phases (CSPs) such as protein-bonded, cyclodextrin-bonded, synthetic polymer and ligand exchange phases. The CSPs form transient diastereomeric complexes with the solute enantiomers. The diastereomeric complexes have different stabilities which causes a difference in retention time and hence separation of the enantiomers (Dappen, et al., 1986).

Proteins can undergo enantioselective interaction with several pharmacologically active compounds. Two such protein CSPs, based on bovine serum albumin (BSA) and α1-acid glycoprotein (AGP), are commercially available. The AGP stationary phase developed by Hermansson (Hermansson, 1983) is comprised of coated AGP cross-linked on a silica matrix. The first generation of AGP columns (EnantioPac®) had a silica matrix which was unstable at a pH below 3 and above 7. The second generation of AGP columns (Chiral AGP®) have a much more stable silica matrix over a wide pH range.

The major disadvantages of protein columns are that the sensitivity of the enantiomer separations varies depending on such factors as ionic strength, temperature, pH and concentration of the organic modifier.
1.5.2 Analytical methods

The purpose of developing a method for separation and quantitation of labetalol stereoisomers was to be able to characterize the disposition, pharmacokinetics and metabolism of individual isomers in pregnant and non-pregnant sheep. At the start of this project, a method which allowed determination of the concentration of individual isomers of labetalol during infusion to steady state in biological fluids was not available in the literature. Because individual isomers of labetalol have been shown to possess widely different pharmacological activities, there was a need to characterize their pharmacokinetic and metabolic profiles.

While attempts have been made in the past to separate the isomers of labetalol by HPLC (Schill, et al. 1986A; Schill, et al. 1986B; Lalonde, et al. 1990), details of the methods and their applicability in pharmacokinetic studies were not adequately discussed. Schill demonstrated the potential of α₁-acid glycoprotein as a stationary phase for the chiral separation of a number of racemic drugs, including labetalol A and B (the diastereomers of labetalol). Lalonde suggested stereoselectivity in the clinical pharmacokinetics of labetalol in healthy male volunteers who received either 1.2 mg/kg intravenously or 200 mg orally. However, the stereoselectivity was based on the proportion of the individual isomers in one sample point corresponding to the highest concentration. Details of the assay including degree of resolution or a representative chromatogram were not provided. Desai et al. (Desai and Gal, 1992) reported a method for the separation of labetalol isomers which involves derivatization with chiral ((4S-cis)-2,2-dimethyl-5-isothiocyano-4-phenyl-1,3-dioxane) and non-chiral (benzyl isothiocyanate and 1-naphthalenemethyl isothiocyanate) reagents. However, a
direct separation of labetalol stereoisomers, applicable to pharmacokinetic studies has not been reported in the literature.

1.6 USE OF LABETALOL IN PREGNANCY

1.6.1 Preeclampsia

Approximately 80% of women take medication at some point during pregnancy. About 5 to 7% of very young or mature primigravid pregnant women suffer from preeclampsia (Patterson, 1978). Preeclampsia is associated with the onset of acute hypertension after the twenty fourth week of gestation (Mosby, 1990). It is characterized by a generalized disturbance in endothelial physiology, wherein endothelial dysfunction is thought to be the final common pathway in the pathogenesis of this disease. Several hypotheses exist for the etiology of preeclampsia. Among them are placental ischemia, immune maladaptation, VLDL / TxA (very low density lipoprotein / toxicity preventing activity) and genetic hypotheses (Dekker, 1994). Other researchers support heterogeneous cause hypotheses in which placental and maternal disorders (hypertension, renal disorder, obesity and diabetes) and preexisting pregnancy are implicated in the etiology of preeclampsia. (Ness and Roberts, 1996). Preeclamptic women exhibit an increased sensitivity to the pressor effects of norepinephrine and renin-angiotensin, and exhibit much lower levels of prostaglandins (Genest et al., 1983; Amery et al. 1982; Page, 1987). Symptoms of preeclampsia include hypertension, proteinuria and edema (Knor, 1987). Uteroplacental blood flow in preeclamptic women is 50 to 70% of normal (Riley, 1981). Severe preeclampsia, characterized by blood pressure greater than 160/90, and proteinuria of 5 g or more/day, may result in fetal and/or maternal death.
1.6.2 Effects of Maternal Administration of Chiral Drugs on the Fetus.

A large number of drugs that are used in the treatment of a variety of conditions in pregnancy are chiral. Examples of some of the more commonly used chiral drugs in pregnancy are:

<table>
<thead>
<tr>
<th>Drug of Choice</th>
<th>Condition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>phenylephrine</td>
<td>allergic rhinitis</td>
<td>Marshall, 1988</td>
</tr>
<tr>
<td>meperidine</td>
<td>analgesia</td>
<td>McLaughlin, 1985</td>
</tr>
<tr>
<td>corticosteroids</td>
<td>hemolytic anemia</td>
<td>Cheung, 1988</td>
</tr>
<tr>
<td>benzodiazepines</td>
<td>anxiety disorders</td>
<td>Loudon, 1987</td>
</tr>
<tr>
<td>salbutamol</td>
<td>asthma</td>
<td>Chung, 1987</td>
</tr>
<tr>
<td></td>
<td>premature labor</td>
<td></td>
</tr>
<tr>
<td>methyldopa</td>
<td>hypertension</td>
<td>Cheung, 1988</td>
</tr>
<tr>
<td>propranolol</td>
<td>hypertension</td>
<td>Cheung, 1988</td>
</tr>
<tr>
<td>idoxuridine</td>
<td>viral infection</td>
<td>Wise, 1987</td>
</tr>
<tr>
<td>rifampin</td>
<td>tuberculosis</td>
<td>Holdiness, 1987</td>
</tr>
<tr>
<td>pseudoephedrine</td>
<td>nasal congestion</td>
<td>Marshall, 1988</td>
</tr>
</tbody>
</table>

All β-adrenoceptor antagonists contain at least one chiral center located in the propanolamine side chain. Almost all of these compounds are used clinically as racemate, although only one enantiomer exhibits significant β-blocking activity (Morris and Kaumann, 1984).
The kinetic, pharmacodynamic and metabolic fate of the racemic forms of these drugs are well established. Although there are a few reported cases of measurement of individual isomers of chiral drugs used in pregnancy, there are no data in the literature about the disposition and metabolism of these isomers in the fetus. Hence, there is almost nothing known about the fate of the individual stereoisomers of chiral drugs in the mother and the fetus. The placental transfer of the stereoisomers of these drugs (except in a few cases) is also largely unknown. The only reported data are from experiments which employed isolated, perfused placentas or single point measurements of stereoisomers at parturition. In one such experiment, the placental transfer of R and S-celiprolol, determined by the maternal and umbilical plasma concentrations at delivery, was found to be non-stereoselective (Kofahl, et al., 1993). The enantiomeric ratio R/S-celiprolol was found to be constant in the plasma samples obtained from four hypertensive pregnant women who had received 200 mg of celiprolol HCL per day. Similarly, the transfer of the stereoisomers of two valproic acid metabolites (4-en-VPA and 4-yn-VPA), following administration of 500 mg of the sodium salts of the racemate, across the mouse placenta has also been shown to be non-stereoselective (Nau, et al., 1991). In contrast, the uptake of γ-aminobutyric acid (GABA) across the isolated perfused human placenta was reported to be stereoselective for the S isomer (Challier, et al., 1992). The authors suggested the operation of a stereoselective sodium-dependent-GABA placental uptake system or stereoselective binding to placental GABA transaminase.

Aside from the possibility of stereoselectivity in terms of placental transfer of chiral drugs, there is also the potential for the stereoselective metabolism of the drug in the mother or fetus to result in differing concentrations of the enantiomers in the two compartments. For example, if maternal stereoselective metabolism
leads to differing steady-state levels of the enantiomers in maternal blood, the isomers would be transferred to the fetus in part in proportion to their concentrations in the mother. Thus, the fetus could receive the enantiomers in proportions different from that in the racemate. Moreover, if fetal metabolism of the drug also operates in a stereoselective manner, there could be further divergence of the enantiomer concentrations in fetal blood, and particularly in the tracheal, amniotic and allantoic fluids into which labetalol and other basic drugs are excreted from the fetus (Rurak et al., 1991). Furthermore, because since drugs in these fluids are preferentially recirculated to the fetal circulation, the fetus could be exposed on a long-term basis to markedly different concentrations of the individual enantiomers. Furthermore, if the isomers had different biologic activities, this could result in a different spectrum of pharmacological activities in the fetus, in response to maternal administration of the racemic drug, than would be observed in an adult given the same preparation. Currently, there do not appear to be any data that provide unequivocal support for the above scenario. However, some of the previous results with labetalol in pregnant sheep (Yeleswaram et al, 1992) are consistent with the hypothesis. Thus, when a 100 mg bolus dose of labetalol was administered iv to pregnant ewes, there was lactic acidemia in both the ewe and fetus. However, the severity and duration of this effect were much greater in the fetus in spite of limited fetal exposure to the drug. The $\text{AUC}_f/\text{AUC}_M$ was 14% and the fetal drug concentrations were about an order of magnitude lower than those in the mother.
1.6.3 Clinical Use of Labetalol in Pregnancy

The clinical use of labetalol and its efficacy in lowering hypertension in pregnancy have been reported in several studies over the past 20 years. These studies have shown labetalol to be an effective antihypertensive drug in women with hypertension during late pregnancy (Michael, 1979 and 1982; Pickles et al., 1989; Saotome and Minoura, 1993; el-Qarmalawi et al., 1995; Mervil et al., 1997). Labetalol used in the management of preeclampsia (Frishman and Chesner, 1987; Rogers, et al., 1990; Hjertberg, et al., 1993; Mahmoud, et al., 1993) is found to be more effective than methyldopa (Riley, 1981; Sibai, et al., 1990) and equally as effective but with less side effects, than hydralazine (Frishman, 1987; Harper & Murnaghan, 1991) in controlling blood pressure in preeclamptic women. Labetalol, unlike hydralazine, does not decrease uteroplacental blood flow, which in preeclamptic women could cause further deterioration of the fetus (Pirhonen, et al., 1991; Morgan, et al., 1993). However, there have also been reported cases of labetalol toxicity in pregnancy, including hemodynamic collapse (Olsen & Beier-Holgersen, 1992b), cardiac arrest in the newborn (Sala, et al., 1993), increased risk of neonatal hypoglycemia (Munshi, et al., 1992), hepatotoxicity (Clark, et al., 1990; Stumpf, 1991; Chon & Middleton, 1992) and fetal death (Olsen & Beier-Holgersen, 1992a).

Labetalol is generally well-tolerated in pregnant women and has no significant maternal toxicity (Cruickshank et al., 1992) and only occasional gastrointestinal complaints (Darmansjah et al., 1995). The prenatal safety of labetalol has been shown to be similar or better than that of methyldopa (Lamming et al., 1980; Plouin et al., 1988; el-Qarmalawi et al., 1995). In comparative studies, labetalol has exhibited lower incidences of fetal growth retardation than atenolol.
(Lardoux et al., 1983) and fetal distress than hydralazine (Sibai et al., 1987). The incidence of neonatal adrenergic blockade (resulting in hypoglycemia, bradycardia and hypotension) following a single maternal dose of labetalol shortly before delivery has been reported (Klarr et al., 1994).

The Canadian Hypertension Society in a recent report (Rey, et al., 1997) recommended the use of labetalol in pregnancy: 1) in hypertensive emergencies; systolic blood pressure greater than 169 mm Hg or a diastolic pressure greater than 109 mm Hg; 2) at a threshold systolic pressure of 140 mm Hg or diastolic pressure of 90 mm Hg in women with gestational hypertension without proteinuria or pre-existing hypertension before 28 weeks’ gestation; 3) in gestational hypertension and proteinuria at any time during the pregnancy; and 4) as a second-line drug in non-severe hypertension.

1.7 DILEVALOL

Dilevalol, the RR stereoisomer of labetalol, is a non-selective β-adrenoceptor antagonist with partial β2-agonist and virtually negligible α1-antagonist activity (Brittain, et al., 1982). It has 3 to 6 times the β-adrenoceptor blocking and approximately 7 times more partial β2-receptor agonist potency than labetalol (Sybertz, et al., 1981). Clinical trials have revealed that dilevalol is equivalent in antihypertensive efficacy to the β1-antagonist metoprolol, the calcium antagonist, nifedipine, and the angiotensin converting enzyme inhibitors, captopril and enalapril (Rodriguez-Saavedra, et al., 1989; Materson, et al., 1989; Walley, et al., 1993). The antihypertensive effect of dilevalol is mediated primarily through a reduction in peripheral vascular resistance (Clifton, et al., 1988).
Dilevalol is completely absorbed after oral administration and the peak plasma concentration of unchanged drug is attained between 50 minutes to an hour (Auer, et al., 1988). This isomer, as with labetalol, exhibits high hepatic clearances with significant first-pass effect and low absolute bioavailability following oral administration (Kramer, et al., 1988). The apparent volume of distribution of dilevalol is about 25 L/kg in healthy human subjects (Chrisp and Goa, 1990). Despite being approximately 75% bound to plasma proteins (Sugeno, et al., 1987a), the drug is extensively distributed into extravascular tissue, as indicated by the high apparent volume of distribution. The metabolism of dilevalol appears to be similar in all species studied thus far (man, dog, rat, monkey, sheep), consisting of glucuronidation and sulphate conjugation via the benzylic or phenolic hydroxyl group. Glucuronidation is extensive and approximately 80% of the total dilevalol recovered from the urine of rats, dogs, and monkeys is in the form of this conjugate (Sugeno, et al., 1987b,d; Alton, et al., 1993).

1.8 RATIONALE

In the preceding discussion, the following points have been made:

- A large number of drugs used in pregnancy—either prescribed, over the counter, or abused, are chiral. These drugs are administered as racemates, an equicomponent mixture of their stereoisomers. Although in most cases the stereoisomers have been shown to possess unique pharmacological properties, the current body of data on the pharmacokinetics, pharmacodynamics and metabolism as well as placental transfer of these drugs has been generated from the measurement of the racemic drug. There are virtually no such data available
on the above parameters of individual stereoisomers of these drugs in pregnancy.

- Stereoselective disposition, metabolism and placental transfer of drugs may result in differing concentrations of the stereoisomers in the mother and the fetus. The fetus could potentially receive proportionally different concentrations of each stereoisomer than the mother. This could result in unpredictable, unwanted and potentially disastrous consequences to the mother or fetus.

- Previous data from the labetalol pregnant sheep studies suggest a stereoselective pharmacokinetic or metabolic process. More pronounced lactic acidemia is observed in the fetus following maternal administration of labetalol.

- In this project, labetalol (which contains two chiral centers, resulting in four stereoisomers) is used as a model compound to study the disposition, metabolism and placental transfer of individual stereoisomers of a chiral drug in pregnancy. The pharmacological properties of these four isomers differ widely from one another, so that essentially the treatment involves the administration of four distinct drugs.

- Labetalol is widely recommended for the treatment of pregnancy-related hypertension.
1.9 SPECIFIC AIMS

1. To develop a direct and sensitive stereoselective assay for the separation and quantitation of each of the labetalol stereoisomers in various biological fluids such as plasma, urine, amniotic and tracheal fluid.

2. To determine the pharmacokinetic parameters of the labetalol isomers in pregnant sheep following intravenous infusion of the racemic drug.

3. To examine the extent of fetal exposure to each isomer following maternal infusion of labetalol.

4. To determine the pharmacokinetic parameters of the dilevalol in non-pregnant sheep following the intravenous bolus administration.

5. To determine the pharmacokinetic parameters of labetalol isomers in the fetal lamb following direct infusion to the fetus.

6. To examine whether glucuronidation conjugative metabolism of labetalol isomers is stereoselective in the pregnant sheep and the fetal lamb.

7. To study the hemodynamic and metabolic effects of continuos infusions of labetalol in the pregnant sheep and the fetus.
2. EXPERIMENTAL

2.1 Materials

Unless otherwise noted, all materials received were used without further purification or modification:

Labetalol hydrochloride (>99% purity) (lot # 85F-0253) (Sigma Chemical Co., St. Louis, MO); injectable labetalol hydrochloride (Trandate®) (5 mg/mL); the internal standard for labetalol assay, 5-[2-[4-(4-methyl phenyl)-2-butylamino]-1-hydroxyethyl] salicylamide hydrochloride hemihydrate (SCH) (>99% pure) and dilevalol hydrochloride (RR-isomer of labetalol) (>99% pure) (gifts from Schering Corporation, Bloomfield, NJ);

The isomers of labetalol (SR, SS, RS and RR) were generously supplied by Schering Plough (Bloomfield, NJ). Potassium dihydrogen orthophosphate, tris(hydroxymethyl)aminomethane (TRIS free base) and disodium hydrogen orthophosphate were purchased from BDH Inc. (Toronto, Ont.). Reagent grade phosphoric acid and ethyl acetate (HPLC grade) were purchased from Caledon Laboratories Ltd. (Georgetown, Ont.). The cationic modifier tetrabutyl ammonium phosphate buffer, TBAP, (0.5 M, pH 7.5) was obtained from Regis Chemical Co. (Morton Grove, IL). Ethyl acetate (HPLC grade 256) was purchased from Caledon Laboratories LTD (Georgetown, Ont.). β-glucuronidase (Glucurase®, Sigma G-4882) obtained from bovine liver, and arylsulphatase, from Aerobacter aerogenes (Sigma S-1629), were purchased from Sigma Chemical Co. (St. Louis, MO).

Thiopental sodium (1g/vial) (Pentothal®); ampicillin (250 mg/vial) (Penbritin®); gentamicin sulphate (40 mg/vial) (Garamycin®); atropine sulphate (0.6 mg/mL);
halothane (Fluothane®); heparin (Hepalean®); sodium chloride for injection USP (Abbott Laboratories, Montreal, PQ) were all obtained from the Pharmacy Department, B.C. Women’s Hospital, Vancouver, B.C.

The following analytical solvents and gases were obtained:

Diethyl ether, ethyl acetate, and toluene (all glass distilled, pesticide grade), and acetonitrile (Caledon Laboratories, Georgetown, Ont.); methanol (Omnisolv®), BDH Chemicals (Toronto, Ont.). Nitrogen USP (Union Carbide Canada Ltd., Toronto, Ont.); pre-purified helium (for degassing HPLC mobile phase) and ultra-pure grade helium (for GC-MSD) (Matheson Gas Products Canada Ltd., Edmonton, Alta.).

Surgical and experimental supplies included the following:

Needles and plastic disposable Luer-Lok® syringes for drug administration and sample collection (Beckton-Dickinson Canada, Mississauga, Ont.); membrane filters (0.45µm) (Millipore, Mississauga, Ont.); disposable plastic pipette tips (National Scientific, San Rafael, CA); borosilicate glass Pasteur pipettes (John Scientific, Toronto, Ont.); heparinized blood gas syringes (Marquest Medical Products Inc., Englewood, CO); heparinized Vacutainer® tubes (Vacutainer Systems, Rutherford, NJ); 15 mL Pyrex® disposable culture tubes (Corning Glass Works, Corning, NY); polytetrafluoroethylene (PTFE) lined screw caps (Canlab, Vancouver, B.C.); polystyrene tubes (Evergreen Scientific International Inc., Los Angeles, CA) and silicone rubber tubing for catheter preparation (Dow Corning, Midland, MI).
2.2 Preparation of Standard Solutions and Buffers

Standard stock solutions (1 mg/mL) of labetalol hydrochloride, the internal standard (SCH) and the labetalol isomers were prepared by dissolving accurately weighed quantities of these compounds in methanol. The stock solutions were stored at 4°C and were used within three months of preparation. No evidence of degradation was found within these time periods. The stock solutions were diluted with distilled water to yield 1 µg/mL, 100 ng/mL and 10 ng/mL concentrations.

For the labetalol assay, the phosphate buffer (0.015 M, pH 3.10) was prepared by dissolving potassium dihydrogen orthophosphate (monobasic) in distilled water. The pH of the solution was adjusted to 3.10 with phosphoric acid. For the labetalol isomer assay, the mobile phase was a 0.02 M phosphate buffer containing 0.015 M TBAP. The mobile phase was degassed with helium for 30 minutes prior to the adjustment of the pH to 7.10 with phosphoric acid.

Carbonate buffer (1M, pH 9.5) was prepared by dissolving potassium carbonate and sodium bicarbonate in distilled water. The pH was adjusted to 9.5 (range: 9.40-9.65) using either concentrated hydrochloric acid or 5 M sodium hydroxide. The pH of the buffer was determined daily prior to use.

Ammonium hydroxide solution (4%) was prepared by diluting ammonium hydroxide USP with distilled water.

Sodium acetate solution (0.2 M) was prepared by dissolving sodium acetate in distilled water and adjusting the final pH to 5.0 using glacial acetic acid.

Tris(hydroxymethyl)aminomethane (TRIS) (0.05 M) was prepared by dissolving TRIS free base in distilled water at room temperature (≈22 °C) and adjusting to a final pH of 7.5 using 1 M hydrochloric acid.
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HPLC grade water was obtained through a Milli-Q® water system (Millipore, Bedford, MA).

2.3 Equipment and Instrumentation

2.3.1 General Instruments

The following equipment was used:

An automatic Speed Vac® concentrator (Model AS 290, Savant, Farmingdale, NY); a vortex-type mixer (Vortex-Genie®) and incubation oven (Isotemp® model 350) (Fisher Scientific Industries, Springfield, MA); IEC model 2K centrifuge (Damon/IEC division, Needham Hts., MA); rotating type mixer (Labquake® model 415-110, Lab Industries, Berkeley, CA); infusion pump (Harvard model 944) (Harvard Apparatus, Millis, MA); Pye Unicam SP8-400 spectrophotometer (Pye Unicam Ltd., Cambridge, UK).

2.3.2 High-Performance Liquid Chromatography

A Hewlett Packard (HP) 1090 liquid chromatographic system (Hewlett Packard, Palo Alto, CA) equipped with an autoinjector, autosampler, a sample loop injector of 250 μl capacity and a HP 79994A Pascal analytical workstation (model 310) was used for analysis. For the labetalol assay a Hypersil-ODS reversed phase microbore column (200 x 2.1 mm, 5μ particle size), a Hypersil-ODS reverse phase microbore guard column (20 x 2.1 mm, 5μ), an on-line solvent filter assembly, and flexible microbore stainless steel tubing (0.12 mm i.d.) (Hewlett Packard Ltd., Palo Alto, CA) were used. Labetalol isomers were
separated on a Chiral AGP® column (10 cm x 4 mm id, 10 µm sorbent particle size) obtained from Regis Chemical Co. (Morton Grove IL). A 10 x 3.0 mm Chiral AGP® guard column (Regis Chemical Co. (Morton Grove IL) was placed before the Chiral AGP® analytical column.

A HP programmable fluorescence detector (model 1046A, Hewlett Packard, Palo Alto, CA) was used with a 370 nm emission cut-off filter, and a 2 mm wide excitation slit and two 4 mm wide emission slits.

2.4 Sheep Experiments

2.4.1 Animal Preparation

Ethical approval for animal experiments was obtained from the Animal Care Committee of the University of British Columbia and the procedures used were in accordance to the guidelines of the Canadian Council on Animal Care. Pregnant (115 to 125 days of gestation; term approximately 145 days) and non-pregnant ewes of Suffolk, Finn and Dorset mixed breed were used in these studies. At least 1 week prior to surgery the animals were brought into the animal unit at the B.C. Research Institute of Children’s and Women’s Health, and kept in groups of 2 or more in large pens in full view of one another where they received a standard diet and had free access to water.

2.4.2 Surgical Procedures

2.4.2.1 Pregnant Sheep

Food was withheld for about 24 h prior to surgery. Prior to surgery 3 mg of atropine was administered to control salivation and approximately 15 minutes later
anesthesia was induced with 1 g intravenous sodium pentothal. The animals were then intubated with an endotracheal tube and anesthesia was maintained with a mixture of halothane (1-2%), nitrous oxide (70%) and oxygen. The ewe's abdomen was shaved, and the surgical area was sterilized with 10% povidone-iodine topical solution. An intravenous bolus injection of 500 mg ampicillin and 5% dextrose solution, at a rate of 1.0-1.5 mL/min, via an intravenous drip through the jugular vein, was administered to the ewe. After a midline abdominal incision was made in the ewe, the uterus was identified and access to the head of the fetus was gained through an incision of the uterine wall. Through a small incision, the fetal trachea was exposed and a silicone catheter (2.2 mm o.d.) was inserted through a small incision 1-2 cm below the larynx between two rings of cartilage and advanced 4-5 cm into the trachea. The catheter did not interfere with fluid efflux from the lung. A 3-0 silk suture was used to anchor the catheter to the skin and a drop of tissue glue (Krazyglue®) was applied on the catheter's point of entry to the trachea. A catheter was placed in the amniotic fluid compartment and sutured to the fetal skin. Following this, the uterine incision was closed and then oversewn. The fetal hindlimbs were subsequently exposed through a second small uterine incision. The femoral artery was exposed through an incision of about 3 cm in length made above the femoral arterial pulse in the groin. The vessel was tied off distally and temporarily constricted proximally. A partial cut was made on the vessel and approximately 5 to 6 cm of the catheter was threaded proximally so that the tip was in the descending aorta. Two silk ties were placed around the vessel and the catheter was anchored to the adjacent muscle on either side of the incision. Both arteries were catheterized.

A silicone rubber catheter was placed in the right and left lateral tarsal veins with approximately 11 to 12 cm of the silastic catheter being inserted into each
vessel to reach the inferior vena cava. For fetal urine collection, a catheter was inserted into the fetal bladder at the apex via a midline abdominal incision. A second amniotic fluid catheter was also implanted and anchored to the abdominal skin of the fetus. The fetus was then gently returned to the uterus. The amniotic fluid that was lost during surgery was replaced with irrigation saline (approximately 1-1.5 L). The uterine incision was closed with a continuous 2-0 gut chromic suture and then oversewn. Finally, the maternal abdominal incision was closed in layers. The maternal femoral artery and vein were catheterized in a similar fashion. The catheters were tunneled subcutaneously and exited through a small incision on the maternal right flank. They were stored in a denim pouch taped to the flank of the ewe. The catheters were filled with heparinized saline and capped when not in use.

At the end of surgery, 500 mg ampicillin was administered to the ewe (via intramuscular injection), fetus (intravenously) and into the amniotic fluid. Gentamicin was also given by the same routes (40, 10 and 20 mg, respectively).

2.4.2.2 Non-Pregnant Sheep

Non-pregnant Dorset or Suffolk breed ewes were premedicated with atropine and then anesthetized with i.v. sodium pentothal (1-1.5 g) and anesthesia was maintained with a mixture of halothane (1-2%), nitrous oxide (70%) and oxygen. Silicone rubber catheters were implanted in the femoral artery and vein of one limb for blood sampling and in the jugular vein for drug administration. A 6R-series transit-time blood flow transducer (Transonic Corp., Watham, Mass.) was placed on the femoral artery of the other hind limb for blood flow measurements. An abdominal incision to the right of the umbilicus was made to gain access to the
gall bladder, where a catheter was placed through a purse string suture to allow for bile collection. The catheters were exteriorized, tunneled subcutaneously through an incision on the flank and secured in a denim pouch. The catheters were filled with heparinized saline and capped when not in use. Ampicillin (500 mg) and gentamicin (40 mg) were administered i.m. prophylactically on the day of surgery and for the following four days. The ewes were allowed to recover for at least five days before they were used in the experiments. Just before the beginning of each experiment, a Foley bladder catheter (16 FR) was inserted for total urine collection.

2.4.3 Post-Surgery Maintenance

All vascular catheters were flushed daily with 2 mL normal saline containing 12 U/mL of heparin. Ampicillin (500 mg) and gentamicin (40 mg) were administered to the ewe for the first four days following surgery. In pregnant sheep ampicillin (500 mg) and gentamicin (20 mg) were also administered into the amniotic cavity on a daily basis until delivery.

2.4.4 Monitoring of Physiological Parameters

The ewes were allowed to recover for at least 3 days following surgery in the company of the other sheep. Before experimentation, the ewe was placed in a monitoring pen adjacent to and in view of the companion sheep.

Maternal and fetal arterial pressures, tracheal and amniotic pressures were monitored with disposable pressure transducers (DTX, Spectramed, Oxnard, CA), while maternal and fetal heart rates were continuously monitored from the arterial
pulse by the use of a cardiotachometers (Sensomedics, Model 9857, Anaheim, CA).

To measure fetal urine flow, the fetal bladder catheter was allowed to drain continuously by gravity into a sterile 10 mL syringe barrel positioned at floor level adjacent to the ewe. The hydrostatic pressure in the fluid column was measured with a disposable pressure transducer and the signal was digitized and sampled by the computer as described for the other variables. When the 10 sec average pressure rose above a preset level (usually 3 mm Hg), the computer turned on a calibrated peristaltic pump, which pumped urine from the syringe barrel via an amniotic fluid catheter into the amniotic fluid compartment. The volume pumped each minute (which equals urine flow) was stored on floppy diskette.

Femoral arterial blood flow was continuously measured using a Transonic T201 transit-time flowmeter (Transonic Corp., Watham, Mass.). All the above parameters were continuously recorded using a Beckman R-711 polygraph recorder (Sensomedics Corp., Anaheim, CA). In addition, an analog to digital conversion board (Daisi Electronics, Newton Square, PA) was used to simultaneously convert the analog signals to digital form through an on-line Apple IIe computer (Apple Computers Inc., Cupertino, CA) equipped with a clock board (Mountain Computers Inc., Scotts Valley, CA). The sampling rate was 2-4 Hz. Average values were displayed on the monitor at 10 second intervals and after each minute. The minute average measurements were automatically transferred to floppy diskettes every 30 minutes for subsequent analysis.
2.5 Animal Experiment Protocols

2.5.1 Maternal Infusion

Maternal blood was collected prior to the administration of the drug in heparinized syringes (about 100 mL) for fetal blood replacement during the experiment. A 100 mg i.v. bolus dose of labetalol (20 mL of 5 mg/mL of Trandate®) was administered through the maternal femoral venous catheter followed immediately by an infusion of 0.5 mg/mL for 6 hours. The loading bolus dose and the infusion rate were estimated from the labetalol pharmacokinetic parameters obtained from previous i.v. bolus experiments in pregnant sheep (Yeleswaram et al., 1992). These values were determined in order to obtain average steady-state concentrations of about 350 ng/mL which is similar to those reported in clinical studies following multiple oral administration of labetalol (McNeil et al., 1982; Chung et al., 1986). Samples (approximately 1.5 mL) for labetalol analysis were collected from the maternal and fetal femoral arterial and amniotic and fetal tracheal catheters at -30, -15, 15, 30, 60, 90, 120 min, and every 1 hour until 6 h. (these times correspond with the sample collection times of the previous labetalol bolus study Yeleswaram et al., 1993). At 6 hours the infusion was stopped and samples were collected at 15, 30, 45, 60, 90, 120 min and every 1 hour until 18 hours post-infusion. Samples (approximately 0.5 mL) for blood gas, glucose and lactate analysis were also collected at these times.

The fetal blood withdrawn at each sample was replaced via the tarsal vein with an equal volume of drug-free maternal blood, collected at the beginning of each experiment. The blood samples were immediately transferred to heparinized tubes on ice. The blood samples were centrifuged at 3000 g for 15 min to obtain the plasma. The plasma, as well as the amniotic and tracheal fluid samples, were
transferred to clean PTFE-lined screw capped glass tubes and stored at -20°C until assayed.

Immediately prior to the start of an experiment, a Foley® urine catheter was inserted into the maternal bladder for continuous urine collection. The urine was stored in a sterile collection bag. At hourly intervals (up to 18 hours post-infusion), the total collected urine volume was measured and a small aliquot was taken for drug analysis. Urine samples were also stored at -20°C until assayed.

2.5.2 Dilevalol Bolus in Nonpregnant Sheep

A 25 mg i.v. bolus dose of dilevalol (in 10 mL normal saline) was administered through the jugular venous catheter over a one minute period to six non-pregnant sheep. Blood samples (3 to 4 mL) for dilevalol analysis were collected from the femoral arterial and venous catheters at -30, -15, 3, 10, 15, 20, 30, 45 and 60 min, every 30 min until 4 h and then at 6, 8, 10, 12 and 24 h. Arterial and venous blood samples (approximately 0.5 mL) were also taken for blood gas analysis immediately following the collection of samples for drug analysis. The blood samples collected for drug measurement were immediately transferred to heparinized tubes and centrifuged at 3000 g for 15 min to obtain the plasma. Plasma samples were stored at -20°C until analysis.

2.5.3 Fetal Infusion

A 4 mg i.v. bolus followed by 15 µg/min of labetalol was administered for 6 hours through the fetal tarsal venous catheter. Maternal arterial, fetal arterial, amniotic, tracheal and fetal urine samples were collected. Sample times and
volumes for labetalol, glucose, lactate and blood gas analysis were the same as those used in the maternal infusion experiments. Fetal blood was replaced with an equal volume of drug-free maternal blood following each sample collection. Fetal urine was collected cumulatively at hourly intervals until the end of infusion. Volume of each urine sample was measured and approximately 5 mL and 10 mL of urine was stored for analysis from the infusion and post infusion periods, respectively. The remainder of the urine was returned to the amniotic cavity via an amniotic catheter. Occasionally during post-infusion sampling there was no urine left to return to the amniotic cavity. All other experimental protocols (volume and time of samples) are identical to the maternal infusion experiments.

2.5.4 Blood Gas Analysis

Hemoglobin concentration and blood oxygen saturation were measured with the use of a Hemoximeter® (Radiometer, Copenhagen). Blood pH, pO2, pCO2, base excess and bicarbonate were measured by an IL 1306 pH/blood gas analyzer (Instrumentation Laboratories, Walham, Mass.). The samples were measured at 37° C and the values were corrected to temperatures of 39° C and 39.5° C by the instrument for the ewe and fetus, respectively. Fetal and maternal blood samples were analyzed for glucose and lactate using a YSL analyzer (Yellow Springs Instruments, Yellow Springs, Colorado).
2.6 Quantitation of Labetalol Stereoisomers in Biological Fluids

2.6.1 Chiral Column

Two different types of $\alpha_1$-acid glycoprotein stationary phase columns were examined during the development of the chiral assay. The first column was a chiral stationary phase protein column, EnantioPac®, which was used in preliminary trials for the separation of labetalol isomers. However, after extensive trials with several combinations of chromatographic conditions, it was clear that baseline separation of labetalol stereoisomers was not possible with this column.

A search for another column led to a report by Narayanan (Narayanan, 1992) on the use of immobilized proteins for chiral resolution. The report described, second generation CSP protein columns, Chiral AGP®, as a higher performance CSP column with improved stability over first generation columns. In Chiral AGP® the protein is covalently immobilized on 5 $\mu$m spherical silica particles with small pore size of 12 nm (In EnantioPac® the protein is immobilized by adsorption on diethylaminoethyl silica with particle size of 10 $\mu$m and pore size of 25 nm).

2.6.2 Optimization of the Mobile Phase

The effects of several parameters on retention and stereoselectivity of the labetalol stereoisomers were studied. These parameters were:

i) Nature of the mobile phase

ii) pH

iii) Nature and concentration of the organic modifier

iv) Flow rate
A conventional method for attaining a suitable mobile phase composition, described widely in the literature, is the use of a phosphate buffer (0.01-0.025 M) modified with up to 10% of an organic solvent (2-propanol or ethanol). The mobile phase compositions suggested in the literature did not separate the labetalol stereoisomers.

Changes in the pH and concentration of the cationic modifier in the mobile phase have been shown to greatly influence the enantioselectivity and hence retention of several different classes of drugs by AGP (Hermansson, 1984; Gorog and Herenyi, 1990; Narayanan, 1992). While changes in the pH of the mobile phase affect enantioselectivity by altering the conformation of the protein stationary phase, cationic modifiers such as TBAP and TBABr influence chiral separation through ion-pair formation and increase in the steric bulk of the molecule (Schill, et al., 1986A; Narayanan, 1992). The separation of labetalol isomers was achieved with a 0.02 M phosphate buffer containing 0.015 M TBAP as mobile phase. The pH of the mobile phase was adjusted to 7.10 (which was below labetalol pKa of 7.30).

2.6.3 Optimization of Detection

To obtain the best signal-to-noise ratio for the detection of the labetalol isomers the optimum excitation and emission wavelengths were determined. A 60 μL aliquot of a 100 ng/mL aqueous solution of labetalol was injected into the column with the fluorescence detector on line. The pump was turned off when the isomer peaks began to elute to trap the isomer peaks in the detector cell. First the excitation wavelength was set at 190 nm and the emission wavelength was varied in increments of 10 nm from 190 to 600 nm. The optimum emission wavelength
was determined from the plot of fluorescence intensity vs emission wavelength plot. The optimum excitation wavelength was obtained by setting the emission wavelength at zero and repeating the above procedure. Optimum excitation (determined for the corresponding emission wavelength) and emission wavelength were 230 and 400 nm, respectively.

2.6.4 HPLC Operating Conditions

For the separation of labetalol from endogenous compounds a Hypersil-ODS reverse phase microbore column (200 x 2.1 mm, 5μ particle size) was connected in series to a Hypersil-ODS reverse phase microbore guard column (20 x 2.1 mm, 5μ) and an on-line solvent filter was used. For labetalol, isomers a 10 cm x 4 mm id, 10 μm sorbent particle size Chiral AGP®, a 10 x 3.0 mm Chiral AGP® guard column and flexible microbore stainless steel tubing (0.12 mm i.d.) was used. The fluorescence intensity of labetalol and its isomers was measured at excitation wavelengths of 196 nm and 230 nm and emission wavelengths of 412 nm and 400 nm, respectively. Both systems included a 370 nm emission cut-off filter, a 2 mm wide excitational slit and two 4 mm wide emission slits. The signal attenuation ranged from 16 to 18.

The mobile phase for the achiral labetalol assay was a 55:45 mixture of 0.015 M phosphate buffer and acetonitrile with pH adjusted to 3.1.

For the chiral assay, the mobile phase was a 0.02 M phosphate buffer containing 0.015 M TBAP. The mobile phase was degassed with helium for 30 minutes prior to adjusting the pH to 7.10 with phosphoric acid. The flow rate was set at 0.5 mL/min. Separation of the isomers was accomplished at ambient temperature.
2.7 Analysis of Labetalol Isomers in Biological Fluids

Total concentration of labetalol was determined by the method of Yeleswaram et al. (Yeleswaram, et al., 1991). Briefly, to 50 to 500 µL of the plasma sample, the internal standard, SCH, HPLC grade water (to 750 µL total volume) and 0.5 mL of pH 9.5 carbonate buffer (1M) were added. The mixture was extracted with 6 mL ethyl acetate. The ethyl acetate layer was separated and re-extracted with 600 µL 0.01M phosphoric acid and an aliquot (60 µL) of the aqueous layer was injected into the HPLC. For determination of the individual isomers, aliquots of the samples were extracted by the procedure that was used in the labetalol assay, but without the addition of the internal standard. The extraction without the internal standard was necessary because the internal standard interfered with labetalol isomer peaks. To calculate the concentration of the isomers addition of the internal standard was not necessary, because in the chiral assay the percentage of each isomer in the racemate was determined and not the absolute concentration. Because there was not enough of the pure isomers available to construct a standard curve for each individual isomer on a routine basis, the achiral labetalol assay method previously described was used to determine the total labetalol concentration in the samples, after which the concentration of each isomer was determined from the following relationship:

\[ C_i = \left( \frac{\% \text{ isomer} \times C_L}{100} \right) \]

where:
- \( C_i \) Concentration of the individual isomer
- \( \% \text{ isomer} \) Percent of the individual isomer determined by the chiral assay
2.8 Assay Characteristics

To obtain the concentration of individual isomers, the proportion of each isomer in the sample (equal to the peak area of the isomer divided by the total area of all four peaks) was multiplied by the total concentration of labetalol determined by the achiral assay as indicated above. The average percent peak area and coefficient of intra- and inter-day variation were calculated at 0.6, 1.5, 3.0, 6.0, 9.0, 12.0, and 15.0 ng labetalol. Linear least-squares regression analysis was used to obtain the correlation coefficient for each isomer over the range of 0.6-15.0 ng of injected labetalol. Resolution of the peaks, $R_s$, was calculated from the equation $R_s=\frac{(2t_R1-2t_R2)}{(1.7W_{h1}+1.7W_{h2})}$, where $t_R$ and $W_h$ are retention time and peak width at half height, respectively, and 1 denotes the peak with longer retention time. Minimum quantifiable limit was defined as 4 times the baseline noise. The coefficient of variation (C.V.) was calculated as: $CV=100 \cdot \frac{\sigma}{\chi}$, where $\sigma$ and $\chi$ are standard deviation and mean values, respectively. The intra- and inter-day CVs were determined by preparing 9 standard curves in one and over 5 days, respectively.

2.9 Analysis of glucuronide and sulphate conjugates of labetalol and its isomers

To measure the concentrations of the conjugated metabolites of labetalol and its isomers, urine samples were treated with $\beta$-glucuronidase (Glucurase®) and
aryl sulfatase according to the procedure described by Brashear (Brasher et al., 1988). Briefly, the urine (50 μL) and bile (250 μL) samples were treated with 0.5 mL pH 5.0 sodium acetate buffer (0.2 M) and 0.5 mL of Glucurase®, for the determination of the glucuronide, and with 0.5 mL pH 7.5 Tris buffer (0.05 M) and 30 μL of the aryl-sulphatase preparation for sulphate determination. The enzyme-treated samples were incubated overnight at 37 °C in a water bath with gentle shaking and the samples were then cooled to room temperature. The concentrations of labetalol in the enzyme-treated samples were determined by the method described previously. Glucuronide and sulphate conjugate concentrations were expressed as the difference between the post-incubation and pre-incubation labetalol or isomer concentrations.

2.10 Data Analysis

2.10.1 Pharmacokinetic Analysis

A suitable weighting factor to be used in curve-fitting was chosen by the method of Albert and coworkers (Albert et al., 1974). Briefly, the concentration-time data from the individual sheep were pooled and estimates of mean concentrations at each time point and the associated variances were used to calculate the weighing factor. The plot of natural logarithm (ln) of variance against ln of concentration is a straight line described by the following equation:

$$\ln \sigma^2 = \ln a + n \ln C$$

where $\sigma^2$ is the variance, $C$ is the mean concentration and $a$ and $n$ are constants. The weighing factor is determined from the value for the slope ($-n$) of the above line. Therefore any concentration $C_t$ is weighted as $(C_t)^n$. 
To select the most appropriate model to describe the decline of labetalol and dilevalol concentrations in plasma and to obtain initial estimates of exponents (e.g. $\alpha$, $\beta$, and $\gamma$), and constants (e.g. A, B, and C), the individual weighted plasma drug concentration-time data were fitted by the computer programs JANA® (Statistical Consultants Inc., Lexington, KY) and PCNONLIN® (version 3.0) (Statistical Consultants Inc. Lexington, KY). JANA were used to estimate the pharmacokinetic model and to provide initial parameter estimates. The initial parameter estimates were then refined by PCNONLIN®. The area under the plasma concentration-time curve ($\text{AUC} = \text{AUC}_0 t_{\text{last}} + C_{t_{\text{last}}/\gamma}$) and area under the first moment curve ($\text{AUMC} = \text{AUMC}_0 t_{\text{last}} + t_{\text{last}} * C_{t_{\text{last}}/\beta} + C_{t_{\text{last}}/\beta^2}$) were used to calculate the Mean Residence Time, MRT ($\text{MRT} = \text{AUMC}_0^\infty / \text{AUC}_0^\infty$), where $t_{\text{last}}$ represents the time of the last sample. The terminal elimination half-life ($t_{1/2\gamma}$), total body clearance (CL), clearance at steady-state (CLss) and the apparent volumes of distribution ($V_d_{\text{area}}$ and $V_d_{\text{ss}}$) were calculated as follows:

\[
t_{1/2\gamma} = 0.693/\gamma
\]

\[
\text{CL} = \text{Dose} / \text{AUC}
\]

\[
\text{CLss} = k_0 / \text{Css}
\]

\[
V_d_{\text{area}} = \text{Dose} / (\text{AUC} \cdot \gamma)
\]

\[
V_d_{\text{ss}} = \text{Dose} \cdot \text{AUMC} / (\text{AUC})^2
\]

2.10.2 Hemodynamic and Metabolic Data Analysis

Blood flow, heart rate and arterial blood pressure were averaged over 30 minute periods. The fluxes of glucose and lactate across the maternal hind limb were calculated as $[(\text{arterial concentration} - \text{venous concentration}) / \text{hind limb}]$.
blood flow]. The minute average measurements of the hindlimb blood flow corresponding to the sampling time were used.

2.10.3 Calculation of *in utero* fetal weight

The weight of the fetuses at the day of experiments were determined by the method of Gresham (Gresham *et al.*, 1972) using the birth weight and the number of days between the experiment and birth (ND):

\[
\log (\text{in utero fetal weight}) = \log (\text{birth weight}) - 0.153 \times (\text{ND})
\]

2.10.4 Statistical Analysis

The concentrations of labetalol stereoisomers were analyzed for statistical significance from each other by Two-Way Analysis of Variance with repeated measures. Student's T-test was used to test for differences in the pharmacokinetic parameters obtained for the plasma concentrations. The blood gas parameters, concentrations of glucose, lactate, hind limb fluxes of glucose and lactate following dilevalol administration were also analyzed for statistical significance against changes from pre-experimental values by Two-Way Analysis of Variance with repeated measures. Changes in arterial pressure, heart rate and flow values following dilevalol administration were tested for significant difference from pre-experimental values by Fisher's Least Square Difference. Values are expressed as mean ± standard error of the mean (SEM) and the level of significance used in all cases was equal to 0.05.
3. RESULTS

3.1 DEVELOPMENT OF A METHOD FOR DIRECT SEPARATION OF LABETALOL STEREOISOMERS IN BIOLOGICAL FLUIDS.

3.1.1 Chromatographic Optimization

Resolution, peak shape and retention times of the labetalol isomer peaks were significantly affected by the pH and the concentration of the organic modifiers in the mobile phase. Baseline resolution defined as $R_s \geq 1.5$ was achieved by manipulation of the pH, TBAP concentration and flow rate of the mobile phase. The retention times for SR, SS, RS and RR peaks were 19.7, 25.1, 31.3 and 36.3 minutes, respectively (Fig 2A). The identities of the isomer peaks were established with the aid of authentic standards. The chromatograms of the individual isomers are shown in Fig 2B, 2C, 2D and 2E.

The resolution of the SR and SS isomers was particularly sensitive to a change in the pH of the mobile phase, while the resolution of the RS and RR isomers was more sensitive to changes in the concentration of TBAP. At a pH of 7.10 and a TBAP concentration of 0.005 M, the SR and SS isomers eluted as separate peaks ($R_s = 1.6$), but the RS and RR isomers coeluted (Fig 3). At pH 6.9 and a TBAP concentration of 0.015 M the RS and RR peaks were completely separated ($R = 1.7$) but SR and SS peaks were not (Fig 4). Complete separation of the isomers was achieved with 0.02 M phosphate buffer containing 0.015 M TBAP with the pH adjusted to 7.10.
Figure 2. Retention times of labetalol stereoisomers SR, SS, RS and RR in order of elution (0.02 M phosphate buffer containing 0.015 M TBAP with the pH adjusted to 7.10 at a flow rate of 0.5 mL/min).
Figure 3. Separation of labetalol isomers at pH of 7.10 and 0.005 M TBAP

Figure 4. Separation of labetalol isomers at pH of 6.9 and 0.015 M TBAP.
3.1.2 Detection of Isomers

The labetalol isomer peaks were trapped in the detector cell and scanned for optimum excitation and emission wavelengths. As in the case of labetalol (Yeleswaram, 1991), the background noise was reduced by the use of the emission cut-off filters and the signal-to-noise ratio was improved by using a 2 mm excitation slit and two 4 mm emission slits. The detector was set at an excitation wavelength of 230 nm and emission wavelength of 400 nm to monitor the nascent fluorescence intensity of the isomers of labetalol.

3.1.3 Calibration and reproducibility

Calibration curve characteristics corresponding to injection of 0.6 to 15 ng of racemic labetalol stock solution are shown in Table 1. The percent peak area of the isomers and the coefficient of variation over the range of the amount of labetalol injected are shown in Table 2. The intra- and inter-day coefficient of variations ranged from 0.59 to 7.72 and 0.23 to 5.48%, respectively. The limit of quantitation (signal to noise ratio=4) was 0.6 ng of labetalol or 0.15 ng of each isomer injected. The maximum amount of labetalol that could be resolved at the baseline was 15.0 ng injected.

3.1.4 Determination of Labetalol Isomers in Biological Fluids

Labetalol was extracted from plasma obtained from chronically instrumented non-pregnant sheep and from blank fluids, spiked with labetalol,
obtained from pregnant sheep from previous experiments. Sample chromatograms from blank fetal tracheal, maternal plasma and amniotic fluid spiked with labetalol are shown in Fig 5A, 5B and 5C, respectively. The 4 isomers were well separated from the endogenous substances in all the fluids examined (plasma, tracheal and amniotic fluid). The chromatogram of isomers extracted from plasma obtained from non-pregnant sheep during the post-infusion period is shown in Fig 6.

<table>
<thead>
<tr>
<th>Isomer</th>
<th>Correlation Coefficient</th>
<th>Slope ± S.D.*</th>
<th>Intercept ± S.D.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR</td>
<td>0.9998</td>
<td>0.221 ± 0.001</td>
<td>0.009 ± 0.018</td>
</tr>
<tr>
<td>SS</td>
<td>0.9999</td>
<td>0.261 ± 0.001</td>
<td>-0.001 ± 0.011</td>
</tr>
<tr>
<td>RS</td>
<td>0.9999</td>
<td>0.265 ± 0.001</td>
<td>-0.006 ± 0.008</td>
</tr>
<tr>
<td>RR</td>
<td>0.9997</td>
<td>0.252 ± 0.002</td>
<td>-0.003 ± 0.025</td>
</tr>
</tbody>
</table>

* Standard Deviation
Table 2.
Average Percent Peak Area and Inter- and Intra-Day C.V. (%) for Each Isomer Following Injection of Racemic Labetalol (n=9).
(CV Values in parentheses)

<table>
<thead>
<tr>
<th>Amount of Labetalol (ng)</th>
<th>SR</th>
<th>SS</th>
<th>RS</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6</td>
<td>22.80</td>
<td>26.00</td>
<td>26.28</td>
<td>24.92</td>
</tr>
<tr>
<td>Inter-Day CV</td>
<td>(5.48)</td>
<td>(0.85)</td>
<td>(2.93)</td>
<td>(4.01)</td>
</tr>
<tr>
<td>Intra-Day CV</td>
<td>(7.72)</td>
<td>(6.45)</td>
<td>(7.56)</td>
<td>(4.24)</td>
</tr>
<tr>
<td>1.5</td>
<td>22.17</td>
<td>25.90</td>
<td>26.38</td>
<td>25.55</td>
</tr>
<tr>
<td>Inter-Day CV</td>
<td>(2.97)</td>
<td>(0.53)</td>
<td>(1.92)</td>
<td>(0.79)</td>
</tr>
<tr>
<td>Intra-Day CV</td>
<td>(6.81)</td>
<td>(2.49)</td>
<td>(1.73)</td>
<td>(3.48)</td>
</tr>
<tr>
<td>3.0</td>
<td>22.31</td>
<td>26.40</td>
<td>26.15</td>
<td>25.14</td>
</tr>
<tr>
<td>Inter-Day CV</td>
<td>(2.36)</td>
<td>(1.87)</td>
<td>(2.42)</td>
<td>(2.52)</td>
</tr>
<tr>
<td>Intra-Day CV</td>
<td>(2.12)</td>
<td>(1.89)</td>
<td>(2.50)</td>
<td>(3.23)</td>
</tr>
<tr>
<td>6.0</td>
<td>22.42</td>
<td>25.75</td>
<td>26.46</td>
<td>25.36</td>
</tr>
<tr>
<td>Inter-Day CV</td>
<td>(0.47)</td>
<td>(0.70)</td>
<td>(0.50)</td>
<td>(0.72)</td>
</tr>
<tr>
<td>Intra-Day CV</td>
<td>(1.29)</td>
<td>(0.78)</td>
<td>(1.42)</td>
<td>(1.96)</td>
</tr>
<tr>
<td>9.0</td>
<td>22.57</td>
<td>26.21</td>
<td>26.63</td>
<td>24.59</td>
</tr>
<tr>
<td>Inter-Day CV</td>
<td>(0.88)</td>
<td>(0.76)</td>
<td>(1.33)</td>
<td>(2.34)</td>
</tr>
<tr>
<td>Intra-Day CV</td>
<td>(1.85)</td>
<td>(0.98)</td>
<td>(0.59)</td>
<td>(1.59)</td>
</tr>
<tr>
<td>12.0</td>
<td>22.25</td>
<td>26.16</td>
<td>26.43</td>
<td>25.17</td>
</tr>
<tr>
<td>Inter-Day CV</td>
<td>(2.97)</td>
<td>(1.32)</td>
<td>(1.04)</td>
<td>(1.96)</td>
</tr>
<tr>
<td>Intra-Day CV</td>
<td>(2.17)</td>
<td>(0.91)</td>
<td>(1.39)</td>
<td>(1.03)</td>
</tr>
<tr>
<td>15.0</td>
<td>22.04</td>
<td>26.06</td>
<td>26.58</td>
<td>25.31</td>
</tr>
<tr>
<td>Inter-Day CV</td>
<td>(2.01)</td>
<td>(0.23)</td>
<td>(0.70)</td>
<td>(1.94)</td>
</tr>
<tr>
<td>Intra-Day CV</td>
<td>(1.68)</td>
<td>(0.86)</td>
<td>(0.69)</td>
<td>(1.33)</td>
</tr>
</tbody>
</table>
Figure 5. Separation of labetalol isomers in fetal tracheal fluid (A), maternal plasma (B) and amniotic fluid (C) samples spiked with labetalol. Lower traces are from control samples. [0.02 M phosphate buffer containing 0.015 M TBAP with the pH adjusted to 7.10 at a flow rate of 0.5 mL/min].
Figure 6. Separation of labetalol isomers in adult sheep plasma (Post-infusion sample). Lower trace from a control sample. [0.02 M phosphate buffer containing 0.015 M TBAP with the pH adjusted to 7.10 at 0.5 mL/min].
3.2 Maternal Infusion Studies

3.2.1 Animals

The details on the age and weight of the animals used in the maternal labetalol infusion studies are summarized in Table 3. These studies were performed in five chronically instrumented pregnant ewes. The gestational age of the ewes during the maternal infusion experiment averaged 129.0 ± 1.4 days.

3.2.2 Pharmacokinetics of Labetalol.

The mean disposition profile of labetalol in maternal and fetal femoral arterial plasma following a 100 mg maternal i.v. bolus and immediate infusion of 0.5 mg/min for 6 hours is shown in Fig 7. A total of 280 mg of labetalol was administered by the end of the experiment. The steady-state concentration of labetalol in maternal and fetal femoral arterial plasma was 423.4±87.8 and 59.6±22.3 ng/mL, respectively. The maternal and fetal pharmacokinetic parameters of labetalol are shown in Table 4.

Labetalol exhibited a triexponential elimination profile (\( Ae^{-at} + Be^{-bt} + Ce^{-ct} \)) in maternal plasma and tri- or bi-exponential decline in fetal plasma. The maternal terminal elimination rate constant (0.189 ± 0.017 h\(^{-1}\)) was significantly higher than that observed in the fetal plasma (0.110 ± 0.013 h\(^{-1}\)) corresponding to mean half-life of 3.66 ± 0.92 hr, which was significantly lower than that in the fetus (6.28 ± 1.4 h) (Student's t test). Maternal clearance and apparent volume of distribution at steady-state were 15.80 ± 4.25 mL/min/kg and 5.16 ± 0.65 L/kg, respectively.
Significant fetal exposure to labetalol following maternal administration was observed with the drug being detectable in the fetal arterial plasma for up to 6 hours post-infusion. The exposure to labetalol, calculated as the ratio of the fetal

### Table 3. Maternal infusion animal experimental details

<table>
<thead>
<tr>
<th>EWE Number</th>
<th>Maternal Weight (kg)</th>
<th>Gestational age during experiment (days)</th>
<th>No. of Fetuses</th>
<th>Fetal birth weight (kg)</th>
<th>Weight of fetus during experiment (kg)</th>
<th>Term (days)</th>
<th>Samples*</th>
</tr>
</thead>
<tbody>
<tr>
<td>229</td>
<td>69.1</td>
<td>126</td>
<td>2</td>
<td>2.890</td>
<td>1.893</td>
<td>138</td>
<td>MA, FA, AMN, TR, Um</td>
</tr>
<tr>
<td>1136</td>
<td>71.7</td>
<td>127</td>
<td>1</td>
<td>3.538</td>
<td>2.577</td>
<td>136</td>
<td>MA, FA, AMN, TR, Um</td>
</tr>
<tr>
<td>1242</td>
<td>75.5</td>
<td>130</td>
<td>2</td>
<td>3.353</td>
<td>2.530</td>
<td>138</td>
<td>MA, FA, AMN, TR, Um</td>
</tr>
<tr>
<td>313</td>
<td>69.9</td>
<td>129</td>
<td>2</td>
<td>3.22</td>
<td>2.516</td>
<td>136</td>
<td>MA, FA, AMN, TR, Um</td>
</tr>
<tr>
<td>1118</td>
<td>87.5</td>
<td>133</td>
<td>2</td>
<td>2.646</td>
<td>2.219</td>
<td>138</td>
<td>MA, FA, AMN, TR, Um</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>74.74 ± 3.37</td>
<td>129.0 ± 1.3</td>
<td></td>
<td>3.13 ± 0.16</td>
<td>2.35 ± 0.13</td>
<td>137.2 ± 0.49</td>
<td></td>
</tr>
</tbody>
</table>

*Samples
MA - Maternal Femoral Arterial Blood
FA - Fetal Femoral Arterial Blood
AMN - Amniotic Fluid
TR - Fetal Tracheal Fluid
Um - Maternal Urine
Fig 7. Semilogarithmic disposition of labetalol in maternal and fetal arterial plasma (maternal 100 mg i.v. bolus and 6 h 0.5 mg/min infusion n=5, mean ± SEM).
**TABLE 4. Labetalol pharmacokinetics in pregnant ewe and fetal lamb following maternal infusion (mean±SEM; n=5).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Maternal</th>
<th>Fetal</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{ss}$ (ng/mL)</td>
<td><strong>423.4 ± 87.8</strong></td>
<td><strong>59.6 ± 22.3</strong></td>
</tr>
<tr>
<td>AUC (mg h/L)</td>
<td><strong>4.13 ± 0.74</strong></td>
<td><strong>1.37 ± 0.15</strong></td>
</tr>
<tr>
<td>$Cl_{ss}$ (ml/min/kg)</td>
<td><strong>15.80 ± 4.25</strong></td>
<td>****</td>
</tr>
<tr>
<td>$\gamma$ (1/h)</td>
<td><strong>0.19 ± 0.02</strong></td>
<td><strong>0.11 ± 0.02</strong></td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td><strong>3.66 ± 0.92</strong></td>
<td><strong>6.28 ± 1.40</strong></td>
</tr>
<tr>
<td>$Vd_{ss}$ (L/kg)</td>
<td><strong>5.16 ± 0.65</strong></td>
<td>****</td>
</tr>
</tbody>
</table>

** Cannot be estimated following maternal dosing
to maternal plasma AUC, was 33.06 ± 5.83%. Labetalol accumulated in the fetal tracheal fluid with concentrations comparable to that in fetal plasma after 3 hours (Fig 7 and 8). Labetalol appeared at a slower rate and at lower concentrations in the amniotic fluid compared to the fetal tracheal fluid (Fig 9). The drug continued to accumulate in the amniotic fluid for up to 4 hours post-infusion and was detectable for up to 24 hours post-infusion in some animals.

3.2.3 Pharmacokinetics of the stereoisomers of Labetalol

The semilogarithmic disposition profiles of labetalol stereoisomers in maternal and fetal femoral arterial blood following maternal dosing are shown in Figs 10 and 11, respectively. The pharmacokinetic parameters of each isomer are shown in Table 5. In maternal plasma, the concentration of the RR isomer at steady state was 132.31 ± 18.79 ng/mL, which was almost twice that of the RS isomer (74.83 ± 11.48 ng/mL). The concentration of the SR and SS isomers were 104.22 ± 17.09 and 112.45 ± 15.96 ng/mL respectively. The RR isomer exhibited significantly higher concentrations than the other isomers throughout the infusion and post-infusion periods. The concentrations of the SR and RR isomers were significantly different from the racemic value (25%) throughout the infusion and post-infusion period (Student’s t-test). The terminal elimination half-life ($t_{1/2}$) of the RR isomer (5.60 ± 1.00 hr) was significantly higher than those of the other isomers whereas the $t_{1/2}$ of the SR isomer was significantly lower (1.75 ± 0.40) (Paired t-test). The steady-state clearance ($Cl_{ss}$) of the RR isomer (12.63 ± 2.05 ml/min/kg) was significantly lower than those for the SR and RS isomers.
Fig 8. Disposition of labetalol in fetal tracheal fluid (maternal 100 mg i.v. bolus and 6 h 0.5 mg/min infusion n=5, mean ± SEM).

Fig 9. Accumulation of labetalol in amniotic fluid (maternal 100 mg i.v. bolus and 6 h 0.5 mg/min infusion n=5, mean ± SEM).
Fig 10. Semilogarithmic disposition profile of labetalol stereoisomers in maternal arterial plasma (maternal 100 mg i.v. bolus and 6 h 0.5 mg/min infusion n=5, mean concentration values. Error bars are not shown for purpose of clarity).
Fig 11. Semilogarithmetic disposition profile of labetalol in fetal arterial plasma
(maternal 100 mg i.v. bolus and 6 h 0.5 mg/min infusion n=5, mean concentration values. Error bars are not shown for purpose of clarity).
TABLE 5. Pharmacokinetics of labetalol stereoisomers in the pregnant ewes and fetal lamb following maternal infusion (mean±SEM; n=5).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MATERNAL</th>
<th>FETAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SR</td>
<td>SS</td>
</tr>
<tr>
<td>C_{ss} (ng/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>104.22±17.09</td>
<td>112.45±15.96</td>
</tr>
<tr>
<td>% Isomer @ Steady State</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24.57±1.83</td>
<td>26.77±1.66</td>
</tr>
<tr>
<td>Cl_{ss} (mL/min/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16.05±2.81</td>
<td>14.87±2.30</td>
</tr>
<tr>
<td>k (1/hr)</td>
<td>0.395±0.63</td>
<td>0.183±0.027</td>
</tr>
<tr>
<td>t_{1/2} (hr)</td>
<td>1.75±0.40</td>
<td>3.78±0.72</td>
</tr>
<tr>
<td>Vd_{ss} (L/kg)</td>
<td>2.51±0.26</td>
<td>5.02±0.44</td>
</tr>
<tr>
<td>F_{AUC/M_{AUC}}</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

a. Significantly different from racemic level  
b. Significantly different from the other isomers  
c. Cannot be determined following maternal dosing  
N/A. Not applicable

The RR and RS isomers exhibited a significantly higher volume of distribution, Vd_{ss}, than those of the SS and SR isomers (Student’s t-test).
In fetal plasma, the concentration of the RR isomer at steady-state was 19.74 ± 4.31 ng/mL, which was significantly higher than the other three isomers (11.00 ± 2.38, 13.02 ± 3.04 and 12.05 ± 2.85 ng/mL for SR, SS and RS, respectively). The terminal elimination half-life (t_{1/2}) and the mean residence time (MRT) of the RR isomer were 13.07 ± 4.02 h, and 17.19 ± 5.63 h, respectively. These values were approximately 3 times higher than those obtained for the t_{1/2} and MRT values of the other 3 isomers. The ratio of the fetal to maternal AUC of the RR isomer (0.748 ± .072) was significantly higher than those of the SR (0.405 ± .0530), SS (0.32 ± 0.040) and RS (0.513 ± 0.066) isomers.

### 3.2.4 Glucuronide Conjugation

The mean rate of maternal urine output during the 6 hours of labetalol infusion was 0.011 ± 0.003 mL/min/kg (48.8± 6.9 mL/h), which was not significantly different than the pre-experimental urine output of 39.4 ± 10.4 mL/h (Table 6). The mean maternal urine pH during the infusion (7.05 ± 0.16) was significantly lower than the pre-experimental value of 7.46 ± 0.05 (Student’s t-test). Urine pH was not measured in previous labetalol studies. The change in pH was not observed in previous control experiments (identical protocol, but with saline infusion) in our laboratory (Gordon, 1995). The cumulative urinary excretion of unchanged and glucuronide conjugated labetalol are shown in Fig 12. Of the 280 mg of labetalol administered, 73.4 ± 6.4 mg (26.2 ± 2.3%) were recovered, of which 10.10 ± 0.8 mg (3.6 ± 0.3%) were excreted as unconjugated drug and 63.35 ± 5.94 mg (22.6 ± 2.1%) was excreted as the glucuronide (Table 7). The cumulative urinary excretion of the unchanged and glucuronide isomers of labetalol are shown in Figs 13 and 14, respectively. Of the 10.10 ± 0.8 mg
unconjugated labetalol, 31.7 ± 4.0% of the was in the form of the RR isomer (compared to 22.8 ± 3.0% for the other isomers) whereas only 14.0 ± 1.9% of the conjugated labetalol was the RR isomer (compared to 28.5 ± 3.2% for the other isomers).

**TABLE 6. Mean maternal hourly urine output and pH during the 6 hours of labetalol infusion.**

<table>
<thead>
<tr>
<th>Ewe #</th>
<th>Maternal Urine Output [Pre-experimental] (mL/h)</th>
<th>Maternal Urine Output During Infusion (mL/h)</th>
<th>Maternal Urine pH [Pre-experimental]</th>
<th>Maternal Urine pH During Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>229</td>
<td>34</td>
<td>39</td>
<td>7.39</td>
<td>7.14</td>
</tr>
<tr>
<td>1136</td>
<td>47</td>
<td>69</td>
<td>7.45</td>
<td>7.21</td>
</tr>
<tr>
<td>1118</td>
<td>25</td>
<td>33</td>
<td>7.49</td>
<td>6.78</td>
</tr>
<tr>
<td>313</td>
<td>51</td>
<td>42</td>
<td>7.51</td>
<td>7.06</td>
</tr>
<tr>
<td>1242</td>
<td>40</td>
<td>61</td>
<td>7.49</td>
<td>7.06</td>
</tr>
<tr>
<td>mean ± SEM</td>
<td>39.4 ± 4.7</td>
<td>48.8 ± 6.9</td>
<td>7.46 ± 0.02</td>
<td>7.05 ± 0.07*</td>
</tr>
</tbody>
</table>

* Significantly different from pre-experimental value
Fig 12. Cumulative maternal urinary excretion of labetalol and its glucuronide conjugate (maternal 100 mg i.v. bolus and 6 h 0.5 mg/min infusion n=5, mean ± SEM).
Fig 13. Cumulative maternal urinary excretion of unconjugated labetalol stereoisomers (maternal 100 mg i.v. bolus and 6 h 0.5 mg/min infusion n=5, mean values. Error bars omitted for the purpose of clarity).

Fig 14. Cumulative maternal urinary excretion of glucuronide conjugates of labetalol stereoisomers (maternal 100 mg i.v. bolus and 6 h 0.5 mg/min infusion n=5, mean values. Error bars omitted for the purpose of clarity).
TABLE 7.

Total Amount of Labetalol and Its Isomers Recovered in Urine of Pregnant Sheep Following 100 mg Bolus and 6 Hour Infusion of Racemic Labetalol

<table>
<thead>
<tr>
<th></th>
<th>AMOUNT (mg)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unchanged</td>
<td>Glucuronide Conjugate</td>
<td>Total</td>
<td>% Total Dose</td>
</tr>
<tr>
<td>Labetalol</td>
<td>10.1±0.8</td>
<td>63.3±5.9</td>
<td>73.4±6.4</td>
<td>26.2±2.3</td>
</tr>
<tr>
<td>SR</td>
<td>2.2±0.2</td>
<td>19.0±2.3</td>
<td>21.2±2.1</td>
<td>7.6±0.8</td>
</tr>
<tr>
<td>SS</td>
<td>2.4±0.2</td>
<td>19.0±1.7</td>
<td>21.4±1.6</td>
<td>7.6±0.6</td>
</tr>
<tr>
<td>RS</td>
<td>2.2±0.3</td>
<td>16.2±1.9</td>
<td>18.4±1.8</td>
<td>6.6±0.6</td>
</tr>
<tr>
<td>RR</td>
<td>3.2±0.4*</td>
<td>8.9±1.2*</td>
<td>12.1±1.0*</td>
<td>4.3±0.4</td>
</tr>
</tbody>
</table>

* Significantly different than corresponding values of the other isomers (p<0.05)
3.2.5 Sulphate Conjugation

The chiral assay was not sensitive enough to reliably measure sulphate conjugates of labetalol isomers.

3.2.6 Hemodynamic and Metabolic consequences of Maternal Labetalol Infusion.

3.2.6.1 Hemodynamic effects.

There were no statistically significant changes in the heart rate or arterial blood pressure of the mother or the fetus following maternal administration of labetalol (Figs 15 and 16). However, heart rate and arterial blood pressure of only two mothers were recorded. The pre-experimental maternal and fetal heart rates were 107.9 and 163.5 ± 4.6 bpm, respectively. The hemodynamic changes in the mother (initial tachycardia and hypotension), while not significantly different, were similar to those reported in the adult non-pregnant sheep (Yeleswaram et al., 1992). In the fetus, there was a trend for tachycardia during and after infusion, but this was not statistically significant. There was no fetal hypotension associated with the maternal administration of labetalol. There were also no hemodynamic changes reported following the infusion of saline to pregnant sheep (n=2, Gordon, 1995).
Fig 15. Changes in maternal and fetal heart rate (maternal 100 mg i.v. bolus and 6 h 0.5 mg/min infusion n=5, mean ± SEM).

Fig 16. Changes in fetal arterial pressure (maternal 100 mg i.v. bolus and 6 h 0.5 mg/min infusion n=5, mean ± SEM).
3.2.6.2 Metabolic Effects:

3.2.6.2.1 Maternal and fetal arterial Po₂, Pco₂, %O₂ saturation, pH and base excess.

Arterial blood gas, lactate and glucose levels were significantly altered following maternal labetalol administration. The control values and the largest altered value are shown in Table 8. Mean maternal arterial Po₂ (Fig 17) decreased significantly below pre-experimental values (125.20 ± 5.29 mmHg) by 1.0 hour to 113.60 ± 2.68 mmHg. In the fetus the arterial Po₂ (Fig 17) was significantly below pre-experimental (21.08 ± 1.48) by 15 minutes of infusion and remained, on average, 4 mmHg below control until 18 hours post-infusion (except at 1 hour post-infusion). The recovery of the fetal arterial Po₂ was slower than that of the mother.

Maternal arterial (MA) Pco₂ (Fig 18) decreased from control (34.8 ± 1.0 mmHg) during the infusion and fell significantly below by 4 hours (29.9 ± 1.4 mmHg). The MA Pco₂ values reached their lowest level (28.9 ± 0.8 mmHg) by 0.5 hours post-infusion and increased to control levels by 4 hours post-infusion. There was an initial increase in the fetal arterial (FA) Pco₂ from control (51.7 ± 1.7 mmHg), but in general the values stayed within control levels for up to 4 hours of infusion and then declined significantly below control by 6 hours. The FA Pco₂ (Fig 18) gradually increased to 53.76 ± 1.00 mmHg by 6 hours post-infusion.

Maternal O₂ saturation (Fig 19) gradually decreased from control (97.64 ± 0.47 mmHg) to its lowest level (significantly below control) by 3 hours of infusion. In contrast the fetal O₂ saturation (Fig 19) fell rapidly from a control value of 39.1 ± 4.3% to its lowest level of 22.7 ± 1.4% at 2 hours into the infusion, and remained
significantly lower throughout the infusion and up to 18 hours after.
Maternal arterial pH (Fig 20) declined slowly and gradually recovered but the

TABLE 8. Control and maximum altered values for arterial blood gases, pH, base excess and glucose and lactate concentrations in the pregnant ewe and fetus (mean ± SEM, n=5)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MATERNAL</th>
<th>FETAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MA Control</td>
<td>MA Max Changed</td>
</tr>
<tr>
<td>Po₂ (mmHg)</td>
<td>125.2 ± 5.3</td>
<td>113.6 ± 2.7*</td>
</tr>
<tr>
<td>Pco₂ (mmHg)</td>
<td>34.82 ± 1.0</td>
<td>28.9 ± 0.8*</td>
</tr>
<tr>
<td>%O₂ Saturation</td>
<td>97.6 ± 0.5</td>
<td>96.8 ± 0.5</td>
</tr>
<tr>
<td>pH</td>
<td>7.48 ± 0.02</td>
<td>7.45 ± 0.02</td>
</tr>
<tr>
<td>Base Excess (mEq/L)</td>
<td>4.0 ± 1.2</td>
<td>-1.6 ± 0.7*</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>0.72 ± 0.11</td>
<td>6.3 ± 0.6*</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>2.84 ± 0.20</td>
<td>7.73 ± 0.35*</td>
</tr>
</tbody>
</table>

* Significantly different than the control values (p<0.05)
Fig 17. Changes in maternal and fetal arterial Po$_2$ (maternal 100 mg i.v. bolus and 6 h 0.5 mg/min infusion n=5, mean ± SEM). * Significantly different from control values.

Fig 18. Changes in maternal and fetal arterial Pco$_2$ (maternal 100 mg i.v. bolus and 6 h 0.5 mg/min infusion n=5, mean ± SEM). * Significantly different from control values.
Fig 19. Changes in maternal and fetal arterial oxygen saturation (maternal 100 mg i.v. bolus and 6 h 0.5 mg/min infusion n=5, mean ± SEM). * Significantly different from control values.

Fig 20. Changes in maternal and fetal arterial pH (maternal 100 mg i.v. bolus and 6 h 0.5 mg/min infusion n=5, mean ± SEM). * Significantly different from control values.
the change was not significant. Fetal arterial pH (Fig 20) declined rapidly and significantly from control values of 7.31 ± 0.01 to 7.27 ± 0.03 at 2 h of infusion. Thereafter, it remained stable until 4 - 6 h of infusion when a further decline occurred. It was still significantly below control at 6 hours post-infusion.

Base excess in both MA and FA (Fig 21) showed significant decreases during the infusion. The decline in base excess was temporally associated with the fall in pH (Fig 20), indicating metabolic acidemia. While the maternal base excess returned to control values at 4 hours post-infusion, the fetal values remained significantly below control for up to 18 hours. The mean MA and FA base excess at steady state were 0.60 ± 0.86 and -3.89 ± 1.11 mEq/L, respectively.

Control saline infusion experiments using the same experimental protocol previously performed in pregnant ewes did not produce any significant alterations in any of the above variables (Gordon, 1995)

3.2.6.2.2 Maternal and fetal arterial plasma lactate and glucose levels.

Maternal and fetal arterial plasma lactate and glucose concentrations at the control and maximum altered levels are shown in Table 8. Both maternal and fetal lactate concentrations (Fig 22) increased significantly above control (0.72 ± 0.11 and 1.0 ± 0.1 mM, respectively) by 15 minutes into the infusion. In the mother, lactate increased continuously and by almost 9 fold by the end of the infusion and then declined throughout the post-infusion period reaching control levels by the end of the experiment. In the fetus, lactate increased 5 fold by 3 hours into the infusion and remained at these levels until the end of the infusion. In contrast
Fig 21. Changes in maternal and fetal base excess (maternal 100 mg i.v. bolus and 6 h, 0.5 mg/min infusion n=5, mean ± SEM). * Significantly different from control values.

Fig 22. Changes in maternal and fetal lactate concentration (maternal 100 mg i.v. bolus and 6 h 0.5 mg/min infusion n=5, mean ± SEM). * Significantly different from control values.
Fig 23. Changes in maternal and fetal glucose concentration (maternal 100 mg i.v. bolus and 6 h 0.5 mg/min infusion n=5, mean ± SEM). All values, except those at 24 hours, are significantly above pre-experimental measurements.
to the mother, the mean fetal lactate increased by a further 25% from the end of infusion until 6 hours post-infusion. Lactate levels were still significantly higher (almost by 5 fold) in the fetus by 18 hours post-infusion.

Maternal and fetal arterial glucose concentrations (Fig 23) rose significantly above control from 2.84 ± 0.20 to 0.63 ± 0.04 mM, respectively, by 15 minutes of infusion and continued to increase until the end of the infusion (by 3 fold in both cases). Glucose concentrations declined in both the mother and fetus throughout the post-infusion period and reached control levels by the end of the experiment (18 hours post infusion). There were no significant changes in lactate or glucose levels in control saline infusion experiments (Gordon, 1995).

3.3 Dilevalol Intravenous Bolus Studies

3.3.1 Pharmacokinetics

The mean arterial plasma concentrations of dilevalol in adult non-pregnant ewes (n=6) following a 25mg i.v. bolus dose are shown in Fig 24. The disposition of dilevalol exhibited a pattern best described by a triexponential equation of the type $Ae^{-at} + Be^{-bt} + Ce^{-ct}$. The mean pharmacokinetic parameters of dilevalol are listed in Table 9. These parameters are compared to the corresponding values for racemic labetalol obtained from previous studies (Yeleswaram, et al., 1993). The mean terminal elimination half-life was 3.48 ± 1.01 h while the total body clearance was 48.7 ± 7.8 mL/min/kg. The estimates of the apparent volume of distribution $Vd_{area}$ and $Vd_{ss}$ (non-parametric), were 13.09 ± 2.78 and 5.32 ± 0.80 L/kg, respectively. Of the dilevalol pharmacokinetic variables, CL, $Vd_{ss}$ $Vd_{area}$ were significantly different from those determined previously for labetalol.
TABLE 9:
Pharmacokinetics of dilevalol (25 mg) and labetalol\textsuperscript{a} (100 mg) in non-pregnant ewes. (mean ± SEM)

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>DILEVALOL</th>
<th>LABETALOL\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N=6)</td>
<td>(N=5)</td>
</tr>
<tr>
<td>CL (mL/min/kg)</td>
<td>48.7 ± 7.80 *</td>
<td>29.00 ± 2.67</td>
</tr>
<tr>
<td>t\textsubscript{1/2}y (h)</td>
<td>3.48 ± 1.01</td>
<td>2.41 ± 0.30</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>1.92 ± 0.33</td>
<td>1.85 ± 0.09</td>
</tr>
<tr>
<td>V\textsubscript{dss} (L/kg)</td>
<td>5.32 ± 0.80 *</td>
<td>3.22 ± 0.31</td>
</tr>
<tr>
<td>V\textsubscript{darea} (L/kg)</td>
<td>13.09 ± 2.78 *</td>
<td>6.19 ± 1.13</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Yeleswaram \textit{et al}., 1992.

* Denotes a significant difference (p<0.05) from the corresponding value for labetalol.
FIG 24:  Semilogarithmic disposition profile of dilevalol in femoral arterial plasma of adult sheep following a 25 mg i.v. bolus dose (n=6, mean ± SEM)
3.3.2 Conjugative Metabolism

The cumulative urinary excretion of unchanged dilevalol, glucuronide and sulfate conjugates over a 12 h period following the administration of 25 mg i.v. bolus were 0.52 ± 0.12, 1.34 ± 0.31 and 0.35 ± 0.14 mg, respectively. The amount of unchanged drug collected in the urine accounted for 2.08 ± 0.48% of the dose, whereas the glucuronide and sulfate conjugates accounted for 5.36 ± 1.24 and 1.4 ± 0.56% of the dose, respectively (Fig 25).

An estimation of the cumulative biliary excretion of dilevalol and its conjugates was not possible, because bile could not be continuously collected due to potentially harmful effects of bile salt depletion. The concentration of unchanged dilevalol and its associated glucuronide and sulfate conjugates in bile are shown in Fig 26. The average concentration of the glucuronide conjugates (2.33 ± 0.52 µg/mL) was significantly higher than that of the sulphate conjugates (0.31 ± 0.05 µg/mL) throughout the sampling period. In both urine and bile, the glucuronide conjugate was present in the highest concentration and this was most extreme for bile.
FIG 25: Cumulative urinary excretion of dilevalol and its glucuronide and sulphate conjugates following administration of a 25 mg i.v. bolus dose. (n=6, mean ± SEM)
FIG 26: Concentrations of dilevalol and its glucuronide and sulphate conjugates in bile (n=6, mean ± SEM).
3.3.3 Pharmacodynamics

The effects of i.v. bolus administration of 25 mg of dilevalol on heart rate and mean arterial pressure are shown in Figs 27 and 28, respectively, whereas those on the vascular resistance ([mean arterial pressure-hind limb venous pressure] ÷ hind limb flow) and femoral arterial blood flow are shown in Figs 29 and 30, respectively. There was a significant increase in heart rate (by 50 ± 11%) and femoral blood flow (by 100 ± 26%) and also a significant decrease in arterial pressure and vascular resistance. Maximum hypotension and tachycardia occurred about 2.5 h following the administration of the drug. Femoral blood flow returned to control levels 9 hours after drug administration whereas the control values for the heart rate and arterial pressure were regained after about 6 hours. Vascular resistance remained significantly lower than pre-experimental levels at 12 hours following the administration of dilevalol. Prior to drug administration, arterial Po₂, Pco₂, and pH averaged 138 ± 12 mm Hg, 36 ± 5 mm Hg and 7.45 ± 0.04, respectively. There were no significant changes in these variables following dilevalol administration. In contrast, the arterial and venous base excess decreased significantly (from 4.4 ± 1.1 to 1.2 ± 0.9 mEq/L) from 2 to 5 h, but returned to normal levels within 5 to 6 hours after administration of dilevalol. Changes in the arterial and venous blood glucose and lactate concentrations are shown in Figs 31 and 32, respectively. Maximum concentrations of glucose (5.3 ± 0.9 mM) were detected between 4 to 6 hours after dilevalol administration, whereas maximum lactate levels (5.4 ± 0.4 mM) were reached between 3 to 4 hours. The concentrations of glucose and lactate approached control values 9 hours following
Fig 27. Changes in the heart rate following i.v. bolus administration of 25 mg of dilevalol (n=6, mean ± SEM). Asterisks denote significant difference (p<0.05) from pre-experimental values.

Fig 28. Changes in the mean arterial pressure following i.v. bolus administration of 25 mg of dilevalol (n=6, mean ± SEM). Asterisks denote significant difference (p<0.05) from pre-experimental values.
Fig 29. Changes in the femoral arterial blood flow following i.v. bolus administration of 25 mg of dilevalol (n=5, mean ± SEM). Asterisks denote significant difference (p<0.05) from pre-experimental values.

Fig 30. Changes in the femoral vascular resistance following i.v. bolus administration of 25 mg of dilevalol (n=5, mean ± SEM).

All values are significantly (p<0.05) below pre-experimental values.
the administration of dilevalol. These plots indicate significant hyperglycemia and lactic acidemia following administration of an i.v. bolus dilevalol. No change in arterial and venous oxygen content was observed. The net uptake of glucose and lactate by the hind limb was calculated via the Fick method, which is described by the following equation: 

\[ [(C_{\text{arterial}} - C_{\text{venous}}) \times \text{Femoral Blood Flow}] \]

These results indicate a slight increase in the glucose uptake (not statistically significant) two hours after the administration of dilevalol (Fig 33A) and significant lactate release from the hindlimb (Fig 33B). Control saline i.v. bolus experiments previously conducted (n=5) did not show any significant change in any of the blood gas parameters (Yeleswaram, 1993). These experiments also showed that the injection of saline has no effect on arterial blood flow, lactate and glucose concentrations nor any effects on hind limb glucose and lactate fluxes.
Fig 31. Effect of i.v. bolus administration of 25 mg of dilevalol on arterial blood glucose concentration (n=6, mean ± SEM). Asterisks denote significant difference (p<0.05) from pre-experimental values.
Fig 32. Effect of administration of 25 mg of dilevalol on lactate concentration (n=6, mean ± SEM). Asterisks denote significant difference (p<0.05) from pre-experimental values.
Fig 33A. Changes in the arterio-venous flux of glucose across the hindlimb following the administration of 25 mg of dilevalol. Positive values indicate net glucose uptake from the hind limbs.
Fig 33B. Changes in the arterio-venous flux of lactate across the hindlimb following the administration of 25 mg of dilevalol. Negative values indicate net lactate release from the hind limbs. Asterisks denote significant difference (p<0.05) from pre-experimental values.
3.4 Fetal Infusion Studies

Labetalol fetal infusion studies were performed in four chronically instrumented pregnant ewes. Details on the age and weight of the animals used in these studies are summarized in Table 10. The birth weights of the operated fetus and gestational ages at the time of the experiments averaged 3.05 ± 0.11 kg and 133 ± 1 days, respectively.

3.4.1 Pharmacokinetics

The disposition profile of labetalol in fetal arterial blood is shown in Fig 34. A total of 9.4 mg of labetalol was administered by the end of the infusion. The steady-state concentration of labetalol in fetal arterial plasma was 150.19 ± 9.37 ng/mL. The drug exhibited a bi-exponential elimination profile \( \text{Ae}^{-at} + \text{Be}^{-bt} \) in fetal plasma following the cessation of the infusion. The fetal pharmacokinetic parameters of labetalol and its stereoisomers are shown in Table 11. The terminal elimination rate constant \((0.074 ± 0.009 \text{ h}^{-1})\) was significantly lower than that observed in maternal \((0.19 ± 0.02 \text{ h}^{-1})\) and fetal plasma \((0.11 ± 0.02 \text{ h}^{-1})\) following maternal infusion (Section 3.2.2., Table 4). Hence the mean half-life of labetalol in fetal plasma \((9.4 ± 2.4)\) was significantly higher than that observed in the fetus \((6.28 ± 1.4 \text{ hr})\) and mother \((3.66 ± 0.92 \text{ hr})\) following maternal infusion (Student’s \(t\) test).

Labetalol accumulated in the fetal tracheal fluid at concentrations comparable to those in fetal plasma after 0.5 hours (Fig 35). Labetalol appeared at a slower rate (at 2 hours) and at lower concentrations in the amniotic fluid.
compared to the fetal tracheal fluid (Fig 36). In contrast to the maternal infusion experiments the drug did not continue to accumulate in the amniotic fluid following the cessation of the infusion.

The disposition of the labetalol isomers in fetal plasma are shown in Fig 37. In fetal plasma, the concentration of the RR isomer at steady-state was 53.9 ± 7.3 ng/mL, which was significantly higher than the other three isomers ([SR]= 28.2 ± 3.7 ng/mL, [SS]= 31.7 ± 3.5 ng/mL, [RS]=33.6 ± 4.8; Table 11). The terminal elimination half-life ($t_{1/2}$) of the RR isomer was 8.62 ± 2.85 h, which was significantly lower than that obtained following maternal infusion of labetalol. In contrast the $t_{1/2}$ of other three isomers SR, SS, and RS (11.03 ± 3.64, 8.13 ± 2.41, 6.64 ± 1.88 h, respectively) were significantly higher (approximately 3 times) than their corresponding values obtained in maternal infusion experiments (Section 3.2.3., Table 5). The steady-state clearance of the RR isomer (22.79 ± 3.44 mL/min/kg) was significantly lower than that of the SR (43.61 ± 5.57 mL/min/kg), SS (38.80 ± 5.14 mL/min/kg) and the RS isomer (36.56 ± 4.64 mL/min/kg). This was similar to steady-state clearance of the RR isomer in the mother following maternal infusion.
### Table 10. Fetal infusion animal experimental details

<table>
<thead>
<tr>
<th>EWE Number</th>
<th>Maternal Weight (kg)</th>
<th>Gestational age during experiment (days)</th>
<th>Fetal Weight at Birth (kg)</th>
<th>Fetal Weight during experiment (kg)</th>
<th>Term (days)</th>
<th>No. of fetuses</th>
<th>Samples*</th>
</tr>
</thead>
<tbody>
<tr>
<td>229</td>
<td>69.1</td>
<td>135</td>
<td>2.89</td>
<td>2.60</td>
<td>138</td>
<td>2</td>
<td>MA, FA, AMN, TR, Uf</td>
</tr>
<tr>
<td>2241</td>
<td>67.7</td>
<td>131</td>
<td>3.12</td>
<td>2.61</td>
<td>136</td>
<td>2</td>
<td>MA, FA, AMN, TR, Uf</td>
</tr>
<tr>
<td>2177</td>
<td>75.0</td>
<td>133</td>
<td>2.87</td>
<td>2.24</td>
<td>140</td>
<td>1</td>
<td>MA, FA, AMN, TR, Uf</td>
</tr>
<tr>
<td>4227</td>
<td>85.9</td>
<td>133</td>
<td>3.33</td>
<td>2.79</td>
<td>138</td>
<td>2</td>
<td>MA, FA, AMN, TR, Uf</td>
</tr>
</tbody>
</table>

| Mean ± SEM | 74.42 ± 4.14         | 133                                    | 3.05 ± 0.11              | 2.56 ± 0.12                        | 138         |                |         |

*Samples*  
MA - Maternal Femoral Arterial Blood  
FA - Fetal Femoral Arterial Blood  
AMN - Amniotic Fluid  
TR - Fetal Tracheal Fluid  
Um - Maternal Urine  
Uf - Fetal Urine
Fig 34. Semilogarithmic disposition profile of labetalol in fetal arterial plasma (fetal 4 mg i.v. bolus and 6 h15 μg/min infusion n=4, mean ± SEM).
**TABLE 11. Pharmacokinetics of Labetalol and its stereoisomers in the fetal lamb following fetal infusion (mean±SEM; n=4).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Labetalol</th>
<th>SR</th>
<th>SS</th>
<th>RS</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AUC (ng*h/L)</strong></td>
<td>4863.5±624.7</td>
<td>907.6±102.8</td>
<td>1187.8±176.3</td>
<td>1038.2±146.1</td>
<td>1730.0±211.5*</td>
</tr>
<tr>
<td><strong>Cₜₜ (ng/mL)</strong></td>
<td>150.2±9.4</td>
<td>28.2±3.7</td>
<td>31.7±3.5</td>
<td>33.6±4.8</td>
<td>53.9±7.3*</td>
</tr>
<tr>
<td><strong>Clₜₜ (mL/min/kg)</strong></td>
<td>33.39±4.54</td>
<td>43.61±5.57</td>
<td>38.80±5.14</td>
<td>36.56±4.64</td>
<td>22.79±3.44*</td>
</tr>
<tr>
<td><strong>β (1/h)</strong></td>
<td>0.074±0.009</td>
<td>0.063±0.008</td>
<td>0.085±0.011</td>
<td>0.104±0.014</td>
<td>0.080±0.010</td>
</tr>
<tr>
<td><strong>t₁/₂ (h)</strong></td>
<td>9.4±2.4</td>
<td>11.03±3.64</td>
<td>8.13±2.41</td>
<td>6.64±1.88</td>
<td>8.62±2.85</td>
</tr>
</tbody>
</table>

* Denotes significant difference (p<0.05) from corresponding value for the other isomers.
Fig 35. Disposition of labetalol in fetal plasma and tracheal fluid (fetal 4 mg i.v. bolus and 6 h 15 μg/min infusion n=4, mean ± SEM).
Fig 36. Disposition of labetalol in amniotic fluid (fetal 4 mg i.v. bolus and 6 h 15 μg/min infusion n=4, mean ± SEM).
Fig 37. Semilogarithmic disposition profile of labetalol stereoisomers in fetal arterial plasma (fetal 4 mg i.v. bolus and 6 h 15 µg/min infusion n=4. Error bars omitted for the purpose of clarity.
3.4.2 Glucuronide Conjugation

The mean rate of fetal urine output during the 6 hours of labetalol infusion was 15.1 ± 3.0 mL/h, which was not significantly different than the pre-experimental value of 12.5 ± 10.0 (Table 12). Furthermore, the mean fetal urine pH during the infusion was 6.88 ± 0.10 which was significantly lower than the pre-experimental mean pH of 7.10 ± 0.03. The change in pH was not observed in previous control experiments (identical protocol, saline infusion) in our laboratory (Gordon, 1995). The cumulative urinary excretion of unchanged and glucuronide conjugated labetalol is shown in Fig 38. Of the 9,400 ug of labetalol administered, 73.8 ± 9.6 ug (0.80 ± 0.10%) was excreted as unconjugated drug while 133.4 ± 25.2 ug (1.4 ± 0.3%) was excreted as the glucuronide (Table 13). The cumulative urinary excretion of the unchanged and glucuronide isomers of labetalol are shown in Figs 39 and 40, respectively. The total labetalol recovered (207.2±34.8 ug) represented 2.2 ± 0.3% of the total dose administered. This amount was significantly lower than that obtained in the maternal infusion experiments. 35.3 ± 3.8% of the unchanged labetalol was in the form of the RR isomer (compared to 22.5 ± 3.7% for the other isomers) while only 6.4 ± 0.4% of the conjugated labetalol was the RR isomer (compared to 27.8 ± 5.1% for the other isomers).
TABLE 12. Mean fetal hourly urine output and pH during the 6 hours of labetalol infusion.

<table>
<thead>
<tr>
<th>Ewe #</th>
<th>Fetal Urine Output (Pre-Experimental) (mL/h)</th>
<th>Fetal Urine Output During Infusion (mL/h)</th>
<th>Fetal Urine pH (Pre-Experimental)</th>
<th>Fetal Urine pH During Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>2177</td>
<td>8.0</td>
<td>14.2</td>
<td>7.12</td>
<td>6.78</td>
</tr>
<tr>
<td>2241</td>
<td>5.6</td>
<td>12.7</td>
<td>7.06</td>
<td>6.97</td>
</tr>
<tr>
<td>4227</td>
<td>24.0</td>
<td>18.5</td>
<td>7.13</td>
<td>6.88</td>
</tr>
<tr>
<td>mean ± SEM</td>
<td>12.5 ± 5.8</td>
<td>15.1 ± 1.7</td>
<td>7.10 ± 0.02</td>
<td>6.88 ± 0.06*</td>
</tr>
</tbody>
</table>

* Significantly different from pre-experimental values
Fig 38. Cumulative fetal urinary excretion of un-conjugated and glucuronide conjugated labetalol (fetal 4 mg i.v. bolus and 6 h 15 μg/min infusion n=3, mean ± SEM).
Fig 39. Cumulative fetal urinary excretion of unconjugated labetalol stereoisomers (fetal 4 mg i.v. bolus and 6 h 15 µg/min infusion n=3, mean ± SEM).
Fig 40. Cumulative fetal urinary excretion of glucuronide conjugated labetalol stereoisomers (fetal 4 mg i.v. bolus and 6 h 15 μg/min infusion n=3, mean ± SEM).
Table 13. Amount of Labetalol and Its Isomers Recovered in Fetal Urine Following 4 mg i.v. Bolus and 6 Hour Infusion (15 ug/min) of Racemic Labetalol (mean ± SEM; n=4)

<table>
<thead>
<tr>
<th>AMOUNT (ug)</th>
<th>Unchanged</th>
<th>Glucuronide</th>
<th>Total</th>
<th>% Total Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conjugate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Labetalol</td>
<td>73.8±9.6</td>
<td>133.4±25.2</td>
<td>207.2±34.8</td>
<td>22.0±3.7</td>
</tr>
<tr>
<td>SR</td>
<td>14.3±2.2</td>
<td>43.3±10.0</td>
<td>57.6±12.2</td>
<td>6.13±1.3</td>
</tr>
<tr>
<td>SS</td>
<td>15.8±2.6</td>
<td>45.3±9.1</td>
<td>61.1±11.7</td>
<td>6.5±1.2</td>
</tr>
<tr>
<td>RS</td>
<td>17.6±2.1</td>
<td>36.4±5.9</td>
<td>54.0±8.0</td>
<td>5.7±0.9</td>
</tr>
<tr>
<td>RR</td>
<td>26.1±2.8*</td>
<td>8.5±0.4*</td>
<td>34.6±3.2*</td>
<td>3.7±0.7</td>
</tr>
</tbody>
</table>

* Denotes a significant difference (p<0.05) from the corresponding value for the other isomers.
3.4.3 Hemodynamic effects.

The effects of fetal labetalol administration on fetal heart rate and arterial blood pressure are illustrated in Figs 41 and 42. During the first 30 minutes of infusion fetal heart rate increased significantly from a control value of 177.8 ± 6.5 bpm to 193.9 ± 7.8 bpm and then declined to significantly below control by 2.5 hours (156.5 ± 8.0 bpm). The heart rate appeared to stabilize near control levels during steady-state, but within two hours of stopping the infusion it fell significantly to 152.5 ± 10.4 bpm. Following this decrease, fetal heart rate gradually increased to control levels during the balance of the post-infusion period.

There appeared to be a moderate hypotension associated with the initial tachycardia observed above (not statistically significant). The fetal arterial blood pressure appeared to decrease from control (51.7 ± 7.9 mmHg) during the first 2 hours of the infusion to 39.0 ± 6.0 mmHg (these changes were not significantly significant). The pressure gradually increased and stabilized for the rest of the infusion and post-infusion periods.

Previous control saline infusion (24 hours) to the fetus using the same animal preparation and administration protocol (n=6) did not produce any significant change in the fetal heart rate and arterial blood pressure (van der Weyde, et al., 1992).
3.4.4 Metabolic Effects:

3.4.4.1 Maternal and fetal arterial Po$_2$, Pco$_2$, %O$_2$ saturation, pH and base excess

Infusion of labetalol to the fetus resulted in various effects on fetal arterial blood gas, base excess, lactate and glucose levels. The control values and the largest altered value are shown in Table 14. Maternal arterial Po$_2$ (Fig 43) did not change significantly from control. In the fetus, however, the arterial Po$_2$ (Fig 43) fell significantly below control (20.0 ± 1.4 mmHg) by 15 minutes of infusion to 15.9 ± 0.08 mmHg but then returned to control values by 30 minutes. The Po$_2$ values were not significantly different from control for the remainder of the infusion, but appeared to decrease from 4 hours to 18 hours post-infusion. Maternal arterial Pco$_2$ did not change significantly from control (32.7 ± 0.35mmHg) throughout the experiment. In contrast, fetal Pco$_2$ was significantly higher from control (48.4 ± 1.0 mmHg) at 1.0 hours of infusion and remained, on average, 2.5 mmHg higher up to 8 hours post-infusion (Fig 44). There was no change in maternal O$_2$ saturation. In contrast fetal O$_2$ saturation (Fig 45) fell rapidly and significantly from control (45.2 ± 2.8 mmHg) by 15 minutes of infusion and except at 30 minutes all values were significantly below control even at 18 hours post-infusion.

While there was no change in the maternal arterial pH, there was a significant decrease in fetal arterial pH (Fig 46), starting at 30 minutes. It declined rapidly and significantly from control values of 7.34 ± 0.03 to reach its lowest levels of 7.22 ± 0.04 at 3 hours post-infusion, and subsequently recovered to within control values by 18 hours post-infusion.
Table 14. Pre-experimental and maximum changed values of fetal blood gas, pH, base excess, lactate and glucose following fetal infusion of labetalol (mean ± SEM; n=4).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MA Control</th>
<th>MA Max Changed</th>
<th>FA Control</th>
<th>FA Max Changed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Po2 (mmHg)</td>
<td>134.8 ± 3.6</td>
<td>115.8 ± 1.5</td>
<td>20.0 ± 1.4</td>
<td>15.9 ± 0.08</td>
</tr>
<tr>
<td>Pco2 (mmHg)</td>
<td>32.7 ± 0.35</td>
<td>31.1 ± 2.2</td>
<td>48.4 ± 1.0</td>
<td>51.4 ± 0.4</td>
</tr>
<tr>
<td>%O2 Saturation</td>
<td>98.6 ± 0.5</td>
<td>97.9 ± 0.5</td>
<td>45.2 ± 2.8</td>
<td>25.5 ± 0.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.49 ± 0.03</td>
<td>7.44 ± 0.04</td>
<td>7.34 ± 0.03</td>
<td>7.22 ± 0.04</td>
</tr>
<tr>
<td>Base Excess (mEq/L)</td>
<td>4.0 ± 0.8</td>
<td>2.9 ± 0.7</td>
<td>2.4 ± 0.9</td>
<td>-5.2 ± 1.2</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>1.01 ± 0.2</td>
<td>1.41 ± 0.6</td>
<td>1.44 ± 0.2</td>
<td>7.22 ± 1.73</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>3.11 ± 0.10</td>
<td>3.53 ± 0.02</td>
<td>0.58 ± 0.08</td>
<td>1.22 ± 0.01</td>
</tr>
</tbody>
</table>
Fig 41. Changes in fetal heart rate (fetal 4 mg i.v. bolus and 6 h 15 μg/min infusion n=4, mean ± SEM). * Significantly different (p<0.05) from control values.

Fig 42. Changes in fetal arterial pressure (fetal 4 mg i.v. bolus and 6 h 15 μg/min infusion n=4, mean ± SEM). Astrisks denote significant difference (p<0.05) from control values.
Fig 43. Changes in maternal and fetal arterial $\text{PO}_2$ (fetal 4 mg i.v. bolus and 6 h 15 $\mu$g/min infusion n=4, mean ± SEM).

Fig 44. Changes in maternal and fetal arterial $\text{PCO}_2$ (fetal 4 mg i.v. bolus and 6 h 15 $\mu$g/min infusion n=4, mean ± SEM).
Fig 45. Changes in fetal arterial oxygen saturation (fetal 4 mg i.v. bolus and 6 h 15 μg/min infusion n=4, mean ± SEM). All values, except that at 0.5 hours, are significantly below the pre-experimental.

Fig 46. Changes in maternal and fetal arterial pH (fetal 4 mg i.v. bolus and 6 h 15 μg/min infusion n=4, mean ± SEM).

Fetal pH values all significantly (p<0.05) below control except at 0.5 h.
Maternal base excess (BE) (Fig 47) tended to increase in the first hour of the infusion and to decline during steady-state (not significant). It remained within the range of control values during the post-infusion period. In the fetus, base excess declined rapidly and significantly from control (2.4 ± 0.9 mEq/L) by 30 minutes of infusion and remained significantly below for the rest of the experiment (Fig 47). The fetal BE gradually improved during post-infusion interval, but did not return to control values before the end of the experiment.

No changes in the fetal blood gas parameters have been observed following fetal infusion of control saline in our laboratory (van der Weyde, et al., 1992).

3.4.4.2 Maternal and fetal arterial plasma lactate and glucose levels.

Maternal and fetal arterial plasma lactate and glucose concentrations at the control and maximum altered levels are shown in Table 14. There was no significant change in the maternal lactate concentration. In contrast, fetal lactate (Fig 48) rose significantly above control (1.44 ± 0.20 mM) by 30 minutes of infusion. It continued to increase up to 6 hours post-infusion (5 fold) and remained significantly elevated until the end of the experiment. The mean fetal lactate increased by 19.6 ± 3.2 % from the end of infusion until 6 hours post-infusion. These observations were similar to those observed in fetal plasma following maternal infusion of labetalol.

Similar to lactate, there was no significant change in the maternal arterial glucose concentration. Fetal arterial glucose concentration (Fig 49) rose significantly above control (0.58 ± 0.08 mM) by 15 minutes of infusion and remained elevated by 2.0 ± 0.3 fold (about 1.0 mM) to the end of the experiment. No significant changes in the glucose or lactate levels or glucose and
Fig 47. Changes in maternal and fetal base excess (fetal 4 mg i.v. bolus and 6 h 15 μg/min infusion n=4, mean ± SEM). Fetal base excess was significantly below pre-experimental values from 30 minutes to the end of the study period.
Fig 48. Changes in maternal and fetal lactate concentration (fetal 4 mg i.v. bolus and 6 h 15 μg/min infusion n=4, mean ± SEM). Fetal lactate concentrations were all significantly above pre-experimental values from 30 minutes of infusion.

Fig 49. Changes in fetal glucose concentration (fetal 4 mg i.v. bolus and 6 h 15 μg/min infusion n=4, mean ± SEM). All values significantly above pre-experimental.
uptake were observed in the fetal saline infusion experiments (van der Weyde, *et al.*, 1992).

4. DISCUSSION

4.1 Development of a method for direct separation of labetalol stereoisomers in biological fluids.

The purpose of developing a method for the separation and quantitation of labetalol stereoisomers was to enable quantification of the individual isomers of labetalol in the lower nanogram range. Furthermore, a method which allowed for the determination of the concentration of individual labetalol isomers during infusion to steady state, in biological fluids was not available in the literature.

A sensitive assay using microbore high-performance liquid chromatography and low-dispersion fluorescence detection was developed in this laboratory for the quantitation of labetalol in various biological fluids of the pregnant sheep (Yeleswaram *et al.*, 1991). This method represented significant improvement over previously published assays (Hidalgo and Muir, 1984; Wang *et al.*, 1985; Abernethy *et al.*, 1986; Ostrovska *et al.*, 1988) in terms of the volume of sample required, precision of quantitation and minimum quantitation limit. However, the above methods are achiral and measure total (*i.e.*, racemic) labetalol. To date, there is no validated report on the quantitation of the individual isomers of labetalol and no data on the pharmacokinetics of these isomers in any pregnant or non-pregnant animal model (except for the RR isomer, dilevalol, when given as
single pure isomer). Schill and coworkers (Schill et al. 1986a&b) were the first to demonstrate the potential of $\alpha_1$-acid glycoprotein as a stationary phase for the chiral separation of a number of racemic drugs, including labetalol A and B (the diastereomers of labetalol). Lalonde (Lalonde et al., 1990) also published a report describing the pharmacokinetics of labetalol stereoisomers, but details of the assay, including the degree of resolution or a representative chromatogram, were not provided. More recently, Desai (Desai et al., 1992) reported a method for the separation of labetalol isomers which involves derivatization with chiral ((4S-cis)-2,2-dimethyl-5-isothiocyanato-4-phenyl-1,3-dioxane) and non-chiral (benzyl isothiocyanate and 1-naphthalenemethyl isothiocyanate) reagents. This indirect or “derivatizing” method has a few disadvantages over direct methods of chiral separation. These include the requirement of highly optically pure derivatizing agent (because any enantiomeric contamination could lead to false determination) and an extra treatment step of the sample to reclaim the starting enantiomers.

Because of the disadvantages of the indirect methods, the use of protein columns for direct separation of labetalol stereoisomers was investigated. The mechanism of separation of enantiomers by $\alpha_1$-acid glycoprotein (AGP) chiral stationary phases is based on the principles of bioaffinity (Dappen, 1986). In a series of papers, Allenmark and coworkers (Allenmark and Bomgren 1982; Allenmark et al., 1983; 1984) have shown that the stereo-differentiating properties of these proteins are based on:

- **Hydrophobic interactions**

  Increasing the hydrophobicity of structurally similar compounds have been shown to greatly enhance their retention on protein columns, i.e. increasing the hydrophobicity of an aroyl substituent by changing a benzoyl- to a 2-naphthoyl group was shown to enhance the retention of the compound.
(Allenmark, 1983). Similarly the resolution of the enantiomers in general parallels their hydrophobicity, i.e. the enantiomer with greater propensity for hydrophobic interactions is retained longer.

- **Interaction of polar groups**
  Another type of bonding interaction between the stationary phase and the solute which affects the resolution of enantiomers is that of the polar groups (Allenmark, 1984). Polar groups may interact strongly with the protein and enhance the affinity of the protein for one enantiomer over another.

- **Steric effects**
  Steric effects influence the difference in affinity of structurally similar compounds for protein columns. In general, steric effects of bulkier groups increases the retention time of the compound.

Our initial objective was to develop a rapid and sensitive assay for direct separation of the stereoisomers of labetalol in biological fluids. Based upon the previous findings reported by Schill and Allenmark (Schill, et al., 1986 A&B, Allenmark, 1983 and 1984) we decided to use Chiral stationary phase (CSP) protein columns to achieve this separation.

The CSP protein column, EnantioPac®, used in previous reports, was used in preliminary trials for the separation of the labetalol isomers. However, after extensive trials with several combinations of chromatographic conditions, it was clear that baseline separation of labetalol stereoisomers was not possible with this column. At a pH higher than 7.0, the matrix in the first generation column, EnantioPac®, was unstable.

A search for a more suitable column was helped by to a report by Narayanan (Narayanan, 1992) on the use of immobilized proteins for chiral
resolution. The second generation protein columns (Chiral AGP®) described in Narayanan’s report covalently immobilized protein on 5 μm spherical silica particles with much smaller pore size (12 nm) over the first generation columns. This arrangement provided a more stable matrix that allowed for the greater variation in the chromatographic conditions such as pH.

We examined the effects of several variables on retention and stereoselectivity of labetalol stereoisomers using the Chiral AGP® column:

i) Nature of the mobile phase
ii) pH of the mobile phase
iii) Nature and concentration of the organic modifier
iv) Flow rate

Our first trials with the Chiral AGP® column produced consistent results, allowing for better optimization of chromatographic conditions for the separation of labetalol isomers. Two main variables examined extensively were pH and concentration of the cationic modifier. Changes in the pH and concentration of the cationic modifier in the mobile phase have been shown to greatly influence the enantioselectivity and hence retention of several different classes of drugs by AGP (Hermansson, 1984; Gorog and Herenyi, 1990; Narayanan, 1992). Whereas changes in the pH of the mobile phase affect enantioselectivity by altering the conformation of the protein stationary phase (Narayanan, 1992), cationic modifiers such as TBAP and TBABr influence chiral separation through ion-pair formation and increase in the steric bulkiness of the molecule (Schill, et al., 1986a&b). In addition, at the optimum pH range for this assay (7.10 - 7.15), labetalol exists in both ionized and unionized forms causing broadening and changes in the symmetry of the peaks.
Separation of labetalol isomers in biological fluids using the Chiral AGP® protein column proved to be challenging because aside from pH and TBAP concentration, the separation was extremely sensitive to changes in

a) Temperature: slight changes (a few degrees) in the ambient temperature affected the temperature of the mobile phase as well as the temperatures of the column. At slightly cooler temperatures the retention times were longer and at a few degrees higher, the peaks were not separated.

b) composition of the biological fluid: the variability in the composition of the plasma, urine, tracheal and amniotic fluid affected the separation of the isomers from endogenous compounds as well as from each other. In addition, the changes in the composition of the amniotic fluid produced great variability in retention times and resolution of labetalol isomer peaks. Therefore, unlike the labetalol achiral assay in which a great number of samples could be analyzed in the same run under the same conditions, the chiral assay required major changes in the sample preparation procedure, HPLC detector settings (such as signal attenuation) and, in some cases, changes in flow rate (urine samples).

c) amount of sample: different amounts of sample are used to adjust for changes in concentration of the drug. This change in the volume of samples extracted different amounts of endogenous compounds within the same run. Generally, the greater the amount of endogenous compounds the lower the retention times of the isomers. This phenomenon might be the result of the interaction of the endogeneous compounds with the same site on the protein column as those of the isomers. In some instances, at low concentrations labetalol isomer peaks had very short retention times and co-eluted with endogenous compounds of the sample.
Using a Chiral AGP® column in combination with a suitable mobile phase cationic modifier a direct and sensitive HPLC assay for the separation of labetalol stereoisomers was developed. In addition to optimization of HPLC conditions described above, several variables (described previously by Yeleswaram et al., 1991) relating to the detector, such as a low volume flow cell of 5 µL capacity to reduce peak diffusion and emission cut-off (370 nm) and excitation and emission slits to reduce baseline noise and optimize signal/noise ratio, were used.

A conventional method for attaining a suitable mobile phase composition, described widely in the literature, is the use of a phosphate buffer (0.01-0.025 M) modified with 0 to 10% of an organic solvent (2-propanol or ethanol) (Schill, et al., 1986a). The mobile phase compositions suggested in the literature did not separate the labetalol stereoisomers. The optimum composition and flow rate of the mobile phase for baseline separation of these isomers were:

1. 0.02 M phosphate buffer (0.0033 M KH$_2$PO$_4$ and 0.0167 M Na$_2$HPO$_4$).
2. pH = 7.10
3. Modifier = 0.015 M tetrabutylammonium phosphate buffer
4. Flow = 0.5 mL/min

The four isomers, in order of elution, were SR, SS, RS and RR with retention times (in methanol and water) of 19, 23, 28 and 34 min, respectively (Fig 2A). The limit of reliable detection of each isomer using 50 to 500 µL of biological fluid was 0.15 ng (0.6 ng of racemic labetalol injected). This sensitivity allowed for measurement of labetalol isomers in all samples collected up to 10
hours post-infusion. The achiral assay previously developed had sensitivity which allowed for the measurement of labetalol up to 18 hours post-infusion.

4.2 Maternal Labetalol Infusion Studies

Whereas the pharmacokinetic behavior of racemic labetalol and dilevalol has been studied in humans and animals, there are no comprehensive pharmacokinetic data for the other individual isomers SR, SS and RS, due in part to the lack of availability of the individual isomers for scientific study and the lack of a sensitive chiral assay. Thus far, the analytical techniques used in the study of the pharmacokinetics of labetalol have not been stereoselective. Therefore, the current pharmacokinetic profile of labetalol is based on the result of simultaneous administration of four stereoisomers with different pharmacological properties, and most likely, different pharmacokinetic characteristics, as well.

The maternal-fetal pharmacokinetics and pharmacodynamics of labetalol have been studied in pregnant sheep (Yeleswaram, et al., 1993a, 1993b). Limited fetal exposure to labetalol was observed (AUC_F/AUC_M = 14.4%) following maternal iv bolus administration. In sheep, the total body clearance, elimination half life and the volume of distribution of labetalol in the fetus were significantly higher than those in the mother. The metabolic effects of labetalol in the mother and the fetus, following maternal bolus administration, included significant hyperglycemia and lactic acidemia (with the latter being more pronounced in the fetus). Fetal O_2 content, in the same experiments, was lowered by 28% from the base level and remained significantly reduced for 5 hours. The hemodynamic effects included a significant increase in femoral blood flow, hypotension and tachycardia. Significant hyperglycemia and lactic acidosis was also observed in the fetus after a 4 mg direct fetal i.v. bolus of labetalol.
Although labetalol has been shown to undergo extensive glucuronidation both in man and sheep (Martin, et al., 1976; Yeleswaram, et al., 1993), there are no such data on the extent of glucuronidation of individual labetalol isomers. In humans less than 5% of labetalol and dilevalol are excreted as unchanged drug in the urine and about 20% of labetalol and 80% of dilevalol were recovered as the glucuronide conjugate (Martin, et al., 1976; Sugeno, et al., 1987a). In sheep, very little is excreted as unchanged drug (1%) or as the sulfate (1.5%) whereas glucuronide excretion appears to be significant at 11.4% of the dose (Yeleswaram, et al., 1993a).

Although there are a few reported cases of the measurement of individual isomers of chiral drugs used in pregnancy, there are no data in the literature about the disposition and metabolism of these isomers in the fetus. The only reported data are from experiments which employed isolated, perfused placentas or single point measurements of stereoisomers at parturition (Nau, et al., 1991; Challier, et al., 1992; Kofahl, et al., 1993).

We chose the chronically instrumented pregnant sheep as our animal model. There were several advantages in using this model, the most important of which for our study was the ability to repeatedly sample from the mother, fetus and the fluid compartments in utero. The primary objectives of the labetalol maternal infusion experiments were: 1. To determine the pharmacokinetics, glucuronide conjugative metabolism and disposition characteristics of the four labetalol stereoisomers in the pregnant sheep and fetal lamb; and 2. To compare the disposition profile of the four isomers in the mother to that in the fetus in order to determine whether the fetus is exposed to the four isomers in proportions different from those observed in the mother.
4.2.1 Maternal and Fetal Pharmacokinetics of Labetalol

Labetalol followed a triexponential decline in the maternal plasma and tri- or biexponential decline in fetal plasma following a 100 mg i.v. bolus and immediate infusion (0.5 mg/min) for 6 hours. The mean steady-state concentrations of labetalol (423.4 ± 87.8 ng/mL) were similar to those obtained in the non-pregnant sheep (454.19 ± 111.05 ng/mL) (Yeleswaram et al., 1992) and to those obtained in clinical trials (350 ng/mL). Clearance of labetalol at steady-state (15.80 ± 4.25 mL/min/kg) was similar to that obtained in non-pregnant sheep (17.17 ± 2.98 mL/min/kg) (Yeleswaram et al., 1993a) but significantly lower than that obtained in the labetalol i.v. bolus experiments in pregnant sheep (27.83 ± 3.46 mL/min/kg) (paired t-test) (Yeleswaram, et al., 1992a). Similar observations have also been made with other β-blockers in which, at steady-state, clearance is significantly reduced (Evans and Shand, 1973; Chauvin et al., 1987) due to saturable first-pass hepatic extraction.

In fetal plasma, labetalol exhibited a tri- or bi-exponential decline with a mean apparent elimination half-life of 6.28 ± 1.4 h which was significantly longer than that observed in the mother (3.66 ± 0.92 hr) (Student t-test). The half life of labetalol was also reported to be significantly longer in the fetus following i.v. bolus administration, although not to the same extent as the continuous infusion administration (Yeleswaram et al., 1993). This phenomenon could be due to various factors working simultaneously in the maternal-fetal circulation, including: recirculation of labetalol from the amniotic, tracheal and allantoic fluid compartments back to the fetus (labetalol persists much longer in these sites than in plasma) and extensive binding of labetalol to fetal proteins and tissues. A prolonged elimination half life has also been reported (up to 24 hours) in
premature infants following maternal oral drug administration of labetalol (Haroldsson and Geven, 1989).

There was a significant transfer of labetalol across the sheep placenta. The exposure of the fetus (33.06 ± 5.83%), which was determined by the ratio of the fetal to maternal plasma AUC, was more than double that observed in previous i.v. bolus studies (Yeleswaram et al., 1992). In humans, fetal exposure to labetalol has been reported to be even higher at around 50% (Rogers et al., 1990). The difference in part could be due to the epitheliochorial sheep placenta having lower permeabilities to hydrophilic polar substances than the hemochorial human placenta (Faber and Thornberg, 1983). Another factor which contributes to the difference between human and animal maternal-fetal drug relationship data is the fact that the human results are based on single time-point determination which cannot provide an accurate assessment.

4.2.2 Pharmacokinetics of Stereoisomers of Labetalol

This appears to be the first study to characterize and report the pharmacokinetic parameters of labetalol stereoisomers. The stereoisomers of labetalol exhibited a tri-exponential decline in maternal plasma. The concentrations of the RS and RR isomers were significantly different from the racemic value (25%) throughout the infusion and post-infusion periods. Specifically, at steady-state the concentrations of the RR (132.31±18.79 ng/mL) and the RS isomers (78.43±11.48 ng/mL) were significantly different than that of the racemic labetalol (106±9.14 ng/mL), suggesting a selective process in the disposition of these isomers in sheep. Our observations, however, are not consistent with results from human studies which have shown that following both single and
multiple oral doses, the RR isomer has a lower systemic bioavailability than labetalol (McNeill et al., 1982; Kramer, et al., 1988; Tenero, et al., 1989). Our results are also in contrast to observations by Lalonde and coworkers (Lalonde, et al., 1990) who reported a lower $C_{\text{max}}$ for the RR isomer following oral administration of labetalol to nine healthy subjects. One reason for the above differences between humans and our sheep experiments might be the route of administration of labetalol. The apparent differences observed between the stereoselective disposition of labetalol isomers in man and sheep are not unique to labetalol. Other chiral drugs have also been shown to exhibit different stereoisomer disposition patterns in different species. For example, humans and dogs show opposite stereoselectivity in the clearance of propranolol stereoisomers (Bai, et al., 1983a; Walle, et al., 1985). In the dog, S-propranolol has been shown to be eliminated more rapidly than the R isomer following a single oral dose (Bai, et al., 1983B).

The ratio of the AUC of the RR isomer over the AUC of the SR, SS and RS isomer, ranged from 1.5 to 1.65. This was coupled with a significantly lower steady-state clearance (12.63 ± 2.05 mL/min/kg) and longer half-life (5.6±1.00 h) for the RR isomer than those of the RS isomer (21.32 ± 3.21 mL/min/kg and 3.63±0.76 h, respectively). The RR isomer also displayed a significantly longer half-life than those of the SR and the SS isomers (1.75±0.40, and 3.78±0.72 h, respectively). These observations are consistent with the glucuronide conjugation data in which the RR isomer was not conjugated to the same extent as the other isomers. However, in the dilevalol bolus experiments the clearance of dilevalol (48.70 ± 7.80 mL/min/kg) is higher than that of labetalol (29.00 ± 2.67 mL/min/kg) obtained from labetalol bolus experiments (Yeleswaram, et al., 1992). One possible explanation is that there might be the competition of the other isomers for the active site of UDP-glucuronyltransferase enzyme involved in glucuronide conjugation of
labetalol isomers. In the absence of the other isomers (such as in dilevalol bolus experiments), the RR isomer is conjugated to a greater extent and hence has a higher clearance. In fact, in the labetalol infusion experiments the extent of glucuronide conjugation of the RR isomer was only 3.17 ± 0.43% of the dose whereas in the dilevalol bolus experiments conjugation was 5.36 ± 1.24% of the dose (significantly different).

Because the volume of distribution of the RR isomer (485.04±38.80 L/kg) was not significantly different from that of the RS isomer (517.35±37.22 L/kg), there does not appear to be a stereoselective process in terms of protein binding in sheep. The enantioselective differences in plasma concentrations appear to be due to differences in metabolism, specifically in glucuronide conjugation of the isomers (see below).

In fetal plasma, the RR, RS and the SS isomer exhibited a bi-exponential decline, while the SR isomer appeared to undergo a tri-exponential decline. The concentrations of the SR and RR isomers were significantly different from the racemic value (25%) throughout the infusion and post-infusion periods. The ratio of the AUC of the RR isomer over the AUC of the SR, SS and RS isomers ranged from 2.6 to 2.9. As in maternal plasma, the concentration of the RR isomer in fetal plasma was also significantly higher than the other isomers at steady-state and throughout the post-infusion period. However, in the fetus the concentrations of the RR isomer relative to the others was proportionally higher (51.6 ± 8.5% to 79.4 ± 6.3%) than was observed in the mother (17.6 ± 4.3% to 68.7 ± 8.2%). This is in contrast with the data obtained for celiprolol, a β₁-adrenoceptor antagonist used in the treatment of all forms of hypertension including pregnancy-induced hypertension. There was no significant difference between the plasma concentrations of celiprolol stereoisomers in umbilical cord venous and arterial
blood following a single 200 mg dose to the mother immediately after parturition (Kofahl, et al., 1993).

The pattern of disposition of the labetalol isomers in the fetus appeared to be different from that observed in the mother. While in maternal plasma the lowest concentration was that of the RS isomer, in fetal plasma it was the SR isomer that had the lowest concentration throughout the sampling period. In addition in fetal plasma at steady-state, the concentration of the SR, SS and the RS isomers were significantly different from each other; a pattern that was different from that observed in the maternal plasma.

The half-life data of the isomers also suggest stereoselective pharmacokinetics or metabolism of labetalol isomers in sheep. The RR isomer had the longest half-life of any of the isomers in both maternal and fetal plasma. However in the fetus the RR half-life (13.07 ± 2.33 h) was almost 2.5 times longer than in the mother (5.60 ± 1.0).

4.2.3 Glucuronide Conjugation

As mentioned previously, glucuronidation is an important pathway in the metabolism of labetalol in man, sheep and other species (Yeleswaram et al., 1993). For chiral molecules this pathway is often stereoselective. Stereoselective glucuronidation has been reported for various classes of chiral drugs in several in vitro studies using liver microsome preparations from different species. In rat and man, UDP-glucuronyltransferase selectively conjugates R-naproxen (a widely used anti-inflammatory drug) and R-flurbiprofen (a non-steroidal antiinflammatory drug) at a much higher rate than the S-enantiomer (el-Mouelhi, et al., 1993; Hamdoune, et al., 1995). In rabbit and man, the enzyme selectively conjugates S-
oxazepam (a C3-hydroxylated benzodiazepine) over the R-enantiomer (Yost and Finley 1985; Patel, et al., 1995). While it is evident that all the labetalol stereoisomers are excreted to a large extent as glucuronide conjugates, there was a significant difference in the extent of glucuronidation of labetalol isomers in sheep. The extent of glucuronidation of the RR isomer was less than the other three isomers. There was 41.2 ± 13.6% more unchanged RR isomer excreted (3.2±0.4 mg) than any other unchanged isomer (2.3±0.2 mg). Accordingly, there was approximately half as much RR glucuronide excreted compared to the other isomers. The difference in glucuronidation of the isomers may, in part, be due to the stereoselective reaction of the UDP-glucuronyltransferase enzyme involved in this reaction. The spatial molecular arrangement of the SR, SS and RS isomers might be a better fit for the active site of the enzyme than that of the RR isomer. The stereoselectivity might be due to the difference in the affinity of the glucuronosyltransferase for the isomers (apparent $K_M$) or the difference in the maximal glucuronidation velocity between the isomers (apparent $V_{max}$).

Conformational dissimilarities in the orientation of the hydroxyl groups may result in the difference in the apparent $V_{max}$. Therefore, the rate of transfer of the glucuronic acid moiety onto each isomer or dissociation of the glucuronidated isomer from the enzyme would likely be different. A difference in $V_{max}$ may also be due to the involvement of multiple glucuronosyltransferase isoforms in the glucuronidation of isomers (Sweeny and Nellans, 1994). Although the enzymatic glucuronidation reaction is not regioselective ( labetalol and dilevalol are glucuronide conjugated at both the alcoholic and phenolic hydroxy groups. Niemeijer, et al., 1991; Alton, et al., 1994), it is possible that the RR isomer is more readily glucuronidated at the alcoholic as opposed to the phenolic hydroxy group with the situation being reversed for the SR, SS and the RS isomers. Given
that the C—O bond of an alcoholic glucuronide may be more easily cleaved than the C—O bond of a phenolic glucuronide (Fenselau and Johnson, 1980), there would be an abundance of the SR, SS and the RS phenolic glucuronides (Alton, et al., 1994).

Another reason for lower levels of RR glucuronide excretion might be the preferential excretion of this conjugate in the bile. In the dilevalol bolus studies, we determined that there in fact is a preferential biliary excretion of dilevalol glucuronide conjugate over that of labetalol (See Section 4.3). It is possible that the RR glucuronide is preferentially excreted in the bile over the glucuronide conjugates of the other isomers and causing a lower RR glucuronide excretion in urine. However, the labetalol isomer glucuronide data are in agreement with the observations of the plasma concentrations of the isomers. Because the SS, SR and RS isomers are selectively glucuronidated over the RR isomer, they exhibit lower plasma concentrations and have higher clearances and shorter half-lives.

4.2.4 Hemodynamic and metabolic effects

In previous studies, we have examined the disposition and pharmacodynamics of labetalol in non-pregnant and pregnant sheep (Yeleswaram et al., 1993a&b). In the pregnant animals, no significant changes in the maternal cardiovascular parameters (heart rate and mean arterial pressure) were reported and only minimal changes were observed in the maternal blood pH and base excess. However, a significant, reversible hyperglycemia and lactic acidosis were observed in both the fetus and ewe, following the administration of a 100 mg i.v. bolus dose of labetalol to the mother (Yeleswaram et al., 1992). The elevation in lactate concentration was more pronounced and persistent in the fetus than in the
mother, despite marginal placental transfer of labetalol, suggesting an indirect or mediated effect. The metabolic effects of labetalol reported in these studies resembled those of a potent $\beta_2$-agonist. In non-pregnant sheep studies, significant increases in femoral blood flow, hypotension, reflex tachycardia, hyperglycemia, and lactic acidemia with a substantial output of lactate (6.25 ± 1.35 g) from the hind limb were observed following the administration of 100 mg i.v. bolus labetalol (Yeleswaram et al., 1993).

Given the long-term use of labetalol in the clinical management of pre-eclampsia as well as our previous i.v. bolus studies in adult pregnant and non-pregnant sheep, which indicated severe metabolic disturbances in both the ewe and the fetus, these effects were also monitored in our 6 hour infusion studies.

4.2.4.1 Hemodynamic Effects

No significant or consistent changes were observed in the heart rate or the arterial pressure in the ewe following maternal administration of labetalol. Heart rate and arterial pressure were obtained from all the fetuses, although, such data were obtained only from two ewes. Heart rate data from the two mothers indicated initial tachycardia which was consistent with observations from previous adult sheep experiments (Yeleswaram, et al., 1993). In the fetus, there was apparent tachycardia for up to three hours, but there was no hypotension associated with the administration of labetalol. Previous labetalol i.v. bolus experiments did not report any significant change in fetal heart rate even at higher doses (Eisenach, et al., 1991; Yeleswaram, et al., 1993). The rise in fetal heart rate in the maternal infusion experiments could be due to the higher dose and prolonged exposure of the fetus to labetalol or perhaps to the prolonged lactic acidemia, given that direct
i.v. infusion of lactate to fetal lambs results in tachycardia (Bocking, \textit{et al.}, 1991). It is possible that the maternal and fetal cardiovascular changes are dose- and mode of administration-dependent. Significant changes in the arterial pressure of normotensive sheep have been reported both at doses of 3 mg/kg (Eisenach, \textit{et al.}, 1991) and prolonged exposure of 100 mg over 5 minutes (Mohan, \textit{et al.}, 1990).

4.2.4.2 Metabolic Effects

Labetalol exerted significant metabolic effects in pregnant sheep. Mean maternal arterial $P\text{O}_2$ and $P\text{CO}_2$ decreased significantly below control for up to 2 and 4 hours, respectively. Both values recovered to control levels before the infusion had stopped. In the fetus, the arterial $P\text{O}_2$ was significantly below control by 15 minutes of infusion and had a slower recovery than that observed in the mother. The recovery of the maternal $P\text{O}_2$ is perhaps due to hyperventilation, whereas the fetus in the absence of any respiratory compensatory mechanism does not recover as quickly. Many factors can contribute to the fall of $P\text{O}_2$ and to the general decline of the fetal blood gas status; these include hyperglycemia and hyperinsulinemia, an increase in fetal oxygen consumption and the development of acidemia in the fetus. In this study, we observed significant hyperglycemia in the fetus. Induced hyperglycemia and hyperinsulinemia have been shown to cause hypoxemia (reduction of $P\text{O}_2$ by more than 5 mm Hg) in the fetal lamb (Milley, 1984; Hay and Meznarich, 1986), the mechanism of which appears to be a rise in fetal oxygen demand. Other adrenegic agonists such as norepinephrine (Lorijn and Longo, 1980), clenbuterol (Eisemann, \textit{et al.}, 1988) and ritodrine (van der Weyde \textit{et al.}, 1992) have been shown to increase fetal oxygen consumption resulting from an increase in an accelerated fetal oxidative metabolism. This increase in the fetal
oxidative metabolism in turn is the result of an increase in plasma glucose and lactate in the fetal lamb.

Whereas the maternal O$_2$ saturation declined gradually, the fetal O$_2$ saturation fell rapidly and significantly due to the pH-induced shift in the oxyhemoglobin dissociation curve to the right (Bohr effect).

4.2.4.3 Maternal and Fetal Lactic Acidemia and Hyperglycemia

Lactate is produced in virtually all tissues in the cell’s cytosol. At physiological pH, lactate (pKa 3.8) is completely dissociated into lactate anion and hydrogen ions resulting in acidemia. In the fetal lamb, lactate is produced from both glucose and non-glucose (e.g. alanine) sources (Kitts and Krishnamurti, 1982) in the lung and carcass (Singh et al., 1984; Simmons and Charlton, 1988) while it is consumed by the heart, liver and kidney (Fischer, et al., 1982; Gleason., et al., 1985; Iwamoto and Rudolph, 1985). After glucose, lactate is the second major carbohydrate unutilized in fetal lamb (Hay et al., 1983). Approximately 22% of fetal oxygen uptake is from the oxidation of lactate (Battaglia and Meschia, 1986), the rate of which is concentration dependent, with increased oxidation occurring at higher concentrations (Hay et al., 1983). The placenta has a relatively low permeability to lactate but it produces at least ¼ of the lactate consumed by the fetus (Sparks et al., 1982). Fetal lactate levels exceed those of the mother and the fetus is a net consumer of lactate. Despite higher lactate concentrations in the fetus, larger quantities of lactate are delivered into the fetal circulation from the placenta as opposed to the maternal circulation (Battaglia and Meschia, 1986).
Various metabolic effects such as glycogenolysis and increased cAMP synthesis result from the stimulation of $\beta_2$ adrenoceptors (Whitsett et al., 1980). In previous studies, exposure of pregnant and non-pregnant ewes and humans to $\beta_2$-adrenoceptor agonists have resulted in elevated levels of glucose, lactate, pyruvate and insulin in the mother and the fetus (Schreyer et al., 1980; Siimes and Creasy, 1980; Basset et al., 1985; van der Weyde et al., 1992; Yeleswaram, et al., 1993).

Arterial lactate and glucose levels were significantly elevated in the mother and fetus following maternal labetalol administration. Metabolic acidosis was observed in both the mother and fetus, where the decline in the base excess was associated with the fall in pH. In the mother, the blood lactate concentration increased continuously by almost 9 fold by the end of the infusion and declined throughout the post-infusion period reaching control levels by the end of the experiment. In the fetus, the arterial lactate level increased 5 fold by 3 hours of infusion and appeared to remain at the same levels until the end of the infusion. In contrast to the mother, the mean fetal lactate increased by 25% from the end of infusion until 6 hours post-infusion. Although the magnitude of the lactate increase is larger in the mother (9 fold), the duration of the lactate elevation is longer in the fetus. The $\beta_2$-adrenergic agonist, ritodrine, also results in prolonged lactate acidemia in the fetal lamb. This has been postulated to be the result of increased fetal production and slow elimination of lactate (Basset et al., 1985; van der Weyde et al., 1992). Another reason for the persistence of lactate in the fetal lamb, could be the elevated levels of the RR isomer and its $\beta_2$-agonist actions in the fetal tissue and placenta (See Dilevalol Bolus Studies, Sec. 4.3). The pharmacokinetic data of the individual isomers of labetalol support this hypothesis. The RR isomer which has the total beta-2 agonist activity of labetalol is present in
significantly higher concentrations, and has a higher half-life and lower elimination rate than the other isomers.

Both maternal and fetal tissues as well as the placenta are thought to be responsible for the release of lactate. The sheep placenta has been shown to contain β2-receptors (Padbury et al., 1981) which may in part be responsible for the increased lactate production in sheep following administration of ritodrine (Wright et al., 1992; van der Weyde et al., 1992).

Significant hyperglycemia was observed in both the mother and the fetus. Hyperglycemia appears to be the result of labetalol’s β2-agonist activity (which stimulates glycogenolysis) and has been reported in several studies involving other β2-agonist active compounds such as ritodrine (Basset et al., 1985, van der Weyde 1992, Schreyer et al., 1980). In previous maternal labetalol administration studies, fetal hyperglycemia was thought to be the direct result of maternal hyperglycemia because there is little or no glycogenolysis in the fetus under normoxic conditions. Because we observed hyperglycemia in only the fetus in the fetal infusion experiments (see below), it is possible that fetal hyperglycemia is the direct result of labetalol β2-stimulated glycogenolysis activity in the fetus.

4.3 Dilevalol Bolus Studies

4.3.1 Pharmacokinetics and metabolism

Dilevalol is the RR stereoisomer of labetalol which is suspected to possess virtually all of the β-adrenergic activity associated with the racemic drug. However, little is known about the metabolic effects of this drug when it is given
as the individual isomer. This study was undertaken to characterize the disposition, conjugative metabolism and pharmacodynamics of dilevalol in the adult non-pregnant sheep and to compare the hemodynamics and metabolic effects of dilevalol with those of labetalol observed in the same animal model (Yeleswaram, et al., 1993). Furthermore, a specific objective of this study was to compare the pharmacokinetics of dilevalol given as pure isomer to those obtained for dilevalol from racemic labetalol administration in previous studies.

In non-pregnant sheep, the total body clearance of dilevalol (48.7 ± 7.8 mL/min/kg) was significantly higher than that observed for labetalol (29.00 ± 2.67 mL/min/kg). The terminal elimination half-life of dilevalol (3.48 ± 1.01 h) was similar to that of labetalol (2.41 ± 0.30 h), whereas the estimates of volume of distribution of dilevalol (Vd_{area} = 13.09 ± 2.78 L/kg; Vd_{ss} = 5.32 ± 0.80 L/kg) appeared to be higher than for labetalol (Vd_{area} = 6.19 ± 1.13 L/kg; Vd_{ss} = 3.22 ± 0.31 L/kg). This latter finding is similar to data reported in previous clinical pharmacokinetic studies (Kramer, et al., 1988; Tenero, et al., 1989) in which the estimates of the volume of distribution of dilevalol (16.6 - 24.6 L/kg) were higher than those reported for labetalol (2.5 - 15.7 L/kg). The difference in the estimates of volume of distribution of these two compounds may be due to the differences in the binding of dilevalol to extravascular tissues and protein in plasma compared to the other labetalol isomers. In clinical studies dilevalol was shown to have a higher degree of apparent protein binding (75%) than labetalol (50%) (Donnelly and Macphee, 1991).

Analysis of the urine and bile samples indicated that, like labetalol, dilevalol undergoes both glucuronide and sulphate conjugation. The extent of sulphation of dilevalol (1.4 ± 0.5 %) was similar to that of labetalol (1.5 ± 0.7 %) whereas the extent of its glucuronidation (5.36 ± 1.24%) was about half of that
observed for labetalol (11.46 ± 2.82%). In the labetalol maternal infusion studies, a similar ratio of the extent of glucuronidation was observed between labetalol and dilevalol (Section 3.2.4). In the maternal infusion studies in which 280 mg of labetalol (70 mg of dilevalol) was administered over a 6 hour period, 22.6 ± 2.1% of labetalol and 12.7 ± 1.7% of dilevalol were excreted as glucuronide conjugate, respectively. The data from maternal infusion studies suggest that the difference in the extent of glucuronidation of labetalol and dilevalol could be due to a possible stereoselective process being involved in their respective conjugation reactions. This observation was supported by the fact that the extent of glucuronidation of each individual isomer was approximately twice that of dilevalol.

These results also indicate that the mechanism(s) of conjugative metabolism of dilevalol in sheep may be different than those observed in man. In healthy human volunteers, the fraction of the dose of the dilevalol excreted in urine was 0.5% for the unchanged drug, 23% for the glucuronide and 8% for the sulphate (Neubeck, et al., 1993). Thus, the ratios of the glucuronide and sulphate conjugates to the unchanged drug (excreted as a percentage of the dose) in man are 46:1 and 16:1, respectively, while similar ratios in sheep were 3:1 and 1:1, respectively.

In bile, the concentrations of the glucuronide conjugates of dilevalol were significantly higher than those of the sulphate conjugates throughout the sampling period (Fig 26). The ratio of the glucuronide to sulphate conjugate in bile was about 9 while the same ratio for labetalol was 0.5 (Yeleswaram, et al., 1993). This difference in the ratio of biliary excretion of the conjugates suggest that the glucuronide conjugate of dilevalol is preferentially excreted in the bile.

The metabolic effects of dilevalol appear to be similar to those caused by labetalol (the result of β2-agonist activity, see above). The 25 mg dilevalol i.v. bolus dose caused significant hyperglycemia (5 fold increase) and lactic acidosis (10 fold increase), the magnitude of which was similar to that observed with a 100
mg labetalol bolus. These observations suggest that the metabolic effects of labetalol seen previously in non-pregnant sheep were caused by the RR isomer and not by the other three isomers. There was a significant net release of lactate from the hind limb with the administration of dilevalol (Fig 32). [The mechanism of lactate release is described above in the Maternal Infusion Studies, Sec. 4.2]. This was similar in magnitude and duration to the release of lactate from the hindlimb following the administration of a 100 mg i.v. bolus of labetalol (Yeleswaram et al., 1993). Because lactate cannot be metabolized by skeletal muscle (Connor and Woods, 1982), the hyperlactacidemia observed in the mother and fetus in maternal and fetal infusion studies may be mostly caused by the release of lactate from this tissue.

The hemodynamic changes observed following the administration of dilevalol included hypotension, delayed onset of tachycardia (50% increase in heart rate) and a significant increase in femoral blood flow (by as much as 100%). These results suggest that dilevalol causes reflex tachycardia and peripheral vasodilation, but it shows no β₁-, or α₁- antagonist activity. The exact mechanism involved in peripheral vasodilation induced by dilevalol is, however, not clear. The magnitude of these effects were similar to those observed with labetalol. Because the amount of dilevalol administered was 1/4 of the amount of labetalol, it could suggest that virtually all of the hemodynamic effects of labetalol are due to the presence of dilevalol.
4.3.2 Comparative analysis of dilevalol pharmacokinetics and metabolism in dilevalol and labetalol experiments.

Individual enantiomers of chiral drugs are considered separate compounds. When these compounds are administered as a racemate, enantiomer-enantiomer interactions may alter the process of absorption, distribution, metabolism, protein binding and excretion of each compound. These interactions may have serious therapeutic consequences.

In our studies dilevalol was administered to adult non-pregnant sheep as a single bolus dose whereas the RR isomer, comprising 25% of labetalol, dilevalol was administered to pregnant sheep as a bolus dose followed by 6 hours of infusion. Although both the mode of administration and the animal model are different, studies have shown that labetalol pharmacokinetic parameters do not significantly differ between pregnant and non-pregnant sheep and between bolus and infusion studies, with the exception of the steady-state clearance (Yeleswaram, et al., 1992a, 1993a and unpublished data). Therefore, a comparative analysis of these parameters will, in part, assist in understanding the effects of the other isomers (SS, SR and RS) in the disposition and metabolism of dilevalol.

The pharmacokinetics and conjugative metabolism of dilevalol appear to be significantly different between racemate dosing (RD) and individual enantiomer dosing (ED) experiments. In ED experiments, dilevalol exhibits significantly higher clearance, lower half life, and higher glucuronide conjugate formation (as a percentage of the dose). Clearance of dilevalol in ED studies was 48.7 ± 7.8 mL/min/kg, almost 4 times of that obtained in RD studies (12.63 ± 2.05 mL/min/kg) while the half lives in the ED and RD experiments were 3.48 ± 1.01 and 5.60 ± 1.00 h, respectively. The higher clearance and lower half life in ED studies are most likely due to higher formation of dilevalol glucuronide conjugate.
In ED experiments 5.36 ± 1.24% of the dose was excreted as glucuronide compared to 3.17 ± 0.43% of the dilevalol dose in RD experiments. As previously discussed (Sec. 4.2.3), in RD experiments, there was a significant difference in the extent of glucuronidation of labetalol isomers in sheep, with dilevalol being the least conjugated isomer. Because in ED experiments, dilevalol is conjugated to a higher extent as a percentage of the dose, there is a possibility that in RD experiments other isomers of labetalol compete for the glucuronidation step thus lowering its clearance.

Such interactions have been shown to affect the metabolism and disposition of enantiomers of other chiral drugs such as propranolol and propafenone. Enantiomers of propranolol undergo stereoselective metabolism in the dog, rat and man (Von Bahr, et al., 1982; Wilson and Thompson, 1984, Fujita, et al., 1993). Propranolol enantiomers have been shown to undergo concentration-dependent metabolic inhibition by their optical isomers at both phase I (hydroxylation) and phase II (conjugation) metabolic pathways (Wilson and Thompson, 1984; Masubuchi, et al., 1993). These inhibitions are partly responsible for higher clearance of the more potent S isomer in the rat and dog. Similarly, R-propafenone has been shown to impair the cytochrome P450 IID6-mediated 5-hydroxylation of active S-propafenone in humans, thereby significantly lowering the clearance of the S isomer (Kroemer, et al., 1991). Thus the beta-blocking effects of the 150 mg of racemic propafenone in man is reported to be more pronounced than those of the 150 mg of S-propafenone alone (Kroemer, et al., 1994).
4.4 Fetal Infusion of Labetalol

The possibility of fetal exposure to drugs following maternal administration has been studied for several decades. Studies in pharmacokinetics and metabolism of drugs in the fetus have been initiated since the early 1970's (Levy and Hayton, 1973). By far the majority of the data available are for racemic drugs and they do not address the pharmacokinetics and metabolism of individual isomers of chiral drugs in the fetus. In the case of labetalol, a few reports have addressed the wide range of single point cord blood concentrations (10-260 ng/mL) that have been observed following administration of labetalol for the clinical management of pre-eclampsia (Michael, 1979; Michael, 1982; Rogers et al., 1990). Single point determinations do not accurately reflect the in utero exposure of the fetus to labetalol. Previous studies in this laboratory (Yeleswaram, et al., 1993b) investigated the pharmacokinetics and metabolism of labetalol in the near term fetal lamb. The conclusions of the study were that labetalol causes significant metabolic effects in the fetus and the magnitude of these metabolic effects was not proportional to the extent of fetal exposure to labetalol. In addition, they reported a longer terminal elimination half life, longer total body clearance and volume of distribution for labetalol in the fetus compared to the ewe following either maternal and fetal administration. The present study was conducted to address some of these questions through the study of the pharmacokinetics and metabolism of individual isomers of labetalol and direct comparison with similar parameters obtained for labetalol from fetal bolus studies. In particular, the pharmacokinetics and metabolism of the RR isomer of labetalol in the fetus, which causes the metabolic effects, were to be investigated.
4.4.1 Pharmacokinetics of Labetalol and its Stereoisomers

Labetalol exhibited a bi-exponential elimination profile ($Ae^{-at} + Be^{-bt}$) in fetal plasma. The terminal elimination half-life was $9.4 \pm 2.4$ hr, which was longer than any such value observed or reported in previous experiments (Yeleswaram, et al., 1993). Consistent with previous experiments, these data suggest a difference in the disposition and elimination of labetalol, between the mother and the fetus. Studies in man also indicate a prolonged half-life for labetalol in the fetus. In a study with 6 week maternal oral administration of labetalol, a half life of 24 hours was reported in the new born infant (Haroldsson and Geven, 1989).

Accumulation and recirculation of labetalol in amniotic and tracheal fluid might also be a factor in the persistence of labetalol in the fetus. In our study, labetalol appeared in fetal tracheal fluid almost immediately (Fig 35) and in concentrations equal to those in plasma (Fig 34). Similar observations have been reported for other amine drugs such as ritodrine, diphenhydramine and metoclopramide (Riggs et al., 1987; Wright et al., 1991b). Two factors may be important in the extensive appearance of labetalol in tracheal fluid: 1) uptake of drug by the fetal lung and release into the tracheal fluid (Rurak, et al., 1991), and 2) a labetalol β2-mediated reduction in the production and resorption of tracheal fluid (Warburton et al., 1987), thus increasing the concentration of solutes in this fluid. Similar to previous experiments (Yeleswaram et al., 1992a, 1993b) labetalol appeared at a slower rate (at 2 hours) and at lower concentrations in the amniotic fluid (Fig 36) and unlike maternal infusion experiments, did not extensively accumulate in amniotic fluid. This could have been due to the collection of fetal urine for drug analysis. Fetal urine that is excreted in the amniotic fluid is a substantial source of labetalol. We collected approximately 5 to
10 mL of fetal urine for the analysis of labetalol and its stereoisomers and their glucuronide conjugates (the remainder of the urine was returned to the amniotic cavity). At most sampling points there was no excess urine to be returned to the amniotic cavity. From the approximately 78 ug of labetalol that was excreted in the urine, about 39 ug or 50% of that would have accumulated in the amniotic fluid (the other 50% in the allantoic fluid since the fetal urine is almost equally distributed between the amniotic and allantoic fluid cavities (Wlodek et al., 1988). Because the volume of the amniotic fluid is estimated at 1,000 mL, the concentration of labetalol in the amniotic fluid would have been greater by about 39 ng/mL. In fetal infusion experiments, labetalol concentration in the amniotic fluid at 6 hours post-infusion was 26.81 ± 3.84 ng/mL. If all the labetalol that was collected was returned to the amniotic cavity, the concentration of labetalol in the amniotic fluid would have been approximately 66 ng/mL, which is closer to the average steady-state concentration of 73.25 ± 6.75 ng/mL. Therefore, the depletion of as a result of fetal urine sampling likely, in part, accounts for the loss of the drug from this fluid compartment.

In the fetal infusion experiments, the concentration of the RR isomer at steady-state was 53.9 ± 7.3 ng/mL, which was significantly higher than the other three isomers (Fig 37). The higher concentrations of the RR isomer were mainly due to the reduced glucuronide conjugation of this isomer (See. 4.4.2 below). The half lives of the isomers were not significantly different from one another, which was in contrast to the results obtained in maternal infusion experiments. The terminal elimination half-life (t_{1/2}) of the RR isomer was 8.62 ± 2.85 h, which was significantly lower than that obtained following maternal infusion of labetalol (13.07 ± 2.33 h). This was in contrast to the half life of the other isomers (SR, SS, and RS ; 11.03 ± 3.64, 8.13 ± 2.41, 6.64 ± 1.88 h, respectively) and labetalol which
were all higher in the fetal infusion experiments. Furthermore, the observation did not agree with the difference in terminal elimination half life values observed between the maternal and fetal bolus experiments. In those experiments, the half life of labetalol was higher following fetal i.v. bolus administration (Yeleswaram, et al., 1993).

4.4.2 Glucuronide Conjugation

With fetal labetalol infusion, 73.79 ± 9.64 µg (0.80%) of the 9,400 µg of labetalol administered was excreted as unconjugated drug while 133.44 ± 25.20 µg (1.4%) was excreted as glucuronide (Table 13). Both amounts were significantly lower in percentage than those obtained in maternal urine following maternal infusion. The total amount recovered (2.2% of the dose) was significantly lower than the 14% from adult sheep in maternal infusion experiments and 12% in non-pregnant bolus experiments (Yeleswaram, et al., 1993). The lower recovery of labetalol from the fetal urine maybe due to the excretion of the drug into other fetal compartments such as amniotic, tracheal and allantoic fluids as well as the fetal to maternal transfer of the drug. Previous studies have shown that approximately 50% of the fetal clearance of labetalol is due to maternal transfer (Yeleswaram et al., 1993b)

Approximately 35.3% of the unchanged labetalol was in the form of the RR isomer (compared to about 21% for the other isomers) whereas only about 6.4% of the conjugated labetalol was the RR isomer (compared to about 30% for the other isomers). The percentage of the unchanged labetalol in the form of RR isomer recovered (35%) was similar to that obtained in maternal infusion experiments (32%), while the conjugated percent recovered (6.4%) was significantly lower
(14%) suggesting a better ability of the maternal liver to conjugate the RR isomer. Studies have shown that the glucuronide conjugation capacity, and indeed the metabolizing capacity, of the sheep fetal liver cannot be generalized. While the efficiency of the glucuronidation of para-nitrophenol (PNP) by the near term fetal sheep liver appears to be comparable to that of the adult (Ring, et al., 1996) other factors such as capacity to transport the conjugate from the hepatocytes to bile is not. On the other hand, using acetaminophen as an example the kinetic properties of UDP-glucuronosyltransfrase were shown to be significantly different between the near term fetus and adult sheep (Wang, et al., 1986). In this study, it was shown that in near term fetuses (113, 135 days; term approximately 145 days) the activity of the UDP-glucuronosyltransfrase Vmax was significantly lower in fetal microsomes. However, the differences between fetal and adult enzymes appear to diminish immediately prior to parturition (141 day old fetuses). The apparent lesser ability of the fetal liver to conjugate the active isomer of labetalol appears to further enhance the adverse metabolic effects of this isomer in the fetus.

4.4.3 Changes in urine composition

Fetal urine was not collected during maternal infusion experiments. Maternal and fetal urine pH was significantly reduced following maternal and fetal infusion of labetalol. In the maternal infusion experiments, the maternal urine pH fell significantly from 7.46 ± 0.05 to 7.05 ± 0.16 while during fetal infusion of labetalol, fetal urine pH fell significantly from 7.10 ± 0.03 to 6.88 ± 0.10 (Table 6 &12). The drop in the urine pH is consistent with the metabolic lactic acidosis observed in both the mother and fetus. Elevated blood lactate levels result in the enhance the urinary excretion of lactate producing in the drop in urine pH. Similar
studies involving the administration of i.v. lactic acid to fetal lamb have shown a marked decrease in the urine pH (Daniel et al., 1975; Wood, et al., 1990).

During the infusion of labetalol, mean average hourly maternal and fetal urine output increased by 9.4 mL/h and 2.6 mL/h, respectively (Tables 6 & 12). Although these changes were not statistically significant, they were consistent with the findings of other studies involving the elevation of plasma lactate (Powell and Brace, 1991) and long term infusion of fluids in sheep (Brace, 1989).

5. SUMMARY

A direct and sensitive HPLC assay for the separation of labetalol stereoisomers was developed. The four isomers in order of elution were SR, SS, RS and RR with retention times of 19, 23, 28 and 34 min, respectively. The limit of reliable detection of each isomer using 50 to 500 µL of biological fluid was 0.15 ng (0.6 ng of racemic labetalol injected).

The pharmacokinetics of labetalol stereoisomers appeared to be stereoselective in both the mother and the fetus. In both cases, the RR isomer was detected at significantly higher concentrations at steady state and throughout post-infusion than the other isomers. In sheep, it appears that stereoselective metabolism of the labetalol isomers in the mother results in differing concentrations of the enantiomers in the two compartments (mother and fetus). The maternal stereoselective metabolism appears to lead to differing steady-state levels of the enantiomers in maternal blood, and the isomers are then transferred to the fetus, in part, in proportion to their concentrations in the mother. Thus, the
fetus received the enantiomers in proportions different from those in the racemic drug mixture. The data also suggest the possibility of stereoselective fetal metabolism of the drug because the pattern of stereoselectivity for the active isomer is enhanced in the fetus.

In fetal infusion experiments, the terminal elimination rate constant of labetalol was significantly lower than that observed in maternal infusion experiments. Hence, the mean half-life of labetalol in fetal plasma was significantly higher than that observed in the fetus and mother following maternal infusion. In fetal plasma, the concentration of the RR isomer at steady-state was significantly higher than the other three isomers. The terminal elimination half-life ($t_{1/2}$) of the RR isomer was significantly lower than that obtained following maternal infusion of labetalol. The total labetalol recovered represented $2.2 \pm 0.3\%$ of the total dose administered. This amount was significantly lower than that obtained in the maternal infusion experiments. A significant amount ($2/3$) of unchanged labetalol excreted was in the form of the RR isomer.

In dilevalol bolus experiments, the estimates of the pharmacokinetic parameters of this isomer were within the ranges previously observed with labetalol, with the exception of CL, $V_{dss}$ and $V_{area}$ which were higher for dilevalol. The metabolic and hemodynamic effects of dilevalol were similar to those of labetalol, suggesting that the observed pharmacological effects of labetalol previously observed in sheep were elicited by dilevalol. These observations may explain the enhancement of the metabolic effects of labetalol in the fetus following maternal bolus administration in previous experiments.
There are similar pharmacokinetics and metabolic pathways for labetalol in sheep and man. It is possible, then that the stereoselective pharmacokinetics and metabolic behavior of labetalol stereoisomers observed in sheep may also be apparent in man. The findings from our studies have contributed to our understanding of the role of individual isomers of labetalol in hemodynamic and metabolic effects in the adult sheep and fetal lamb. Therefore, similar studies of labetalol stereoisomers in man may result in better dosing and monitoring of each stereoisomer in pregnant women to reduce the drug's side effects.

6. CONCLUSIONS

i. Baseline separation of labetalol stereoisomers was achieved using an α₁-acid glycoprotein stationary phase. The limit of detection of the individual isomers was 0.15 ng (0.6 ng of injected racemic labetalol).

ii. Labetalol undergoes stereoselective disposition in maternal and fetal units following maternal and fetal infusion.

iii. The RR isomer which has the total beta-2 agonist activity of labetalol has significantly higher concentration, longer half-life and lower elimination rate than the other isomers.

iv. Labetalol isomers undergo stereoselective glucuronidation. The RR isomer constitutes approximately 1/3 of the unchanged excreted labetalol.
v. The disposition pattern of the labetalol isomers in the fetus are different from that observed in the mother and the fetus is exposed to labetalol isomers in proportions different to those seen in the mother.

vi. There is significant lactic acidemia and hyperglycemia in the mother and fetus following labetalol infusion.

vii. The hemodynamic and metabolic effects of dilevalol, including the net release of lactate from the hindlimb, were also similar to those observed with labetalol.

viii. Virtually all of the previously observed pharmacological effects of labetalol in non-pregnant sheep were elicited by dilevalol.

ix. Enhancement of the metabolic effects in the fetus following maternal bolus administration of labetalol in previous experiments is mainly due to higher levels of dilevalol present in the fetus.

7. Proposed Future Studies

Future studies that can further contribute to our understanding of the mechanism of stereoselective pharmacokinetics and metabolism of labetalol isomers include:

i. Determination of the mechanism of stereoselective conjugation of labetalol stereoisomers. To examine the mechanism of stereoselective glucuronidation of labetalol isomers, \textit{in vitro} studies can be conducted using maternal and fetal liver microsomal preparations.
ii. Structure elucidation of glucuronide conjugates of labetalol stereoisomers. To determine the molecular site of glucuronidation of labetalol stereoisomers, the glucuronide conjugate of each isomer has to be separated. The glucuronides of the individual isomers are diastereomers and therefore can be separated using non-chiral columns. An HPLC method based on the chromatographic conditions described by Niemeijer (Niemeijer, et al., 1989) could be used to separate the glucuronides. The method uses a Nucleosil 5-C\textsubscript{18} column and an acetonitrile / sodium dihydrogen phosphate buffer. The chromatographic conditions (pH, flow rate, etc.) will have to be optimized to separate the glucuronides from the endogenous substances of biological fluids. The site of conjugation on the labetalol molecule could be deduced by the use of a LC/MS/MS method.

iii. Investigation of the pharmacokinetics of other isomers of labetalol given as individual isomers in order to examine whether their disposition differs from that of dilevalol. This study will require synthesis of pure isomers of labetalol.
8. REFERENCES


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