EFFECTS OF ACUTE MODERATE HYPOXEMIA ON THE PHARMACOKINETICS OF METOCLOPRAMIDE AND ITS METABOLITES IN CHRONICALLY INSTRUMENTED SHEEP

by

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We accept this thesis as conforming to the required standard

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Hypoxemia is known to induce various physiological changes which can result in pharmacokinetic changes. To examine the effect of acute, moderate hypoxemia in metoclopramide (MCP) pharmacokinetics, a continuous infusion [14 hours] of MCP was administered during pre-hypoxemia (2hr), hypoxemia (6hr) and post-hypoxemia (6hr) in non-pregnant sheep. Hypoxemia was achieved by lowering the ewe’s inspired O₂ concentration. During the experiment, arterial blood and urine samples were collected. MCP and its mono- and di-deethylated metabolites were measured in these fluid samples using a gas chromatography-mass selective detector (GC-MSD) method. Steady-state concentrations of MCP were achieved in each of the three periods. During hypoxemia, MCP plasma steady-state concentration increased significantly from 50.72 ± 1.06 to 63.62 ± 1.79 ng/mL, and later decreased to 55.83 ± 1.15 ng/mL during the post-hypoxemic recovery period. Plasma mdMCP concentration (32.78 ± 1.73 ng/mL) also increased, compared to the control group (21.20 ± 1.39 ng/mL), during hypoxemia and the subsequent normoxemic period. Renal excretion of MCP and its metabolites significantly decreased during hypoxemia. Increased urine flow with decreased urine osmolality was also observed. Thus the results indicate that acute, moderate hypoxemia affects MCP pharmacokinetics.
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LIST OF ABBREVIATIONS

\(\phi\) determining power
\(\sigma^2\) population variance
A.R.E. amount remained to be excreted
ACS American Chemical Society
ANF atrial natriuretic factor
ANOVA analysis of variance
AUC area under the curve
AVP arginine vasopressin
BMY 4-amino-5-chloro-2(2-butanone-3-yl)-oxy-N,N-diethylaminoethyl benzamide
BSP sufobromophthalein
Cl\(_t\) total body clearance
COPD chronic obstructive pulmonary disease
\(C_{ss}\) apparent arterial steady-state concentration
CV coefficient of variation
ddMCP N,N-dideethyl-metoclopramide
\(D_u\) accumulated drug/metabolite in urine
EI electron impact
\(f'_{u}\) fractional renal metabolite elimination constant
\(f_m\) metabolite formation fraction constant
\(f_{mu}\) renal metabolite elimination fraction constant
\(f_u\) fractional renal elimination constant
GC-ECD gas chromatography-electron capture detector
GC-MSD gas chromatography-mass selective detector
GFR glomerular filtration rate
GX glycinexylidide
HCl hydrochloric acid
HCO\(_3^-\) bicarbonate
HFB heptafluorobutyryl
HFBA heptafluorobutyric anhydride
I.D. inner diameter
ICG Indocyanine Green
\( k_{i0} \) apparent drug elimination rate constant from the central compartment
\( k_{12}, k_{21} \) apparent first order inter-compartmental distribution rate
\( K_E \) apparent first order drug elimination rate constant
\( k_f \) metabolite formation rate constant
\( K_mO_2 \) Michaelis affinity constant of oxygen
\( k_{mu} \) renal metabolite elimination rate constant
\( k_o \) infusion rate
\( k_u \) renal elimination rate constant
LD i.v. bolus loading dose
LOQ limit of quantitation
MA maternal arterial blood sample
\( M_B \) amount of metabolite in the body
MCP metoclopramide
mdMCP N-monodeethyl-metoclopramide
MEGX N-monoethyl-glycinexylidide
MSD mass selective detector
NaOH sodium hydroxide
\( \text{PaCG} \) arterial carbon dioxide partial pressure
\( \text{PaG} \) arterial oxygen partial pressure
\( \text{PO}_2 \) oxygen partial pressure
PTFE polytetrafluorethylene
r coefficient of determination
\( r^2 \) coefficient of correlation
SCAN mass scanning mode
SD standard deviation
SEM standard error of the mean
SIM selective ion monitoring mode
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<tr>
<td>SMZ</td>
<td>sulphamethazine</td>
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<tr>
<td>TBC</td>
<td>total body clearance</td>
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<tr>
<td>TEA</td>
<td>triethylamine</td>
</tr>
<tr>
<td>TRIS</td>
<td>2-amino-2-hydroxymethyl-1,3-propanediol</td>
</tr>
<tr>
<td>UHP</td>
<td>ultra-high purity</td>
</tr>
<tr>
<td>UR</td>
<td>urine total collection</td>
</tr>
<tr>
<td>$V_c$</td>
<td>apparent volume of distribution of the central compartment</td>
</tr>
<tr>
<td>$V_d$</td>
<td>apparent volume of distribution</td>
</tr>
<tr>
<td>$X_B$</td>
<td>amount of drug in the body</td>
</tr>
<tr>
<td>Scheme</td>
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1. **INTRODUCTION**

1.1 **Pharmacology and Clinical Use**

Metoclopramide (MCP), 4-amino-2-methoxy-5-chloro-N-(2-diethylaminoethyl) benzamide, is a potent antiemetic and gastric motility modifier that has been used to treat nausea and vomiting associated with uremia and to reduce the incidence of vomiting and pulmonary aspiration associated with emergency anaesthesia (Davies and Howells, 1973; Dundee et al., 1974). MCP is structurally related to procainamide, but is devoid of antiarrhythmic or local anaesthetic activity at antiemetic clinical doses (Harrington et al., 1983). However, MCP-elicited prolactin secretion increase and extrapyramidal symptoms have been reported (Pinder et al., 1976). The pharmacological actions of MCP are most pronounced in the gastrointestinal tract where generalized increases in motility are seen after oral and i.v. administration (Pinder et al., 1976; Schultze-Delrieu, 1981).

\[
\begin{align*}
\text{Metoclopramide} & : \\
\text{Procainamide} & : 
\end{align*}
\]
Metoclopramide is effective in reducing postoperative vomiting (Lind and Breivik, 1970). At high doses (5 doses of 2 mg/Kg administered intravenously over 8 hours), MCP is effective in reducing the nausea and vomiting associated with the use of cisplatinum (Gralla et al., 1981) and other chemotherapeutic agents (Strum et al., 1984). MCP also increases lower oesophageal sphincter tone, oesophageal peristalsis and gastric emptying, all of which would relieve patients with gastro-oesophageal reflux (Bright-Asare and El-Bassoussi, 1980; Winnan et al., 1980). MCP reduces the nausea associated with migraine and increases the rate of absorption of oral analgesic agents, including aspirin (Matts, 1974; Volans, 1978).

Metoclopramide also significantly reduces the emesis of early pregnancy (Singh and Lean, 1970) and during labour (McGarry, 1971; Vella et al., 1985). It significantly increases the gastric emptying rate of women in labour (Howard and Sharp, 1973) and increases the tone of the lower esophageal sphincter, which is impaired during pregnancy and labour (Brock-Utne et al., 1978). These actions and the ability to diminish the frequency of vomiting during labour have led to its more frequent use in elective and emergency obstetrical anaesthesia (Cohen et al., 1984; Shaughnessy, 1985). MCP also prevents nausea and vomiting associated with epidural anaesthesia during elective Caesarean section (Chestnut et al., 1987). The antiemetic effects of MCP are thought to be mediated through both peripheral (gastrointestinal) and central sites. The drug is believed to raise the threshold of the chemoreceptor trigger zone through antagonism of dopamine receptors, and to decrease the sensitivity of visceral nerves which transmit...
afferent impulses from the gastrointestinal tract to the emetic centre in the lateral reticular formation (Pinder et al., 1976).

A decrease in renal plasma flow of about 20% has been reported in oncology patients receiving high doses of MCP (1-2.5 mg/kg) (Israel et al., 1986), and also a decrease in hepatic blood flow was noted in the rat at MCP doses of >25 mg/kg, resulting in dose-dependent kinetics (Tam et al., 1981a). MCP also stimulates the release of various hormones in man and in animals. Prolactin release was observed in the rat, healthy adults, pregnant women, children (Harrington et al., 1983; Arvela et al., 1983), neonates (Ruppert et al., 1986) and pregnant ewes (Fitzgerald and Cunningham, 1982). This effect is likely mediated by antagonism of the dopamine-mediated inhibition of prolactin secretion by the pituitary or hypothalamus. An increased level of plasma aldosterone was reported in rats, and man (Harrington et al., 1983; Sommers et al., 1988; Vonmoos et al., 1990) as a result of inhibition of central dopamine receptors (Albibi and McCallum, 1983) or acetylcholine release from post-ganglionic cholinergic nerve terminals within the adrenal cortex (Sommers et al., 1988). Stimulation of vasopressin secretion has also been reported in healthy human volunteers (Norbiato et al., 1986).

1.2. Pharmacokinetics of Metoclopramide

A two-compartment model adequately describes the disposition of MCP following i.v. administration in humans (Bateman et al., 1980). The drug is rapidly distributed with a relatively high volume of distribution in humans (2.2 to 3.4 L/Kg) indicating extensive extravascular distribution, which may be expected for a lipid-soluble,
basic drug. Rapid placental transfer occurs in man (Bylsma-Howell et al., 1983; Cohen et al., 1984) and in sheep (Riggs et al., 1988; 1989). MCP is extensively metabolised via sulphate and glucuronide conjugation in rabbit and humans (Arita et al., 1970; Cowan et al., 1976; Bateman et al., 1980), and by O-demethylation, N-deethylation and amide hydrolysis in rabbit, rat, and dog (Arita et al., 1970; Bakke and Segura, 1976; Cowan et al., 1976). The elimination half-life is ≈120 min in the dog (Bateman et al., 1980) and ≈50 min in the rat with a dose dependent increase at doses exceeding 15 mg/kg (Tam et al., 1981a). The dose dependent kinetics at higher doses are likely due to altered hepatic blood flow (Tam et al., 1981a), an observation confirmed using the Indocyanine Green (ICG) clearance method. The plasma elimination half-life in non-pregnant, maternal, and fetal sheep is 63 ± 14, 88 ± 17, and 113 ± 29 min, respectively (mean ± SEM), after an i.v. bolus dose (Riggs et al., 1988). The volume of distribution in sheep is higher (5.5 to 7.0 L/Kg) than in humans (2.2 to 4.0 L/Kg) (Riggs et al., 1988).

**Hepatic clearance and metabolism:** Within 24 hours of i.v. bolus MCP administration in humans, about 80% of the dose was excreted in the urine as free drug (=25% of the dose), glucuronide (=2-5% of the dose) and sulphate (=50% of the dose) conjugates (Teng et al., 1977; Bateman et al., 1980). The ratio of conjugates excreted from humans and a variety of animals is significantly different, suggesting significant species differences in MCP metabolism. Sulphate conjugation and glucuronic acid conjugation are the two major metabolic pathways for MCP in humans and the rabbit (Cowan et al., 1976). However, the sulphate and glucuronide conjugates of MCP have not been found in the dog and the rat, where the deethylation of MCP is the dominant metabolic pathway (Teng et al.,
In humans, the total body clearance approximates hepatic plasma flow (11.61 mL/min/Kg), with renal clearance (2.6 mL/min/Kg) accounting for ≈20% of total body clearance, suggesting the clearance is flow-limited rather than metabolic capacity-limited (Bateman et al., 1980). In sheep, the total body clearance is considerably higher (86.7 mL/min/Kg) compared to humans, which coincides with the higher hepatic blood flow in sheep (0.5-3.0 L/min) than humans (0.5-1.5 L/min). The liver is thought to be the major site of MCP elimination (Desmond and Watson, 1986). The elimination half-life of MCP in humans is 2.6 to 4.6 hours (Bateman et al., 1980; Graffner et al., 1979), whereas in sheep, it is much shorter (1.1-1.6 hours) (Riggs et al., 1988). A high total body clearance in sheep has also been observed with drugs such as diphenhydramine (Yoo et al., 1986), meperidine (Szeto et al., 1978), and lidocaine (Bloedow et al., 1980).

Renal failure: Studies of MCP kinetics in renal failure showed a significant decrease in total body clearance (Bateman et al., 1981; Tam et al., 1981b; Wright et al., 1988) with an increased terminal half-life in both humans and rat. However, the contribution of renal clearance of MCP as intact drug as a fraction of total body clearance is ≈20%, thus, it is not easy to explain the observed 2-4 fold decrease in total body clearance noted in renal failure both in humans and the rats. A renal failure-induced change in extrahepatic metabolism was proposed to explain the decreased total body clearance (Tam et al., 1981b), however, Kapil et al. (1984) ruled out significant extrahepatic metabolism using tissue metabolism studies in vitro. Diminished hepatic MCP metabolism secondary to renal failure was also suggested (Bateman., 1981; Kapil et al., 1984; Wright et al., 1988). Wright et al. (1988) speculated the presence of an unidentified substance in the plasma of
uremic patients which might inhibit MCP metabolism. Another possible explanation could be that the accumulation of metabolites, including glucuronide and sulphate conjugates, might reduce the activity of the metabolic enzymes involved in the elimination of the drug through a negative feedback mechanism (Stryer, 1988).

Hepatic cirrhosis: Increased MCP plasma concentrations are observed in hepatic cirrhosis patients (Hellstern et al., 1987; Magueur et al., 1991; Albani et al., 1991). A reduction of functional hepatic blood flow due to intra- and extra-hepatic shunting is the likely cause of the altered MCP kinetics with this condition (Magueur et al., 1991), although reduced hepatic metabolism cannot be ruled out. Plasma concentrations of α₁-acid glycoprotein, the major binding protein of MCP, is stable in cirrhosis (Kremer et al., 1988) and the plasma binding of MCP is relatively low, i.e. ≈40% (Webb et al., 1986). Therefore, the protein binding (i.e. free fraction) and tissue distribution of MCP are unlikely to be affected. Phase II pathways of metabolism i.e. conjugation reactions, which account for the majority of MCP elimination in humans, are not significantly affected by liver dysfunction (Magueur et al., 1991).

Therefore, from the data describing MCP kinetics associated with renal and hepatic dysfunction, it is suggested that disease-induced changes in MCP kinetics may be due to altered blood flow distribution and changes in intrinsic capacity for drug metabolism.
1.3. **Hypoxemia and Associated Physiological Changes**

1.3.1. *Hypoxemia and hypoxia*

Hypoxemia is a reduction in blood PO$_2$, whereas hypoxia is a fall in tissue O$_2$ utilization when tissue oxygen delivery is not sufficient to meet normal metabolic demands. Hypoxia can be a consequence of severe hypoxemia, but can also occur without a reduction in blood PO$_2$, as in the case of carbon monoxide poisoning or severe anemia. Hypoxia is usually accompanied by metabolic/lactic acidosis due to anaerobic metabolism. When hypoxemia causes PO$_2$ to drop below the level that is required to maintain the oxygen diffusion gradient from capillary blood to mitochondria, lactic acidosis will occur due to anaerobic glycolysis (Denison, 1986). Oxidative phosphorylation accounts for about 85 per cent of total body oxygen consumption (Weibel, 1984). However, oxidative phosphorylation is not likely affected by local PO$_2$, since the Michaelis affinity constant ($K_m$O$_2$) of cytochrome a$_3$ oxidase for oxygen is below 1 mm Hg. Other enzymes involved in the oxygen-consuming processes such as hydroxylases, oxygenases, and oxidases (except cytochrome a$_3$), have much higher and widely differing $K_m$O$_2$, ranging from 5 to 250 mm Hg, therefore these processes are much more vulnerable to hypoxemia. When the body is deprived of oxygen progressively, quantitatively minor but qualitatively important processes will fail long before there is a noticeable impairment of total body oxygen uptake or oxidative phosphorylation (Denison, 1986). Thus, for example, some oxidative drug metabolism may be affected in relative mild hypoxia/hypoxemia (Jones, 1981).
### 1.3.2. Physiological changes during hypoxemia

Compensatory response mechanisms during hypoxemia consist of:

1. *increased oxygen extraction and decreased oxygen demand in tissues.*
2. *increased cardiac output.*
3. *preferential redistribution of available oxygen supply.*

The rate at which oxygen is delivered to the tissues by the arterial blood is the product of cardiac output and the arterial oxygen concentration. When arterial O\(_2\) concentration is reduced as in hypoxemia, oxygen delivery can be maintained, or at least its fall can be limited, by:

A. *increasing oxygen extraction and decreasing oxygen demand in tissues:* Cain and Chapler (1979) examined the oxygen extraction (i.e. increasing the arterial-venous oxygen concentration difference) by the canine hind limb during hypoxic hypoxia (i.e. hypoxia due to reduced inspired oxygen content). A significant increase in oxygen extraction during hypoxemia was observed (i.e. ranging from \(=20\%\) in normoxemia to \(=60\%\) in moderate and to \(=80\%\) in severe hypoxemia). Oxygen uptake by tissue was maintained during moderate hypoxemia, therefore no metabolic vasodilatory effect was developed. However, during severe hypoxemia, a significant decrease in oxygen uptake, an increase in cardiac output and limb blood flow, and decrease in total and peripheral resistance were observed (Cain and Chapler, 1979). This suggests that prolonged severe hypoxemia (>20 min) causes an increased blood flow by local metabolic vasodilation in spite of any centrally mediated constrictor action [autoregulatory escape] (Granger et al., 1975;1976). An increase in O\(_2\) extraction causes a fall in vascular PO\(_2\). Therefore, this
compensatory mechanism will likely be limited by the onset of autoregulatory escape i.e. local metabolic vasodilation, since a critical arterial oxygen level (PaO₂ = ~10-12 mm Hg) in tissues is needed to maintain an adequate O₂ diffusion gradient from blood to mitochondria (Connett et al., 1990).

B. increasing cardiac output: An elevated aortic blood flow is associated primarily with an increase in heart rate which is mediated in large part by enhanced sympathoadrenal activity (Krasney, 1967), and a fall in systemic vascular resistance (Kontos et al., 1965; Sylvester et al., 1979). A net increase in cardiac output and arterial pressure (Kontos et al., 1967) is mediated by the peripheral chemoreceptor interactions, such as a pulmonary vasodilator reflex (Daly and Robinson, 1968), the local vasodilating action of hypoxia (Daugherty et al., 1967), and in addition, by the direct central nervous system action of hypoxia (Downing et al., 1963).

The preferential redistribution of cardiac output during hypoxia was reported in the dog (Krasney, 1971; Adachi et al., 1976) and in the sheep (Nesarajah et al., 1983). Redistribution of blood flow is accomplished by local effects of hypoxia, which produces vasodilation in coronary and cerebral vessels, and by chemoreceptor reflex, which produces vasoconstriction in skeletal muscle and the splanchnic bed and dilation in coronary vessels (Heistad and Abboud, 1980). Superior caval flow was enhanced at the expense of inferior caval flow during hypoxia, but this hypoxic redistribution of systemic flow was abolished by chemoreceptor denervation (Krasney, 1971; Malo et al., 1984). Carotid and aortic chemoreceptors provide for reflex vasoconstrictive support of the circulation during systemic hypoxia (Chalmer et al., 1965; Daly and Scott, 1964), which
leads to preferential redistribution of blood flow to the cephalic region during hypoxia, with a decline in splanchnic blood flow. Local flow is altered variably in hypoxia depending upon the relative balance of vasoconstrictor and vasodilator components, which is influenced by the level of oxygen in the blood (Adachi et al., 1976). For example, the pulmonary circulation is very sensitive to the decrease in the \( \text{PO}_2 \), which alters the regional distribution of pulmonary arterial blood flow [hypoxic vasoconstriction] (Benjamin and Gorlin, 1952; West, 1988). In contrast, the coronary circulation exhibits a very sensitive vasodilator response to the local decrease in \( \text{PO}_2 \) (Gregg, 1950; Adachi et al., 1976). The main determinants of the distribution of cardiac output are the relative arterial flow resistance and venous pressures (Mitzre and Goldberg, 1975), and these changes in the distribution of cardiac output reflect alteration in the arterial pressure-flow relationship of each vascular bed (Malo et al., 1984).

The preferential redistribution of cardiac output and reduced oxygen demand in non-essential tissues (e.g. digestive system and skeletal muscles) will temporarily compensate for a reduced oxygen supply up to a certain limit. However, this mechanism is also limited by the development of systemic and local lactic acidosis as a consequence of the increased anaerobic glycolysis in tissues, which in turn causes local vasodilation (Nesarajah et al., 1983). Furthermore, acidosis will cause a decrease in the hemoglobin \( \text{O}_2 \) saturation by the Bohr effect, which is followed by reduced oxygen delivery and a further drop in pH, ultimately compromising the oxygen delivery to the vital organs (Nesarajah et al., 1983)
Hepatic haemodynamics and function: Moderate hypoxemia in cats (Larsen et al., 1976) and in dogs (Hughes et al., 1979; Scholtholt and Shiraishi, 1970) does not significantly alter either hepatic arterial or portal venous conductance. However, more severe hypoxemia in the dog significantly increases systemic arterial pressure and reduces hepatic arterial blood flow, indicating increased hepatic arterial vascular resistance (Hughes et al., 1979). Portal venous blood flow seems to be unaffected by hypoxemia (Scholtholt and Shiraishi, 1970; Larsen et al., 1976, Ishikawa et al., 1974; Hughes et al., 1979), but Mathie and Blumgart (1983) showed a small but significant increase in portal venous flow. These different observations are likely related to the precise timing of measurement and the level of hypoxemia chosen for the investigation. Overall, the effects of hypoxemia on hepatic haemodynamics seem to be minor.

Alternatively, Roth and Rubin (1976) showed a significant decrease in total hepatic and portal venous flow, with a slight increase in hepatic arterial flow in a study of hypoxic hypoxia. This discrepancy is likely due to the hypocapnia which results from hyperventilation. Hypercapnia and hypocapnia have been shown to affect total hepatic flow significantly (Mathie and Blumgart, 1983; Hughes et al., 1979). Hypercapnia tends to increase total hepatic and portal venous flow, due to decreased mesenteric vascular resistance (Mathie and Blumgart, 1983).

It has been suggested that hepatic function is more sensitive to hypoxemia than other organs in spite of minor alteration in haemodynamics, because ≈70% of hepatic blood supply is venous i.e. low blood oxygen tension (Preisig et al., 1972). On the other
hand, the liver receives nearly 25% of the total cardiac output and has a low oxygen extraction ratio \( i.e. \approx 15-20\% \) \( (\text{Rowell et al., 1984}) \). Studies on humans and other species \( (\text{Rowell et al., 1968; 1984}) \) showed that the liver can extract nearly 100\% of the available \( \text{O}_2 \) and this increase in oxygen extraction is the major adjustment observed in response to reduced hepatic \( \text{O}_2 \) delivery \( (\text{Larsen et al., 1976; Tashkin et al., 1972}) \). Despite this efficient \( \text{O}_2 \) extraction, abnormalities in sulfobromophthalein (BSP) retention \( (\text{Shorey et al., 1969}) \) and bile flow \( (\text{Larsen et al., 1976}) \) were observed during moderate hypoxemia. The extraction of Indocyanine Green was also reduced during both moderate and severe hypoxemia \( (\text{Blackmon and Rowell, 1986; Marleau et al., 1987}) \). However, Blackmon and Rowell \( (1986) \) showed that acute hypoxemia in humans did not affect splanchnic \( \text{O}_2 \) uptake in spite of a fall in hepatic venous \( \text{O}_2 \) content. In addition, hepatic glucose release and lactate uptake were not affected by acute hypoxemia in this study.

**Renal function:** Hypoxemia influences the renal excretion of sodium and water. It has been long known that acute moderate hypoxemia \( (\text{PaO}_2 > 40 \text{ mm Hg}) \) causes an increase in urine volume (diuresis) and renal excretion of sodium and chloride in healthy volunteers \( (\text{Burrill et al., 1945; Berger et al., 1949; Kilburn et al., 1971}) \). Acute moderate hypoxemia also causes renal vasodilation, thereby decreasing renal vascular resistance. Thus, mean renal blood flow is maintained despite reduced mean arterial pressure. There are significant changes in intrarenal haemodynamics without significant changes in excretory functions such as glomerular filtration rate and sodium excretion \( (\text{Zillig et al., 1978}) \). Diuresis during moderate hypoxemia seems to be related to decreased plasma aldosterone \( (\text{Colice and Ramirez, 1985}) \) and angiotensin II \( (\text{Vonmoos et al., 1990}) \) levels,
and increased atrial natriuretic factor concentrations (du Souich et al., 1987). Basic renal metabolism is also maintained in moderate hypoxemia in spite of reduced oxygen delivery (Sinagowitz et al., 1976). In patients with chronic obstructive pulmonary disease, renal physiological functions such as renal perfusion, glomerular filtration and sodium excretion were reduced abruptly during severe hypoxemia (PaO₂ < 40 mm Hg) or hypercapnia (PaCO₂ > 60 mm Hg) (Kilburn et al., 1971; Bruns, 1978).

1.4. Respiratory Disorders and Drug Disposition and Metabolism

The effects of respiratory disorders including hypoxemia on drug disposition and metabolism are the subject of several reviews (du Souich et al., 1978; Farrell, 1987; Jones et al., 1989; and Taburet et al., 1990). The onset of hypoxemia is the common pathway of most of the frequently observed respiratory disorders such as asthma and chronic obstructive pulmonary disease (COPD). Changes in PaCO₂ and blood pH are also associated with hypoxemia. In severe pneumonia, pulmonary edema and pulmonary embolism, hypoxemia is associated with hypocapnia and respiratory alkalosis (West, 1988), whereas in COPD, hypoxemia is associated with hypercapnia and respiratory acidosis. The increased adverse effects and toxicity associated with clinical dose administration of digoxin (Smith, 1975; Doherty et al., 1977) and theophylline (Zwillich et al., 1975; Hendeles et al., 1977) to patients with pulmonary disorders suggests that changes in drug clearance is of major significance.
1.4.1. *Effects of acute and chronic hypoxemia on drug metabolism*

Merritt and Medina (1968) observed that hexobarbital sleeping time in mice was reduced at altitude. A low plasma concentration of hexobarbital at altitude was reported in the study. The *in vitro* metabolism of hexobarbital in hepatic microsomes was higher in the preparation from the rats at altitude than those from the rats at ground level. Similar results were shown in many drugs undergoing oxidative metabolism such as zoxazolamine, phenobarbital, and phenylbutazone (Medina and Merritt, 1970).

In contrast, Cumming and Mannering (1970) reported increased hexobarbital half-life at low $P_O_2$ (≈45 mmHg) in both isolated liver and *in vivo*, and proved reduced hepatic metabolism at lower $P_O_2$. Roth and Rubin (1976) further examined the relationship between hepatic blood flow and hexobarbital metabolism in hypoxic-hypoxemia and carbon monoxide (CO) induced hypoxemia. Both CO-induced and hypoxic hypoxemia decreased $P_aO_2$ and oxygen saturation, whereas $P_aCO_2$ is reduced only in hypoxic hypoxemia (hypocapnia due to hyperventilation). A redistribution of cardiac output induced by hypoxemia (*i.e.* 33% reduction in hepatic blood flow) was observed in hypoxic hypoxia/hypocapnia, resulting in reduced hepatic metabolism of hexobarbital. These experiments seemed to give contradictory results from the previous studies (Merritt and Medina, 1968; Medina and Merritt, 1970), however, we should consider that rats at altitude in the studies of Medina and Merritt were exposed to chronic hypoxemia (*i.e.* 5 days), whereas the studies of Cumming and Mannering (1970) and Roth and Rubin (1976) involved acute hypoxemia.
These studies demonstrated the different effects of acute and chronic hypoxemia. Acute hypoxia is usually associated with impaired hepatic drug metabolism (Jones, 1981), whereas chronic hypoxia may actually stimulate oxidative drug metabolizing enzyme systems [induction] (du Souich et al., 1978). Therefore, the pharmacokinetic study during hypoxemia must take into account the different effects of acute and chronic hypoxemia on drug metabolism and, in turn, drug kinetics.

1.4.2. Effects of hypoxemia on hepatic drug metabolism

It is valuable to examine the studies on antipyrine kinetics during hypoxemia, since antipyrine elimination seems to be proportional to the non-specific hepatic microsomal enzyme activity (Kolmodine et al., 1969). In addition, antipyrine is distributed evenly throughout body water, and metabolized in the liver, without any extensive excretion by the kidney in an unchanged form. (Laybourn et al., 1986). Cumming (1976) observed that patients with low PaO₂ (less than 55 mmHg) had a prolonged antipyrine half-life (18.4 hr) compared with those whose PaO₂ were greater than 55 mm Hg (8.4 hr). Laybourn et al. (1986) also found lower antipyrine clearance \( (i.e. \approx 18\%)} \) in patients with pulmonary disease than in healthy volunteers. In contrast, Agnihotri et al. (1978) observed enhanced antipyrine clearance in patients with COPD. Some patients showed hypercapnia, which could cause an increase in hepatic blood flow (Hughes et al., 1979) and, in turn, hepatic drug clearance. Patients with COPD are also likely to suffer chronic hypoxemia, thus leading to the induction of hepatic enzyme
systems (du Souich et al., 1978). Therefore, these contradicting observations may have resulted from different clinical conditions in these studies.

1.4.3. Effects of hypoxemia on drug disposition and protein binding

Du Souich et al. (1978) suggested that the effects of respiratory disease on the efficacy of most long term antibiotic usage is likely to be minimal, since the therapeutic indices are wide. However, hypoxemia causes significant alteration in aminoglycoside serum pharmacokinetics. Myers et al. (1977) showed a significant increase in half-life of amikacin in hypoxic (PaO$_2$ < 50 mm Hg) infants (7.3 hr) compared to normoxic infants (4.8 hr). The decreased clearance in hypoxemia is likely due to a reduced renal blood flow, with a concomitant decrease in glomerular filtration rate from renal vasoconstriction (Bruns, 1978). Hypoxic stimulation of aortic chemoreceptors results in renal vasoconstriction (Korner, 1963). A reduction in amikacin and gentamicin clearance during hypoxemia was observed in rat and rabbit (Mirhij et al., 1978). The apparent volume of distribution was not affected by hypoxemia, thus the change in drug clearance is directly related to a change in drug elimination. Since aminoglycoside excretion is almost entirely mediated by glomerular filtration (Levy et al. 1975), the alteration in renal excretion during hypoxemia could account for the change in amikacin elimination.

Very different results were observed with sulphamethazine (du Souich and Couteau, 1984). Significant increases in the apparent volume of distribution and non-renal clearance were observed in rabbits during hypoxemia and metabolic acidosis. An increase in the apparent volume of distribution during hypoxemia is probably related to
decreased protein binding of this drug, thus leading to an increase in sulphamethazine free fraction. Since the pKa of sulphamethazine is 7.4, a change in plasma pH associated with pulmonary disorders will affect the ionization of sulphamethazine. With hypercapnia and metabolic acidosis, the proportion of non-ionized sulphamethazine increased (the ratio of ionized to non-ionized sulphamethazine decreased by 42%), and this subsequently may affect the distribution and clearance of sulphamethazine. However, the changes in blood pH and subsequent alteration in renal elimination will have various results, since the increase in non-ionized drug may not only enhance drug diffusion (increasing volume of distribution, glomerular filtration rate and non-renal clearance), but also increase tubular re-absorption (decreasing renal clearance). Since these changes in disposition can counter balance each other, the consequence appears that the changes in pH associated with hypercapnia only slightly affects sulphamethazine kinetics. In contrast, hypoxemia, which has little effect on blood pH, significantly increased sulphamethazine free fraction, thereby significantly increasing non-renal clearance and exerting a greater effect on drug kinetics (du Souich et al., 1978; du Souich and Couteau, 1984). Drugs such as phenobarbital (Waddell and Butler, 1957), the salicylates (Hill, 1971) and sulphaethidole (Kostenbauder et al., 1962; Dettli et al., 1967) show an increased volume of distribution and decreased renal elimination during hypoxemia.
1.4.4. *Digoxin kinetics in hypoxemia*

An increased sensitivity to digitalis in patients with COPD/hypoxemia (Baum *et al.*, 1956; Baum *et al.*, 1959; Morrison and Killip, 1971) and in dogs with hypoxia (Beller and Smith, 1972; Harrison *et al.* 1968) has been reported. It was suggested that hypoxemia decreases digoxin plasma concentration, but increases myocardial digoxin levels in anaesthetized and artificially ventilated dogs (Harron *et al.*, 1978), however, this observation (increased myocardial digoxin level) was later contradicted (Saito *et al.*, 1981; du Souich *et al.*, 1985a,b). Du Souich *et al.*, (1985a) showed plasma concentrations of digoxin were lower in hypoxemic and hypercapnic dogs. This decreased digoxin plasma concentration is probably due to an increase in the apparent total body clearance (~45%) and the volume of distribution (~36%), mostly in the peripheral compartments. Digoxin tissue concentrations were increased during hypoxemia-hypercapnia, especially in liver (~15%), which suggests that the increase in total body clearance is mainly due to an increase in hepatic clearance. An increase in hepatic blood flow has been reported during hypercapnia (Dutton *et al.*, 1976; Hughes *et al.*, 1979), further supporting the speculation of increased hepatic clearance, since many drugs exhibit blood flow-limited metabolic rate. Increased digoxin binding to erythrocyte membranes was also reported during hypoxemia/hypercapnia (du Souich *et al.*, 1985a), and there seems to be a direct relationship between erythrocyte digoxin receptor and cardiac glycoside receptor (Aker, 1977) and volume of distribution (Aronson and Graham-Smith 1976; 1977). Therefore, it is speculated that the change in the distribution pattern is partly due to the change in receptor binding during hypoxemia and hypercapnia.
The influence of hypoxemia on digoxin kinetics and tissue distribution was further examined using tritiated digoxin by du Souich *et al.* (1985b). Similar to the hypoxemia-hypercapnia study (du Souich *et al.*, 1985a), a significant change in volume of distribution was observed during hypoxemia (du Souich *et al.*, 1985b). However, this increase in the volume of distribution was largely due to an increase in the size of the central compartment rather than the peripheral compartment. Therefore, the increase in the volume of distribution is likely due to a significant increase in plasma protein binding of digoxin during hypoxemia. Total body clearance of digoxin was not significantly changed, but an increase in renal clearance and a decrease in non-renal clearance occurred during hypoxemia (du Souich *et al.*, 1985b). Unlike with the hypoxemia-hypercapnia study (du Souich *et al.*, 1985a), no hepatic accumulation of digoxin was observed. These results on hepatic digoxin accumulation are consistent with the findings regarding hepatic blood flow in hypoxemia (Larsen *et al.*, 1976; Richardson and Withrington, 1981) and hypoxemia/hypercapnia (Hughe *et al.*, 1979; Mathie and Blumgart, 1983). Increased hepatic blood flow during hypoxemia-hypercapnia resulted in hepatic accumulation of digoxin, whereas hypoxemia alone did not alter hepatic blood flow or hepatic digoxin accumulation.

1.4.5. Theophylline kinetics and respiratory disorders

There has been extensive study of theophylline kinetics during respiratory disorders, because theophylline is a potent bronchodilator used in the management of asthma and chronic obstructive airway diseases (Hendeles *et al.*, 1983,1985,1986;
Ogilvie, 1978). Kolbeck et al. (1979) examined the influence of acute respiratory acidosis and alkalosis on the volume of distribution and half-life in dogs. No changes in volume of distribution were noted, but there was a reduced half-life (i.e. increased elimination rate) during respiratory acidosis. Since only 8% of a dose of theophylline was recovered in urine as the parent compound (Ogilvie, 1978), the change in clearance was likely due to the change in metabolic clearance. However, Clozel et al. (1981) found no significant change in theophylline kinetics during respiratory acidosis in dogs, which is contrary to the finding of Kolbeck et al. (1979). A reduced theophylline clearance was observed in hepatic cirrhosis patients (Mangione et al. 1978), while smokers were found to have increased clearance (Powell et al., 1977;1978). These results further suggest that theophylline is mainly eliminated by hepatic microsomal oxidative enzymes.

Letarte and du Souich (1984) observed increased theophylline serum concentrations during hypoxemia and/or hypercapnia, but not with metabolic acidosis in rabbits. The decreased clearance was mainly due to a reduction in non-renal theophylline clearance, thus suggesting a reduction in theophylline biotransformation. A decreased volume of distribution was also observed with hypoxemia, therefore the elimination rate constant was not significantly affected during hypoxemia unlike with hypercapnia and hypoxemia/hypercapnia. Saunier et al. (1987) further examined the effects of acute and chronic hypoxemia on theophylline disposition in conscious dogs. In contrast to the previous studies, no changes in theophylline disposition were observed. Furthermore, the recovery of theophylline and its metabolites, 1,3-dimethyluric acid and 3-methylxanthine, in urine was not significantly different from control.
1.4.6. Lidocaine kinetics/metabolism during hypoxemia and similarity with MCP

The available data on lidocaine kinetics during hypoxemia/hypercapnia may provide some insight about the effects of these perturbations on MCP kinetics, since these two drugs share some similar chemical structure and \textit{in vivo} metabolism and pharmacokinetic properties, despite their rather different pharmacological effects.

Marleau \textit{et al.} (1987) examined the effects of hypoxemia and/or hypercapnia on lidocaine and Indocyanine Green kinetics in rabbits. In humans, lidocaine is extensively and rapidly metabolised in the liver \textit{i.e.} it is characterized by a hepatic extraction ratio of 70\% (Stenson \textit{et al.}, 1971), thus the hepatic clearance is likely to be a flow-limited process, just as with ICG and MCP. The apparent volume of distribution and the total body clearance of lidocaine were not significantly affected at lower lidocaine doses (130 mg/min/Kg, infusion). The serum to cerebrospinal fluid ratio of lidocaine was not modified with a low lidocaine dose, but slight decreases in lidocaine clearance were observed with higher doses (infusion, 260 mg/min/Kg). An increased serum level of one metabolite, N-monoethyl-glycinexylidide (MEGX), was observed with hypercapnia and hypoxemia-hypercapnia (Marleau \textit{et al.}, 1987). The increased plasma MEGX concentration is more likely due to a decrease in the 3-hydroxylation of MEGX, rather than due to a decrease in the deethylation of MEGX to glycinexylidide (GX) (Suzuki \textit{et al.}, 1984), therefore the elimination of MEGX is reduced while the GX plasma concentration was similar in hypercapnia and hypoxemia-hypercapnia.
Scheme I  Comparison of the N-deethylation reactions of MCP and lidocaine

This biotransformation *i.e.* deethylation of lidocaine to MEGX and GX may share similar characteristics with the metabolism of MCP to mono- and di-deethyl MCP. Du Souich *et al.* (1992) observed increased plasma MEGX and GX concentrations during hypoxemia in a lidocaine infusion study in dogs, whereas plasma lidocaine concentrations remained unchanged. The radioactive microsphere method was used to estimate hepatic, renal and cerebral blood flows three hours after the end of lidocaine infusion. With acute moderate hypoxemia, there was an increase in cerebral blood flow, but renal and hepatic blood flows were not affected. It was concluded that acute moderate hypoxemia decreased the rate of elimination of both active metabolites of lidocaine without
modifying the perfusion to the organs responsible for their elimination (du Souich et al., 1992).

1.5. Rationale

There is substantial evidence that pulmonary dysfunction causing hypoxemia influences drug disposition and metabolism. These changes are likely either a direct consequence of the perturbations in blood gas and acid-base status (e.g. respiratory alkalosis due to hyperventilation and lactic acidosis) or a result of the cardiovascular and metabolic responses to hypoxemia (e.g. redistribution of cardiac output). There is also a possibility that therapeutic agents administered will interfere with the compensatory response to hypoxemia. A number of pharmacokinetic parameters were altered in hypoxemic conditions. However, the changes were highly variable and dependent on the chemical characteristics of the specific drug, the prevailing blood gas/acid-base conditions, protein binding and the species used in the experiments. These changes were likely caused by various physiological changes induced by hypoxemia/acidosis as described in Section 1.4.

The study of MCP kinetics and pharmacodynamics during hypoxemia will result in an improvement of our understanding in the following areas:

1. A pharmacokinetic study during hypoxemia will provide information on the changes in both MCP disposition and metabolism.
2. A study of metabolite kinetics will provide information on alteration in drug metabolism during hypoxemia. The assessment of drug metabolism is important, since there is increasing evidence that the metabolites of some drugs (e.g. morphine glucuronides (Mulder, 1992) and mono- and di-deethylated lidocaine, MEGX and GX (Narang et al., 1978)) have their own pharmacological activity (agonistic and/or antagonistic) and, in selected instances, toxicity. This toxicological response may be altered during hypoxemia and hypoxia.

The past studies of MCP have provided a solid base of pharmacokinetic, metabolic and pharmacodynamic information in normoxemic non-pregnant and pregnant sheep. The present study will examine of the effect of hypoxemia/hypoxia on all the salient parameters characterizing MCP in non-pregnant sheep. Such a study will provide essential ground work for future studies of the effects of hypoxemia on maternal and fetal MCP disposition and metabolism during pregnancy.

1.6. **Objectives**

The objectives of the present study are:

1. to confirm the applicability in a hypoxemia study of an existing GC-MSD assay method for MCP and its metabolites, mono- and di-deethyl MCP.
2. to determine the extent to which the pharmacokinetics of MCP and its metabolites are altered by reduced oxygen supply.
3. to examine physiological changes induced by hypoxemia during steady-state drug administration.
2. EXPERIMENTAL

2.1 Materials and Supplies

2.1.1 Chemicals

4-amino-5-chloro-2-methoxy-N,N-diethyl-aminoethy-l-benzamide monohydrochloride monohydrate (MCP-HCl-H\textsubscript{2}O) and MCP-HCL 5 mg/mL (Reglan® Injectable, 2 and 5 mL ampoules) were obtained from A.H. Robins Research Laboratories (Richmond, VA, USA) and A.H. Robins Canada Inc. (Montreal, PQ). 4-amino-5-chloro-2-methoxy-N-ethyl-aminoethyl-benzamide monohydrochloride monohydrate (monodeethyl MCP-HCl-H\textsubscript{2}O) and 4-amino-5-chloro-2-methoxy-aminoethyl-benzamide monohydrochloride monohydrate (dideethyl MCP-HCl-H\textsubscript{2}O) were synthesized in our laboratory (Riggs et al., 1994). The internal standard, 4-amino-5-chloro-2(2-butanone-3-yl)-oxy-N,N-diethylaminoethyl benzamide monohydrochloride monohydrate (BMY-HCl-H\textsubscript{2}O) was supplied by Bristol-Myers Squibb Co. (Wallingford, CT, USA).

2.1.2 Reagents

Sodium acetate and 2-amino-2-hydroxymethyl-1,3-propanediol (TRIS free base) were obtained from BDH Chemicals, Toronto, ON. American Chemical Society (ACS) reagent grade Sodium Hydroxide pellets were obtained from Fisher Scientific Co., Fair Lawn, NJ. ACS reagent grade Hydrochloric acid 37% was obtained from American Scientific and Chemical, Seattle, WA. Ammonia Solution 25% was obtained from Fisher
Scientific Co. Heptafluorobutyric anhydride (HFBA) and triethylamine (TEA) sequanal Grade were obtained from Pierce Chemical Co., Rockford, IL, USA.

2.1.3 Enzymes

Glucuronidase (Glucurase®: β-D-Glucuronide glucuronosohydrolase: EC 3.2.1.3 (bovine liver) approx. 5000 Sigma units/mL, acetate buffered to pH 5.0 at 25°C) and sulfatase (Arylsulfatase, aryl-sulfate sulfohydrolase, phenolsulfatase; EC 3.1.6.1 19 units/mL partially purified enzyme in 50% glycerol-0.01 M Tris solution, pH 7.5 [Lot 42H6814]) were purchased from Sigma Chemical Co., St. Louis, MO, USA.

2.1.4 Solvents

Toluene, and dichloromethane (distilled in glass) were purchased from Caledon Laboratory. Inc., Georgetown, Ont.. Deionized water was produced on site using a Milli-Q® system, Millipore Corp., Bedford, MA.. ACS reagent grade methanol and acetone were obtained from BDH Chemicals, Toronto, ON.

2.1.5 Gases

Hydrogen Ultra High Purity (UHP), helium UHP and argon/methane (95:5) were obtained from Matheson Gas Products Canada Ltd., Edmonton, AB.
2.1.6. Supplies for animal studies

The following supplies was used in the animal studies: needles and plastic disposable Lure-Lok® syringes for drug administration and sample collection (Becton-Dickinson Canada, Mississauga, ON); heparinized Vacutainer® tubes (Vacutainer Systems, Rutherford, NJ, USA); 15 mL Pyrex® disposable glass culture tubes (Corning Glass works, Corning, NY, USA); polytetrafluoroethylene (PTFE) lined screw caps (Canlab, Vancouver, BC); silicone rubber tubing for catheter preparation (Dow Corning, Midland, MI, USA).

2.2. Stock and Reagent Solutions

Metoclopramide•HCl•H₂O (=11.82 mg of MCP•HCl•H₂O is equivalent to =10 mg of MCP free base), monodeethyl MCP•HCl•H₂O (=12.00 mg of mdMCP•HCl•H₂O is equivalent to =10 mg of mdMCP free base) and dideethyl MCP•HCl•H₂O (=12.24 mg of ddMCP•HCl•H₂O is equivalent to =10 mg of ddMCP free base) were accurately weighed and dissolved in HPLC grade water in individual volumetric flasks. Using serial dilution, these stock solutions were added to a combined stock solution of final concentration of = 0.04 μg/mL each. The internal standard, BMY•HCl•H₂O (=11.03 mg of BMY•HCl•H₂O is equivalent to =10 mg of BMY free base) was accurately weighed and dissolved in deionized water using serial dilution to a final concentration of =0.2 μg/mL. These stock solutions were stored at 4°C for up to two months.

Sodium hydroxide (NaOH), as 1M and 5M solutions, was prepared by dissolving NaOH pellets in deionized water. Aqueous ammonia (4%) solution was prepared by
diluting ammonia solution strong (27%) in deionized water. Triethylamine 0.0125 M in toluene was prepared by diluting triethylamine with toluene and subsequently adding four or five NaOH pellets to the resulting solution.

A 0.2 M (pH 5.0) sodium acetate buffer, for glucuronidase incubation, was prepared by dissolving sodium acetate in deionized water and adjusting this solution to a final pH of 5.0 using glacial acetic acid. TRIS buffer, 0.05 M (pH 7.5), for sulphatase incubation was prepared by dissolving TRIS free base in deionized water and adjusting the final pH of this solution to 7.5 using a 1 M HCl solution.

2.3 Sample Preparation, Extraction and Derivatization

The procedure for sample extraction and derivatization remained the same as published by Riggs et al. (1994).

All glassware used in the preparation of stock solutions and extraction was pre-washed with detergent in an automatic dishwasher and then soaked in chromic acid for a minimum of six hours. Thereafter, it was thoroughly rinsed with tap water over 6 hours using a mechanical rinser and finally with distilled/deionized water before being dried overnight.

2.3.1 Sample extraction

All extractions were carried out in 15 mL and 10 mL glass culture tubes with PTFE-lined screw caps. Plasma (100-200 μL) and urine (100-200 μL of 50x dilution in deionized water) obtained from animal studies were added to clean 15 mL tubes
containing 0.5 mL 1M NaOH (pH \approx 14) and 0.3 mL of BMY internal standard solution (\approx 0.2 \mu g/mL). The aqueous phase was then adjusted to a final volume of 2.2 mL with deionized water. Six mL of dichloromethane was added, tubes capped, and MCP and its selective metabolites and BMY were extracted into the organic layer by shaking for 20 min on a rotary shaker (Labquake® model 415-110, Lab Industries, Berkeley, CA, USA). After shaking, tubes were stored in a freezer (\approx -20°C) for 10 min. Following centrifugation at 2500 rpm for 5 min, the tubes were removed and shaken lightly to break any emulsion that may have formed during the extraction process. Following a further 5 min of centrifugation, the aqueous layer was vacuum aspirated and discarded. The remaining organic layer was transferred to clean 10 mL tubes using Pasteur pipettes and taken to dryness using an AS290 Automatic SpeedVac® concentrator (Savant Instruments Inc., Farmingdale, NY, USA) under controlled vacuum. The dried residue was reconstituted with 0.8 mL of toluene containing 0.0125 M TEA. A 20 \mu L volume of heptafluorobutyric anhydride (HFBA) was added, tubes capped, vortex-mixed (Vortex-Genie®, Fisher Scientific Industries, Springfield, MA, USA) and these mixtures were placed in an oven at 55°C for 60 min. After cooling to room temperature, the excess derivatizing agent (HFBA) was removed by hydrolysis with 0.5 mL of deionized water (vortex-mix for 10 sec) and neutralized with 0.5 mL of 4% ammonia solution (vortex-mix for 10 sec). Following centrifugation of the tubes at 2500 rpm for 1 min, the toluene layer was immediately transferred to a clean autosampler vial with a glass insert and capped with a PTFE-lined aluminum seal. A 2 \mu L volume aliquot was injected into the GC-MSD for the final assay measurement.
Scheme II  Extraction procedure for MCP and its metabolites

Plasma and urine samples (0.1-0.2 mL)  Blank plasma or urine samples spiked with MCP/metabolite standard

1M NaOH, 0.5 mL  BMY (I.S.), 0.3 mL  Water q.s. 2.0 mL  Dichloromethane, 6 mL  Shake, 20 min  Centrifuge, 2 X 5 min

Organic layer

Aspirate aqueous layer  Evaporate to dryness under reduced pressure at 40°C

Dried extract

0.0125 M TEA in toluene, 0.8 mL  HFBA, 20 μL  1 hour at 55°C

Cool to room temperature

Water, 0.5 mL, vortex 10 sec  4% aq. ammonia, 0.5 mL, vortex 10 sec  Centrifuge, 1 min

Discard aqueous layer  Inject into GC/MSD (2 μL)
2.3.2 Analysis of glucuronide and sulphate conjugates

The presence of glucuronide and sulphate conjugates of MCP, mdMCP and ddMCP in urine was determined by enzymatic hydrolysis. The hydrolysis procedure used in the present study was a modified version of Brasher et al. (1988) for the determination of ritodrine conjugates in humans. Urine samples were divided into 3 aliquots of 0.1 mL to determine the concentrations of the compounds in non-conjugated form [Set I], glucuronide-conjugated form [Set II] and sulphate-conjugated form [Set III].

Non-conjugated [Set I]: A volume of 0.9 mL of deionized water was added to each aliquot.

Glucuronide-conjugated [Set II]: Volumes of 0.4 mL 0.2 M sodium acetate buffer (pH 5.0) and 0.5 mL of glucuronidase were added to each aliquot to provide a glucuronidase activity of 2500 U/mL.

Sulphate-conjugated [Set III]: A volume of 0.9 mL of diluted sulphatase solution in 0.05 M TRIS buffer (pH 7.5) was added each aliquot to give a final sulphatase activity of 0.25 U/mL.

All the aliquots were incubated overnight at 37°C in a water bath. Following incubation, the samples were cooled to room temperature and extracted as described in Section 2.3.1. Non-conjugated MCP/mdMCP/ddMCP were measured from the samples in Set I. The concentrations of glucuronide-conjugated MCP/mdMCP/ddMCP were
calculated by deducting non-conjugated concentrations from the samples in Set II. The concentration of sulphate-conjugates was calculated similarly from the samples in Set III.

2.4 Standard Curve Preparation

Volumes of 0.02, 0.04, 0.08, 0.16, 0.32, 0.64 and 0.80 mL of mixed stock solution of MCP, mdMCP and ddMCP were pipetted into 15 mL tubes containing 0.5 mL 1 M NaOH, 0.3 mL of internal standard BMY solution (0.3 µg/mL) and corresponding blank biological fluids. The aqueous phase was adjusted to a final volume of 2.2 mL with deionized water and then extracted and derivatized as described in Section 2.3. A standard curve for each of MCP, mdMCP and ddMCP was calculated and plotted by the peak area ratio of heptafluorobutyryl (HFB) derivatives of MCP, mdMCP and ddMCP over BMY versus the known concentration of MCP, mdMCP and ddMCP free base. A weighting factor 1/Y(area ratio) was used for linear regression of the standard curves according to the method used in Riggs et al. (1994).

2.5 Instruments and Equipment

2.5.1 Gas chromatography (GC-MSD)

A Model 5890 Series II Hewlett-Packard gas chromatograph equipped with a HP 5971A Mass Selective Detector (MSD), split-splitless capillary inlet system, and a Windows® GC ChemStation on a HP Vectra® 25T 486 computer (Hewlett-Packard Co., Avondale, PA) was used for the MSD assay. A 25 m x 0.2 mm I.D. cross-linked 5% phenylmethylsilicone (Ultra-2®) 0.33 µm film thickness fused-silica capillary column
(Hewlett-Packard Co., Avondale, PA), 78 mm x 2 mm I.D. borosilicate glass inlet liners
(Hewlett-Packard Co., Avondale, PA), and Thermogreen® LB-2 silicone rubber septa
were also used.

2.5.2 *Operating conditions for the GC and MSD*

The operating conditions for the GC/MSD system were as follows: injection port
temperature, 260°C; purge time, 30 sec; split vent flow, 30 mL/min; initial column
temperature, 100°C; column head pressure, 10 p.s.i.; carrier gas (helium) flow rate, 3
mL/min; temperature program: 100°C hold for 0.8 min, temperature ramp at 40°C/min to
270°C then hold constant for 5 min, and then a further increase to 290°C at 70°C/min and
hold for 3 min, followed by a further increase to 300°C at 70°C/min and a final hold for
3.5 min to clean out the column after each run; Total run time, 16.98 min; MSD transfer
line and source temperature, 310°C. Aliquots of 2 μL are injected onto the column.

A HP 5971A Mass Selective Detector was used in electron impact (EI) ionization
mode with -70 eV ionization energy and 300μA emission current. The MSD was
manually tuned to the ions of *m/e* 100, 264 and 414 using the standard
perfluorotributylamine. Chromatograms were generated in both mass scanning (SCAN)
and selective ion monitoring (SIM) modes in the study. For the quantitative assay, MCP
and its metabolites were optimally detected in SIM mode using the total ion current of
*m/e* 380 for ddMCP and mdMCP (Group 1), *m/e* 86 and 380 for MCP (Group 2) and *m/e*
86 and 366 for BMY (Group 3). The dwell time was set at 700 msec. per group, which
provided 1.37 scan cycle/sec for Group 1 and 0.697 scan cycle/sec for Groups 2 and 3.
2.6 Animal preparation

2.6.1 Animal handling

The ewes (Dorset, Suffolk, or crossbred) were brought to the Animal Unit in the Children's Variety Research Centre about one week prior to surgery to allow them to become acclimatized. The ewes were fasted overnight before surgery and each received a 3-mg i.v. injection of atropine sulfate (Astra Pharmaceuticals Inc., Mississauga, ON) 10-15 min prior to induction of anaesthesia with Pentothal (1 mg/kg, i.v.; Abbott Laboratory, Montréal, Qué.). Following endotracheal intubation, anaesthesia was maintained by ventilating the ewes throughout the surgery with 1.0-1.5 % halothane (Ayerst Laboratory, Montréal, Qué.) and 60-70% N₂O in oxygen.

2.6.2 Surgical Preparation

Aseptic techniques were employed throughout surgery. Sterile silicone rubber catheters (Dow Corning, Corning, NY), filled with heparinized 0.9% saline, were implanted in the femoral vein and artery. In some animals, an ultrasound transit-time flow probe (Transonic Systems Inc., Ithaca, NY, USA) was implanted on the left femoral artery. Then, an oblique incision was made in the abdomen to the right of the umbilicus, and the liver and bowel were retracted to expose the gall bladder. The gall bladder was held steady with an artery forceps, and a small incision was made in the apex, through which a silicone rubber catheter was inserted. The catheter was secured by a purse string suture which also closed the gall bladder incision. Except during experiments, this
catheter was kept sealed to allow for the normal drainage of bile through the bile duct. However, these flow probes and bile catheters were used in the other studies.

All catheters were filled with heparinized saline (12 U/mL), tunneled subcutaneously, and exteriorized through a small incision in the right flank of the ewe where they were stored in a cloth pouch. The abdominal incision was closed in layers. A catheter for nitrogen infusion (10.0 mm o.d.) (Fisher Scientific, Cambridge, MA, USA) was then implanted in the trachea, between adjacent tracheal rings, 5-6 cm below the larynx, and inserted for 4-5 cm; this catheter did not affect normal breathing by the ewe (Gleed et al., 1986). During surgery, an i.v. drip of 5% dextrose solution (500 mL; Baxter Canada, Toronto, ON) was administered. Immediately following surgery, ampicillin (500 mg; Novopharm Ltd., Toronto, ON) and gentamicin (40 mg; Garamycin®, Schering, Pointe Claire, Qué.) were administered i.m. to the ewe. Following surgery, the ewes were kept in holding pens with other sheep and given free access to food and water. Ampicillin (500 mg) and gentamicin (40 mg) i.m. were also given prophylactically to the ewe for 5 days following surgery. All animals were allowed to recover for a minimum of 3 days before experiments.
2.7 **Experimental Protocol**

On experimental days, the ewe was placed in a monitoring cage adjacent to the holding pen in full view of companion ewes and with free access to food and water. A Foley® catheter (Bard Urological Div., CR Bard Inc., Conington, GA, USA) was inserted via the urethra for continuous urine collection. Metoclopramide hydrochloride (Reglan® injectable 5 mg/mL, A.H. Robins, Montreal, Que.) was diluted with sterile isotonic saline to 3 mg/mL. The infusion [rate = 0.21 mg (0.07 mL)/min] was preceded by a loading dose of 15 mg (5 mL) given via the femoral vein. After reaching steady-state during normoxia (2 hrs.), hypoxemia was induced by giving 7 L/min of nitrogen through the implanted tracheal catheter. The blood gas status was monitored by taking arterial blood samples at the intervals described in the sampling schedule. The nitrogen flow rate was adjusted to maintain the hypoxemia at the desired level of PaO$_2$ (i.e. 55-60 mm Hg). The hypoxemic period was continued for 6 hours. Then, the nitrogen flow was stopped and the infusion was continued for another 6 hours. The total infusion time of MCP was thus 14 hours. Arterial blood (2.5 mL) samples for MCP determination along with blood gas samples (~0.7-1.0 mL) were taken as shown in the protocol [Scheme IV]. The blood samples for MCP determination were transferred immediately to a heparinized Vacutainer® (Becton-Dickinson, Rutherford, NJ) and centrifuged at 3500 rpm for 10 min. Urine samples were collected according to the protocol. The volume and pH of urine were measured with a graduated cylinder and a Fisher Accumet® pH meter model 620 (Fisher Scientific Inc., Cambridge, MA, USA). Plasma and urine samples were
transferred to disposable borosilicate glass culture tubes (Corning Glass, Corning, NY) with Teflon or PTFE-lined screw caps and stored at -20°C until the time of assay.

Control experiments were also carried out, using the same MCP infusion and sampling protocol, but without hypoxemia. The following are the schematic diagrams of experimental protocol used in the present study.

Scheme III  Schematic diagrams of the experimental protocol

1. Experimental Group

   Hour 0 2 8 14
   Pre-hypoxemic (normoxemic) Hypoxemic Post-hypoxemic (normoxemic)

2. Control Group

   Hour 0 2 8 14
   Pre-hypoxemic (normoxemic) Normoxemic Post-hypoxemic (normoxemic)
Scheme IV  MCP infusion-hypoxemia sampling protocol

[Pre-hypoxemic period (2 hours)]
- 0:05  BL  BG  : blood controls
0:00  Loading dose (15 mg)/Infusion (rate = 0.21 mg/min.)
U  : fluid controls
0:05  BL  BG
0:15  BL
0:30  BL  BG  U
0:45  BL
1:00  BL  BG  U
1:15  BL
1:30  BL
2:00  BL  BG  U  : hypoxemia starts

[Hypoxemic period (6 hours)]  * rate of N\textsubscript{2} flow = 7 L/min. initially and increased to 11 L/min.
2:05  BL  BG
2:30  BG
3:00  BL  BG  U
4:00  BL  BG  U
5:00  BL  BG  U
6:00  BL  BG  U
7:00  BL  BG  U
7:15  BL  BG
7:30  BL  BG
8:00  BL  BG  U  : hypoxemia ends

[Post-hypoxemic period (6 hours)]
8:05  BL  BG
8:30  BG
9:00  BL  BG  U
10:00  BL  BG  U
11:00  BL  BG  U
12:00  BL  BG  U
13:00  BL  BG  U
13:15  BL  BG
13:30  BL  BG
14:00  BL  BG  U  : infusion ends

* BL : plasma sample, BG: blood gas sample, U : urine sample.
2.8  **Recording Procedures and Blood Gas Analysis**

For 24 hours before, during and 24 hours following MCP administration, arterial pressure was measured using a disposable strain-gauge DTX transducer (Statham model P23Db, Gould Inc., Oxnard, CA). Heart rate was measured from the arterial pulse pressure using a cardiotachometer (Model 9857, Sensormedic Corp., Anaheim, CA). These variables were recorded on a polygraph recorder (Beckman R612 recorder, Beckman, Schikker Park, IL or Gould TA4000 thermal array recorder, Gould Inc., Valley View, OH). The analog signals of arterial pressure and heart rate were converted simultaneously to digital data using an on-line computer system consisting of an Apple Ile® computer, Interactive systems (Daisy Electronics, Newton Square, PA), and A-D converter and clock card (Mountain Software, Scott's Valley, CA) with a sampling rate of 15 Hz and averaged over 1 min. The recordings were analysed to provide an estimate of arterial pressure and heart rate averaged over 1 min intervals.

Samples (≈ 1 mL) for blood gas analysis and glucose/lactate measurement were taken simultaneously with those for drug analysis. Arterial PaO$_2$, PaCO$_2$, base excess and pH were measured with an IL 1306 pH/blood gas analyzer (Allied Instrumentation Laboratory, Milan, Italy) set at a temperature of 37.0°C and corrected to 39.0°C. Oxygen saturation and hemoglobin content were measured, in duplicate, using an OSM2 Hemoximeter® (Radiometer, Copenhagen, Denmark). Blood glucose and lactate concentrations were measured, in triplicate, using a glucose/lactate 2306 STAT plus analyzer (YSI Inc., Yellow Spring, OH, USA). Osmolality of urine was measured by
freezing-point depression method using Advanced DigiMatic® Osmometer Model 3D2 (Advanced Instruments, Norwood, MA, USA).

2.9 Data Analysis

2.9.1. Determination of steady-state drug concentration

Steady-state MCP concentration was determined according to the following procedures and criteria:

1. Visual inspection: the plot of plasma concentration versus time was visually inspected for a plateau portion using a straight edge ruler.

2. Coefficient of variance: the coefficient of variation for the data set of MCP concentrations in the plateau portion was calculated. With a maximum criterion of CV = 10 %, all extreme outlier(s) were eliminated from the data set.

3. Student's t-test/Analysis of Variance (ANOVA) for the regression: the slope of linear regression line from the data set of plasma MCP concentration in the plateau portions was analyzed using a two-tailed t-test and ANOVA for the correlation with the null hypothesis (Ho): slope = 0 against the alternative hypothesis (Ha): slope ≠ 0 with alpha = 0.05. In these tests, the rejection of Ho suggests that the plasma drug concentration tends to either increase or decrease in a given period (i.e. a steady-state was not achieved).
2.9.2. Calculation of pharmacokinetic parameters

Total body clearance (Clb) was estimated as:

\[ Cl_t = \frac{k_o}{C_{ss}} \]

where \( k_o \) is the infusion rate and \( C_{ss} \) is the apparent arterial steady-state concentration.

Renal clearance values of MCP and mdMCP were calculated from 1) dividing the accumulated drug/metabolite recovered in urine \( (D_u) \) by area under the plasma drug/metabolite concentrations curve (AUC) as a function of time during the hypoxemic and normoxemic periods \[ D_u(t_2-t_1)/AUC(t_2-t_1) \] and 2) calculation using the slope of the accumulated drug/metabolite in urine \( (D_u) \) versus AUC (see Appendix A for the equation derivation).

The fractional renal excretion constant of MCP \( (f_u = k_u/K_E) \) was calculated by dividing the slope of the asymptote of the accumulated MCP in urine versus time curve with the infusion rate, \( k_o \).

\[ D_u = (k_u * k_o / K_E) t - (k_u * k_o / K_E^2) \]

where \( k_u \) is the renal excretion rate, and \( D_u \) is the cumulative amount of intact drug in urine. Since \( k_o \) is given, the ratio \( (k_u / K_E) \) can be calculated. The detailed equation derivations are shown in appendix A and theoretical and practical discussion is described in the section 4.4.2.

The fractional renal metabolite excretion constants for mdMCP, ddMCP and conjugates were also calculated in the study. These parameters are the product of two
fractional constant $f_{m(\text{metabolite})}$ and $f_{m(\text{metabolites})}$. The metabolite formation fraction constant $f_{m(\text{metabolite})}$ is the fraction of $k_{f(\text{metabolite})}/ K_{E(\text{parent drug})}$, which represents the metabolic elimination proportion (to a specific metabolite) of the total parent drug elimination. The second fractional constant, the renal metabolite excretion fraction $f_{m(\text{metabolite})}$ is the fraction of $k_{m(\text{metabolite})}/ K_{m(\text{metabolite})}$, which represents the renal excretion proportion of the total metabolite elimination. Therefore the composite fractional constant, the fractional renal metabolite excretion constant $f_{u(\text{metabolite})} = f_{m(\text{metabolite})} f_{m(\text{metabolite})}$, represents the proportion of renal excretion of a specific metabolite from the total drug elimination.

2.9.3 Statistical tests

Statistical evaluations were performed on various pharmacokinetic and physiological parameters using either Student's t-test (paired or unpaired), F-test, ANOVA (Analysis of Variance) and Tukey test. The level of significance was chosen $p < 0.05$. Theory and formulae used for statistical analysis were obtained from Zar (1984), and Microsoft Excel® for Windows® program (Microsoft Corp., Redmond, WA, USA) with Analysis Tools® and SlideWrite Plus® for Windows® (Advanced Graphics Software Inc., Carlsbad, CA, USA) were used for data processing/analysis and graphical presentation. The mean values in the text and tables are presented as the mean ± standard error of the mean (SEM), unless otherwise described.
3. **RESULTS**


3.1.1. *GC-MSD method for the quantitative analysis of MCP and selected metabolites.*

For the quantitative analysis of MCP, monodeethyl MCP (mdMCP) and dideethyl MCP (ddMCP), a GC-MSD method of Riggs et al. (1994) was used for its high sensitivity, selectivity and reproducibility. Representative chromatograms of MCP, mdMCP and ddMCP (1 ng/mL each) in plasma and blank extracts from plasma and urine are shown in Figure 1. The step-like shifting of the baseline of the chromatograms at 10.25 min represents a change in the background ion current at the SIM group switching (i.e. from Group 1 [m/e 380] to Group 2 [m/e 86 and 380]). An additional group switching occurs with Group 3 [m/e 86 & 366] at 11.50 min with no visible change in baseline. In spite of these changes in the selective ion monitoring group, the baseline remained stable throughout the assay. Chromatograms from urine and bile are similar to those from plasma. The individual retention times of the HFB-derivatives of ddMCP, mdMCP, MCP and the internal standard BMY were 9.29 min, 10.13 min, 10.38 min and 12.33 min, respectively. No sign of significant interference from the endogenous compounds was shown in any of the biological fluids collected in the study. Figure 2 (A-D) shows the mass spectra of MCP, monodeethyl MCP (mdMCP), dideethyl MCP (ddMCP) and the internal standard, BMY, respectively. Both MCP and BMY undergo extensive fragmentation resulting in a base peak of m/e 86 (Figure 2, C and D). In addition to the ion of m/e 86, a less abundant ion of m/e 380 and 366,
respectively, was also monitored for MCP and BMY, to enhance the selectivity. The base peak of ion \textit{m/e} 380 was used for ddMCP and mdMCP.

![Representative total ion chromatograms (SIM mode) of 1 ng/mL ddMCP, mdMCP, MCP and 33.3 ng/mL BMY added to 0.3 mL of plasma. Superimposed chromatograms of blank plasma and urine are also shown.](image)

Figure 1.
3.1.2. Calibration (standard) curve

Calibration (standard) curves were obtained by analyzing blank plasma, urine or bile spiked with varying amounts of MCP, mdMCP and ddMCP (1, 2, 4, 8, 16, 24, 32 and 40 ng/mL of each) and plotting the area ratio of the heptafluorobutyric (HFB) derivatives of MCP/BMY, mdMCP/BMY and ddMCP/BMY against the indicated MCP, mdMCP and ddMCP concentrations, respectively [Figure 3]. The data for a representative calibration used in the quantitation of MCP, mdMCP and ddMCP from plasma are presented in Table 1. Linearity is observed over the concentration range studied (1 - 40 ng) with a coefficient of variation (CV) < 15% for all the data points composing the curves of ddMCP, mdMCP and MCP. The limit of quantitation (LOQ) was 1 ng/mL for all three compounds in the biological fluids collected in the study. The coefficient of variation was relatively lower in the mid-range of the calibration curve and higher at either the low- or high-end range of the curve. The coefficients of regression and the line of best-fit through the data points was determined from linear regression with a weighting factor of 1/Y.

3.1.3. Enzyme incubation

The enzyme activities of glucuronidase and sulphatase were tested following the standard procedures provided by the supplier under the experimental conditions described in Section 2.3. In order to evaluate the effect of glucuronidase and sulphatase incubation on the quantitation of MCP and its metabolites, 3 sets of calibration curves (no enzyme incubation, glucuronidase incubation and sulphatase incubation) were prepared and extracted using the same procedures listed in Section 2.3. Since biological samples spiked with standard drugs
do not contain any conjugates of MCP, mdMCP and ddMCP, the calibration curves from each set would not show any difference unless enzyme incubations have affected the samples. Table 2 lists the results of the weighted linear regression of 3 sets. The slopes and intercepts from enzyme incubation of glucuronidase and sulphatase (Set II and III) were analyzed against Set I which was incubated without any enzyme using Student's t-test for linear regression. No significant difference in slopes or intercepts was observed among these calibration curves. Therefore, these results demonstrate that the enzyme incubation procedure does not affect the quantitation and linearity of the assay method.

Plasma and urine samples from the experiments were incubated with glucuronidase and sulphatase to determine the existence of these conjugates of MCP, mdMCP and ddMCP, however, no significant amount of conjugate was detected.
Figure 3. Representative calibration (weighted) curves for MCP, mdMCP and ddMCP from spiked plasma. [mean ± SD]
Table 1. Weighted calibration curve data (mean peak area ratio ± SD) for ddMCP, mdMCP and MCP in plasma.*

<table>
<thead>
<tr>
<th>Conc. (ng/mL)</th>
<th>ddMCP</th>
<th>mdMCP</th>
<th>MCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>3.95 ± 0.32 (8.11%)</td>
<td>2.23 ± 0.25 (11.03%)</td>
<td>1.65 ± 0.07 (4.48%)</td>
</tr>
<tr>
<td>32</td>
<td>2.86 ± 0.21 (7.31%)</td>
<td>1.75 ± 0.17 (9.69%)</td>
<td>1.26 ± 0.04 (2.93%)</td>
</tr>
<tr>
<td>24</td>
<td>2.20 ± 0.20 (9.09%)</td>
<td>1.25 ± 0.08 (6.00%)</td>
<td>0.93 ± 0.05 (4.95%)</td>
</tr>
<tr>
<td>16</td>
<td>1.47 ± 0.12 (8.09%)</td>
<td>0.85 ± 0.07 (8.32%)</td>
<td>0.62 ± 0.05 (8.39%)</td>
</tr>
<tr>
<td>8</td>
<td>0.70 ± 0.02 (3.50%)</td>
<td>0.38 ± 0.04 (9.58%)</td>
<td>0.32 ± 0.03 (7.78%)</td>
</tr>
<tr>
<td>4</td>
<td>0.34 ± 0.03 (8.99%)</td>
<td>0.18 ± 0.02 (10.38%)</td>
<td>0.16 ± 0.01 (4.43%)</td>
</tr>
<tr>
<td>2</td>
<td>0.18 ± 0.01 (3.10%)</td>
<td>0.10 ± 0.01 (12.07%)</td>
<td>0.08 ± 0.01 (11.48%)</td>
</tr>
<tr>
<td>1</td>
<td>0.10 ± 0.01 (5.95%)</td>
<td>0.06 ± 0.01 (6.64%)</td>
<td>0.05 ± 0.01 (12.38%)</td>
</tr>
<tr>
<td>slope</td>
<td>0.0934</td>
<td>0.0541</td>
<td>0.0397</td>
</tr>
<tr>
<td>intercept</td>
<td>-0.0068</td>
<td>-0.0099</td>
<td>0.0042</td>
</tr>
<tr>
<td>r²</td>
<td>0.9932</td>
<td>0.9930</td>
<td>0.9976</td>
</tr>
<tr>
<td>Average CV</td>
<td>6.77%</td>
<td>9.21%</td>
<td>7.10%</td>
</tr>
</tbody>
</table>

* The values shown in the parenthesis are the coefficient of variance.
Table 2. Weighted calibration curve data (slope, intercept and $r^2$) for ddMCP, mdMCP and MCP with enzyme incubation study in urine.

<table>
<thead>
<tr>
<th>Set</th>
<th>slope</th>
<th>intercept</th>
<th>$r^2$</th>
<th>slope</th>
<th>intercept</th>
<th>$r^2$</th>
<th>slope</th>
<th>intercept</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (no enzyme)</td>
<td>0.0976</td>
<td>-0.0176</td>
<td>0.9969</td>
<td>0.0539</td>
<td>-0.0252</td>
<td>0.9937</td>
<td>0.0411</td>
<td>-0.0073</td>
<td>0.9995</td>
</tr>
<tr>
<td>II (glucuronidase)</td>
<td>0.0903</td>
<td>-0.0082</td>
<td>0.9978</td>
<td>0.0524</td>
<td>-0.0073</td>
<td>0.9941</td>
<td>0.0393</td>
<td>0.0001</td>
<td>0.9998</td>
</tr>
<tr>
<td>III (sulphotase)</td>
<td>0.0937</td>
<td>-0.0071</td>
<td>0.9980</td>
<td>0.0489</td>
<td>-0.0085</td>
<td>0.9914</td>
<td>0.0396</td>
<td>0.0030</td>
<td>0.9996</td>
</tr>
</tbody>
</table>
3.2 Physiological Changes Associated with Hypoxemia

Table 3 lists the identification and the body weight of ewes on which experiments were performed, and the types of samples collected and the duration of the infusion. The body weight of the experimental group averaged 61.4 ± 3.5 Kg (Mean ± SEM) and the control group averaged 61.3 ± 2.1 Kg. Arterial plasma samples for drug assay and blood gas/pH/lactate/glucose measurement, were collected according to the experiment protocol shown in appendix A. In addition, urine and bile samples were also collected according to the animal experimental protocol.

Table 3. Identification and weight of ewes, samples collected and nitrogen/MCP infusion.

<table>
<thead>
<tr>
<th>Ewe No.</th>
<th>Weight (Kg)</th>
<th>Sample*</th>
<th>Nitrogen Infusion</th>
<th>MCP Infusion*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(L/min)</td>
<td>Change (hr)</td>
</tr>
<tr>
<td><strong>Experimental</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>102</td>
<td>69.5</td>
<td>MA, UR</td>
<td>Initial 7 to 10 at hr 7.5</td>
<td>14</td>
</tr>
<tr>
<td>139</td>
<td>60.4</td>
<td>MA, UR</td>
<td>Initial 7 to 8 at hr 6</td>
<td>14</td>
</tr>
<tr>
<td>989</td>
<td>69.5</td>
<td>MA, UR</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>1154</td>
<td>53.1</td>
<td>MA, UR</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>1158</td>
<td>54.5</td>
<td>MA, UR</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>61.4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SEM</strong></td>
<td><strong>3.53</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>220</td>
<td>61.7</td>
<td>MA, UR</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>327</td>
<td>55.4</td>
<td>MA, UR</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>328</td>
<td>63.6</td>
<td>MA, UR</td>
<td>0</td>
<td>15**</td>
</tr>
<tr>
<td>1159</td>
<td>64.5</td>
<td>MA, UR</td>
<td>0</td>
<td>9***</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>61.3</strong></td>
<td>p &gt; 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SEM</strong></td>
<td><strong>2.05</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* MA: maternal arterial blood sample, UR: urine total collection. ** MCP infusion was temporary disrupted. *** MCP infusion was discontinued at hour 9.
^ MCP infusion rate (0.21 mg/min) with 15 mg loading dose at hour 0.
# weight compared between the two groups according to Student's t-test.
3.2.1. *Arterial blood gas status and pH*

The mean (± SEM) arterial blood gas partial pressure of CO₂ and O₂ (PaCO₂ and PaO₂) and blood pH of the experimental and control groups are shown in Figure 4a and 4b, respectively. Mean (± SEM) values for arterial blood gas pressure, pH, oxygen saturation, base excess and bicarbonate concentration are presented in Table 4.

*PaO₂ and O₂ saturation:* The initiation of nitrogen infusion through the intra-tracheal catheter at hour 2 in the experimental group decreased arterial PaO₂, in less than 5 min. Mean PaO₂ was significantly decreased (≈48%) from 116.35 ± 2.83 to 60.31 ± 1.57 mm Hg (p<0.0001) during the hypoxic period. O₂ saturation was also significantly decreased (≈18%) from 100.16 ± 0.40% to 82.78 ± 1.53% during hypoxemia. In some animals (ewe nos. 102 and 139), PaO₂ increased slightly (≈70-80 mmHg) during the late hypoxic period, at which point the nitrogen flow was increased to 9-11 L/min to maintain the PaO₂ close to 60 mmHg. After 6 hours of hypoxemia, the intra-tracheal nitrogen infusion was stopped. PaO₂ and O₂ saturations have returned to 116.38 ± 1.26 mmHg and 100.15 ± 0.20 %, respectively, levels which are similar to the pre-hypoxic level (p>0.05). No changes in PaO₂ or O₂ saturation was observed throughout the periods in the control group.

*PaCO₂, bicarbonate concentration and base excess:* During hypoxemia, the mean PaCO₂ also significantly decreased (≈10%) from 41.71 ± 0.39 to 37.35 ± 0.48 mmHg (p<0.0005), a reduction of a much lesser degree than PaO₂. Base excess was decreased significantly from 3.4 ± 0.29 (pre-hypoxic) to 2.5 ± 0.29 meq/L (post-hypoxic period). Initially, the base excess was slightly increased at the onset of hypoxemia, from 3.9 (at hour
2) to 4.8 meq/L (at 2:05), and gradually decreased. Blood bicarbonate (HCO$_3^-$) concentration was also decreased during hypoxemia from 26.51 ± 0.32 meq/L to 25.21 ± 0.25 meq/L, but without an initial increase as shown in base excess. After nitrogen infusion was stopped, the PaCO$_2$ returned to 40.82 ± 0.52 mmHg, a level similar to the pre-hypoxemic period (p>0.05). However, after cessation of the nitrogen infusion, base excess and bicarbonate (HCO$_3^-$) concentrations remained lower than the pre-hypoxemic levels. In the control group, no significant change was observed in PaCO$_2$, bicarbonate concentration or base excess.

*Blood pH and hemoglobin concentration*: Arterial blood pH showed a very slight apparent increasing trend from 7.424 ± 0.0051 during the pre-hypoxemic period (normoxemia) to 7.442 ± 0.0057 during the hypoxemic period, although this did not reach statistical significance. During the post-hypoxemic period, blood pH decreased to 7.409 ± 0.0053 (p < 0.0005), a level significantly lower than those of the pre-hypoxemic and hypoxemic periods. This delayed acidosis appears to coincide with the delayed lactic acid accumulation in the blood, as shown in Table 5. During the hypoxemic period, the hemoglobin concentration also increased slightly (statistically not significant; p= 0.058) from 7.97 ± 0.23 g % (pre-hypoxemic period) to 8.40 ± 0.15 g % (hypoxemic period), and was restored to 7.83 ± 0.18 g % during the post-hypoxemic period.
Table 4. Mean (± SEM) arterial blood pH, gas partial pressure (PaCO$_2$ and PaO$_2$), bicarbonate (HCO$_3^-$) concentration and base excess. $^1$

<table>
<thead>
<tr>
<th></th>
<th>pre-hypoxemia</th>
<th>hypoxemia</th>
<th>post-hypoxemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>7.424 ± 0.0053</td>
<td>7.442 ± 0.0057</td>
<td>7.409 ± 0.0053 *</td>
</tr>
<tr>
<td>Control</td>
<td>7.501 ± 0.0076</td>
<td>7.495 ± 0.0068</td>
<td>7.492 ± 0.0063</td>
</tr>
<tr>
<td><strong>PaCO$_2$</strong> (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>41.27 ± 0.39</td>
<td>37.35 ± 0.48 **</td>
<td>40.83 ± 0.58</td>
</tr>
<tr>
<td>Control</td>
<td>37.14 ± 0.41</td>
<td>37.70 ± 0.65</td>
<td>37.22 ± 0.50</td>
</tr>
<tr>
<td><strong>PaO$_2$</strong> (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>116.9 ± 1.15</td>
<td>60.7 ± 1.57 ***#</td>
<td>116.5 ± 0.71</td>
</tr>
<tr>
<td>Control</td>
<td>117.7 ± 1.90</td>
<td>116.1 ± 1.88</td>
<td>120.0 ± 1.91</td>
</tr>
<tr>
<td><strong>O$_2$ sat (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>100.16 ± 0.40</td>
<td>82.78 ± 1.53 ***#</td>
<td>100.15 ± 0.20</td>
</tr>
<tr>
<td>Control</td>
<td>97.07 ± 0.17</td>
<td>96.60 ± 0.22</td>
<td>96.59 ± 0.14</td>
</tr>
<tr>
<td><strong>HCO$_3^-$</strong> (meq/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>26.51 ± 0.32 ****</td>
<td>25.21 ± 0.25</td>
<td>25.57 ± 0.25</td>
</tr>
<tr>
<td>Control</td>
<td>29.29 ± 0.38</td>
<td>28.86 ± 0.40</td>
<td>28.35 ± 0.46</td>
</tr>
<tr>
<td><strong>Base Excess (meq/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>3.4 ± 0.27 ****</td>
<td>2.5 ± 0.29</td>
<td>2.1 ± 0.28</td>
</tr>
<tr>
<td>Control</td>
<td>7.3 ± 0.46</td>
<td>6.7 ± 0.42</td>
<td>6.3 ± 0.47</td>
</tr>
<tr>
<td><strong>Hemoglobin (g %)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>7.95 ± 0.23</td>
<td>8.40 ± 0.15</td>
<td>7.83 ± 0.18</td>
</tr>
<tr>
<td>Control</td>
<td>7.71 ± 0.13</td>
<td>7.76 ± 0.13</td>
<td>7.75 ± 0.17</td>
</tr>
</tbody>
</table>

* Significantly lower than pre-hypoxemia and hypoxemia according to ANOVA (p < 0.0025) and Tukey test (p < 0.005).
** Significantly lower than pre-hypoxemia and post-hypoxemia according to ANOVA (p < 0.0005) and Tukey test (p < 0.001).
*** Significantly lower than pre-hypoxemia and post-hypoxemia according to ANOVA (p < 0.0001) and Tukey test (p < 0.0005).
**** Significantly higher than hypoxemia and post-hypoxemia according to ANOVA and Tukey test (p < 0.05).
# Significantly lower than control group according to Student's t-test (p < 0.0001).
$^1$ n = 5 for the experimental group, n = 4 for the control group.
Figure 4a. Mean (± SEM) arterial blood pH and gas partial pressure (PaCO₂ and PaO₂) over the duration of the experiment [experimental group, n = 5].

→ pH, ○ PaO₂, ● PaCO₂.
3.2.2. Arterial blood lactate and glucose concentration during hypoxemia

Tables 5 and 6 list the mean (± SEM) lactate and glucose concentrations of the experimental and control groups. Visual inspection of the plots of arterial blood lactate concentration versus time for the experimental group (Figure 5a) shows that there were noticeable changes in lactate and glucose concentration within the hypoxemic and post-
hypoxemic periods. Each of the hypoxemic and post-hypoxemic periods were subdivided into three 2-hour intervals for the statistical analysis. In the experimental group, the arterial blood lactate concentrations gradually increased (= 60 % by hour 8) during the hypoxemic period (statistically significantly at the late hypoxemic period i.e. hour 6:00-8:00) and remained elevated after cessation of the nitrogen infusion. The lactate levels had returned to the pre-hypoxemic level by the late post-hypoxemic period (hour 12:00-14:00). Thus, the lactate concentrations from 6:00 to 12:00 (i.e. during late hypoxemia and subsequent recovery period) were significantly (p < 0.05) higher than the remaining experiment periods [baseline-level]. In the control group, lactate concentration remained at a level similar to that during the pre-hypoxemic period through the entire experiment (p > 0.85) [Figure 5b].

Arterial blood glucose concentration also increased, to a lesser degree (= 10 % higher at hour 8), in the late hypoxemic period, in a manner similar to the increase seen in the lactate concentration. During the post-hypoxic period, the glucose level remained higher than that of the pre-hypoxemic period in the experimental group. In contrast, in the control group, the glucose concentration remained relatively constant. Figure 5b shows a decreasing trend through the hypoxemic and post-hypoxemic periods, but this decrease was not statistically significant.
Figure 5a. Mean (± SEM) arterial blood lactate and glucose concentration (mmol/L) over the duration of the experiment [experimental group, n = 5]. —○— blood lactate and —●— glucose.

Figure 5b. Mean (± SEM) arterial blood lactate and glucose concentration (mmol/L) over the duration of the experiment [control group, n = 4]. —○— blood lactate and —●— glucose.
Table 5. Mean (± SEM) blood lactate concentration (mmol/L).

<table>
<thead>
<tr>
<th>Period</th>
<th>Experimental</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-hypoxemia 0:00-2:00</td>
<td>0.558 ± 0.024</td>
<td>0.524 ± 0.047</td>
</tr>
<tr>
<td>Hypoxemia 2:00-4:00</td>
<td>0.578 ± 0.035</td>
<td>0.476 ± 0.035</td>
</tr>
<tr>
<td>4:00-6:00</td>
<td>0.644 ± 0.054</td>
<td>0.504 ± 0.062</td>
</tr>
<tr>
<td>6:00-8:00</td>
<td>0.849 ± 0.044*</td>
<td>0.565 ± 0.052</td>
</tr>
<tr>
<td>Post-Hypoxemia 8:00-10:00</td>
<td>0.879 ± 0.049*</td>
<td>0.614 ± 0.043</td>
</tr>
<tr>
<td>10:00-12:00</td>
<td>0.804 ± 0.064*</td>
<td>0.533 ± 0.069</td>
</tr>
<tr>
<td>12:00-14:00</td>
<td>0.615 ± 0.025</td>
<td>0.446 ± 0.029</td>
</tr>
</tbody>
</table>

* Significantly higher than baseline group (pre-hypoxemia, 2:00-6:00 and 12:00-14:00) according to ANOVA (p < 1E-10) and Tukey test (p < 0.05).

n = 5 for the experimental group and n = 4 for the control group.

Table 6. Mean (± SEM) blood glucose concentration (mmol/L).

<table>
<thead>
<tr>
<th>Period</th>
<th>Experimental</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-hypoxemia 0:00-2:00</td>
<td>2.74 ± 0.05</td>
<td>3.24 ± 0.14</td>
</tr>
<tr>
<td>Hypoxemia 2:00-4:00</td>
<td>2.81 ± 0.05</td>
<td>3.23 ± 0.17</td>
</tr>
<tr>
<td>4:00-6:00</td>
<td>2.81 ± 0.07</td>
<td>3.09 ± 0.23</td>
</tr>
<tr>
<td>6:00-8:00</td>
<td>2.91 ± 0.04*</td>
<td>3.17 ± 0.18</td>
</tr>
<tr>
<td>Post-Hypoxemia 8:00-10:00</td>
<td>3.03 ± 0.04*</td>
<td>2.92 ± 0.16</td>
</tr>
<tr>
<td>10:00-12:00</td>
<td>2.97 ± 0.09*</td>
<td>2.78 ± 0.17</td>
</tr>
<tr>
<td>12:00-14:00</td>
<td>2.98 ± 0.05*</td>
<td>2.80 ± 0.11</td>
</tr>
</tbody>
</table>

* Significantly higher than baseline group (pre-hypoxemia and 2:00-6:00) according to ANOVA (p < 3E-05) and Tukey test (p < 0.05).

n = 5 for the experimental group and n = 4 for the control group.
3.2.3. Plasma electrolyte concentration, urine flow, osmolality and pH and renal osmolal excretion rate.

Table 7 lists mean (± SEM) plasma electrolyte (Na⁺, K⁺ and Cl⁻) concentrations, hemoglobin content and urine flow (volume collected per hour), osmolality and pH. The mean urine flow in the experimental group was increased significantly during hypoxemia, from an average of 80.20 ± 12.65 mL/hr during the pre-hypoxemic period to 131.90 ± 19.53 mL/hr during the hypoxemic period, and later was restored to 78.30 ± 7.58 mL/hr during the post-hypoxemic period (p < 0.025). As shown in Figure 6a, hypoxemia-induced diuresis was observed in the early to middle hypoxemic period (between hour 2:00 to 6:00) and returned to the values close to the normoxemic level during the late hypoxemic and post-hypoxemic periods. However, in two of the animals in the experimental group (ewe no. 1154 and 1158), hypoxemia-induced diuresis was not observed. In the control group, there was no significant change in urine flow throughout the entire experimental period [Figure 6b].

In addition, urine osmolality was also significantly decreased from 1141.15 ± 37.22 mOsmol/Kg (pre-hypoxemic) to 808.13 ± 92.99 mOsmol/Kg during hypoxemia, and restored to 1172.03 ± 40.77 mOsmol/Kg after hypoxemia. Again, similar to urine flow, the urine osmolality in ewe no. 1154 and 1158 did not change during hypoxemia. These results describing urine flow and osmolality suggest that there may be some correlation between these two parameters. Thus, the plot of urine osmolality versus urine flow was plotted as shown in Figure 7. As seen in the plot, there is an apparent inverse relationship between these two parameters (r = 0.80). In addition, the renal osmolal elimination rate constant, the product of urine osmolality and urine flow, was also significantly decreased from 88.23
mOsmol/h in the pre-hypoxemic period to 61.32 mOsmol/h during the hypoxemic period, and later restored to 89.37 mOsmol/h in the post-hypoxemic period. Therefore, the negative correlation between urine osmolality and flow and hypoxemia-induced reduction in osmolal excretion suggests that the increased urine flow during hypoxemia is due to increased water excretion rather than a secondary urine flow increase by elevated electrolyte/osmolal excretion (Walker, 1982; Colice et al., 1991).

Similar to the findings with urine volume and osmolality, the urine pH significantly decreased from 7.532 ± 0.0218 in the pre-hypoxemic period to 7.468 ± 0.0144 in the hypoxemic period. Later, the urine pH was restored to 7.519 ± 0.0102 in the post-hypoxemic period, which is a level similar to the pre-hypoxemic period in the experimental group. Increased renal lactate excretion may be related to the urinary acidosis during hypoxemia, but no lactate measurement was conducted during the experiment. Therefore, no direct correlation could be made in the present study.

In the control group, the physiological parameters such as urine flow, pH and osmolality remained fairly constant throughout the experimental periods (p >> 0.05). However, urine flow (p <0.05), urine pH and osmolality (p << 0.05) in the experimental group were significantly different from the control group during the hypoxemic period (hour 2 to 8).
Figure 6a. Mean (± SEM) urine flow (mL/hr) and osmolality (mOsm/Kg) over the duration of the experiment [experimental group, n = 5].
- •- urine osmolality and —— urine flow.

Figure 6b. Mean (± SEM) urine flow (mL/hr) and osmolality (mOsm/Kg) over the duration of the experiment [control group, n = 4]
- ○- urine osmolality and —— urine flow.
Figure 7. Correlation of urine osmolality (mOsm/Kg) and urine flow (mL/hr). Correlation coefficient (r) = 0.80.

Plasma sodium (Na\textsuperscript{+}) concentration was not significantly changed during hypoxemia. Na\textsuperscript{+} concentration tends to increase slightly as the infusion proceeds, from 146.6 ± 1.02 mM during the pre-hypoxemic period to 150.1 ± 0.59 mM during the hypoxemic period. However, this apparent tendency towards an increase was observed in both the experimental and control group, and yet, no difference was found between the two groups. Therefore, it suggests that hypoxemia does not affect the plasma Na\textsuperscript{+} concentration, in spite of changes in urine flow and osmolality.

However, plasma potassium (K\textsuperscript{+}) concentration was increased significantly during hypoxemia from 4.12 ± 0.030 mM (pre-hypoxemic) to 4.24 ± 0.022 mM (hypoxemic) [p <
0.05], and returned to 4.15 ± 0.034 mM during the post-hypoxemic period. In contrast, plasma K\(^+\) concentration in the control group remained constant during the hypoxemic period, 4.11 ± 0.030 mM and 4.17 ± 0.025 mM, and significantly increased to 4.41 ± 0.037 mM during the post-hypoxemic period.

Plasma chloride ion (Cl\(^-\)) concentration was significantly increased during the hypoxemic period in the experimental group, from 111.4 ± 0.48 mM (pre-hypoxemic) to 114.1 ± 0.53 mM (hypoxemic), and remained elevated throughout the post-hypoxemic period (113.4 ± 0.57 mM). In the control group, plasma Cl\(^-\) concentration was also increased during the hypoxemic period from 110.7 ± 0.24 mM to 112.0 ± 0.18 mM, but to a lesser degree (significantly lower, p < 0.01) than the experimental group.
Table 7. Mean (± SEM) plasma electrolyte concentration, hemoglobin content and urine flow, urine pH and osmolality.

<table>
<thead>
<tr>
<th></th>
<th>pre-hypoxemia</th>
<th>hypoxemia</th>
<th>post-hypoxemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urine Flow</strong> (mL/hr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>80.20 ± 12.65</td>
<td>131.90 ± 19.53**</td>
<td>78.30 ± 7.58</td>
</tr>
<tr>
<td>Control</td>
<td>76.00 ± 15.38</td>
<td>92.58 ± 10.54</td>
<td>94.96 ± 10.52</td>
</tr>
<tr>
<td><strong>Urine pH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>7.532 ± 0.0218</td>
<td>7.468 ± 0.0112***</td>
<td>7.535 ± 0.0148</td>
</tr>
<tr>
<td>Control</td>
<td>7.551 ± 0.0134</td>
<td>7.514 ± 0.0144</td>
<td>7.519 ± 0.0102</td>
</tr>
<tr>
<td><strong>Osmolality</strong> (mOsmol/Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>1141.15 ± 37.22</td>
<td>808.13 ± 92.99***</td>
<td>1172.03 ± 40.77</td>
</tr>
<tr>
<td>Control</td>
<td>1241.69 ± 63.64</td>
<td>1239.88 ± 46.28</td>
<td>1256.92 ± 29.08</td>
</tr>
<tr>
<td><strong>Plasma Na⁺</strong> (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>146.6 ± 1.02</td>
<td>150.1 ± 0.59</td>
<td>149.1 ± 0.98</td>
</tr>
<tr>
<td>Control</td>
<td>148.4 ± 0.25</td>
<td>150.2 ± 0.27</td>
<td>150.1 ± 0.18</td>
</tr>
<tr>
<td><strong>Plasma K⁺</strong> (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>4.12 ± 0.030</td>
<td>4.24 ± 0.022^</td>
<td>4.15 ± 0.034</td>
</tr>
<tr>
<td>Control</td>
<td>4.11 ± 0.030</td>
<td>4.17 ± 0.025</td>
<td>4.41 ± 0.037^^ ***</td>
</tr>
<tr>
<td><strong>Plasma Cl⁻</strong> (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>111.4 ± 0.48***</td>
<td>114.1 ± 0.53###</td>
<td>113.4 ± 0.57</td>
</tr>
<tr>
<td>Control</td>
<td>110.7 ± 0.24###</td>
<td>112.0 ± 0.18</td>
<td>112.5 ± 0.19</td>
</tr>
</tbody>
</table>

* Significantly higher; p < 0.025 according to ANOVA and p < 0.025 from post-hypoxemia and p < 0.10 from pre-hypoxemia in Tukey test.
** Significantly lower; p < 0.001 according to ANOVA and p < 0.001 from post-hypoxemia and p < 0.01 from pre-hypoxemia in Tukey test.
# Significantly lower; p < 0.05 from control group according to Student's t-test.
## Significantly lower; p < 0.005 from control group according to Student's t-test.
### Significantly higher; p < 0.0001 from control group according to Student's t-test.
#### Significantly higher; p < 0.05 from experiment group according to Student's t-test.
^ Significantly higher; p < 0.01 from control group according to Student's t-test.
^^ Significantly higher; p < 0.001 from pre-hypoxemic and hypoxemic period according to ANOVA and Tukey test.
### Significantly lower; p < 0.001 from hypoxemic and post-hypoxemic periods according to ANOVA and Tukey test.

n = 5 for the experimental group and n = 4 for the control group.
3.2.4. *Mean arterial blood pressure and heart rate.*

Mean arterial blood pressure and heart rate were also measured in some of the animals in the study. Tables 8 and 9 list mean heart rate and arterial blood pressure during the pre-hypoxemic, hypoxemic and post-hypoxemic periods. During the hypoxemic period, mean heart rate and arterial blood pressure were significantly increased ($p < 0.05$) to $122.49 \pm 9.15$ beats/min and $96.99 \pm 10.26$ mm Hg from $108.95 \pm 6.61$ beats/min and $90.32 \pm 10.63$ mm Hg (the pre-hypoxemic period) in the experimental group. After the cessation of nitrogen infusion, both arterial pressure and heart rate returned to the levels close to those of the pre-hypoxemic periods ($p > 0.05$). Mean arterial pressure in the control group remained constant throughout the entire experiment. Mean heart rate in the control group appeared to decrease gradually through the experiment period, but it was not statistically significant.

**Table 8. Mean (± SEM) heart rate (beats/min)**

<table>
<thead>
<tr>
<th>Ewe #</th>
<th>pre-hypoxemia</th>
<th>hypoxemia</th>
<th>post-hypoxemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental 139</td>
<td>100.0 ± 1.3</td>
<td>109.3 ± 0.7</td>
<td>89.3 ± 0.9</td>
</tr>
<tr>
<td>989</td>
<td>105.0 ± 1.0</td>
<td>118.1 ± 0.5</td>
<td>93.4 ± 0.4</td>
</tr>
<tr>
<td>1158</td>
<td>121.9 ± 0.8</td>
<td>140.1 ± 0.7</td>
<td>129.7 ± 0.6</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>109.0 ± 6.6</td>
<td>122.5 ± 9.2*</td>
<td>104.2 ± 12.8</td>
</tr>
<tr>
<td>Control 327</td>
<td>112.7 ± 1.7</td>
<td>110.6 ± 0.8</td>
<td>96.4 ± 0.6</td>
</tr>
<tr>
<td>220</td>
<td>119.6 ± 0.7</td>
<td>112.1 ± 0.4</td>
<td>101.1 ± 0.4</td>
</tr>
<tr>
<td>1159</td>
<td>125.0 ± 0.5</td>
<td>123.3 ± 0.3</td>
<td>121.0 ± 0.3</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>119.1 ± 3.6</td>
<td>115.3 ± 4.0</td>
<td>106.1 ± 7.5</td>
</tr>
</tbody>
</table>

* Significantly higher than the pre-HO and post-HO periods according to paired t-tests ($p < 0.05$)
Table 9. Mean (± SEM) arterial blood pressure (mm Hg)

<table>
<thead>
<tr>
<th>Ewe #</th>
<th>pre-hypoxemia</th>
<th>hypoxemia</th>
<th>post-hypoxemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Mean ± SEM</strong></td>
<td><strong>Mean ± SEM</strong></td>
<td><strong>Mean ± SEM</strong></td>
</tr>
<tr>
<td></td>
<td>90.3 ± 10.6</td>
<td>97.0 ± 10.3*</td>
<td>89.6 ± 8.62</td>
</tr>
<tr>
<td><strong>Experimental</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>139</td>
<td>70.8 ± 0.4</td>
<td>77.6 ± 0.2</td>
<td>72.5 ± 0.25</td>
</tr>
<tr>
<td>989</td>
<td>107.3 ± 0.3</td>
<td>112.6 ± 0.3</td>
<td>96.0 ± 0.27</td>
</tr>
<tr>
<td>1158</td>
<td>92.8 ± 0.5</td>
<td>100.8 ± 0.3</td>
<td>100.3 ± 0.33</td>
</tr>
<tr>
<td><strong>Mean ± SEM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>327</td>
<td>85.9 ± 1.0</td>
<td>85.4 ± 0.6</td>
<td>73.5 ± 0.5</td>
</tr>
<tr>
<td>220</td>
<td>76.3 ± 0.3</td>
<td>78.2 ± 0.2</td>
<td>80.6 ± 0.3</td>
</tr>
<tr>
<td>1159</td>
<td>94.6 ± 0.3</td>
<td>95.3 ± 0.2</td>
<td>94.4 ± 0.2</td>
</tr>
<tr>
<td><strong>Mean ± SEM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significantly higher than the pre-hypoxic period according to paired t-tests (p < 0.01).

3.3 Metoclopramide Pharmacokinetics Following i.v. Infusion to Steady-state and Induction of Hypoxemia.

3.3.1. Steady-state plasma MCP concentration and total body clearance (TBC) during normoxemia and hypoxemia.

Infusion parameters for the study were chosen based on results obtained during the previous metoclopramide study in sheep (Riggs, 1989). Similar to the previous study, a visual inspection of plasma MCP concentrations and statistical analysis indicated that the MCP steady-state concentrations were attained between 30 min and 75 min of the infusion duration (infusion rate at 0.21 mg/min) after an initial 15 mg i.v. bolus loading dose in 8 of 9
animals (4 in the experimental group and 4 in the control group) used in the study. One animal in the experimental group (Ewe No. 1154) did not reach the state-state concentration within 2 hours of infusion, despite the administration of a loading dose. Data from this animal was excluded from the final pharmacokinetic data analysis, since the hypoxemic and post-hypoxemic steady-state drug concentrations could not be compared to the pre-hypoxemic steady-state concentrations.

Semi-logarithmic plots of mean (± SEM) plasma MCP and mdMCP concentration versus time profiles obtained following the initial i.v. loading dose and 14-hour infusion are shown in Figure 8a for the experimental group and Figure 8b for the control group. The plasma ddMCP concentration was below the assay quantitation limit in the plasma samples assayed, thus, it was not appropriate to attempt statistical analysis and plotting of the plasma ddMCP data.

Table 10 lists mean (± SEM) plasma metoclopramide concentrations in the experimental and control groups. Plasma metoclopramide reached average steady-state concentrations of 50.72 ± 1.06 ng/mL for the experimental group and 50.84 ± 0.99 ng/mL for the control group in the pre-hypoxemic period (p > 0.05). Steady-state concentrations were obtained 30 - 45 min after initiation of infusion in 3 ewes and by 1 to 1.25 hour in 5 ewes. During the hypoxemic period, the mean MCP steady-state concentration increased significantly from 50.72 ± 1.06 to 63.62 ± 1.79 ng/mL (p < 0.0001) in the experimental group. In the control group, plasma MCP steady-state concentration remained constant (p > 0.05). The elevated MCP steady-state concentration during hypoxemia in the experimental group is significantly higher than that of the control group (p < 0.005).
Table 10. Mean (± SEM) steady-state plasma MCP concentration (ng/mL)

<table>
<thead>
<tr>
<th>Ewe #</th>
<th>pre-hypoxemia</th>
<th>hypoxemia</th>
<th>post-hypoxemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>45.80 ± 1.95*</td>
<td>65.39 ± 1.61**</td>
</tr>
<tr>
<td></td>
<td>139</td>
<td>54.75 ± 2.12*</td>
<td>73.31 ± 3.14</td>
</tr>
<tr>
<td></td>
<td>989</td>
<td>68.26 ± 2.68</td>
<td>74.71 ± 4.03****</td>
</tr>
<tr>
<td></td>
<td>1158</td>
<td>30.95 ± 1.03</td>
<td>43.69 ± 1.94**</td>
</tr>
<tr>
<td>Mean ± SEM¹</td>
<td></td>
<td>49.94 ± 7.83</td>
<td>64.28 ± 7.16^</td>
</tr>
<tr>
<td>Mean ± SEM²</td>
<td></td>
<td>50.72 ± 1.06</td>
<td>63.62 ± 1.79###</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>65.07 ± 3.41</td>
<td>78.39 ± 4.01</td>
</tr>
<tr>
<td></td>
<td>327</td>
<td>57.65 ± 3.81</td>
<td>55.02 ± 1.19</td>
</tr>
<tr>
<td></td>
<td>328</td>
<td>43.91 ± 1.86</td>
<td>38.48 ± 3.02</td>
</tr>
<tr>
<td></td>
<td>220</td>
<td>33.08 ± 1.50</td>
<td>44.41 ± 1.30</td>
</tr>
<tr>
<td>Mean ± SEM¹</td>
<td></td>
<td>49.93 ± 7.12</td>
<td>54.08 ± 8.80</td>
</tr>
<tr>
<td>Mean ± SEM²</td>
<td></td>
<td>50.84 ± 0.99</td>
<td>54.23 ± 2.15</td>
</tr>
</tbody>
</table>

* Significantly lower than hypoxemic and post-hypoxemic period according to ANOVA/Tukey test (p < 0.05).
** Significantly higher than pre- and post-hypoxemic period according to ANOVA/Tukey test (p < 0.05).
*** Significantly higher than pre-hypoxemia and lower than hypoxemic period according to ANOVA and Tukey test (p < 0.05).
**** Significantly higher than post-hypoxemic period according to ANOVA/Tukey tests (p < 0.005).
# Significantly higher than control group according to Student's t-test (p < 0.005).
## Significantly higher than control group according to Student's t-test (p < 0.05).
^ Significantly higher than pre- and post-hypoxemic periods according to paired t-tests (p < 0.05).
1 Mean ± sem calculated from the set of individual plasma MCP steady-state concentrations and analyzed by series of paired t-tests among the periods. (n = 4)
2 Mean ± sem calculated from the mean plasma MCP concentration curve and analyzed by ANOVA and Tukey test.
Figure 8a. Mean (± SEM) plasma MCP and mdMCP concentration (ng/mL) over the duration of the experiment [experimental group, n = 4]. -o- MCP and -●- mdMCP.
Figure 8b. Mean (± SEM) plasma MCP and mdMCP concentration (ng/mL) over the duration of the experiment-[control group, n = 4].
-○- MCP and -●- mdMCP.
During the post-hypoxemic period, the arterial plasma MCP steady-state concentration decreased to a concentration close to that seen in the pre-hypoxemic period (55.83 ± 1.15 ng/mL) in the experimental group. However, this concentration was significantly higher than that of the equivalent period in the control group (p = 0.04). The mean MCP steady-state concentrations in the control group remained the same throughout the entire experimental period (p > 0.05).

There was some individual variability in pharmacokinetic response to the hypoxemic stress. In general, hypoxemia appears to have induced a reduction in drug elimination (i.e. increased plasma steady-state concentration), but there was a noticeable difference in MCP kinetics during the post-hypoxemic recovery period in the experimental group. In ewe number 989 and 1158, the MCP steady-state concentrations increased significantly during hypoxemia and returned to the pre-hypoxemic level during the subsequent post-hypoxemic recovery period. In ewe number 102, the MCP concentration also increased significantly during hypoxemia, but did not return to a pre-hypoxemic level. The MCP concentration during the post-hypoxemic period in this animal remained between the pre-hypoxemic and hypoxemic level (but significantly different from both). Alternatively, the MCP concentration increased significantly during hypoxemia in ewe number 139, and remained high throughout the post-hypoxemic period.

Table 11 lists the plasma MCP total body clearance (TBC) in both the experimental and control groups. In the experimental group, TBC decreased significantly from 274.22 ± 47.99 L/h (4.47 ± 1.04 L/h/Kg) to 205.40 ± 28.17 L/h (3.33 ± 0.66 L/h/Kg) during
hypoxemia. During the post-hypoxemic period, TBC was increased to 245.78 ± 44.24 L/h (4.00 ± 0.96 L/h/Kg). In contrast, the TBC of control group remained relatively constant throughout the experiment period (p > 0.05). Thus, these results from cross comparison experiments suggest that the total body clearance of metoclopramide is reduced during moderate hypoxemia (PaO2 = 60 mmHg) and restored during the subsequent recovery period (post-hypoxemia).
Table 11. Plasma MCP total body clearance (TBC)*.

<table>
<thead>
<tr>
<th>Ewe No.</th>
<th>pre-hypoxemia</th>
<th>hypoxemia</th>
<th>post-hypoxemia</th>
<th>change^</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>275.11</td>
<td>192.70</td>
<td>222.70</td>
<td>-30%</td>
</tr>
<tr>
<td></td>
<td>(3.96)</td>
<td>(2.77)</td>
<td>(3.20)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>230.12</td>
<td>171.87</td>
<td>173.36</td>
<td>-25%</td>
</tr>
<tr>
<td></td>
<td>(3.81)</td>
<td>(2.84)</td>
<td>(2.87)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>184.58</td>
<td>168.64</td>
<td>212.43</td>
<td>-9%</td>
</tr>
<tr>
<td></td>
<td>(2.66)</td>
<td>(2.42)</td>
<td>(3.06)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>407.08</td>
<td>288.40</td>
<td>374.61</td>
<td>-29%</td>
</tr>
<tr>
<td></td>
<td>(7.47)</td>
<td>(5.29)</td>
<td>(6.87)</td>
<td></td>
</tr>
<tr>
<td>Mean ± SEM (L/h)</td>
<td>274.22 ± 47.99</td>
<td>205.40 ± 28.17*</td>
<td>245.78 ± 44.24</td>
<td></td>
</tr>
<tr>
<td>Mean ± SEM (L/h/Kg)</td>
<td>4.47 ± 1.04</td>
<td>3.33 ± 0.66*</td>
<td>4.00 ± 0.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>193.64</td>
<td>160.73</td>
<td>166.96</td>
<td>-16%</td>
</tr>
<tr>
<td></td>
<td>(3.50)</td>
<td>(2.90)</td>
<td>(3.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>218.57</td>
<td>228.76</td>
<td>210.04</td>
<td>+5%</td>
</tr>
<tr>
<td></td>
<td>(3.44)</td>
<td>(3.60)</td>
<td>(3.30)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>380.87</td>
<td>283.70</td>
<td>303.94</td>
<td>-25%</td>
</tr>
<tr>
<td></td>
<td>(5.90)</td>
<td>(4.40)</td>
<td>(4.71)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>286.95</td>
<td>327.45</td>
<td>297.03</td>
<td>+14%</td>
</tr>
<tr>
<td></td>
<td>(4.65)</td>
<td>(5.31)</td>
<td>(4.81)</td>
<td></td>
</tr>
<tr>
<td>Mean ± SEM (L/h)</td>
<td>270.01 ± 41.89</td>
<td>250.16 ± 36.00</td>
<td>244.49 ± 35.53</td>
<td></td>
</tr>
<tr>
<td>Mean ± SEM (L/h/Kg)</td>
<td>4.37 ± 0.58</td>
<td>4.05 ± 0.52</td>
<td>3.96 ± 0.47</td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses show the total body clearance normalized to ewe body weight (L/h/Kg). [n = 4]

* Significantly lower than pre-hypoxemic period (p < 0.05) according to paired t-test.

^ Relative change during hypoxemic period compared to pre-hypoxemic period.
3.3.2. Plasma mdMCP concentration during hypoxemic and normoxemic MCP steady-state.

The mean (± SEM) arterial plasma concentration of mdMCP during MCP steady-state is listed in Table 12. Plasma mdMCP concentration (metabolite) increased significantly (p < 0.005) from the pre-hypoxemic to the hypoxemic periods in both experimental and control groups. This increase in plasma mdMCP concentration, unlike that of plasma MCP concentration, does not appear to be related to the induction of hypoxemia, since the elevation of plasma mdMCP concentration was gradual throughout the pre-hypoxemic period and observed in both experimental and control groups. Therefore, it is likely to be a result of gradual metabolite accumulation during the drug infusion. Visual inspection of the plasma mdMCP concentration-time profiles (Figures 8a and 8b) suggests that mdMCP concentration also reached an apparent steady-state in the ewes in both the experimental and control groups, however, the time to an apparent steady-state was longer than for MCP, ranging from 4 to 5 hours. Plasma mdMCP concentration was not significantly changed during the post-hypoxemic period. The mean mdMCP concentrations during the hypoxemic (32.78 ± 1.73 ng/mL) and post-hypoxemic periods (33.07 ± 1.08 ng/mL) were similar (p >> 0.05).

However, when the plasma mdMCP concentrations of the hypoxemic and post-hypoxemic periods in the experiment group were compared to those of the control group, mdMCP concentrations of experimental group were significantly higher (p < 0.0001), suggesting a higher plasma mdMCP accumulation during hypoxemia.
Table 12. Mean (± SEM) arterial plasma mdMCP concentrations during the MCP infusion (ng/mL)

<table>
<thead>
<tr>
<th>Ewe #</th>
<th>pre-hypoxemia</th>
<th>hypoxemia</th>
<th>post-hypoxemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Experimental</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>102</td>
<td>22.99 ± 1.72</td>
<td>30.35 ± 2.94</td>
<td>27.20 ± 1.68</td>
</tr>
<tr>
<td>139</td>
<td>13.12 ± 1.13*</td>
<td>26.63 ± 0.51</td>
<td>30.20 ± 1.13</td>
</tr>
<tr>
<td>989</td>
<td>21.89 ± 1.37*</td>
<td>39.03 ± 2.44</td>
<td>38.47 ± 1.51</td>
</tr>
<tr>
<td>1158</td>
<td>18.33 ± 3.36*</td>
<td>40.01 ± 1.55</td>
<td>34.99 ± 2.01</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>18.62 ± 1.30*</td>
<td>32.78 ± 1.73#</td>
<td>33.07 ± 1.08#</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>327</td>
<td>13.17 ± 1.75*</td>
<td>23.18 ± 1.97</td>
<td>25.59 ± 1.41</td>
</tr>
<tr>
<td>328</td>
<td>24.01 ± 0.61**</td>
<td>26.89 ± 0.63</td>
<td>27.42 ± 1.23</td>
</tr>
<tr>
<td>220</td>
<td>13.38 ± 0.53</td>
<td>13.72 ± 0.39</td>
<td>16.37 ± 0.59***</td>
</tr>
<tr>
<td>1159</td>
<td>5.40 ± 0.17*</td>
<td>19.31 ± 1.94</td>
<td>26.84 ± 1.12</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>14.26 ± 0.68*</td>
<td>21.20 ± 1.39</td>
<td>22.27 ± 0.81</td>
</tr>
</tbody>
</table>

* Significantly lower than hypoxic and post-hypoxic period according to ANOVA/Tukey test (p< 0.005).
** Significantly lower than hypoxic and post-hypoxic period according to ANOVA/Tukey test (p< 0.05).
*** Significantly higher than pre-hypoxic and hypoxic period according to ANOVA/Tukey test (p< 0.05)
# Significantly higher than control group according to Student’s t-test (p< 0.0001).
3.4. Renal Excretion of Metoclopramide and its Metabolites Following the i.v. Infusion to Steady-state and Induction of Hypoxemia.

3.4.1. Renal clearance of MCP and its metabolites during hypoxemia and normoxemia.

Renal clearance values of MCP and mdMCP were calculated from 1) dividing the amount recovered ($D_{ur}$) by area under drug plasma concentrations curve ($AUC_{ur}$) as a function of time during the hypoxemic and normoxemic periods [$Du(t_2-t_1)/AUC(t_2-t_1)$] and 2) calculation using the slope of the accumulated drug in urine ($Du$) versus ($AUC_u$) (see Figure 9 and Appendix A for the equation derivation). Since the plasma concentration of ddMCP appears to be below the quantitation limit, its renal clearance value could not be calculated. As shown in Tables 13 and 14, the renal clearance values obtained by both methods were relatively consistent. Some discrepancies between the calculated clearance values were observed when there were data point(s) noticeably deviating from the regression line in the plot of $D_{ur}$ versus $AUC_u$.

Tables 13a and 13b show the MCP renal clearance calculated by both method 1 and 2 during the hypoxemic and post-hypoxemic periods. Due to the short duration of the pre-hypoxemic period (i.e. 2 hours with 3 data points), the renal clearance during this period could only be approximated, and the value during hypoxemia was compared to the post-hypoxemic values. In the experimental group, renal MCP clearance during hypoxemia (2.75 ± 0.22 L/h) was significantly lower (by = 64%) than during the post-hypoxemic period (8.15 ± 1.86 L/h). In contrast, the renal clearance remained constant (7.01 ± 0.72 L/h and 6.73 ± 0.59 L/h respectively) during the equivalent times in the control group. In addition, renal clearance in the experimental group was significantly lower than in the control group during
the hypoxemic period, but not afterwards. There was a considerable individual variation in
the reduction of renal clearance during hypoxemia. For example, ewe number 1158 showed
an apparent 75% reduction in the renal clearance during hypoxemia, whereas ewe number
139 showed a 33% decrease.

Tables 14a and 14b list the mdMCP renal clearance during hypoxemia and post-
hypoxemia. The values of mdMCP renal clearance were considerably higher (≈ 6-7 fold)
than those of MCP. Higher urinary accumulation and, to a lessor degree, lower plasma
mdMCP concentration contributed to higher renal clearance values for mdMCP. Similar to
the MCP renal clearance in the experimental group, the mdMCP renal clearance during
hypoxemia (14.60 ± 2.56 L/h) was significantly lower (≈ 62%) than during post-hypoxemia
(38.57 ± 9.09 L/h). In the control group, the renal clearance remained constant (43.90 ± 9.41
L/h and 38.39 ± 7.12 L/h, respectively). Consequently, as with MCP renal clearance, the
value for mdMCP in the experimental group was significantly lower than that in the control
group during the hypoxemic period, but not during post-hypoxemia. There was also a
considerable individual variation in the reduction of mdMCP renal clearance during
hypoxemia, although less than that seen for MCP renal clearance. For example, with ewe no.
1158, there was an apparent 69% reduction in renal clearance during hypoxemia while ewe
no. 139 showed a 39% decrease.
Figure 9  Representative plot of the accumulated drug (or metabolite) in urine ($\Sigma Du$) versus AUC. Slope of the curve represents the renal clearance [L/h] (ewe no. 102 in the experimental group).

$HO = 2.89 \text{ L/h}; \text{post-HO} = 8.99 \text{ L/h}$

$HO = \text{hypoxemia}, \text{post-HO} = \text{post-hypoxemia}.$
Table 13a. MCP renal clearance (L/h)\(^1\) \([CL_{\text{renal(MCP)}}]\)

<table>
<thead>
<tr>
<th>CLrenal(MCP)</th>
<th>ewe no.</th>
<th>hypoxemia</th>
<th>post-hypoxemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>102</td>
<td>3.20</td>
<td>8.25</td>
</tr>
<tr>
<td>Group</td>
<td>139</td>
<td>2.09</td>
<td>2.92</td>
</tr>
<tr>
<td></td>
<td>989</td>
<td>3.33</td>
<td>7.21</td>
</tr>
<tr>
<td></td>
<td>1158</td>
<td>2.94</td>
<td>11.07</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>2.89*</td>
<td>7.36</td>
</tr>
<tr>
<td>± SEM</td>
<td></td>
<td>0.28</td>
<td>1.69</td>
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<tr>
<td>Control</td>
<td>220</td>
<td>6.58</td>
<td>6.97</td>
</tr>
<tr>
<td>Group</td>
<td>327</td>
<td>4.96</td>
<td>5.78</td>
</tr>
<tr>
<td></td>
<td>328</td>
<td>7.59</td>
<td>8.01</td>
</tr>
<tr>
<td></td>
<td>1159</td>
<td>7.73</td>
<td>7.41</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>6.72</td>
<td>7.04</td>
</tr>
<tr>
<td>± SEM</td>
<td></td>
<td>0.64</td>
<td>0.47</td>
</tr>
</tbody>
</table>

* Significantly lower (p<0.03) than post-hypoxemia according to paired t-test.
# Significantly lower (p<0.001) than the control group according to t-test.
1 renal clearance calculated using time-averaged urinary excretion \([Du(t_{2-1})/AUC(t_{2-1})]\).

Table 13b. MCP renal clearance (L/h)\(^2\) \([CL_{\text{renal(MCP)}}]\)

<table>
<thead>
<tr>
<th>CLrenal(MCP)</th>
<th>ewe no.</th>
<th>hypoxemia</th>
<th>post-hypoxemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>102</td>
<td>2.89</td>
<td>8.99</td>
</tr>
<tr>
<td>Group</td>
<td>139</td>
<td>2.09</td>
<td>3.13</td>
</tr>
<tr>
<td></td>
<td>989</td>
<td>2.95</td>
<td>8.39</td>
</tr>
<tr>
<td></td>
<td>1158</td>
<td>3.05</td>
<td>12.08</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>2.75*#</td>
<td>8.15</td>
</tr>
<tr>
<td>± SEM</td>
<td></td>
<td>0.22</td>
<td>1.86</td>
</tr>
<tr>
<td>Control</td>
<td>220</td>
<td>6.83</td>
<td>6.21</td>
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<tr>
<td>Group</td>
<td>327</td>
<td>5.11</td>
<td>5.44</td>
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<tr>
<td></td>
<td>328</td>
<td>7.66</td>
<td>7.06</td>
</tr>
<tr>
<td></td>
<td>1159</td>
<td>8.45</td>
<td>8.19</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>7.01</td>
<td>6.73</td>
</tr>
<tr>
<td>± SEM</td>
<td></td>
<td>0.72</td>
<td>0.59</td>
</tr>
</tbody>
</table>

* Significantly lower (p<0.025) than post-hypoxemia according to paired t-test.
# Significantly lower (p<0.001) than the control group according to t-test.
2 renal clearance calculated using the slope of \([Du(t_{0})/AUC(t_{0})]\) plot.
Table 14a. mdMCP renal clearance (L/h)\(^1\) [\(CL_{\text{renal(mdMCP)}}\)]

<table>
<thead>
<tr>
<th>(CL_{\text{renal(mdMCP)}})</th>
<th>ewe no.</th>
<th>hypoxemia</th>
<th>post-hypoxemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental Group</strong></td>
<td>102</td>
<td>12.38</td>
<td>26.67</td>
</tr>
<tr>
<td></td>
<td>139</td>
<td>12.14</td>
<td>20.90</td>
</tr>
<tr>
<td></td>
<td>989</td>
<td>21.79</td>
<td>53.68</td>
</tr>
<tr>
<td></td>
<td>1158</td>
<td>11.44</td>
<td>40.66</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td>14.44(<em>^</em>)</td>
<td>35.48</td>
</tr>
<tr>
<td><strong>± SEM</strong></td>
<td></td>
<td>2.46</td>
<td>7.35</td>
</tr>
</tbody>
</table>

| **Control Group**            | 220     | 65.96     | 59.97          |
|                              | 327     | 35.75     | 36.23          |
|                              | 328     | 44.25     | 52.12          |
|                              | 1159    | 30.88     | 22.03          |
| **Mean**                     |         | 44.21     | 42.59          |
| **± SEM**                    |         | 7.76      | 8.45           |

\* Significantly lower (p<0.02) than post-hypoxemia according to paired t-test.
\# Significantly lower (p<0.001) than the control group according to t-test.

1 renal clearance calculated using time-averaged urinary excretion \([Du(t2-t1)/AUC(t2-t1)]\).

Table 14b. mdMCP renal clearance (L/h)\(^2\) [\(CL_{\text{renal(mdMCP)}}\)]

<table>
<thead>
<tr>
<th>(CL_{\text{renal(mdMCP)}})</th>
<th>ewe no.</th>
<th>hypoxemia</th>
<th>post-hypoxemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental Group</strong></td>
<td>102</td>
<td>9.84</td>
<td>26.72</td>
</tr>
<tr>
<td></td>
<td>139</td>
<td>13.20</td>
<td>21.69</td>
</tr>
<tr>
<td></td>
<td>989</td>
<td>21.85</td>
<td>61.70</td>
</tr>
<tr>
<td></td>
<td>1158</td>
<td>13.49</td>
<td>44.17</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td>14.60(<em>^</em>)</td>
<td>38.57</td>
</tr>
<tr>
<td><strong>± SEM</strong></td>
<td></td>
<td>2.56</td>
<td>9.09</td>
</tr>
</tbody>
</table>

| **Control Group**            | 220     | 70.91     | 55.85          |
|                              | 327     | 31.65     | 33.64          |
|                              | 328     | 42.58     | 42.10          |
|                              | 1159    | 30.44     | 21.99          |
| **Mean**                     |         | 43.90     | 38.39          |
| **± SEM**                    |         | 9.41      | 7.12           |

\* Significantly lower (p<0.02) than post-hypoxemia according to paired t-test.
\# Significantly lower (p<0.001) than the control group according to t-test.

2 renal clearance calculated using the slope of \(Du(t-0)/AUC(t-0)\) plot.
3.4.2. *Fractional renal excretion constants of MCP and its metabolites*

The fractional renal excretion constants were approximated for MCP and two of its metabolites, mdMCP and ddMCP, in the present study. The fractional renal excretion constants (*e.g.* $fu(MCP) = ku/KE$) were calculated by dividing the slope of the asymptote of the accumulated drug/metabolites in urine versus time curve with the infusion rate, $ko$ (Figure 10), and these fractional renal excretion constants are dimensionless (*i.e.* they represent a proportion of the total excretion constant in terms of a fraction). The detailed equation derivations are shown in appendix A and theoretical and practical discussion is described in section 4.4. The fractional renal excretion constant was used to determine relative changes in the renal excretion of the parent drug and its metabolite (*i.e.* the proportion of the renal excretion to the total body elimination of the drug/metabolite).

**Scheme V**  Diagram of metabolic and renal elimination of a drug

where $X_B$ and $M_B$ represent the amount of drug and metabolite in the body, respectively, $X_u$ and $M_u$ represent the drug and metabolite excreted in urine, respectively. $ku$ and $kf$ are renal and metabolite elimination rate constants and the sum of these two rate constants is equal to $KE$, apparent first order drug elimination rate constant. $kmu$ represents renal elimination rate constant of the metabolite.
Table 15 lists the fractional renal MCP excretion constant \( (fu(MCP) = ku/K_E) \). Due to the short duration of the pre-hypoxemic period (i.e. 2 hours with 3 data points), the fractional renal excretion constant, \( fu(MCP) \), was not calculated during the pre-hypoxemic period. As with the MCP and mdMCP renal clearance values, the \( fu(MCP) \) value during hypoxemia was compared to the post-hypoxemic estimate. During the hypoxemic period, the \( fu(MCP) \) is significantly lower by 58\%, \( (0.0138 \pm 0.0019) \) than during the post-hypoxemic period \( (0.0329 \pm 0.0053) \) in the experimental group. In control group, the \( fu(MCP) \) remained fairly constant throughout these periods, \( 0.0296 \pm 0.0032 \) and \( 0.0282 \pm 0.0040 \), respectively. Consequently, the \( fu(MCP) \) value during the hypoxemic period in the experimental group was significantly lower than corresponding estimate in the control group.

The fractional renal metabolite excretion constants for mdMCP and ddMCP were also calculated in the study. Because the individual parameters such as metabolite formation and elimination rate constants cannot be determined with the present study design, unless the metabolites are injected individually, these parameters are the product of two fractional constants \( fm(metabolite) \) and \( fmu(metabolites) \). The metabolite formation fraction constant \( fm(metabolite) \) is the fraction of \( k_f(metabolite)/K_E(parent\ drug) \), which represents the metabolic proportion (to a specific metabolite) of the total parent drug elimination. The second fractional constant, the renal metabolite elimination fraction \( fmu(metabolite) \) is the fraction of \( k_{mu(metabolite)}/Km(metabolite) \), which represents the renal excretion proportion of the total metabolite elimination. Therefore, the composite fractional constant, the renal metabolite excretion fraction \( f^*u(metabolite) = fm(metabolite) x fmu(metabolite) \), represents the proportion of renal excretion of a specific metabolite from the total drug elimination. For example, if the value of \( f^*u(metabolite\ A) = \)
\( f_{m}(\text{metabolite A}) \times f_{mu}(\text{metabolite A}) \) was 0.01 = 0.05 * 0.2, then it would mean that 5% of parent drug was bio-transformed into the metabolite A and 20% of the metabolite was excreted in the urine (\text{i.e.} overall 1% of the parent drug is excreted in the urine in the form of metabolite A).

![Graph](image)

**Figure 10.** Representative plot of the accumulated drug (or metabolite) in urine (\( \Sigma Du \)) versus time (h). The slope of the curve represents the product of the fractional renal excretion rate constant for MCP (or metabolite) and the infusion rate constant of MCP. (Ewe no. 102 in the experimental group). HO = hypoxemia, post-HO = post-hypoxemia.

The fractional renal metabolite excretion constants of mdMCP and ddMCP are listed in Tables 16 and 17. The \( f_{u}(\text{mdMCP}) \) was significantly lower (\~{}65\%) during hypoxemia (0.0378 ± 0.0098) compared to that of the post-hypoxic period (0.1080 ± 0.0339) in the experimental group. In the control group, the \( f_{u}(\text{mdMCP}) \) remained constant, 0.0719 ± 0.0085 and 0.0711 ± 0.0125, respectively. Thus, the \( f_{u}(\text{mdMCP}) \) value during hypoxemia in the
experimental group was also significantly lower than that of the control group. Similar to the renal clearance values from the previous section, the $f_{u(mdMCP)}$ values were about 2 to 4 fold higher than those of $f_u(MCP)$. In some cases (ewe numbers 989 and 1158 and 328), this process has accounted for more than 10% of the total drug elimination.

The $f_{u(ddMCP)}$ was also significantly lower (~60%) during hypoxemia (0.0223 ± 0.056) compared to that of the post-hypoxemia (0.0562 ± 0.0152) in the experimental group. In the control group, the $f_{r(mdMCP)}$ remained constant, 0.0259 ± 0.0053 and 0.0259 ± 0.0052, respectively. However, unlike $f_{u(MCP)}$ and $f_{u(mdMCP)}$, the $f_{u(ddMCP)}$ value during hypoxemia in the experimental group was not significantly different from that of the control group.

Table 15. MCP fractional renal excretion constants $[f_u(MCP)]^a$

<table>
<thead>
<tr>
<th>$f_u(MCP)$</th>
<th>ewe no.</th>
<th>hypoxemia</th>
<th>post-hypoxemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>102</td>
<td>0.0151</td>
<td>0.0401</td>
</tr>
<tr>
<td>Group</td>
<td>139</td>
<td>0.0124</td>
<td>0.0182</td>
</tr>
<tr>
<td></td>
<td>989</td>
<td>0.0183</td>
<td>0.0410</td>
</tr>
<tr>
<td></td>
<td>1158</td>
<td>0.0094</td>
<td>0.0325</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.0138**</td>
<td>0.0329</td>
</tr>
<tr>
<td>± SEM</td>
<td></td>
<td>0.0019</td>
<td>0.0053</td>
</tr>
<tr>
<td>Control</td>
<td>220</td>
<td>0.0205</td>
<td>0.0214</td>
</tr>
<tr>
<td>Group</td>
<td>327</td>
<td>0.0312</td>
<td>0.0250</td>
</tr>
<tr>
<td></td>
<td>328</td>
<td>0.0349</td>
<td>0.0399</td>
</tr>
<tr>
<td></td>
<td>1159</td>
<td>0.0319</td>
<td>0.0266</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.0296</td>
<td>0.0282</td>
</tr>
<tr>
<td>± SEM</td>
<td></td>
<td>0.0032</td>
<td>0.0040</td>
</tr>
</tbody>
</table>

* Significantly lower (p<0.02) than the post-hypoxemic period according to paired t-test.

* Significantly lower (p<0.005) than the control group according to t-test.

$^a$ Renal elimination fraction constant $f_u(MCP)$ denotes the ratio of $ku/KE$ of MCP.
Table 16. mdMCP fractional renal excretion constants $^a$ $[f_u(\text{mdMCP})]^a$

<table>
<thead>
<tr>
<th>$f_u(\text{mdMCP})$</th>
<th>ewe no.</th>
<th>hypoxemia</th>
<th>post-hypoxemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>102</td>
<td>0.0248</td>
<td>0.0560</td>
</tr>
<tr>
<td>Group</td>
<td>139</td>
<td>0.0273</td>
<td>0.0530</td>
</tr>
<tr>
<td></td>
<td>989</td>
<td>0.0668</td>
<td>0.1955</td>
</tr>
<tr>
<td></td>
<td>1158</td>
<td>0.0323</td>
<td>0.1274</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.0378**</td>
<td>0.1080</td>
</tr>
<tr>
<td>± SEM</td>
<td></td>
<td>0.0098</td>
<td>0.0339</td>
</tr>
<tr>
<td>Control</td>
<td>220</td>
<td>0.0766</td>
<td>0.0758</td>
</tr>
<tr>
<td>Group</td>
<td>327</td>
<td>0.0638</td>
<td>0.0614</td>
</tr>
<tr>
<td></td>
<td>328</td>
<td>0.0933</td>
<td>0.1032</td>
</tr>
<tr>
<td></td>
<td>1159</td>
<td>0.0539</td>
<td>0.0442</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.0719</td>
<td>0.0711</td>
</tr>
<tr>
<td>± SEM</td>
<td></td>
<td>0.0085</td>
<td>0.0125</td>
</tr>
</tbody>
</table>

* Significantly lower (p<0.05) than post-hypoxemia according to paired t-test.
# Significantly lower (p<0.05) than the control group according to t-test.

$a f_u(\text{mdMCP})$ denotes the composite fraction constant $f_m(\text{mdMCP}) \times f_{mu}(\text{mdMCP})$, the product of the ratio of $k_f(\text{mdMCP})/K_{E\text{mdMCP}}$ and $k_{mu}(\text{mdMCP})/K_{m\text{mdMCP}}$.

Table 17. ddMCP fractional renal excretion constants $^a$ $[f_u(\text{ddMCP})]^a$

<table>
<thead>
<tr>
<th>$f_u(\text{ddMCP})$</th>
<th>ewe no.</th>
<th>hypoxemia</th>
<th>post-hypoxemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
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<td>0.0127</td>
<td>0.0262</td>
</tr>
<tr>
<td>Group</td>
<td>139</td>
<td>0.0188</td>
<td>0.0369</td>
</tr>
<tr>
<td></td>
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<td>0.0384</td>
<td>0.0926</td>
</tr>
<tr>
<td></td>
<td>1158</td>
<td>0.0194</td>
<td>0.0693</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.0223*</td>
<td>0.0562</td>
</tr>
<tr>
<td>± SEM</td>
<td></td>
<td>0.0056</td>
<td>0.0152</td>
</tr>
<tr>
<td>Control</td>
<td>220</td>
<td>0.0365</td>
<td>0.0377</td>
</tr>
<tr>
<td>Group</td>
<td>327</td>
<td>0.0153</td>
<td>0.0157</td>
</tr>
<tr>
<td></td>
<td>328</td>
<td>0.0181</td>
<td>0.0190</td>
</tr>
<tr>
<td></td>
<td>1159</td>
<td>0.0335</td>
<td>0.0314</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.0259</td>
<td>0.0259</td>
</tr>
<tr>
<td>± SEM</td>
<td></td>
<td>0.0053</td>
<td>0.0052</td>
</tr>
</tbody>
</table>

* Significantly lower (p<0.02) than post-hypoxemia according to paired t-test.

$a f_u(\text{ddMCP})$ denotes the composite fraction constant $f_m(\text{ddMCP}) \times f_{mu}(\text{ddMCP})$, the product of the ratio of $k_f(\text{ddMCP})/K_{E\text{ddMCP}}$ and $k_{mu}(\text{ddMCP})/K_{m\text{ddMCP}}$. 
4. DISCUSSION

4.1. GC-MSD Method of Analysis of Metoclopramide and its Metabolites

A GC-MSD method for the assay of MCP, mdMCP and ddMCP, previously developed and validated in our laboratory (Riggs et al., 1994), was chosen for its high sensitivity and reproducibility for application to the present study. The GC-MSD method has a number of inherent advantages over the GC-ECD method previously developed in our laboratory (Riggs et al., 1983; 1990), due to the selective nature of mass spectrometry. The simultaneous detection and quantification of MCP and its metabolites at higher (=2x) sensitivity was made possible using MSD technology. Moreover, the extraction procedures are simpler than with the GC-ECD method, thereby reducing the sample preparation time significantly.

The use of narrow bore fused silica capillary columns and GC-MSD with selective ion monitoring enabled us to separate and quantitatively analyze the deethylated metabolites of MCP in the presence of MCP in the biological fluids collected in the study. Two deethylated metabolites of MCP, mdMCP and ddMCP, were analysed from various biological fluids. The use of narrow bore (0.2 mm I.D.) fused silica capillary columns in the place of the conventional 0.33 mm I.D. columns and the solvent trapping effect (Riggs et al., 1994) has provided baseline separation among the metabolites and MCP. This increased separation, especially between MCP and mdMCP (a metabolite often present in quantities greater than the intact MCP) was essential for the accurate quantitation of these compounds in urine. Significant amounts of mdMCP were recovered in urine during the infusion study,
therefore there is a possibility of over-estimation of MCP in these samples if the analysis provides insufficient separation, particularly when GC-ECD is the method of choice. In contrast, the use of selective ion monitoring method in GC-MSD has enabled the detection of the analytes with minimal interference from endogenous compounds. As shown in Figure 1, the interference from contaminants was minimal even with reduced extraction procedures.

Linearity over a 1-40 ng/mL concentration range was observed for MCP, mdMCP and ddMCP extracted from plasma and urine with individual standard curves from each matrix having a coefficient of determination ($r^2$) of at least 0.98. The method has been found to be reliable with an overall average coefficient of variation less than 10% and the limit of quantitation of 1 ng/mL. The standard curves were linear, however, slightly greater variability (*i.e.* slightly higher CV values) was observed in the corresponding drug concentration below 4 ng/mL. Since a slight variation in the peak area ratio at the higher concentrations can greatly affect the y-intercept of the linear regression curve, the lower drug concentrations in the curve will be affected more by this variation. The use of the weighting factor of $1/$(peak area ratio) improved the linearity over the whole range, especially at the lower concentration range. Even though it was not crucial to the infusion study, the GC-MSD method permitted attainment of a minimum quantitation limit of 1 ng/mL, representing 2.5 pg at the detector, a reasonable improvement over the quantitation limit of 2 ng/mL obtained with the previous GC-ECD method.

Thus, the GC-MSD method with selective ion monitoring provides a more stable, straightforward and reliable means for measurement of MCP and its metabolites than previous methods of MCP measurement. Furthermore, it is anticipated that, when the present
work is completed, the GC-MSD method will enable us further investigations of stable isotope labeled MCP in pregnant hypoxemic animals under another animal research protocol.

4.2. **Physiological Changes Associated with Hypoxemia**

4.2.1. *Induction of hypoxemia and Blood gas changes*

Several experimental methods of inducing hypoxemia have been previously used, such as reduced inspired oxygen content and carbon monoxide (CO) administration (Roth *et al.*, 1976). There are several reported methods for reducing inspired O\(_2\) content, including an isolated gas chamber (Medina and Merritt, 1970), a hypobaric chamber (Jacobs *et al.*, 1988), a Plexiglas\(^\circledR\) chamber enclosing the head and neck of the ewe (Rurak *et al.*, 1990) or plastic bag over the head (du Souich *et al.*, 1984) into which a gas mixture can be delivered to cause hypoxemia, and lastly, an intra-tracheal nitrogen infusion (Gleed *et al.*, 1986). The latter method involves infusion of nitrogen (≈4-10 L/min) via a small non-occlusive catheter chronically implanted in the trachea. The nitrogen mixes with the air normally inspired by the animal, resulting in a lowering of O\(_2\) concentration. Various degrees of hypoxemia can be achieved by varying the nitrogen flow rate. A rapid PaO\(_2\) equilibration time (≈3 min) can be obtained after changing the N\(_2\) flow. Intra-tracheal nitrogen infusion was an effective method of lowering PaO\(_2\) in the sheep. The lowered PaO\(_2\) was reached within 5 min. of the onset of hypoxemia (first sampling after nitrogen infusion). Moreover, the animal did not appear to be disturbed by nitrogen infusion. The implantation of an intra-tracheal catheter was a minor surgical procedure and performed at the same time with other surgical procedures during the general anaesthesia. Other methods of inducing hypoxemia in the
experimental animal, such as an isolation gas chamber and placement of the plastic bag over
the head, could potentially cause undue restriction and stress to the animals. The intra­
tracheal infusion method seems to minimize some of the side effects of other experimental
methods used in the sheep. All animals under the study have free access to water and food in
their normal housing before and during experiments. Furthermore, all ewes are maintained in
a relatively close contact with other companion ewes during each experimental period, and,
thus, environmental alterations during and between experiments were minimized. In
addition, the intra-tracheal nitrogen infusion procedure consumes less nitrogen (i.e. ≈7 L/min
vs. >40 L/min for the chamber methods), thus resulting in a substantial cost savings.

Intra-tracheal nitrogen infusion (7 to 11 L/min) rapidly induced a moderate
hypoxemia (PaO₂ ≈ 60 mmHg) in the sheep, within 5 min of the onset of nitrogen infusion.
This is similar to the result reported for pregnant ewes in the study that first described the
nitrogen infusion technique (Gleed et al., 1986). At the same time, a significant decrease in
PaCO₂ (hypocapnia) and a tendency of increased blood pH (respiratory alkalosis) was
observed in the experimental group. Infused nitrogen mixed with normally inspired room air
will result in a reduction of oxygen content in the inspired gas mixture in the lung thus
resulting in reduction of PaO₂. In addition, hypocapnia is caused by hyperventilation, since a
typical response to reduced PaO₂ is increased ventilation (hyperventilation) (Daly, 1963).
This lowered PaO₂ was maintained through intra-tracheal nitrogen infusion (rate = 7 L/min).
However, in some animals, nitrogen infusion was increased to maintain PaO₂ around 60
mmHg. A variation in individual response to hypoxemia was observed, but correlation
between hypoxemic response and physiological values such as urine flow and lactate was not
obvious from a visual survey.
4.2.2. Blood pH and lactate concentration during hypoxemia

Increased blood lactate concentration was observed in the late hypoxemic period in the experimental group and it remained relatively high through the early and middle post-hypoxemic recovery periods. Thus, there seems to be a trend of a gradual increase in the blood lactate concentration during hypoxemia. However, this accumulation of lactate is not likely the result of inadequate oxygen supply to the tissues, since the $O_2$ saturation during hypoxemia in the study remained around 70 to 80 %. In addition, the "excess lactate" level, an indication of tissue $O_2$ adequacy, is estimated to be close to zero at the level of $PaO_2$ obtained in the study (≈ 60 mmHg) (Cain, 1965), thus the change in the lactate concentration in the present study is not likely due to $O_2$ deficit of body. Rather, it is more likely related to hypocapnia and the resulting changes in acid-base balance during hypoxemia (Huckabee, 1958). The increased glucose level during late hypoxemia and hypocapnia during hypoxemic period may also, in part, explain the lactate accumulation.

It has been suggested that the lactate production is not controlled exclusively by the adequacy of cellular oxygenation, and is affected to a very significant extent by the pyruvate changes, hyperventilation or pH alteration of the body and changes in blood glucose concentration (Huckabee, 1957) and sympathetic activity. Takano (1968) has demonstrated that plasma lactate accumulation in dogs was less for isocapnic hypoxia compared to hypocapnic hypoxia. Cain (1969) also showed diminished lactate accumulation during hypoxemia with $\beta$-adrenergic blockade and hypercapnia. Furthermore, Cain (1973) observed lactate accumulation induced by $\beta$-stimulation without oxygen deficit. Zborowska-Sluis and
Dossetor (1967) observed increased blood lactate concentration in hyperventilated dogs with no increase in lactate production across major organ systems (no changes in the A-V lactate concentration difference across liver, muscle, and gut). In this study, a small decrease in hepatic lactate utilization was observed, but it was estimated to account for only a small portion of the lactate increase. A stimulation of red blood cell glycolysis by low PaCO₂ or respiratory alkalosis was also observed (Murphy, 1960; Zborowska-Sluis and Dossetor, 1967). With no effective aerobic metabolic system present, energy production in the red blood cell is almost by anaerobic glycolysis, and thus independent of changes in oxygen availability. Therefore, these studies suggest that lactate accumulation during a moderate hypoxemia is associated with alteration in blood acid-base balance, and/or glucose concentration and sympathetic activation.

Acidosis was also observed in the post-hypoxemic period, but not in the hypoxemic period. This delayed response suggests that acidosis is likely a secondary response to hypoxemia, rather than a direct response. The decrease in blood pH seems to correspond with an increase in lactate level in the late hypoxemic and early/middle post-hypoxemic period (hour 6:00 to 12:00). An increase in hydrogen ion concentration (i.e. acidosis during post-hypoxemia) in the blood is likely a secondary reaction to the accumulation of lactic acid (Frommer, 1983).
4.2.3. *Adrenosympathetic system and blood glucose during hypoxemia*

Increased heart rate and mean arterial blood pressure during the hypoxemic period was observed in the present study. Since hypoxemia is known to cause activation of the adrenosympathetic system and, also, to increase circulating catecholamine concentrations, the increased heart rate and arterial blood pressure in the study may be associated with this adrenosympathetic activation.

Stimulated adrenosympathetic activity and increased plasma catecholamine concentration during hypoxia has been observed in many previous studies. Since Cannon and Hoskins (1911) demonstrated secretion of epinephrine into adrenal venous blood during asphyxia, it has been generally accepted that hypoxia stimulates a sympathoadrenal discharge. Claustre and Peyrin (1982) and Claustre *et al.* (1985) reported a mild adrenosympathetic activation and a significant increase in plasma free catecholamine (epinephrine, norepinephrine and dopamine) concentration during a moderate hypoxia in rats and cats. The increase in plasma epinephrine level in these studies was especially profound during hypoxia, and this increased epinephrine concentration may contribute to physiological changes associated with hypoxia. Johnson *et al.* (1983) also observed elevated adrenal medullary activity and urinary epinephrine excretion during acute moderate hypoxemia independent of cardiac sympathetic activity in rats, thus speculating that the response during acute moderate hypoxemia is primary by adrenal medullary activation. In addition, the activation of the adrenomedullary system by hypoxemia may have some residual effect even after restoration of PaO₂. Critchley *et al.* (1980) showed that the release of catecholamine from the adrenal medulla, in response to prolonged carotid body hypoxemia, outlasted the stimulus by more
than 30 min. They also found that a moderate hypoxemia with carotid arterial PaO₂ of 50-60 mmHg was sufficient to evoke the release of catecholamine.

A small, but statistically significant, increase in glucose concentration during hypoxemia was also observed in the present study. The relatively small increase in blood glucose concentration in the present study is likely due to a moderate level of hypoxemia. The development of a modest degree of hyperglycemia under stress such as hypoxia and hypothermia may represent an adaptive response to stress, helping to protect and maintain the supply of an important fuel for the brain and other tissue (Baum and Porte, 1980). Increased blood glucose concentration during stress (stress hyperglycemia) is common in acute hypoxemia (Baum and Porte, 1980). Earlier studies have suggested that the elevated blood glucose concentration was a result of increased hepatic glycogenolysis and the direct hormonal effects of hypoxia (Britton and Kline, 1945; Himwich et al., 1943; Shelley, 1961). There is an increased epinephrine secretion by the adrenal medulla in combination of secondary glucagon release by α-cells of pancreatic islets during hypoxemia. This, in turn, stimulates glycogen breakdown in muscles and liver, thus resulting in the elevation of blood sugar level.

Catecholamines can directly stimulate glucose production by the liver (Rizza et al., 1980). In addition, they can interfere with insulin-mediated glucose uptake (Chiasson et al., 1981; Deibert and DeFronzo, 1980). Therefore, increased plasma catecholamine levels during hypoxemia result in an increased blood glucose level and subsequent shunting of blood glucose to insulin-independent tissues, such as the brain. There is also evidence for a stimulatory effect of catecholamine on glucagon secretion mediated via a β-adrenergic and, in
a lessor degree, α-adrenergic mechanism (Gerich et al., 1973; Gerich et al., 1974; Samols and Weir, 1979). When anaesthetized dogs were made acutely hypoxic, a very large inhibition of insulin secretion and increased plasma glucagon levels were observed (Baum and Porte, 1980).

The sympathetic nervous system, activated at low oxygen tension (Comline and Silver, 1966; Cunningham et al., 1965), has an important role in mediating the hypoxic inhibition of insulin release (Baum and Porte, 1969). α-adrenergic receptor stimulation, known to inhibit insulin secretion, is a major factor in this process (Baum and Porte, 1972). Although both α- and β-adrenergic receptors are stimulated with sympathetic activation, α-adrenergic suppression of insulin predominates in hypoxia because β-adrenergic enhancement is impaired (Baum and Porte, 1976). This suppression of insulin in combination with hepatic glycogenolysis mediated by epinephrine and glucagon during hypoxia may explain the elevated level of blood glucose during hypoxemia and subsequent post-hypoxemia in the present study.

4.2.4. Urine flow and osmolality during hypoxemia and MCP infusion.

In the present study, urine flow was significantly increased during hypoxemia, at the same time, urine osmolality was significantly decreased in the experimental group. Diuresis during acute hypoxemia, either hypocapnic or isocapnic, was demonstrated in the conscious dog (Walker, 1982) and rat (Colice et al., 1991). Studies previous to these, however, have reported a variety of urine flow responses (diuresis and antidiuresis) to acute hypoxemia (Selkurt, 1953; Stickney et al., 1946; Anderson et al., 1978). However, many of these earlier
studies used anaesthetized and/or extensively manipulated animal preparations, which may have complicated results and made interpretation difficult. Acute hypoxemia in humans usually has resulted in a diuretic response (Berger et al., 1949; Burrill et al., 1945; Currie and Ullman, 1961; Ullman, 1961).

Currently, the mechanism of diuresis during hypoxemia is not clearly understood. A number of mechanisms such as increased GFR secondary to increased renal blood flow and arterial blood pressure, and hypoxemia-induced hormonal changes in plasma atrial natriuretic factor (ANF), aldosterone and vasopressin levels have been proposed.

The diuretic response during hypoxemia may be, in part, related to a hemodynamic alteration associated with hypoxemia. In dogs, hypocapnic hypoxemia caused an increase in GFR and renal blood flow secondary to the arterial blood pressure and cardiac output increase (with a lesser degree in isocapnic hypoxemia), while renal vascular resistance remained unaltered (Koehler et al., 1980; Walker, 1982). In addition, glomerular filtration rate (GFR) was also increased during hypoxemia in these studies. However, acute hypoxemia-induced diuresis in the rat has been associated with a fall in systemic arterial blood pressure and cardiac output (Colice et al., 1991; Ou et al., 1989), thereby suggesting the involvement of other diuretic mechanism(s). In the present study in sheep, there was a small, but statistically significant, increase in mean arterial blood pressure during hypoxemia in the experimental group. But, it is not clear whether the hypoxemia-induced diuresis is, in fact, directly related to the increased mean arterial blood pressure, due to lack of information regarding renal blood flow and GFR. However, diuresis during hypoxemia may be, at least in part, due to elevated glomerular filtration rate due to an increase in renal blood flow secondary to arterial blood pressure increase.
Acute hypoxia in anaesthetized dogs has been reported to stimulate arginine vasopressin (AVP) release and an antidiuretic response (Anderson et al., 1978), but acute hypoxia in conscious humans and rats does not affect plasma AVP levels (du Souich et al., 1987; Ashack et al., 1985; Jones et al., 1981). Aldosterone levels fall with acute hypoxia in humans, but only after 30 to 60 min of exposure (Colice and Ramirez, 1985). Thus, these studies suggested that AVP and aldosterone may not be closely related to acute hypoxia-induced diuresis. The involvement of ANF in hypoxemia-induced diuresis was also suggested (Colice et al., 1991; Koller et al., 1990; Shimizu and Nakamura, 1986). It has been reported that acute hypoxemia is a potent stimulus for atrial natriuretic factor (ANF) release (du Souich et al., 1987; Ramirez et al., 1988; Lawrence et al., 1990) in humans, in isolated rat and rabbit hearts (Baertschi et al., 1986) and in rabbits (Baertschi et al., 1988). An immediate ANF release from the heart at the onset of hypoxia was reported (Baertschi et al., 1986), which coincides with the transient diuresis in acute hypoxemia. A correlation between plasma ANF concentration and arterial blood pressure was suggested (du Souich et al., 1987). However the increased plasma ANF levels during hypoxemia are more likely associated with right atrial distention secondary to pulmonary hypertension (Ou et al., 1986) and hyperventilation (Colice et al., 1991), since the distention of cardiac atria is the primary physiological stimulus for the release and synthesis of ANF (Lange et al., 1987).

There was a significant decrease in urine osmolality during hypoxemia, unlike the acute moderate hypoxemia-induced diuresis in the studies of Walker (1982) and Colice et al. (1991) which showed no changes in urine osmolality during hypoxemia. The average renal osmolal excretion rate, the product of urine flow and urine osmolality, was also decreased.
significantly during hypoxemia. The osmolal excretion parameter was used in the study and could be regarded as a rough indicator of the quantity of salt and other soluble compounds excreted in urine in a given time, since urine electrolyte analysis could not be performed satisfactorily in all the urine samples in the present study. Urine electrolyte analysis of the two animals in the experimental group was performed, and there appear to be no significant changes in electrolyte excretion (i.e. no natriuresis or kaliuresis) during hypoxemia in contrast to the finding of Walker (1982) and Colice et al. (1991). Figure 7 shows the relationship between urine osmolality and urine flow. There was a negative correlation with the coefficient of determination ($r$) of 0.80, which means the increased urine flow was accompanied by lowered urine osmolality. These relationships suggest that the increased urine flow during hypoxemia is not likely the result of the increased urine volume excretion secondary to increased electrolyte excretion by the kidney, as shown in the previous studies (Walker, 1982; Colice et al., 1991). Therefore, the diuresis in the current study may be due to enhanced water excretion independent of urinary electrolyte excretion. Since hypoxemia was induced during the continuous MCP infusion, the presence of MCP could be associated with the low urine osmolality.

Since both the administration of MCP and acute hypoxemia are known to affect renal haemodynamics and urine flow in an opposing direction, the present study which combines these two factors raises an interesting perspective. MCP is reported to decrease renal blood flow and urine flow in humans (Israel et al., 1986). Norbiato et al. (1986) reported that MCP decreased free water excretion in man by increased vasopressin secretion. During acute moderate hypoxemia similar to the present study (oxygen saturation = 75-80%), the plasma aldosterone concentration (either normal or elevated by MCP administration) was
significantly reduced and ANF levels were slightly increased in humans (Vonmoos et al., 1990; Lawrence et al., 1990). The hypoxemia-induced increase in plasma ANF levels caused a prominent suppression of plasma aldosterone without natriuresis (Lawrence et al., 1990; Lawrence and Shenker, 1991). In addition, a low-dose ANF infusion, which is a similar level to a moderate hypoxemia-induced ANF increase, has little or no effect on natriuresis (Shenker et al., 1992). These results may explain the absence of natriuresis during hypoxemia in the present study. Unfortunately, the lack of information of plasma hormone levels (e.g. ANF and aldosterone) does not allow us to address this subject in the present study, and thus further studies may be required.

4.3. **Statistical and Practical Aspects of Experiment Design**

4.3.1. **Design of experimental protocol**

The infusion experimental protocol with 3 phases (pre-hypoxemic, hypoxemic and post-hypoxemic periods) in the study was developed in order to address some of problems commonly associated with studying pharmacokinetics during hypoxemia. Most previous pharmacokinetic studies during hypoxemia have been based on the i.v. bolus design with drug administration to the separate hypoxemic and control (normoxemic) groups [Design I] (du Souich et al., 1985 a, b). In some studies, paired experiments were performed on the same subjects [Design II] (Saunier et al., 1987; Clozel et al., 1981). Since there are considerable individual variations in the physiological and pharmacokinetic responses to hypoxemia (du Souich et al., 1978; Taburet et al., 1990), conducting a comparative study of pharmacokinetics on separate hypoxemic and control groups [Design I] poses some potential problems. This is particularly true when the sample size is small and the variance in the
group is large, as has been encountered in previous studies. A larger sample size would be required to test a significant difference between two groups with a higher population variance and small minimum detectable difference between population means (Zar, 1984). Thus, this design may be suitable for conducting experiments in a large population of small animal species such as the rat and rabbit where minimal surgical preparations are involved. This design [Design I] was rejected from consideration due to the practical limitation of conducting a large number of complicated surgeries and pharmacokinetic experiments on larger animals like sheep.

Two experimental protocols were initially considered. One protocol was a paired i.v. bolus study [Design II], where two separate i.v. bolus doses of MCP would be given on separate days under hypoxemic and normoxemic conditions, similar to Saunier et al. (1987). The other was continuous MCP infusion with normoxemic control and hypoxemic periods [Design III]. The i.v. bolus method offers complimentary information to that obtained with the present i.v. infusion method, i.e. the elimination rate constant and the volume of distribution. However, the paired i.v. bolus method poses several practical problems in the pharmacokinetic study of hypoxemia. The present study requires the quantitative comparison of pharmacokinetic parameters between hypoxemic and normoxemic conditions, in order to evaluate the impact of hypoxemia on drug disposition. A certain time delay (i.e. at least 2-3 days) between paired experiments would be required to ensure total elimination of drug and related metabolites from the body between successive experiments and all the conditions except inspired gas content should be identical for both experiments. The i.v. infusion method [Design III] has eliminated these requirements of a paired experiment and reduces the time for experiments. Physiological and pharmacokinetic changes induced by hypoxemia
could be demonstrated within a single experiment. In addition, it would be possible to observe dynamic physiological and pharmacokinetic changes during and after hypoxemia.

Another reason for developing the infusion method was to establish intra-subject controls, since it has been reported that individual tolerance toward hypoxemic stress and the degree of pharmacokinetic changes are highly variable (du Souich et al., 1978; Taburet et al., 1990). The use of an intra-subject control method in the present protocol has greatly improved the statistical analysis of pharmacokinetic and physiological data. For example, the data set of the plasma MCP steady-state concentration from pre-hypoxemic, hypoxemic and post-hypoxemic periods from a single experiment was used as the population and the mean values for each time period were compared using analysis of variance (ANOVA). Whereas, in other methods [Design I and II], it would be necessary to compare the hypoxemic and control group values of pharmacokinetic parameters such as rate of elimination, which have considerably higher variance. This process of comparison of the pharmacokinetic and physiological parameters from the hypoxemic and normoxemic periods within a single animal, instead of the hypoxemic and control groups, greatly decreases $\sigma^2$ [the population variance] and thus resulting in a higher $\phi$ value [the determining power] of the test in a given number of experiments (Zar, 1984). This means that the power of the test in a given number of the experiment subjects was improved in the present study. Therefore, the intra-subject comparison should provide a more reliable interpretation of the effect of hypoxemia and also reduce the number of animals required for the study.

In addition to the intra-subject control periods (Scheme III), the control group experiments were conducted in four of the ewes. In the control group, the infusion study was
conducted according to the same protocol as in the experimental group (Scheme IV), except for the nitrogen infusion-induced hypoxemia. The data sets corresponding to the same periods between experimental and control groups were qualitatively compared and statistically analysed. For example, a parallel comparison of changes in MCP steady-state concentrations between the experimental and control groups enables us to confirm that any changes observed are not an artifact of the experiment, such as might have arisen due to the prolonged infusion. Thus, the comparison of data from the experimental with the control group eliminates any possibility that the results were affected by factors not related to hypoxemia, such as obscure time-dependent effects.

Statistical comparisons using Student's t-test were performed between two groups, however, in some parameters with higher variation, these statistical analysis could not adequately performed due to low \( \phi \) value [the determining power of the test] \( (i.e. \) high variance and low sample number). These comparisons, in a sense, are similar to the comparison in the Design I type experiment, which compares the data from two data sets randomly chosen from the two populations. Therefore, the control group in the present study provides the additional comparison with the physiological and pharmacokinetic data gathered during the hypoxemic period in the experiment group.
4.3.2. Statistical and practical considerations for determining the steady-state drug concentration.

Previous studies on metoclopramide pharmacokinetics in sheep (Riggs et al., 1988: 1990) reported that the half-life of MCP in non-pregnant sheep was about 50 min and that a steady-state concentration could be achieved within 1 hour using an appropriate loading dose. Theoretically, it would require 4-5 hours to reach a steady-state without a loading dose. However, the time to steady-state can be greatly reduced with an appropriate loading dose (see Section 4.3.3.).

Since the protocol used in the present study requires the determination of three steady-state drug concentrations within a single experiment (one for each pre-hypoxemic, hypoxemic and post-hypoxemic periods in the experimental group and the equivalent periods in the control group), it was essential to set up an appropriate assessment procedure for the steady-state drug concentrations. For the assessment of steady-state, the three following criteria were used. These criteria were designed so that the steady-state concentration could be experimentally determined in a practical and statistically sound manner. In addition, it was designed to minimize bias during the data processing procedure:

1. the plasma MCP concentration versus time plot was visually inspected. Plateau sections in the pre-hypoxemic, hypoxemic and post-hypoxemic periods (i.e. the section where plasma concentrations were remained relatively constant) were chosen for further statistical analysis.

2. the data sets from these plateau sections were analysed for the coefficient of variation (CV). The maximum CV limit of 10% was used as criteria for the deviation from mean steady-state concentration. The data set from the pre-hypoxemic period tends to exhibit
higher CV values due to the shorter time allowed to attain steady-state and the smaller number of data points gathered. Consequently, a CV value of 15% was used as the limit in two of the animals. During the data processing procedure, the extreme outliers (more extreme than 2 SD from the mean) from each data set were eliminated. In this case the terminal data points from the later time period were given preference to remain in the set over earlier data points, since the steady-state drug concentration is likely attained during the later time period. In practice, any extreme outlier(s) was carefully examined for possible error(s) and in some cases re-assayed. After this step, a terminal data point from the earliest time period was removed until the CV value is equal or smaller than the limit.

3. the slope ($\beta$) of the regression line was also examined. The slope of the regression line computed from the sample data expressed quantitatively the straight-line dependency of $Y$ on $X$ (i.e. plasma drug concentration on time) in the sample (Zar, 1984). If the slope of the regression line of the plateau section is significantly different from zero, then it would suggest that plasma drug concentration has a trend of either an increase or decrease over the given time (i.e. steady-state was not reached). Therefore, the slope was analysed to determined whether it was different from zero using analysis of variance (ANOVA) and t-test for linear regression. In statistical terms, the linear regression of the data set was performed with the null-hypothesis ($H_0 : \beta = 0$) that the slope of the linear regression line is zero and the alternate hypothesis, $H_A : \beta \neq 0$. The analysis of variance (ANOVA) for linear regression and Student's t-test were used with the level of significance, $\alpha = 0.05$. This procedure was chosen to ensure that there is no tendency for a gradual increase or decrease in the drug concentrations over time. The rejection of the null-hypothesis ($H_0$) would mean that steady-state was not reached.
From this steady-state assessment, in most of animals, the MCP steady-state concentration was found to be reached between 30 min and 75 min with a 15 mg loading dose in the pre-hypoxemic period. One animals (ewe no. 1154) was found not to reach steady-state within 2 hours of infusion with a loading dose. Data from this animal was excluded from the subsequent pharmacokinetic data analysis, since the hypoxemic and post-hypoxemic steady-state concentration could not be compared to the pre-hypoxemic steady-state concentration. After onset of hypoxemia, another steady-state (hypoxemic) was reached between 2 to 4 hours from the onset of hypoxemia. The post-hypoxemic steady-state was reached between 2 to 3 hours after stopping nitrogen infusion. This apparent shorter time to steady-state during the post-hypoxemic period, as compared to that of the hypoxemic period, is likely due to the fact that the half-life of MCP during the post-hypoxemic period is shorter (=20%) than that of the hypoxemic period.
4.3.3. *Theoretical aspects of infusion with loading dose.*

The steady-state drug concentration ($C_{p\infty_{inf}}$) after a continuous drug infusion is the point where the rate of input of drug (infusion rate) is equal to the rate of drug leaving the body (elimination rate). Since the infusion rate is constant, the time to reach steady-state is primarily dependent upon the elimination rate constant.

For the present study, the one-compartment open model and the two-compartment open model with drug elimination from the central or “well perfused” compartment (see the schematic diagrams on the next page), were used for computer-aided simulation, theoretical interpretation and calculation of pharmacokinetic parameters. Other concepts provided utility in data interpretation, including physiological modeling, moment analysis and the non-compartmental clearance concept. Using pharmacokinetic parameters determined by Riggs *et al.* (1989) in non-pregnant sheep, the computer aided simulations of i.v. infusion based on the one- and two-compartment models were performed as shown in the Figure 11. It would take approximately 3.4 hours to reach a steady-state (95%) and 5.2 hours for 99% from the one-compartment model without a loading dose. In the two-compartment model, the time to reach steady-state was slightly longer (4.0 hour for 95% and 6.4 hour for 99%) than that of the one-compartment model. The longer time to reach steady-state in the two-compartment model is likely to be a consequences of the delayed transfer of drug between the central and peripheral compartments, when compared to immediate drug distribution in the one-compartment model.
Scheme V  Diagrams of the compartment models used for computer simulation.

One-compartment Open Model

Two-compartment Open Model with central drug elimination

where ko represents the infusion rate constant of MCP, LD is the i.v. bolus loading dose, Vd is the apparent volume of distribution in one-compartment model, Vc and Vr is apparent volume of the central and peripheral compartment, respectively, Ke is the apparent first order MCP elimination rate constant, k_{12} and k_{21} are the apparent first order inter-compartmental distribution rate constants for MCP and k_{10} is the apparent MCP elimination rate constant from the central compartment.
Figure 11. Simulation of Cp after simple i.v. infusion without loading dose using pharmacokinetic parameters from Riggs et al. (1989)

Figure 12. Simulation of Cp after i.v. infusion with loading dose (one-compartment model)
Figure 13. Simulation of Cp after i.v. infusion with loading dose (two-compartment model) using pharmacokinetic parameters from Riggs et al. (1989)

In the present study, a loading dose was combined with the infusion to reduce time required to reach the steady-state drug concentration. Therefore, further computer simulations of the infusion with a loading dose were performed using the one- and two-compartment model as shown in Figures 12 and 13. An ideal (optimal) loading dose with the one-compartment model is $LD = C_{ss} \cdot V_d$, which theoretically results in instantaneous arrival and maintenance of a steady-state as shown in Figure 12. However, in the actual experimental and therapeutic environment, it is neither practical nor possible to calculate and adjust a loading dose for each individual subject, thus the loading dose calculated from the mean values of $C_{ss}$ and $V_d$ would be used for all the experiment subjects. Therefore, this loading dose regimen could result in either over- or under-estimation of the optimal loading dose. Figure 12 shows a simulated plot of plasma drug concentration (in terms of plasma
steady-state drug concentration) versus time (in terms of half-life), which based on the one-compartment model with first-order kinetics. A loading dose of 90% or 110% of the optimal dose will take approximately 1 half-life to reach the range of steady-state. A loading dose of 80% or 120% of the optimal dose will take approximately 2 half-lives. Therefore, even with some deviation from the optimal loading dose, the time to reach steady-state will be greatly reduced with an appropriate loading dose.

In the two-compartment model, the use of a loading dose is more complicated due to the distribution phase to the peripheral compartment. Unlike the one-compartment model, two loading dose protocols are possible for drugs best described by a two-compartment pharmacokinetic characteristics. A loading dose of \( LD = C_{ss} \times V_c \) will result in an immediate plasma drug concentration equal to the steady-state concentration, however the plasma drug concentration will be decreased below the steady-state concentration and gradually increased as shown in Figure 13. An alternative loading dose of \( LD = C_{ss} \times V_{ss} \) will initially give a higher plasma concentration than the steady-state concentration, but very rapidly decreased to the steady-state concentration. The time to steady-state using the second regimen is usually much shorter than the former. Therefore, a loading dose of 15 mg of MCP was used in the study as suggested by Riggs (1989), which represents the loading dose close to the second protocol. The use of a loading dose also greatly reduced the time to attain steady-state in the two compartment model. This may be a better approximation for MCP, since the MCP pharmacokinetics following the i.v. bolus dose exhibited two-compartment characteristics (Riggs et al., 1988). With an optimal loading dose of \( LD = C_{ss} \times V_{ss} \), it will take about 30 minutes to attain the range of steady-state (95 -105% of C_{ss}). A loading dose of 80% and 120% of the optimal dose will take approximately 1.5 hour to reach the range.
Therefore, these computer-aided simulations theoretically demonstrate that the time to the steady-state can be greatly reduced by an appropriate loading dose. This confirms the finding of Riggs et al. (1989) that steady-state conditions for MCP were achieved within approximately 2 half-lives using a loading dose of 15 mg. In the present study, MCP steady-state concentration was achieved between 0.5 to 1.25 hours after initiation of the infusion and administration of a loading dose, which is in good agreement with the present theoretical derivation and computer-aided simulation.

4.4. Metoclopramide Pharmacokinetics Following the i.v. Infusion to Steady-state and Induction of Hypoxemia

4.4.1. Steady-state plasma MCP concentration and total body clearance of MCP during normoxemia and hypoxemia

The present study shows that acute moderate hypoxemia affects plasma metoclopramide pharmacokinetics in chronically instrumented conscious sheep. The steady-state plasma MCP concentration significantly increased and total body clearance decreased during acute moderate hypoxemia. During the post-hypoxemic period, the steady-state plasma MCP concentration decreased to a level similar to that of the pre-hypoxemic period. The mean plasma MCP concentration during the post-hypoxemic period was slightly higher than the mean plasma MCP concentration during the pre-hypoxemic period, but was not significantly different (p > 0.05). Therefore, these results suggested that acute moderate hypoxemia reduces total body clearance of MCP (i.e. resulting in a higher plasma MCP steady-state concentration).
A reduction in total body drug clearance during acute hypoxemia was observed with a number of drugs such as theophylline (Saunier et al., 1987), aminoglycosides (Mirhij et al., 1978), phenytoin (Babini and du Souich, 1986) and propofol (Audibert et al., 1992). Drugs such as hexobarbital and antipyrine, which undergo oxidative biotransformation by the hepatic mixed oxygenases, are directly affected by reduced oxygen tension (Jones, 1981). Conjugation reactions such as glucuronidation and sulphation, which are the major elimination pathway of many drugs, are also affected by low oxygen tension (Aw and Jones, 1981), but the formation of these conjugates does not appear to be a significant proportion of MCP elimination in sheep. In addition, acute hypoxemia decreases the activity of several isoenzymes of the cytochrome P-450 (Srivastava et al., 1980; Jones et al., 1989; du Souich et al., 1990). There are also observations that moderate hypoxia could promote the formation of oxygen free-radicals (Proulx and du Souich, 1990; Brass et al., 1991) that are able to diminish the activity of certain isozymes of the cytochrome P-450 (Proulx and du Souich, 1990). These reports suggest that acute hypoxemia reduces drug elimination processes, in general, which is in agreement with the findings of the present study.

Alternatively, no change in pharmacokinetics during acute hypoxemia was observed with drugs such as furosemide (du Souich et al., 1985) and diltiazem (du Souich et al., 1993). In some cases, there is an increase in the apparent total body clearance during acute hypoxemia with some drugs such as sulphamethazine (du Souich et al., 1984) and digoxin (du Souich et al., 1985a; 1985b). However, this increase in total body clearance was mainly due to an increase in the apparent volume of distribution (i.e. increased tissue distribution and changes in plasma protein binding), rather than to an actual increase in drug elimination from the body.
No studies on the enzymatic pathway for the oxidative N-deethylation of MCP to mdMCP and ddMCP have been reported, however the N-deethylation of a structurally similar compound, lidocaine, appears to be metabolized by the hepatic P-450 enzyme system (Suzuki et al., 1984; Bargetzi et al., 1989) and there are many reports that N-deakylation reactions such as demethylation, deethylation and depropylation are mediated by the hepatic P-450 enzyme system (Testa and Jenner, 1976). In addition, the liver is thought to be the major metabolic site for MCP (Kapil et al., 1984; Desmond and Watson, 1986). Therefore it is probable that the N-deethylation of MCP is also mediated by the hepatic P-450 enzyme system. In vitro studies have demonstrated that a low partial pressure of oxygen reduced the $K_m$ value of the demethylation of ethyl-morphine (Holtzman et al., 1983; Erickson et al., 1982) and of the hydroxylation of phenytoin (Tsuru et al., 1982), indicating that adequate oxygen pressure may be essential for this route of biotransformation. In conscious rabbits, hypoxemia reduced the demethylation and/or the hydroxylation of theophylline (Letarte et al., 1984) and the hydroxylation of phenytoin (du Souich et al., 1986). In addition, acute hypoxemia appears to decrease the activity of the cytochrome P-450 in rat lung and liver (Srivastava et al., 1980). These studies suggested that hypoxemia may affect several cytochrome P-450 isozymes, and thus may explain, in part, the accumulation in plasma of MCP and mdMCP due to reduced metabolic elimination of the drug. The effect of hypoxemia on plasma ddMCP concentration was not determined, due to the low plasma concentration of the metabolite.

Alternatively, changes in hepatic blood flow during acute hypoxemia-hypocapnia could explain the reduction of MCP clearance. A moderate hypoxemia alone appears to exert only minimal effect in total hepatic blood flow (Lasen et al., 1976; Hughes et al., 1979; du
Souich et al., 1992). However, a hypoxemia in combination with either hypocapnia or hypercapnia may affect total hepatic blood flow more than hypoxemia alone (Hughes et al., 1979; Mathie and Blumgart, 1983). Since hepatic elimination of MCP is essentially modulated by the blood flow to the liver [flow-limited] (Bateman et al., 1980), the effect of acute hypoxemia-hypocapnia on MCP clearance may be explained by the reduction in total hepatic blood flow.

4.4.2. Plasma mdMCP concentration during normoxemia and hypoxemia

Dealkylation of secondary and tertiary amine groups to yield primary and secondary amine groups, respectively, is one of the most important and most frequently encountered reactions in drug metabolism. Biological N-dealkylation occurs without apparent changes in the state of oxidation of the nitrogen atom, but the removed alkyl group is oxidised (Testa and Jenner, 1976). In the present study, considerable plasma mdMCP concentrations (on average ≈30-50% of plasma MCP concentration) were found. Plasma mdMCP concentration also appeared to reach an apparent steady-state in both experimental and control groups. However, it is not possible to confirm that steady-state, in a conventional sense, was achieved in the study, since the input rate constant of mdMCP (i.e. a product to infusion rate of MCP and $k_f(MCP\rightarrow mdMCP)$) could not be evaluated in the current infusion design.

Plasma mdMCP concentration was significantly higher in the experimental group as compared to the control group. The increased mdMCP concentration in the experimental group appears to be related to the induction of hypoxemia, however, mdMCP concentration did not decrease during the post-hypoxemic period. The interpretation of metabolite kinetics, mainly mdMCP in this study, is more complicated than the parent drug, since plasma
mdMCP concentrations depend on: (1) the rate of N-deethylation of MCP that yields mdMCP; (2) the rate of deethylation of mdMCP producing ddMCP; (3) renal elimination rate of mdMCP and (4) other biotransformation rates for mdMCP. The input rate of mdMCP (the rate of N-deethylation of MCP) could not be directly determined in the study. However, it is unlikely increased during hypoxemia, since total body clearance of MCP was decreased, and, as mentioned above, there are reports that the cytochrome P-450 system which mediates N-deethylation reaction is reduced during acute hypoxemia (Srivastava et al., 1980). Therefore, the rates of mdMCP elimination processes, such as renal excretion and mdMCP metabolism, are likely decreased during hypoxemia. Renal elimination of mdMCP was significantly decreased during hypoxemia as shown in Section 3.4. Similar accumulation of MEGX and GX metabolites of lidocaine was also observed during acute hypoxemia (Marleau et al., 1987; du Souich et al., 1992).

4.4.3. Renal elimination of MCP and its metabolites during normoxemia and hypoxemia

Renal elimination of MCP and its metabolites was examined in the present study. Since the infusion protocol was used, the conventional methods of determining urinary excretion parameters such as the plots of A.R.E. (amount remained to be excreted) vs. time or urinary excretion rate vs. time could not be applied in this study. In addition, changes in drug elimination during the hypoxemic and post-hypoxemic periods complicated the estimation of renal drug elimination parameters. Therefore, two modified methods of determining urinary drug excretion during the phased infusion were developed and applied in the study. The first
method was based on the estimation of renal clearance from urinary drug accumulation and AUC [Method 1a and 1b]. The other method was based on the determination of fractional renal drug excretion constant, which was derived and modified from the concept of urinary excretion determination during infusion by Gibaldi and Perrier (1982) [Method 2].

Firstly, the renal clearance of MCP and mdMCP was calculated in the study either from a) dividing the amount of drug/metabolite recovered in urine \((D_u)\) by the area under plasma drug/metabolite concentration curve (AUC) in a given time period [Method 1a], and b) using the slope of the accumulated drug/metabolite in urine \((D_u)\) versus AUC [Method 1b]. As seen in Section 3.4.1., both methods appear to give a similar estimation of renal clearance. The slope from the pre-hypoxemic period could not be determined, since there were only 3 data points in the period, and also only 1 or 2 data points representing a urine collection during MCP steady-state. As a result, in the experimental group, the clearance values obtained during the hypoxemic period were compared with those determined during the subsequent post-hypoxemic segment (totaling 12 of the 14 hour total experiment duration). In addition, renal clearance parameters from the equivalent period in the control group were also compared with the hypoxemic period. Since no significant change in renal clearance in the control group was observed, it is supported that this approach is valid.

Secondly, the fractional renal excretion constants for MCP, mdMCP and ddMCP, were calculated by dividing the slope of the asymptote of the \(D_u\) versus time curve with the infusion rate of MCP. In the case of parent drug, the fractional renal excretion constant \((f_u)\) is the proportion of the renal elimination rate constant over the apparent total drug elimination rate constant \((k_u/K\epsilon)\), which indicates the fraction of total drug elimination due to renal drug
excretion. In addition, the fractional renal excretion constants for metabolites were also calculated. The theoretical derivation of these parameters was similar to those of the parent drug. However, the fractional renal excretion parameters determined from the accumulation plot represent hybrid fractional constants (i.e. a product of fractional renal elimination constant of a metabolite and fractional metabolic elimination constant of the parent drug) [see Section 3.4.2. and Appendix A]. These parameters may appear to be highly complicated, but, in simple terms, represent the fraction/proportion of renal excretion in the form of a specific metabolite out of the total drug elimination. Due to the constraints imposed by the infusion design, it was not feasible to determined absolute (i.e. not fractional constants) renal elimination and metabolic parameters in the study.

Since the present study was designed to detect the changes in the drug kinetics during hypoxemia, it is critical to determine the renal drug elimination parameters during both normoxemic and hypoxemic period within a single experiment (phased infusion). As shown in Section 4.3., any infusion can be theoretically considered as a series of infusions with appropriate loading doses. Therefore, each pre-hypoxemic, hypoxemic and post-hypoxemic period in the study could be considered as “separate” infusions with appropriate loading doses, and renal excretion parameters from each periods could be treated independently.

Previous studies on renal clearance during acute hypoxemia have reported various responses. No changes in renal clearance during acute hypoxemia were observed with many drugs such as theophylline (Letarte and du Souich, 1984), furosemide (Babini and du Souich, 1986), lidocaine (du Souich et al., 1992) and diltiazem (du Souich et al., 1993). Renal clearance of phenytoin decreased during acute hypoxemia (from 0.2 to 0.03 mL/min/Kg), but
it apparently was not statistically significant (du Souich et al., 1986). Alternatively, renal clearance of sulfamethazine appears to be increased, but statistically not significant, during acute hypoxemia (du Souich and Courteau, 1984). Renal clearance of digoxin was significantly increased during acute hypoxemia (du Souich et al., 1985). In one of the studies, renal clearance of metabolites was measured. No significant changes in renal clearances of lidocaine metabolites, MEGX and GX, were observed (du Souich et al., 1992).

There was significant reduction in renal clearance of MCP and mdMCP during the hypoxemic period in the experimental group as shown in Section 3.4.1. The reduction in MCP renal clearance (about 2.75 L/h during hypoxemia and 8.15 L/h during normoxemia) are much more extensive compare to the reduction in total body clearance (205 L/h during hypoxemia and 245 L/h during normoxemia). Even though the renal clearance of MCP accounts for only about 5% of total body clearance during normoxemia (about 8 L/h out of 245 L/h), the reduction of renal clearance during hypoxemia accounts for about 15% of the change in total body clearance during hypoxemia (change of about 5.5 L/h out of 40 L/h). Therefore, the reduction in renal clearance of MCP contributes significantly to the reduction of total body clearance of MCP.

Similarly, the renal clearance of mdMCP was significantly affected by hypoxemia (14.6 L/h during hypoxemia and 38.6 L/h during normoxemia). However, the total body clearance of mdMCP could not be determined in this study, thus quantitative assessment of the contribution of the mdMCP renal elimination to total body clearance of MCP could not be evaluated. Renal elimination of ddMCP was also significantly affected by acute
hypoxemia. Therefore, acute hypoxemia in the study appears to reduce renal elimination of MCP and its deethyl metabolites.

Overall, the present study of the pharmacokinetics of MCP and its deethylated metabolite, mdMCP and ddMCP, during acute hypoxemia has shown that there is a reduced elimination of MCP and its metabolites during acute hypoxemia, and this reduction in systemic clearance is associated with a reduced renal elimination of the compounds.
5. **SUMMARY AND CONCLUSIONS**

A capillary GC-MSD method of Riggs *et al.* (1994) for MCP, mdMCP and ddMCP was adapted and applied to study the effect of acute moderate hypoxemia on the pharmacokinetics of MCP and its deethylated metabolites in plasma and urine from non-pregnant ewes following i.v. infusion to steady-state and induction of hypoxemia via intratracheal nitrogen infusion.

A. Acute hypoxemia induces several physiological changes in non-pregnant ewes during continuous MCP infusion:

1. Reduces arterial blood PaO$_2$ (hypoxemia) and PaCO$_2$ (hypocapnia) during the hypoxemic period in the experimental group.

2. Elevates arterial blood lactate and glucose level during the late hypoxemic period and for a time after restoration of normoxia.

3. Increases urine flow and decreases urine osmolality and pH during the hypoxemic period.

B. Hypoxemia affects the pharmacokinetics of MCP and its deethylated metabolites, mdMCP and ddMCP.

1. Total body clearance (TBC) was decreased during hypoxemia, and restored during the post-hypoxemic recovery period.

2. Plasma mdMCP concentration was increased during the hypoxemic and post-hypoxemic period as compared to the control group.

3. The renal elimination of MCP and mdMCP was decreased during the hypoxemic period. The fractional renal elimination rate constants of MCP, mdMCP and ddMCP were also lower during hypoxemia.
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Appendix A. Theoretical Derivation of Pharmacokinetic Equations used in the study

Part I: One-compartment Open Model

The following are schematic representation of one-compartment open model with metabolism.

The abbreviation used in the following equation derivation and the main text are:

- $kf$ = metabolite/conjugate formation rate constant for the parent drug
- $k_{oeth}$ = non-metabolic elimination rate constant for the parent drug
- $kr$ = renal elimination rate constant of the parent drug
- $kmr$ = renal elimination rate constant of the metabolite
- $kmnr$ = non-renal elimination rate of the metabolite
- $KE = kf + k_{oeth}$ = apparent elimination rate of the parent drug
- $km = kmr + kmnr$ = apparent elimination rate of the metabolite
- $fm = kf / KE$ = metabolic fraction of the parent drug elimination
- $f_{mr} = kmr / km$ = renal fraction of the metabolite elimination
- $fr = kr / KE$ = renal fraction of the parent drug elimination
- $ko$ = infusion rate constant
- $Do$ = loading dose
- $Xp$ = amount of the parent drug in the body
- $Cp$ = concentration of the parent drug in the body
- $Vp$ = apparent volume of distribution of the parent drug in the body
- $Xm$ = amount of the metabolite in the body
- $Cm$ = concentration of the metabolite in the body
- $Vm$ = apparent volume of distribution of the metabolite in the body
- $ti = Do / ko$ = infusion time equivalent loading dose
Theoretical Derivation:

The rate of drug disposition in the parent drug compartment during an infusion defined as below

\[ \frac{dX_p}{dt} = k_o - KE * X_p \]  \hspace{1cm} (1)

with Laplace transformation \((\mathcal{L}(X) = \mathcal{X})\), Eq. 1 becomes

\[ sX_p - X_p(0) = \frac{k_o}{s} - KeX_p \]  \hspace{1cm} (2)

Case 1: a simple infusion without loading dose

since \(X_p(0) = 0\), Eq 2. becomes

\[ sX_p + KE* X_p = \frac{k_o}{s} \]  \hspace{1cm} (3)

\[ X_p = \frac{k_o}{s(s + KE)} \]  \hspace{1cm} (4)

by reverse Laplace transformation,

\[ X_p = \frac{k_o}{KE} * (1 - \exp(-KE * t)) \]  \hspace{1cm} (5)

\[ C_p = \frac{k_o}{KE/Vp} * (1 - \exp(-KE * t)) \]  \hspace{1cm} (6)

Case 2: an infusion with a loading dose

since \(X_p(0) = Do\), Eq 2. becomes

\[ sX_p + KE* X_p = \frac{k_o}{s} + Do \]  \hspace{1cm} (7)

\[ X_p = \frac{k_o}{s(s + KE)} + \frac{Do}{s + KE} \]  \hspace{1cm} (8)

by reverse Laplace transformation,

\[ X_p = \frac{k_o}{KE} * (1 - \exp(-KE * t)) + Do * (1 - \exp(-KE * t)) \]  \hspace{1cm} (9)

\[ C_p = \frac{k_o}{KE/Vp} * (1 - \exp(-KE * t)) + \frac{Do}{Vp} * (1 - \exp(-KE * t)) \]  \hspace{1cm} (10)

Urinary excretion

\[ \frac{dX_u}{dt} = kr * X_p \]  \hspace{1cm} (11)

with Laplace transformation, Eq. 11 becomes

\[ sX_u - X_u(0) = kr * X_p \]  \hspace{1cm} (12)

Case 1: a simple infusion without loading dose (Gibaldi and Perrier, 1984)

since \(X_u(0) = 0\), Eq. 12 becomes

\[ sX_u = kr* \frac{k_o}{s(s+KE)} \]  \hspace{1cm} (13)
\[ X_u = \frac{kr \cdot ko}{s^2/(s + KE)} \]  

(14)

by reverse Laplace transformation,

\[ X_u = \frac{kr \cdot ko \cdot t}{KE} - \frac{kr \cdot ko}{KE^2} \cdot \left(1 - \exp(-KE \cdot t)\right) \]  

(15)

From the plot of \( X_u \) vs. \( t \), when the drug is infused to a steady-state, the term \( \exp(-KE \cdot t) \) approaches zero. The asymptote becomes

\[ X_u = \frac{kr \cdot ko \cdot t}{KE} - \frac{kr \cdot ko}{KE^2} \]  

(16)

The urinary excretion fraction constant \( k_o/KE \) can be determined from the slope of asymptote, \( kr \cdot ko/KE \), by dividing \( ko \).

Case 2: an infusion with a loading dose

since \( X_p(0) = D_o \), Eq. 12 becomes

\[ sX_u = \frac{kr \cdot ko}{s/(s + KE)} + \frac{D_o}{(s + KE)} \]  

(17)

\[ X_u = \frac{kr \cdot ko}{s^2/(s+KE)} + \frac{D_o}{s/(s+KE)} \]  

(18)

by reverse Laplace transformation,

\[ X_u = \frac{kr \cdot ko \cdot t}{KE} - \frac{kr \cdot ko}{KE^2} \cdot \left(1 - \exp(-KE \cdot t)\right) \]  

\[ + \frac{D_o}{KE \cdot \left(1 - \exp(-KE \cdot t)\right)} \]  

(19)

From the plot of \( X_u \) vs. \( t \), when the drug is infused to a steady-state, the term \( \exp(-KE \cdot t) \) approaches zero. The asymptote becomes

\[ X_u = \frac{kr \cdot ko \cdot t}{KE} + \frac{kr \cdot ko}{KE^2} + \frac{D_o}{KE} \]  

(20)

The urinary excretion fraction constant \( k_o/KE \) can be determined from the slope of asymptote, \( kr \cdot ko/KE \), by dividing \( ko \).

By using the t-intercept, \( KE \) can be determined, however the error associated with the intercept is accumulative i.e. any error in each data point will be directly reflected in the t-intercept of the asymptote unlike to the slope, thus does not warrant its use.

The rate of metabolite disposition in the metabolite compartment during an infusion defined as below
\[
dX_n/dt = k_f \cdot X_p - K_m \cdot X_m
\]  
(21)

with Laplace transformation \((\mathcal{L}(X) = \mathcal{X})\), Eq. 21 becomes

\[
s\mathcal{X}_m - X_m(0) = k_f \cdot \mathcal{X}_p - K_m \cdot X_m
\]  
(22)

Case 1: a simple infusion without loading dose

since \(X_m(0) = 0\), Eq 22 becomes

\[
s\mathcal{X}_m = k_f \cdot k_o / (s(s + K_E)) - K_m \cdot \mathcal{X}_m
\]  
(23)

\[
\mathcal{X}_m = k_f \cdot k_o / (s(s + K_E)(s + K_m))
\]  
(24)

by reverse Laplace transformation,

\[
X_m = k_f k_o [1/k_m /K_E + \exp(-K_m t)/k_m/(k_m-K_E) + \exp(-k_E t)/k_E/(k_E-K_m)]
\]  
(25)

\[
C_m = k_f k_o /V_m [1/k_m /K_E + \exp(-K_m t)/k_m/(k_m-K_E)
+ \exp(-k_E t)/k_E/(k_E-K_m)]
\]  
(26)

Case 2: an infusion with a loading dose

\[
s\mathcal{X}_m = k_f k_o / ((s + K_E) - K_m \cdot \mathcal{X}_m)
\]  
(27)

\[
\mathcal{X}_m = k_f k_o / ((s + K_E)(s + K_m)) + k_f D_0 / ((s + K_E)(s + K_m))
\]  
(28)

by reverse Laplace transformation,

\[
X_m = k_f k_o [1/k_m /K_E + \exp(-K_m t)/k_m/(k_m-K_E)
+ \exp(-k_E t)/k_E/(k_E-K_m)] + k_f D_0 / ((s + K_E)(s + K_m))
\]  
(29)

\[
C_m = k_f k_o /V_m [1/k_m /K_E + \exp(-K_m t)/k_m/(k_m-K_E)
+ \exp(-k_E t)/k_E/(k_E-K_m)] + k_f D_0 / ((s + K_E)(s + K_m))
\]  
(30)

Urinary excretion

\[
dX_{mu}/dt = k_m r \cdot X_m
\]  
(31)

with Laplace transformation, Eq. 31 becomes

\[
s\mathcal{X}_{mu} = k_m r \cdot \mathcal{X}_m
\]  
(32)

Case 1: a simple infusion without loading dose

\[
s\mathcal{X}_{mu} = k_m r k_o / ((s + K_E)(s + K_m))
\]  
(33)

\[
\mathcal{X}_{mu} = k_m r k_o / (s^2 + K_E)(s + K_m)
\]  
(34)

by reverse Laplace transformation,
\[ X_{mu} = k_{mr} k_f k_o \left[ t / k_m / K_E \cdot \left( - (K_E + k_m) / k_m^2 / K_E^2 \right) \right. \\
+ C \cdot \exp(-K_E t) + D \cdot \exp(-k_m t)) \right] \]  

where \( C = (k_m + 2K_E) / K_E^2 / k_m^2 - 1 / (K_E k_m (K_E + k_m)) \) 
\( D = - K_E / K_E^2 / k_m^2 - 1 / (K_E k_m (K_E + k_m)) \)  

From the plot of \( X_{mu} \) vs. \( t \), when the drug is infused to a steady-state, the terms \( \exp(-K_E t) \) and \( \exp(-k_m t) \) approach zero. The asymptote becomes

\[ X_{mu} = k_{mr} k_f k_o \left[ t / k_m / K_E \cdot \left( - (K_E + k_m) / k_m^2 / K_E^2 \right) \right] \]  

The metabolite urinary excretion fraction constant \( k_{mr} k_o / K_E / k_m \) can be determined from the slope of asymptote, \( k_o k_{mr} k_o / K_E / k_m \), by dividing \( k_o \).

Case 2: an infusion with a loading dose

\[ sX_{mu} = k_{mr} k_o k_f / s / (s + K_E) / (s + k_m) + k_{mr} k_f D_o / (s + K_E) / (s + k_m) \]  

\[ (37) \]

\[ X_{mu} = k_{mr} k_f k_o \left[ t / k_m / K_E \cdot \left( - (K_E + k_m) / k_m^2 / K_E^2 \right) + C \cdot \exp(-K_E t) + D \cdot \exp(-k_m t)) \right] + k_{mr} k_f (D_o K_E k_m - k_o (K_E + k_m)) / K_E^2 / k_m^2 \]  

where \( C = (k_m + 2K_E) / K_E^2 / k_m^2 - 1 / (K_E k_m (K_E + k_m)) \) 
\( D = - K_E / K_E^2 / k_m^2 + 1 / (K_E k_m (K_E + k_m)) \)  

From the plot of \( X_{mu} \) vs. \( t \), when the drug is infused to a steady-state, the terms \( \exp(-K_E t) \) and \( \exp(-k_m t) \) approach zero. The asymptote becomes

\[ X_{mu} = k_{mr} k_f k_o \left[ t / k_m / K_E \cdot \left( - (K_E + k_m) / k_m^2 / K_E^2 \right) \right] + k_{mr} k_f (D_o K_E k_m - k_o (K_E + k_m)) / K_E^2 / k_m^2 \]  

\[ (40) \]

The metabolite urinary excretion fraction constant \( k_{mr} k_o / K_E / k_m \) can be determined from the slope of asymptote, \( k_o k_{mr} k_o / K_E / k_m \), by dividing \( k_o \).