THE PHARMACOKINETICS OF METOCLOPRAMIDE IN NORMAL RATS AND IN RATS WITH EXPERIMENTAL RENAL AND HEPATIC DYSFUNCTION

by

YUN KAUF TAM

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Department of  

Pharmaceutical Sciences

The University of British Columbia  
2075 Wesbrook Place  
Vancouver, Canada  
V6T 1W5

Date  Dec. 24, 1968
ABSTRACT

Metoclopramide (MCP), 4 amino-5-chloro-2-methoxy-N(2-diethylaminoethyl) benzamide, a procainamide derivative, is a potent anti-emetic and gastric motility modifier. Clinically, MCP is used in gastrointestinal diagnostic examinations, treatment of various types of gastrointestinal disorders and as a pre- and post-operative anti-emetic. Very little pharmacokinetic information was available prior to this study. This thesis reports the pharmacokinetic study of MCP in normal rats and in rats with renal and hepatic dysfunction.

The Pharmacokinetics of MCP in Normal Rats

The pharmacokinetics of MCP have been examined as a function of dose and route of administration. No significant first-pass metabolism was seen in urine and plasma studies (F = 0.9). The area under the plasma concentration vs. time curves (AUC) increased disproportionately while average plasma clearances (ClTB) reduced with increasing dose indicating dose-dependent kinetics for MCP. However, the percentage of dose excreted as intact drug and de-ethylated metabolite (DE-MCP) was constant after administration of a range of intravenous doses (35 fold) indicating dose independent kinetics. The plasma and urine results could not be explained readily by conventional Michaelis-Menten kinetics. The elimination of MCP was proposed to be blood flow dependent. After a high dose of MCP (35 mg/kg), the drug may transiently
reduce perfusion to the eliminating organs (viz., liver, kidney, etc.). This hypothesis was supported by an initial reduction and then a subsequent return to control levels of the clearance of the blood flow indicator, indocyanine green, after MCP administration. Parallel to this observation, it was noted that the plasma half-life of MCP was initially prolonged (104 ± 14 minutes) and followed by a resumption of the normal elimination half-life (58 ± 7 minutes). A question remaining unresolved is why MCP does not undergo first-pass metabolism over the dose range studied.

**Kinetics of MCP in Hepatic Impaired Rats**

The hepatic impairment caused by carbon tetrachloride (CCl₄) increased the half-life and AUCs of MCP by approximately 3 fold \([t_{1/2}]: \text{control} = 52 \pm 15 \text{ min}., \text{test} = 170 \pm 40 \text{ min}; \text{AUC: control} = 290 \pm 50 \text{ mcg-min/ml}, \text{test} = 840 \pm 200 \text{ mcg-min/ml}] while the \(\text{Cl}_{TB}\) was diminished to a similar extent \([\text{control} = 13 \pm 1 \text{ ml/min}; \text{test} = 4.6 \pm 1.0 \text{ ml/min}]. The volume of distribution \((V_d)\) did not change significantly. The renal clearance \((\text{Cl}_R)\) of MCP was reduced slightly probably due to renal damage caused by CCl₄ \([\text{control} = 2.9 \text{ ml/min}; \text{test} = 2.3 \text{ ml/min}]. The reduction of hepatic function caused a significant increase in the percentage of dose excreted as intact drug in urine \([\text{control} = 20 \pm 2\%; \text{test} = 47 \pm 7\%]. However, the \text{DE-MCP} fraction was unaltered \([\text{control} = 11 \pm 3\%; \text{test} = 9.3 \pm 3\%]. These results indicate that the liver is a major metabolic organ for MCP removal but extra-
hepatic metabolism may also occur.

Kinetics of MCP in Renal Impaired Rats

The effect of renal dysfunction on the kinetics of MCP has been studied using two surgically [bilateral ureteral ligation (BUL) and two-step 5/6 nephrectomy (TSN)] and a chemically [uranyl nitrate (UN)] induced method providing a wide range of renal impairment [creatinine clearance: control = 1.7-2.0 ml/ min; test = 0.03- 0.38 ml/min]. Renal damage was the highest in the UN group followed by the BUL and TSN groups. The $t_{1/2}$ of MCP was significantly increased [control = 50-70 min, test = 130-160 min] and $Cl_B$ [control = 11-16 ml/min, test = 3.4-7 ml/min] and $Cl_R$ [control = 2.0-2.9 ml/min, test = 0.2-0.7 ml/min] decreased while the $V_d$ [control = 4.0-4.5 L/kg, test = 3.2-4.4 L/kg] is only slightly decreased in all models studied. A positive correlation was observed between the $Cl_B$, $Cl_R$ and nonrenal clearance with creatinine clearance ($r^2 > 0.97$) indicating that the metabolic and renal clearances of MCP are related to renal function. The findings obtained from the hepatic and renal impairment models indicate that, besides the liver, the kidneys may also play an important role in metabolizing MCP. This may, in part, explain why MCP does not undergo first-pass metabolism in the rat.
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<td>adenosine diphosphate</td>
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<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
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<tr>
<td>BUL</td>
<td>bilateral ureteral ligation (or ligated)</td>
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<td>CCl&lt;sub&gt;4&lt;/sub&gt;</td>
<td>carbon tetrachloride</td>
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<td>CI</td>
<td>chemical ionization</td>
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<tr>
<td>Cl&lt;sub&gt;H&lt;/sub&gt;</td>
<td>hepatic clearance</td>
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<td>Cl&lt;sub&gt;int&lt;/sub&gt;</td>
<td>intrinsic clearance of free drug</td>
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<td>Cl&lt;sub&gt;int&lt;/sub&gt;</td>
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<td>Cl&lt;sub&gt;T&lt;/sub&gt;B</td>
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<td>Cl&lt;sub&gt;NR&lt;/sub&gt;</td>
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<td>Cl&lt;sub&gt;R&lt;/sub&gt;</td>
<td>renal clearance</td>
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<td>CNS</td>
<td>central nervous system</td>
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<td>CTZ</td>
<td>chemoreceptor trigger zone</td>
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<td>DE-MCP</td>
<td>de-ethylated metoclopramide</td>
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<td>E</td>
<td>extraction ratio</td>
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<td>E&lt;sub&gt;H&lt;/sub&gt;</td>
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<td>E&lt;sub&gt;K&lt;/sub&gt;</td>
<td>renal extraction ratio</td>
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<td>ECD</td>
<td>electron capture detector</td>
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<td>EI</td>
<td>electron impact</td>
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<td>GFR</td>
<td>Glomerular filtration rate</td>
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<td>GLC</td>
<td>gas liquid chromatography</td>
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<td>HFB</td>
<td>heptafluorobutyryl</td>
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<td>HFBA</td>
<td>heptafluorobutyryl anhydride</td>
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HPLC  high performance liquid chromatography
HVA  homovanillic acid
i.d.  internal diameter
i.m.  intramuscular
i.v.  intravenous
MCP  metoclopramide
MCP-HCl-H₂O  metoclopramide monohydrochloride monohydrate
MS  mass spectrometry (or spectrometer)
PUN  plasma urea nitrogen
Qₜ  kidney blood flow
PGOT  plasma glutamic oxalo-acetic transaminase
ₜ½  half-life
TSN  two step 5/6 nephrectomy (or nephrectomized)
UN  uranyl nitrate

\[ V_d(\text{area}) \] volume of distribution determined by area method

\[ V_d(\text{ss}) \] volume of distribution determined by steady-state method.

±  one standard deviation
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To Theresa - the dearest person in my life. The thesis is dedicated to her because she has lived through all the hardship with me.
The author would like to thank Dr. Axelsson for providing such a friendly atmosphere during my study. The friendship is going to be an everlasting one. The author is indebted to Dr. W. Godolphin who has assisted us in the biochemical analysis. The author would like to express his sincere thanks to Mrs. B. McErlane who has assisted me in surgery, blood sampling and drug analysis. Dr. Price's constructive criticism in renal aspect of this project is deeply appreciated. The author also appreciate Mr. R. Kapil and K.W. Rigg's help. This project has been made possible by the support of Medical Research Council and the Kidney Foundation.
INTRODUCTION

Metoclopramide (MCP), 4-amino-5-chloro-2 methoxy-N-(2-diethylaminoethyl benzamide) (pKa=9.3), was introduced in 1964 (Justin-Besancon and Laville). The pharmacological properties of this new drug were first evaluated in the early sixties. Unlike its analog, procainamide, MCP has no significant cardiac effects. However, it has been observed that MCP is a very potent anti-emetic agent and that it has profound effects on the gastro-intestinal tract (Justin-Besancon and Laville, 1964; Magrieson et al., 1966; Martin and Scobie, 1967; Eisner, 1968, 1971; James and Humes, 1969; Ramsbottom and Hunt, 1970; Howells et al., 1971; Johnson, 1971a, 1973; Kree1, 1973, and Hancock et al., 1974).

![Metoclopramide structure]

Pharmacodynamic Studies

guinea pig and rat (Marmo et al., 1970 and Hay, 1975) have established the effect of MCP on the gastrointestinal tract. MCP increases the lower esophageal sphincter pressure and the force of peristaltic contractions (Heitmann and Miller, 1970; Bremner and Bremner, 1972; and Guelrud, 1974) without any effect on relaxation in man. MCP increases the peristaltic strength of the gastric antrum, relaxes the pyloric canal and duodenal cap and increases the synchronization of the gastric antral and duodenal motility (Johnson, 1973) in humans. Therefore, the gastric emptying time is reduced (James and Humes, 1969).

Effects resembling those in the esophagus and stomach after MCP have been observed in the small intestine (Johnson, 1971a, 1973). The transit time through the duodenum and jejunum is reduced in man as a result of the drug induced increased motility (James and Humes, 1969 and Magrieson et al., 1966). No marked effect on colonic motor activity in vivo is observed (Eisner, 1971; Banke et al., 1972).

Mechanism of Action - MCP has been postulated to have both peripheral and CNS actions. It has been shown that MCP inhibits emesis in animals to locally acting emetics such as copper sulfate (R) and to centrally acting drugs such as Hydergine and apomorphine (Justin-Besancon and Laville, 1964). However, the actual pathways are yet uncertain.

Esophageal and gastric contractions induced by MCP are
blocked by anticholinergic drugs such as atropine and potentiated by cholinergic drugs such as carbachol and methacholine (Jacoby and Brodie, 1967 and Johnson, 1971b). MCP has no anticholinesterase activities (Eisner, 1968) and its actions are unaffected by ganglion blocking agents such as chlorisondamine.

Eisner (1968) has shown that MCP has no action on the isolated human smooth muscles from the body or the antrum of the stomach, however, MCP sensitizes the preparations to acetylcholine. It is postulated that MCP acts via the intramural cholinergic neurons responsible for modifying gastric motility but not gastric secretion (Eisner, 1968).

A recent study showed that MCP partially and significantly reduces the relaxation effect of adenosine 5-triphosphate (ATP), adenosine diphosphate (ADP) and adenosine, but potentiates the effect of noradrenaline on the atropine pretreated taenia coli, rabbit ileum and rat duodenum (Okwuasaba and Hamilton, 1975). The inhibiting effect of ATP, ADP and adenosine on the peristalsis of guinea-pig ileum was decreased, the effect of adrenaline was potentiated and the effect of theophylline ethylenediamine was not affected by MCP. This specific action of MCP was postulated to be due to its sensitive blockade, at the post-synaptic sites, of the effect of the inhibitory purinergic transmitter (ATP and related nucleotides) released during peristalsis (Okwuasaba and Hamilton, 1975). Such action of MCP in antagonizing the action of the intrinsic inhibitory
mechanism may well be complementary to the documented muscarinic sensitizing action reported by others (Jacoby and Brodie, 1967; Birtley and Baines, 1973 and Fontaine and Reuse, 1973). In man, the gastric emptying time prolonged by L-dopa was reduced by MCP action on the gastro-intestinal tract. This might be partly due to the anti-dopaminergic effect of MCP (Berkowitz and McCallum, 1980).

MCP is reported to raise the threshold of activity in the chemoreceptor trigger zone (CTZ) and decreases the sensitivity of visceral nerve which transmits afferent impulses from the gastrointestinal tract to the emetic center in the lateral reticular formation. Thus, MCP prevents vomiting induced by central emetics (Justin-Besancon and Laville, 1964 and Klein et al., 1968). Cannon (1975) had indicated that drugs which stimulate the CTZ are dopamine-like. The action of MCP in the CTZ is believed to be due to its dopaminergic effect. More evidence has been accumulated recently to support this hypothesis. Peringer et al., (1975) have observed that after injecting MCP intraperitoneally, MCP has no effect on the dopamine concentrations on the whole brain, but it increases the homovanillic acid (HVA) (a dopamine metabolite) concentration both in the corpus striatum and in the mesolimbic area. It has been postulated that MCP blocks dopamine receptors (Peringer et al., 1975 and Blower, 1975) and as a result MCP causes an increase in firing of the dopaminergic neurons. Hence, the turnover of dopamine is increased (Elliott et al., 1977). MCP has been shown to stimulate prolactin secretion both in animals (Carlson et al., 1977).
and Jiro et al., 1977) as well as in humans (Delitala et al., 1976; Ogiha\textsuperscript{ra} et al., 1977; Sowers et al., 1977; and Huizing et al., 1979). This effect is abolished by CB154, a dopaminergic stimulant (Jiro et al., 1977). Behavioral studies (Dolphin et al., 1975) indicate that MCP resembles pimozide in antagonizing the effect of apomorphine or amphetamine in producing turning behavior in mice with unilateral lesions of the nigrostriatal pathway. It also inhibits the apomorphine induced stereotopy and the reversal of reserpine-induced suppression of locomotor activity (Jenner et al., 1975). It appears that MCP is a relatively potent antagonist of striatal dopamine receptors. The specificity of MCP as a dopamine receptor antagonist has also been shown on the peripheral system (Goldberg et al., 1978 and Brodde and Schemuth, 1978). This was indicated by a shift of the dose-response curve for dopamine induced relaxation significantly to the right in a concentration dependent manner (Brodde and Schemuth, 1979) when the isolated rabbit mesenteric arteries were studied with MCP.

Clinical Trials. As a result of the wide clinical implications of its properties, MCP has been tested in various conditions of upper gastro-intestinal distress and disease since the 1960's.

Symptomatic Relief - MCP is very effective in relieving postoperative nausea and vomiting (Trafford, 1967 and Handley, 1967). In a clinical study of 1,500 patients, MCP has been shown to be as effective as phenothiazine but with fewer side effects (Robinson, 1973a) in the treatment of nausea and vomiting. However, pre-operative nausea and vomiting are not significantly
relieved by MCP (Dundee and Clark, 1973). This is probably due to its CNS as well as peripheral effect.

Radiology - Barium meal examination is usually a time consuming procedure, especially for those patients who have pyloric obstruction which makes the visualization of the duodenum more difficult. MCP, with its properties in accelerating gastric emptying by increasing peristaltic activities and relaxing the pyloric canal, reduces the radiological examination time significantly (Howarth et al., 1969; James and Melrose, 1969 and Kreeel, 1970). Furthermore, this agent is particularly useful in small-bowel examination (James and Humes, 1969).

Gastrointestinal Intubation - MCP when given i.m. (Pirola, 1967) or i.v. (Bolin, 1969) shortens the time required to introduce a biopsy capsule or aspirating catheter through the pylorus into proximal jejunum. This is due to the effect of MCP to relax the pyloric sphincter.

Upper Gastrointestinal Endoscopy - Emergency endoscopy for upper gastrointestinal hemorrhage is benefited by giving MCP i.v. This stimulates the passage of blood into the small bowel, cleaning the field for improved inspection (Bader, 1973), by the action of increasing the strength of peristaltis of the gastrointestinal tract.
Gastrointestinal symptoms after vagotomy - Post-vagotomy symptoms like postprandial vomiting, belching, epigastric distress, and diarrhea are alleviated by MCP in patients up to 2 to 3 years after vagotomy (Stadaas and Aune, 1972) simply due to the acceleration of the gastric emptying after MCP.

In Acid-Pepsin Disease -

Gastric Ulcer - Impaired gastric emptying time has been implicated in the pathogenesis of benign gastric ulceration, therefore, an agent which enhances gastric emptying may be beneficial. A clinical study (Hoskin, 1973) showed that MCP is almost equivalent to carbenoxolone in treating a single, chronic, lesser-curve gastric ulcer with fewer side effects. However, the results of controlled trials are not conclusive (Hoskin, 1973). Further well designed studies are awaited to prove the efficacy of MCP in gastric ulcer treatment.

Duodenal Ulcer - MCP is found to be highly effective in preventing relapse of duodenal ulceration, but no beneficial effects are shown with patients who have acute exacerbation of duodenal ulcer (Moshal, 1973). MCP is postulated to exert its effect by reducing gastric emptying time. Thus, it reduces the acid content next to the site of hemorrhage.
Reflux Esophagitis - Despite the action of MCP on the lower esophagus (Heitman and Miller, 1970), it does not improve the condition of patients with severe symptomatic reflux esophagitis (Venables et al., 1973).

In Anesthesia - MCP significantly increases gastric emptying in pregnant women during labor thereby reducing the incidence of vomiting and aspiration during emergency anesthesia, resulting in a reduction in the death rate caused by Mendelson's syndrome (McGarry, 1971; Howard, 1973 and Howard and Sharp, 1973).

Aspiration of stomach contents into the bronchial tree is a major cause of mortality and morbidity in emergency anesthesia. MCP is an effective agent to accelerate gastric emptying in a short time (Davies and Howells, 1973 and Dundee et al., 1974). Thus, the incidence of mortality caused by pulmonary aspiration of stomach contents has been reduced.

In Gastrointestinal Disorders - MCP has been found to be effective in dilating the pylorus of those patients with pyloric stenosis. This enables the patients to avoid emergency operations and a better radiological evaluation and clinical preparation is obtained before surgery. MCP is still not the ultimate treatment of pyloric stenosis (Zer and Dintsman, 1975).

In Migraine - Some beneficial effects on migraine after MCP treatment are observed (Matts, 1974). However, the mechanism of action of MCP in migraine is not known. It has been suggested that
MCP enhances the absorption of analgesics (Nimmo, 1973) with its potent anti-emetic properties (Trafford, 1967). MCP reduces the incidence of nausea and vomiting caused by the analgesics used in migraine (Matts, 1974).

**Side Effects** - In a literature survey (Robinson, 1973b), a total of 1,023 patients showed an incidence of side effects in 11% of the cases. The most common adverse effects are drowsiness and lassitude (4%), bowel disturbances (1.2%), other untoward effects (4.3%) and dizziness or faintness (0.8%). Also facial dyskinesia (Melmed and Bank, 1975) and tetanus-like motor disorder resembling phenothiazine induced "pseudo-tetanus" (Cochlin, 1974) have been documented. Recently, it has been reported that hyperprolactinemia was observed in patients treated with MCP (Aono et al., 1978). This may be, in part, the cause of both galactorrhea and menstrual abnormalities since these symptoms can be reversed by stopping the treatment, provided that patients have not taken the drug for longer than a year (Aono et al., 1978). Also, hyperprolactinemia has been associated with hypogonadism in man (Thorner et al., 1974). MCP was reported to cause a reduction in seminal volume, total sperm count and libido and a loss of spontaneous erections after subchronic administration (Falaschi et al., 1978).

**Influence on Drug Absorption** - Most drugs are absorbed from the gastro-intestinal tract as the unionized form by passive diffusion. The formation of the unionized species is pH dependent. It was
previously assumed that acidic drugs will be absorbed faster in the stomach where the pH in the environment is low (Shore et al., 1957). However, Nimmo et al., (1973) showed that the absorption of the low molecular weight and easily diffusible compounds such as ethanol and weakly acidic drugs such as aspirin, warfarin and pentobarbital is, in fact, slower from the stomach. The site where maximal absorption occurs is the upper small intestine due to the high relative surface area of the small intestine as compared to the stomach. With this in mind, it can be postulated that the time required for a drug to be delivered into the small intestine may affect the rate and/or extent of its absorption. It has been shown by Heading et al., (1973) that the absorption of paracetamol was dependent on the rate of gastric emptying. MCP, an agent which modifies gastric motility, may influence the absorption of those drugs which are administered concomitantly. Specifically, MCP may affect the peak plasma level attained and the area under the curve of the concentration vs. time curve.

Analgesics - The effects of MCP for migraine headache are still uncertain. However, it has been observed that the absorption of aspirin is delayed during a migraine attack (Volans, 1975). MCP, when administered i.m. immediately before the ingestion of aspirin, increases the rate of absorption of the latter (Volan, 1975). Therefore, it is postulated that MCP decreases the gastric emptying time during a migraine attack. This facilitates the absorption of the analgesic. Similar observations have been obtained
by Heading et al., (1973) in the study of paracetamol absorption in man.

**Digoxin** - MCP decreased the steady-state blood level of digoxin (from 0.72 to 0.46 ng/ml) when administered in multiple doses (tablets) to man. Although there is no direct evidence showing that this is a result of increased gastric motility, the digoxin blood level is raised by propantheline which has the opposite effect of MCP (Manninen and Apajalahti, 1973).

**Antibiotics** - It has been observed by (Jamali and Axelson, 1977) that MCP enhances the extent of absorption of griseofulvin given in solution while depressing the extent of absorption of griseofulvin given as a suspension dosage form. MCP accelerates the rate of absorption of tetracycline and pivampicillin in normal subjects as well as convalescent patients (Gothoni et al., 1972). The time to achieve maximum plasma level was significantly reduced. It was suggested that it was due to the effects of MCP on the gastrointestinal tract, to stimulate gastric emptying and intestinal motility.

**Levodopa** - The absorption of L-dopa is variable (Mearrick et al., 1974). MCP effectively increases the gastric motility resulting in an increase in the rate and extent (3-fold increase) of levodopa absorption when tested in Parkinsonian patients (Mearrick et al., 1974)

**Ethanol** - MCP given orally or i.v. before the ingestion of a standard dose of ethanol increases the rate of absorption of ethanol (Johnson 1973; Gibbons and Lant, 1975 and Bateman et al., 1978).
in man. This is due to the effect of MCP on the rate of gastric emptying.

**Isoniazid** - MCP has no apparent effect on the absorption of isoniazid in tuberculosis patients (Savio and Pontiggia, 1965).

**Drug Disposition and Hepatic Blood Flow**

A perfusion limited model was first proposed by Rowland (1973) to describe the effect of changes of physiological factors such as blood flow, enzyme activities and protein binding on the hepatic disposition of drugs. This model was later further elaborated by Wilkinson and Shand (1975) and Nies et al., (1976). The basic assumptions of this model are: 1) the drug is removed solely by the liver 2) the removal processes follow first order kinetics and 3) the hepatic venous drug concentration is in equilibrium with the drug in the liver. The hepatic clearance is described by the following equations (1 and 2)

\[ C_{1_H} = Q \cdot E \]  
\[ C_{1_H} = Q \cdot \frac{f_B C_{1_int}}{Q + f_B C_{1_int}} \]  

where \( C_{1_H} \) is the hepatic clearance, \( E \), the extraction ratio, \( Q \), the hepatic blood flow rate, \( f_B \), the free fraction of drug in the blood and \( C_{1_int} \), the intrinsic clearance of the free drug by the enzyme.
With an appropriate analysis, one can predict changes in drug disposition induced in three fundamental variables.

**Effect of Hepatic Blood Flow on Clearance.** For this discussion, protein binding is assumed to be constant. The effect of protein binding changes in drug disposition will be discussed later. If drug binding to blood is constant, $f_B$ can be incorporated into the intrinsic clearance term, $C_{int}$, which is the intrinsic ability of the liver to clear the drug from the blood. Then equation 1 and 2 become:

$$C_{H} = Q \cdot E = Q \cdot \frac{C_{int}}{Q + C_{int}}$$

(3)

For drugs with low intrinsic clearance when compared to hepatic blood flow, $C_{H}$ depends on the intrinsic clearance, $C_{int}$ (equation 4).

$$C_{H} \approx C_{int}$$

(4)

On the other hand, if $C_{int}$ is much greater than the hepatic blood flow $Q$, then, the hepatic elimination would be sensitive to changes in flow, $Q$ (equation 5).

$$C_{H} \approx Q$$

(5)

Since the hepatic blood flow rate is not infinitely variable and it is within certain physiological limits (0.5 - 2 L/min in man), any alteration in hepatic blood flow rate would not be expected to change hepatic clearance by more than 4 fold (Wilkinson and Shand, 1975).
The wide variation seen in drug extraction ratios depends upon the differences in intrinsic clearance for these drugs. From equation 3 it can be deduced that if the $C_{\text{int}}$ is equal to blood flow, the extraction ratio is equal to 0.5. A plot of hepatic clearance vs. hepatic blood flow (within the physiological limits) shows that when $C_{\text{int}}$ is greater than $Q$, the hepatic clearance would tend to be dependent on flow ($E>0.5$) whereas the opposite is true when $C_{\text{int}}$ is less than flow rate ($E<0.5$). (Fig. 1).

**Indocyanine Green (ICG) - A Blood Flow Rate Indicator**

The tricarbocyanine dye, ICG, has been introduced into clinical medicine by Fox et al., (1957) for measuring cardiac output. Since then, a number of investigators have used this agent to study hepatic blood flow rate. The physiochemical properties, toxicity and distribution of ICG has been extensively reviewed and studied by Paumgartner (1975). ICG (molecular weight = 775) is rapidly and completely bound to plasma proteins. ICG is rapidly distributed and retained in the vascular system. Hepatic uptake and biliary excretion are the routes of elimination. (90-100% of a dose) Enterohepatic cycling has been found to be minimal. The dye is relatively non-toxic ($L.D._{50} = 50 \sim 80$ mg/kg). Distribution to and elimination by the other organs such as the kidneys has not been measurable. Therefore, this dye seems to have all the required qualities of being a cardiac output and hepatic blood flow rate indicator. It has been shown that the ICG clearance at lower dose levels is a good indicator
of the hepatic blood flow and cardiac output (< 0.64 μmoles/100g body weight in rats). However, at higher dose levels (> 1.3 μmoles/100 gm body weight in rats), the hepatic uptake process become saturated and the removal of ICG from blood does not reflect hepatic blood flow rate. In this thesis, a low dose of ICG (5 mg/kg) has been used to measure the changes in the cardiac output and the hepatic blood flow after various treatments.

**Effects of Enzyme Activity on Drug Clearance** - Changes of metabolic and biliary excretory processes will be reflected in \( C_{\text{int}} \) values. Figure 2 illustrates the effect of changes in \( C_{\text{int}} \) on the actual hepatic clearance of a drug. Such changes are the largest when \( C_{\text{int}} \) is small. A change of \( C_{\text{int}} \) from 167 ml/min to 375 ml/min results in a change in the extraction ratio from 0.1 to 0.2 at average liver blood flow rate (1.5 L/min) and a doubling of hepatic clearance. A change in \( C_{\text{int}} \) from 6 to 13.5 L/min would only result in a change of \( E \) from 0.8 to 0.9, an increase of 12.5% in the drug clearance at the normal blood flow (1.5 L/min.). Therefore, it can be summarized that drugs with a high intrinsic clearance such as propranolol (Nies et al., 1973), propoxyphene (Nies et al., 1976), lidocaine (Branch et al., 1973), to name a few, have high extraction ratios and the elimination of these drugs is very sensitive to flow change and least sensitive to enzyme activity changes. Drugs such as tolbutamide (Nies et al., 1976) and antipyrine (Branch et al., 1974) which
Fig. 1. The relationship between liver blood flow and hepatic clearance for drugs with varying extraction ratios (ER). The arrows indicate the range over which liver blood flow can vary and extraction ratios refer to a normal flow of 1.5 L/min.

Fig. 2. The relationship, according to equation 3, between the intrinsic clearance, hepatic extraction and actual hepatic clearance assuming a liver blood flow of 1.5 L/min. The inset indicates on an expanded scale the relationship at low values of Clint.
have low extraction ratios are very sensitive to changes in enzyme activities but insensitive to changes in flow.

**Clearance and Half Life.** The total clearance of a drug is expressed by equation 6 where $C_l_s$ is the systemic clearance, $t_{1/2}$ is the half life of a drug and $V_d$, the volume of distribution of the drug. It is noted in equation 3 that the clearance term reflects the disposition of a drug which is determined by the physiological parameters such as enzyme activity, blood flow and protein binding, only. $C_l_H$ is not affected by any other terms which are not related to the removal processes. On the other hand, the half-life of a drug could be related to changes not only due to clearance but also distributional changes. Therefore, half-life when compared to clearance is not the best index to reflect drug elimination.

**Influence of Route of Administration.** When a drug is given intravenously, the availability, $F$, is assumed to be 1. However, when a drug is given orally (assuming complete absorption and no prehepatic elimination), the availability is related to the extraction ratio in equation 7.

$$F = 1 - E$$ (7)
$\text{Cl}_s$ after an oral dose can be expressed by Equation 8,

$$\text{Cl}_s = \frac{FD_0}{AUC_0} = QE$$  (8)

where $D_0$, is the oral dose and $AUC_0$ is the area under the plasma concentration vs.time curve after an oral dose. Substituting equation 7 into 8, the following relationship is obtained (equation 9):

$$\frac{D_0}{AUC_0} = \frac{QE}{1-E} = \text{Cl}_{\text{int}}$$  (9)

This interesting relationship indicates that the $AUC_0$ and, hence, the average drug concentration is independent of flow regardless of whether the drug has a high or low intrinsic clearance. Changes in $\text{Cl}_{\text{int}}$ will give a reciprocal change in the $AUC_0$ which in turn reflects the changes in $F$. For drugs with low intrinsic clearance, the fraction of an oral dose $F$ reaching the systemic circulation is high whereas the opposite is true for drugs which have a high intrinsic clearance (Equation 7).

MCP has been indirectly shown to undergo significant first pass metabolism both in man (Schupan et al., 1979; Graffner et al., 1979 and Bateman et al., 1980a) and in rabbits (Bakke and Segura, 1976). It is therefore postulated that the elimination of MCP may be blood flow rate limited in the rat. In addition, an impairment of the hepatic function may significantly alter MCP disposition. Part of this thesis is devoted to the study of these elimination characteristics of MCP in rat.
Renal Failure and Drug Accumulation

When renal function is diminished, through acute or chronic renal disease, those drugs which are predominantly eliminated via the kidney tend to be retained in the body and may accumulate to toxic levels with multiple dosing (Welling and Craig, 1976, and Levy, 1977). Some drugs are cleared by the kidneys as the unchanged form while others may undergo extensive metabolism and subsequent urinary excretion. Thus, if a drug is excreted largely in the urine (e.g. MCP - 84% of dose) regardless whether the drug is excreted as intact drug or metabolized and then excreted, its overall pharmacokinetic profile is partially a function of the integrity of renal function.

Besides the risk of toxic accumulation of drugs and/or toxic metabolites in renal insufficiency, certain physiological and anatomical changes also alter the pharmacokinetic parameters of drugs which can make dosage modification even more difficult. The volume of distribution of drugs such as digoxin (Reuning et al., 1973), cephalexin, colistimethate, and insulin (Gibaldi and Perrier, 1972), have been reported to decrease due to the change of distribution characteristics of these drugs in renal impairment. These authors noted a significant increase in half-life for the drugs examined, which was accompanied by a change in volume of distribution (Gibaldi and Perrier, 1972 and Reuning et al., 1973). It was further suggested that a given drug concentration in the serum of patients with renal impairment may produce significantly less intense clinical or toxicological reponse than the same
concentration in normal subjects if the site of action is within the tissue compartment (Gibaldi and Perrier, 1972).

The influence of disease states on protein binding has recently been recognized (Craig et al., 1976). The presence of uremia has been associated with a significant reduction in the protein binding of a number of drugs, in particular organic acids (Reidenberg et al., 1971; Shoeman and Azarnoff, 1972; Andreasen et al., 1973; Reidenberg and Affrine, 1973; and Craig et al., 1974) and a concomitant increase in apparent distribution volume (Reidenberg, 1971; Fischer, 1972; Craig et al., 1973, and Craig et al., 1974). The sera from uremic subjects had lower albumin concentrations than normal. Furthermore, the decreased plasma protein binding in uremia could not be accounted for by hypoalbuminemia alone (Craig et al., 1976). This may be due to the increase in the level of endogenous inhibitors which compete for the binding sites in renal impairment (Dromgoole, 1973; Andreassen and Jacobsen, 1974; Craig and Wagnild, 1974; and Sjoholm et al., 1976) and/or the binding quality of the protein may change in uremia (Shoeman and Azarnoff, 1972 and Boobis, 1977).

The binding of most basic drugs (desmethylimipramine, quinidine, dapsone, trimethoprim, propranolol and tubocurarine) studied to date is normal in uremic patients (Reidenberg et al., 1971; Craig and Kunin, 1973 and Ghoneim, 1973 and Reidenberg, 1977). The drugs triamterene and diazepam have proven to be exceptions to
this observation, however, (Reidenberg et al., 1971 and Reidenberg and Affrime, 1973). Alteration of the erythrocyte concentration can also perturb the volume of distribution of some drugs. For example, gentamicin binding to red blood cells is decreased in anemia and as a consequence plasma concentrations are elevated relative to subjects with normal haemoglobin levels (Riff and Jackson, 1976). Since the hematocrit is often from 17-20% in renal impairment, the presence of anemia may further alter the volume of distribution of some drugs. Physiological changes such as edema and dehydration can alter the volume of distribution of some drugs, as has been shown for the sulphonamides or thiocyanate. The effect of other factors such as drug receptor sensitivities in diseased cases have been reviewed (Fabre and Balant, 1976).

The effect of changes in protein binding may alter the distribution and elimination pattern of a drug. A typical example would be phenytoin. Letteri et al., (1971) observed more rapid elimination of phenytoin in patients with renal failure than in normal subjects. This was confirmed by Odar - Cederlof and Borga (1974) who proved that the rapid elimination of phenytoin in patients with renal dysfunction was due to rapid metabolic clearance of the drug. The increase in the metabolic clearance could be partly explained by an increase in the free circulating concentration of phenytoin available for elimination. MCP has been shown to moderately bind to bovine, rat and rabbit sera (<20%) (Pognini and Dicarlo, 1972). Therefore, a change in protein binding in renal failure is unlikely to
alter the kinetics of MCP.

The influence of kidney disease on drug metabolism has been reviewed (Reidenberg, 1974 and Reidenberg, 1978). Drug metabolism by oxidative pathways appears to be normal in uremic patients. The pentobarbital elimination half-life was shortened in dialyzed patients who are not on dialysis (Reidenberg 1978). This was shown to be due to a reduction in volume of distribution; the actual metabolic clearance of pentobarbital was unaltered. Highly protein bound drugs which have a low intrinsic clearance may have their elimination rates increased because of decreased protein binding and increased clearance of free drug (Wilkinson and Shand, 1975). Antipyrine (characterized by a low intrinsic clearance and weak plasma protein binding) has been shown to have accelerated metabolism in patients with renal dysfunction. This cannot be accounted for by the reduction in plasma protein binding (Reidenberg, 1978). Oxidative drug metabolism was found to decrease in uremic rats (Leber and Schutterle, 1973). There appeared to be a species difference in the oxidative metabolism of drugs in uremia (Reidenberg, 1978).

Pharmacokinetics of MCP – Despite the significant number of papers published on MCP, very little information pertaining to its pharmacokinetics was available prior to the initiation of the present study. The general complaint was the lack of a highly sensitive assay. Tunon et al., (1974) in a study using rats, showed that MCP followed bi-exponential elimination kinetics after an i.v. dose (10 mg/kg). The half-life of the drug reported was extremely short
(13 minutes). A spectrophotometric technique was employed to analyse the blood samples taken from the same rat. In samples after 16 minutes, MCP could not be detected due to the lack of sensitivity of the method. The half-life calculated was almost certainly erroneous due to the use of truncated data. Bakke et al., (1976) observed that MCP followed first order elimination \( (t_{1/2} = 20 \text{ minutes}) \) in rats. The half-life of MCP after oral administration was prolonged but the mechanism was unrecognised. By comparing the area under the plasma concentration versus time curve (AUC), it appeared that the availability of the drug administered orally was only about a tenth of an equivalent i.v. dose. With no evidence of incomplete absorption, first-pass metabolism was postulated. These results, however, would have to be re-examined by using a more sensitive assay method because each datum obtained by Bakke et al., (1976) was obtained by sacrificing individual rats, and the standard deviation (probably due to the inter-individual variations) observed was very high. The TLC-photodensitometry method was capable of detecting 0.25 mcg/ml of MCP provided 1 ml of plasma was used. That is to say the capability of this assay is limited to detect lower nanogram levels of MCP. Continuous sampling in small animals such as rats was prohibited because comparatively large volumes of plasma samples (1 ml) were required. A colorimetric technique was used by Arita et al.,(1970a) to study the metabolites of MCP. The sensitivity of this method was only comparable to the TLC-photodensitometry technique but not superior. A GLC-FID assay (Kaempe, 1974) was employed by forensic investigators to isolate
and identify MCP qualitatively. The sensitivity of this method was not reported.

Recently, a HPLC assay of MCP was reported (Teng et al., 1977). The lowest detectable concentration of this assay is about 5 ng/ml provided 5 ml of plasma is being analysed. Again, this assay was not found to be sensitive enough to be used to analyse small volume samples. In view of the available technology to analyse MCP, the development of a highly sensitive assay was of prime importance to facilitate the study of the pharmacokinetics of this drug. A highly sensitive GLC-electron capture detection (ECD) assay has recently been reported by our laboratory (Tam and Axelson, 1978). This enabled the quantitation of minute amounts of MCP (detection limit = 20 pg) in serial blood samples (0.1-0.2 ml). Subsequent to this development, a modified assay for human plasma and urine analysis has also been reported (Tam and Axelson, 1979). In the past year, a HPLC assay (Graffner et al., 1979) and a GC-mass spectrometric assay (Bateman et al., 1978) have appeared in the literature for MCP quantitation in human biological samples. The sensitivity of these assays, although not superior to our methods, have been found to be adequate for quantitation of MCP in human plasma samples. The applicability of these methods has been shown by using these techniques in studying the kinetics of MCP in normal human volunteers (Bateman et al., 1979; Graffner et al., 1979 and Bateman et al., 1980a).
The kinetics of MCP in man have been found to be dependent on the route of administration (Graffner et al., 1979, Bateman et al., 1980a). Substantial first-pass metabolism has been reported by these authors. The high variability observed in the effect of route of administration study may in part, be due to the tablet dosage form employed (Bateman, et al., 1980a). The bioavailability calculated by Graffner et al., (1979) was achieved by comparing the area under the plasma concentration vs. time curve (AUC) of an unequal intravenous (i.v.) dose (10 mg) and oral doses (20 mg), though no evidence of non-linear kinetics was provided (Graffner, et al., 1979). Although linear kinetics were proposed by Teng et al., (1977) for MCP, a bioavailability study was not reported. A more rigorous method such as comparing the AUCs after identical i.v. and oral (solution) dose is required. A study of the effect of route of administration and dose on the kinetics of MCP in normal human volunteers is presently underway in our laboratory. Preliminary data suggest significant first-pass metabolism.

A preliminary study has been reported describing the pharmacokinetics of MCP in patients with renal impairment (Bateman et al., 1980b). The half-life of MCP has been observed to increase 4-fold whereas the renal and total body clearance were diminished to a similar extent in uremic patients. This observation was unexpected since MCP is extensively metabolized (approximately 20% of an administered dose is excreted intact).
The underlying mechanism has not been elucidated nor has any hypotheses been put forward to attempt explanation of this observation. Since conjugation is one of the major metabolic pathways in man, (40% of dose) whether the unexpected alteration in kinetics is due to an increase in biliary excretion of MCP conjugates which may be hydrolyzed and reabsorbed from G.I. tract remains to be examined. This proposal is analogous to the one proposed by Levy (1979) for Diflunisal. In fact, enterohepatic cycling has been reported for MCP in one of the studies (Donatelli, 1971). Protocols have been designed to study the kinetics of MCP in patients with renal dysfunction and are currently underway in our laboratory.

Metabolism - MCP is extensively metabolized in all animal models studied (Arita et al., 1970b, Bakke et al., 1976, and Cowan et al., 1976). Conjugation is the major metabolic pathway in man (Teng et al., 1977, Bateman et al., 1978, 1980a and Tam et al., 1979), and rabbit (Arita et al., 1970b). De-ethylation is also dominant in the rabbit (Arita et al., 1970b) and the rat (Teng et al., 1977). An in vitro study (Beckett and Hiuzing, 1975) showed that MCP had eight metabolites. Later, Cowan et al., (1976) was able to identify four additional metabolites (scheme I). These metabolites exist in very minute quantities (<1% of a dose).

In this thesis, a GLC-ECD assay is reported to simultaneously quantitate MCP and the de-ethylated metabolite (DE-MCP) in rat urine (Tam and Axelson, 1979b). The pharmacokinetics of MCP has been studied as a function of dose and route of
Scheme I. A schematic of metabolites of metoclopramide recovered from rabbit urine.
administration in the rat. Finally, the kinetics of MCP in renal and hepatic impaired rats have also been reported in this thesis.
ELECTRON-CAPTURE GAS LIQUID CHROMATOGRAPHIC ASSAY (EC-GLC) FOR SIMULTANEOUS DETERMINATION OF MCP AND ITS MAJOR METABOLITE DE-ETHYL-MCP (DE-MCP) IN RAT URINE

Materials

4-Amino-5-chloro-2-methoxy-N-(2-ethylaminoethyl) benzamide (DE-MCP) and 4-amino-5-chloro-2-methoxy-N-(2-diethyl aminoethyl) benzamide monohydrochloride monohydrate (MCP-HCl.H₂O) (Lot no.9207) were supplied by A.H. Robins Co. (Richmond, Va., U.S.A.). Heptafluorobutyric anhydride (HFBA) was purchased from Pierce (Rockford, Ill. U.S.A.). Diazepam was supplied by Hoffmann-La Roche (Montreal, Canada). One normal sodium hydroxide and 4% ammonium hydroxide were prepared from BDH and ACS reagent grade materials respectively. Chloroform (distilled in glass) was obtained from Caledon (Georgetown, Ontario, Canada).

GLC

A Hewlett-Packard gas chromatograph (model 5840) equipped with a 63Ni ECD and a glass column (1.2 m x 2 mm I.D.) containing 3% of OV-225 coated on Supelcoport®(100-120 mesh) was used. The operating conditions for routine analysis were: injection temperature, 250°C; oven temperature 235°C; detector temperature, 350°C; carrier gas [argon-methane (19:1, v/v)] flow-rate, 40 ml/min.

Extraction and Derivative Formation

To 1 ml of blank urine containing DE-MCP and MCP-HCl-H₂O was added 1 ml of 1 N NaOH (pH ≈ 13) and 6 ml of chloroform, and the mixture was shaken on a horizontal shaker for 20 min to extract the DE-MCP.
After centrifugation, 5 ml of the organic phase was transferred to a 15-ml centrifuge tube, and the contents were dried under a gentle stream of nitrogen. The residue was reconstituted with 1 ml of internal-standard solution (1 μg/ml of diazepam in benzene) and 20 μl of heptafluorobutyric anhydride (HFBA) were added. After thorough mixing (vortex-type mixer), the reaction mixture was incubated at 55°C for 20 min, then allowed to cool, and the excess of derivatizing agent was removed by hydrolysis with 0.5 ml of water and neutralization with 0.5 ml of 4% ammonium hydroxide solution.

Mass Spectrometry (MS)

Electron impact (EI). A Varian Mat-111 GLC-EI mass spectrometer was used to study the heptafluorobutyryl (HFB) derivative of DE-MCP and MCP. The following conditions were used: for GLC, the injection and oven temperatures were 250°C and 230°C, respectively, the carrier-gas (helium) flow-rate was 20 ml/min, and a 1.8-m. x 2-mm I.D. glass column packed with 3% of OV-17 coated on Chromosorb W (80-100 mesh) was used. For MS, the ionization energy was 70 eV, the electron-multiplier voltage was 2 kV, the analyzer temperature was 250°C and the separator-oven temperature was 200°C.

Chemical ionization (CI). A Finnigan GLC-CI mass spectrometer (model 4000) was employed to identify the molecular ion of the HFB derivatives. The GLC conditions used were as described for routine analysis except that the glass column (0.6 m x 2 mm I.D.) contained 3% of OV-101 coated on Chromosorb W (80-100 mesh), and methane was used as
a carrier and reagent gas (flow-rate 40 ml/min). The separator oven temperature was 250°.

Quantitative Studies

A 1-μl portion of the HFB derivative solution was injected into the Hewlett Packard Model 5840 reporting GLC-ECD equipped with an automatic sampler. Quantitative estimation of DE-MCP in the urine samples was accomplished by plotting the area ratios (derivative to internal standard) against the concentration of DE-MCP.

ANIMAL HANDLING

Male Wistar rats weighing 200-300 g were used in all of the studies. All newly received animals were held in isolation and allowed to acclimatize to the surroundings of the animal care facilities before handling. The rats were fed with the standard Purina rat chow and tap water was allowed ad libitum. A left jugular vein cannula (Weeks & Davis, 1964) was implanted in the animals involved in plasma level and creatinine clearance studies. A two to three day post-surgery recovery period ensured complete recovery of the animals prior to drug administration. After the surgery and during the studies, the rats were housed individually in stainless steel metabolism cages (9.8 in x 7 in x 7 in).

PHARMACOKINETIC STUDY OF MCP IN NORMAL RATS

I.V. Administration - Isotonic solutions of metoclopramide monohydrochloride monohydrate (MCP.HCl.H₂O) (supplied by A.H. Robins,
Lot 9207) equivalent to 5, 15, 25 and 35 mg/kg MCP base were prepared by adding appropriate amounts of sodium chloride to bring the osmolality of the solutions to 280 mOsm/kg of H₂O. The MCP solution was injected into the jugular vein via the cannula and 0.1-0.2 ml blood samples were taken at 0, 1, 2, 4, 6, 10, 15, 20, 35, 45, 60, 90, 120, 150, 180, 210, 240, 300, 360, and 420 minutes after MCP administration through the jugular vein. The study of the transient non-linear elimination characteristics was carried out in a group of rats which received a 35 mg/kg i.v. MCP dose. The blood sampling schedule was 0, 1, 5, 10, 20, 40, 60, 120, 180, 240, 350, 360, 420, 450, 480, 510, 540, 570, 600, 630, 660, 690 and 720 minutes after the administration of MCP. After each blood sample, the cannula was flushed with 0.1 ml of heparin solution (20 units/ml) to prevent blood clot formation within the cannula. The blood samples were immediately centrifuged and the plasma separated and stored at -20°C until analyzed.

Oral Administration - An aqueous solution of MCP·HCl·H₂O equivalent to 15 mg/kg MCP base in 0.5 ml solution was prepared. MCP was introduced to the rat by oral intubation after light ether anesthesia. Approximately 0.1-0.2 ml of blood was collected through the jugular vein via the cannula at 0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, 360 and 420 minutes after drug administration. The cannula and the blood samples were treated as described in the previous section. This experiment was performed simultaneously with another group of rats which received an identical i.v. dose of MCP.
Urinary Excretion Study - The urinary excretion study was carried out following intraperitoneal (i.p.), oral and i.v. (tail vein) administration of 1, 5, 15, 25 and 35 mg/kg of MCP equivalent to free base. Urine samples free of any fecal contaminants from rats housed in individual metabolism cages (Fig.3) were collected at 24 h intervals up to 72 h. The samples were diluted and aliquots were stored at -20°C. until analyzed.

Plasma Indocyanine Green (ICG) Studies - ICG (Hynson, Wescott and Dunning, Baltimore, Md.) solution for intravenous injection was always freshly prepared in the supplied solvent. The ICG solution (0.5 mg/kg, 0.2 ml) was injected via the jugular vein cannula 30 minutes after saline, 5 mg/kg and 35 mg/kg MCP pretreatment. The same protocol was employed in another study except ICG was administered 720 minutes after saline or 35 mg/kg MCP pretreatment. Exactly 0.3 ml of blood was taken via the cannula 1, 3, 5, 7, 10 and 15 minutes after the dye administration. The blood samples were individually transferred to 15 ml centrifuge tubes containing 1 ml of 2% human serum albumin in normal saline. The samples were immediately centrifuged and the plasma samples were analysed within 3 hours after collection. ICG in the plasma was analysed by the UV spectrophotometric method described by Caesar et al., (1961).

PHARMACOKINETICS OF MCP IN CC14 TREATED RATS

Plasma Level Study - The animals were randomly separated into two groups. The control group received 0.5 ml normal saline and the experimental group received a dose of 1.2 ml/kg carbon
Fig. 3. Diagram to show the device employed in collecting urine samples from rats without fecal contamination.
tetrachloride ($Cl_4$) orally one day prior to MCP administration. An i.v. dose of MCP hydrochloride salt equivalent to 15 mg/kg MCP was administered to rats under light ether anesthesia (between 9-10 am). Approximately 0.1-0.2 ml of blood was taken 1, 3, 6, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360, 420 and 480 minutes after MCP administration. The blood samples were transferred into heparinized Carraway tubes $^{(R)}$, centrifuged and the plasma was separated and stored as described in the previous section.

**Urine Study** The rats were randomly separated into two groups. The control and experimental group received a dose of 0.5 ml saline or 1.2 ml/kg $CCl_4$, respectively, one day prior to drug administration. An i.v. dose of MCP hydrochloride salt equivalent to 15 mg/kg MCP was administered via the tail vein to test and control animals under light ether anesthesia. Each rat was individually housed in a stainless steel metabolism cage which permitted collection of urine free of faecal contamination (Fig. 3). Food and water were allowed ad libitum. Cumulative urine samples were taken every 24 hrs up to 48 hrs, and the samples were diluted to 100 ml with water and aliquots were frozen until assayed.

**Biochemical Study** The extent of renal and hepatic impairment by $CCl_4$ was determined by measuring the creatinine clearance, plasma urea nitrogen (PUN) and plasma glutamic oxalo-acetic transaminase (PGOT) levels. This was accomplished by taking 1.5 ml of blood from the saline and $CCl_4$ pretreated animals at the end of a 24 hour urine collection. The plasma GOT (aspartate amino transferase E.C. -2611) and creatinine were analyzed on a fast centrifugal analyzer (Electro-Nucleonics (GEMSAEC). The plasma GOT was analyzed using Henry's (1960) modification of the Karmen method (1955).
The creatinine levels were determined using the alkaline picrate method. The plasma urea nitrogen was measured using the urease/glutamate dehydrogenase reaction on a Du Pont Automated Clinical Analyzer. The precision (coefficient of variation) for creatinine plasma GOT and urea nitrogen was 5% at 2.3 mg/dL, 3% at 14 IU/L and 4% at 20 mg/dL respectively. These biochemical evaluations were performed by the Division of Clinical Chemistry at the Vancouver General Hospital.

**PHARMACOKINETICS OF MCP IN CHEMICALLY AND SURGICALLY INDUCED RENAL DYSFUNCTION**

**Surgical Methods**

Two step 5/6 nephrectomy (TSN) was performed in the following manner: an incision was made about 1 cm left of the spinal cord and extended 2-2 1/2 cm from the rib cage. The left kidney was exposed and dissected free of the surrounding tissues. Two ligatures were placed tightly around the two poles of the kidney with 2-0 silk so that the kidney was sectioned into thirds. The two poles were removed and bleeding was observed to be minimal. The remaining one third of the kidney was returned to the peritoneum after cessation of bleeding. The right kidney was completely removed and a jugular vein cannula implanted one week later. Two-step sham operated rats which were handled identically served as controls for the TSN rats. MCP administration was performed 24 hrs after the removal of the right kidney.

Bilateral ureteral ligation (BUL) was performed after exposing the kidney in the same manner as for the TSN. Two ligatures were tied around each ureter and were then cut between the ligatures. A group of sham operated rats which were handled identically served as BUL controls. MCP administration was performed no less than 24 hrs after surgery.
Chemical Induction

Uranyl nitrate (UN), 5 mg/kg (in 0.5 ml saline), was administered through the tail vein 6 days prior to MCP administration. Saline treated animals which served as controls were injected with 0.5 ml of saline intravenously.

Plasma Level and Urine Study - MCP (15 mg/kg) was administered intravenously to all the animals between 0900-1000 hrs. Blood samples (0.1-0.2 ml) were taken via the jugular vein cannula, 1, 2, 4, 6, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360 and 420 minutes after MCP administration. The blood samples were centrifuged and the plasma immediately separated and stored at -20° until analyzed.

Cumulative 24 and 48 hr urine samples were collected after MCP administration, as previously described. The samples were diluted to 100 ml with distilled water and an aliquot was stored at -20°C until analyzed.

Assessment of Renal Function

To establish the renal function of the animals which underwent surgical or chemical induction of renal impairment, one ml of blood was taken via the jugular vein cannula on day 0, 1, 2, 4 and 6 from the control and UN, BUL and TSN treated animals. After centrifugation, a 0.5 ml plasma sample was collected and the
plasma creatinine, plasma urea nitrogen and PGOT were analyzed by the Clinical Chemistry Division of the Vancouver General Hospital to assess the progression of renal damage. A 1 ml sample of blood was taken from the animals after MCP treatment and the physiological function was assessed by measuring the PUN, plasma creatinine and PGOT for all three models (TSN, BUL and UN). Creatinine clearance was estimated by measuring the amount of creatinine excreted in 24 hr in the urine and the plasma creatinine concentration at the time of urine collection.

**PHARMACOKINETIC AND STATISTICAL ANALYSES**

Initial estimates of the pharmacokinetic parameters for MCP after an i.v. or oral dose were obtained by computer analysis using the program, AUTOAN (Sedman and Wagner 1976). These estimates were later fitted using the program NONLIN (Metzler et al., 1974). All the data points were weighted equal to the reciprocal of the concentrations. The AUCs were calculated using equation 10.

\[
AUC_0^\infty = AUC_0^t + AUC_t^\infty
\]  

(10)

where \( t \) represents the time when the last sample was taken. The first term \( AUC_0^t \) was calculated using the trapezoidal rule and the second term was calculated using equation 11.

\[
AUC_t^\infty = 1.44 C_t t_{1/2}
\]  

(11)
where $C_t$ is the concentration of the last sample and $t_{1/2}$ is the plasma half-life of MCP. In most instances the AUC obtained from $0-t$ was over 95% of the total AUC, therefore the error in AUC estimation arising from an inaccurate estimation of $t_{1/2}$ is minimal. Analysis of variance was employed to statistically test the difference between groups of data. The level of significance was chosen to be $p = 0.05$ for all the analyses.
RESULTS

GLC

A representative chromatogram obtained from the extract of rat urine is shown in Fig. 4; no endogenous interference was observed in chromatograms from blank urine samples. Peaks with retention times at 3.04 and 3.59 min were the HFB derivatives of DE-MCP and MCP, respectively. Linearity was observed in the range studied (0.30-1.50 μg/ml MCP and 0.40-1.85 μg/ml DE-MCP in urine). Quantitation of MCP and DE-MCP was accomplished by analysing a serial dilution of known concentrations of MCP and DE-MCP in the urine extract (Fig. 5). Regression analysis showed that the best-fit lines through the data points were described by:

\[ y = 0.911 \times + 0.011 \text{ for MCP} \]
\[ y = 1.5 \times + 0.039 \text{ for DE-MCP} \]

with \( r^2 = 0.999 \) in both cases. The HFB derivatives were shown to be stable for at least 24h at ambient temperature.

MS - The identity of the GC peaks were confirmed by using GC mass-spectrometric methods.

EI. The fragmentation pattern of the HFB derivative of DE-MCP is similar to that of the HFB derivative of MCP. For MCP, the base peak is at m/e 86 and the other most abundant peaks are at 99, 380 and 423. (Fig. 6). The base peak for DE-MCP is at m/e 380 and the most intense peaks are at m/e 396, 409 and 422. (Fig. 7).
Fig. 4. A representative chromatogram obtained from the urine extract. The retention times at 3.04, 3.59 and 7.45 minutes were the HFB derivative of MCP and DE-MCP and diazepam respectively.
Fig. 5. Calibrations curves of the urine extracts: a. MCP. b. DE-MCP
Plasma extract after metoclopramide admin.

\[ \#38 - 32 \]

\[ >420 \times 5 \]

Fig. 6. Mass spectra of the HFB-derivative of MCP
a. electron impact  b. chemical ionization
Fig. 3. Mass spectra of the derivative of DE-MCP: (a) El; (b) Cl.

Fig. 7. Mass spectra of the HFB-derivative of DE-MCP
   a. electron impact  b. chemical ionization
The (M+1) peak for the HFB derivative of MCP is at m/e 486 whereas the (M + 1) peak for the HFB derivative of DE-MCP is at m/e 636.

Recovery

The extraction efficiency was evaluated by using a standard curve prepared after dissolving authentic MCP and DE-MCP directly in chloroform. The average recoveries from urine extracts for MCP and DE-MCP were 84±6% (n=5) and 86±5% (n=5) respectively.

Dose Dependent Kinetics of MCP - The plasma levels of MCP after low i.v. doses (< 25 mg/kg MCP) declined in a manner best described by a biexponential equation (Figure 8) (equation 12):

\[ C_p = A e^{-\alpha t} + B e^{-\beta t} \quad (12) \]

where \( C_p \) is the plasma concentration at time \( t \). \( A \) and \( B \) are the intercept values for the \( \alpha \) and \( \beta \) phases, respectively. The constants \( \alpha \) and \( \beta \) describe the slope of the initial distributive phase and the terminal elimination phase, respectively. The pharmacokinetic parameters obtained from these data are shown in Table I. Plasma MCP levels after high i.v. doses (35 mg/kg) became tri-phasic (Fig. 9).

Following the distributive phase, the first log linear portion has an average half-life of 104 ± 14 minutes. Approximately 400 minutes after MCP administration, the plasma levels assumed an average half-life (58 ± 7 minutes) which was similar to the \( t_{1/2\beta} \) obtained after low doses (\( t_{1/2\beta} = 58 ± 10 \) minutes). This
Fig. 8. Representative MCP Log plasma concentration vs. time curves after a range (5-25 mg/kg) of i.v. doses.
## TABLE I

Kinetic parameters obtained after i.v. doses

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>5</th>
<th>15</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>$B_{(min)}^{-1}$</td>
<td>0.015 ± 0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0124 ± 0.003</td>
<td>0.0095 ± 0.0016&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>$t_{1/2}$ (min)</td>
<td>47 ± 9</td>
<td>58 ± 10</td>
<td>72 ± 13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AUC (mcg.min/ml)</td>
<td>110 ± 45</td>
<td>300 ± 26</td>
<td>616 ± 200</td>
</tr>
<tr>
<td>$V_d$ (area) (L/kg)</td>
<td>3.8 ± 1.0</td>
<td>4.6 ± 0.6</td>
<td>4.4 ± 0.9</td>
</tr>
<tr>
<td>$V_{dss}$ (L/kg)</td>
<td>3.3 ± 1.0</td>
<td>4.1 ± 0.6</td>
<td>4.0 ± 0.8</td>
</tr>
<tr>
<td>$Cl_T$ (ml/min)</td>
<td>12.0 ± 3.5</td>
<td>13.0 ± 1.0</td>
<td>9.78 ± 2.6</td>
</tr>
<tr>
<td>$Cl_R$ (ml/min)</td>
<td>2.91</td>
<td>2.59</td>
<td>2.3</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

<sup>a</sup> mean ± standard deviation

<sup>b</sup> one way ANOVA showed significant difference (p > 0.05)
Fig. 9. A representative log plasma concentration vs time curve after a 35 mg/kg dose of MCP (I.V.). The sampling period was extended to 720 minutes.

INSERT: The plasma t 1/2 of the intermediate kinetic phase was evaluated using a truncated and more intensive sampling protocol.
phenomenon clearly indicates that MCP's disposition kinetics are dose-dependent. The non-linear nature of MCP kinetics was further illustrated by the disproportionate increase in AUC with dose at high dose levels (≥ 25 mg/kg) (Fig.10).

The inspection of a typical plasma concentration vs time curve (figure 11) obtained after oral administration shows that MCP (15 mg/kg) is rapidly absorbed. The time required to achieve maximum plasma concentration is between 30-60 minutes. This agrees closely to previous literature values (Bakke and Segura, 1976; Hucker, et al., 1968). The AUC values obtained after a 15 mg/kg oral dose are insignificantly different from the AUC values obtained after an identical i.v. dose (Fig.12). The availability (F) after an oral dose has been calculated using equation 13.

\[
F = \text{availability} = \frac{(AUC)_{\text{oral}}}{(AUC)_{\text{i.v.}}} \quad (13)
\]

F is equal to 0.91 indicating that first-pass metabolism does not occur at this dose level.

**Urinary Excretion Data** - A study of the cumulative excretion products in 48 h rat urine after MCP administration (1-35 mg/kg) after i.v., i.p. or oral dosing, showed no significant differences (p<0.05) in the percentage of intact drug and metabolite recovered (p<0.05). (Table II and III). There was no significant difference in the excretion of intact drug (Table II) and the de-ethylated metabolite (Table III) over a 35 fold dose range given
Fig. 10. A plot of AUC vs dose to illustrate the non-linear nature of the MCP kinetics.
Fig. 11. Representative plasma concentration vs. time plots obtained after i.v. (△) and oral (▲) administration of MCP (15 mg/kg).
Fig. 12. The AUC comparison between i.v. and oral administration of a 15 mg/kg dose of MCP. (The bars represent the mean ± one standard deviation)
TABLE II

% of MCP recovered after 48 hr cumulative urine analysis.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>i.v.</th>
<th>i.p.</th>
<th>oral</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21.6 ± 5.1 (4)</td>
<td>23.6 ± 4.2 (4)</td>
<td>21.9 ± 6.3 (6)</td>
</tr>
<tr>
<td>5</td>
<td>25.1 ± 4.6 (6)</td>
<td>23.9 ± 7.6 (6)</td>
<td>20.1 ± 8.5 (6)</td>
</tr>
<tr>
<td>15</td>
<td>21.0 ± 3.5 (6)</td>
<td>23.2 ± 5.6 (5)</td>
<td>24.4 ± 1.2 (6)</td>
</tr>
<tr>
<td>25</td>
<td>24.9 ± 3.3 (7)</td>
<td>27.3 ± 2.7 (5)</td>
<td>26.4 ± 3.9 (6)</td>
</tr>
<tr>
<td>35</td>
<td>26.1 ± 3.6 (6)</td>
<td>25.6 ± 5.5 (6)</td>
<td>28.0 ± 2.1 (4)</td>
</tr>
</tbody>
</table>

a) mean ± standard deviation
b) (n), number of animals
% of De-ethylated metabolite equivalent to MCP recovered after 48 hrs cumulative urine collection.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>i.v.</th>
<th>i.p.</th>
<th>oral</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.1 ± 1.3&lt;sup&gt;a&lt;/sup&gt;(4)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.8 ± 1.5 (4)</td>
<td>6.3 ± 1.1 (6)</td>
</tr>
<tr>
<td>5</td>
<td>7.0 ± 2.9 (6)</td>
<td>8.4 ± 2.0 (6)</td>
<td>8.8 ± 1.8 (6)</td>
</tr>
<tr>
<td>15</td>
<td>7.3 ± 1.6 (6)</td>
<td>8.6 ± 1.4 (5)</td>
<td>11.1 ± 2.2 (6)</td>
</tr>
<tr>
<td>25</td>
<td>10.0 ± 1.6 (7)</td>
<td>12.8 ± 1.8 (5)</td>
<td>12.5 ± 1.1 (6)</td>
</tr>
<tr>
<td>35</td>
<td>8.6 ± 1.5 (6)</td>
<td>9.4 ± 2.3 (6)</td>
<td>12.1 ± 3.0 (4)</td>
</tr>
</tbody>
</table>

<sup>a</sup> mean ± standard deviation
<sup>b</sup> (n), number of animals
by hepatic (oral, i.p.) and systemic (i.v.) routes of administration. This observation would normally tend to rule out dose-dependent kinetics and/or first-pass metabolism particularly in the presence of confirmatory plasma data showing equivalent AUC values (15 mg/kg, i.v. vs. oral) (Fig. 12). Dose-dependency would be noted if the percentage of dose excreted as the intact drug and/or corresponding metabolite(s) changed with an increase in MCP dose. Had MCP been affected by first-pass metabolism, the administration of MCP by a hepatic route of administration would have yielded less excretion of intact drug than that seen after a systemic route of administration. The apparent lack of first-pass metabolism is confirmed after inspection of plasma data at the 15 mg/kg dose level (i.v. vs oral) (Fig. 12) showing identical AUC values. Similarly, the lack of difference in excretion (Tables II, III) after the oral and intraperitoneal routes tends to rule out significant gut metabolism of MCP. It is clear, however, that the urine data showing apparent linear dose-independent pharmacokinetics for MCP is in conflict with the observed dose-dependent nature of the plasma data.

ICG Clearance - Fig. 13 shows that the plasma ICG clearance 30 minutes after a 5 mg/kg MCP i.v. dose is significantly higher than the plasma clearance of ICG (0.5 mg/kg i.v.) 30 minutes after a 35 mg/kg i.v. dose (p<0.05). There is no significant difference between the ICG clearances of the control and the 5 mg/kg MCP pretreated group (p<0.05). It would appear that the apparent dose dependent kinetics of MCP are due to a reduction in blood flow through the major eliminating organ(s). Further evidence for this
Fig. 13. Comparison of ICG clearance 30 minutes and 7 hours after saline, 5 mg/kg and 35 mg/kg MCP. (The bars represent mean ± standard deviation)
hypothesis is given by the return of ICG clearance to control levels seven hours after the administered 35 mg/kg i.v. dose of MCP (Fig.13) with a concomitant resumption of linear kinetics for MCP (35 mg/kg dose level) (Fig. 9) after the transient nonlinear phase.

CARBON TETRACHLORIDE TREATMENT

Biochemistry. The administration of CCl\textsubscript{4} resulted in extensive liver cell necrosis as indicated by a large increase in the PGOT levels (Table IV). However, the plasma creatinine and PUN level are insignificantly changed (Table IV) when compared to the control. The creatinine clearance was significantly reduced by CCl\textsubscript{4} treatment indicating some damage of kidney tissue (Table IV).

Plasma Study. The plasma levels of both the control and the experimental groups follows a bi-exponential decline (Fig.14). The CCl\textsubscript{4} pretreated group has a higher plasma MCP level and the terminal elimination phase was delayed when compared to the controls (Fig.14). The pharmacokinetic parameters obtained are summarized in Fig.15. The half-life of MCP was increased by approximately 3 fold and the area under the plasma concentration curve (AUC) was also increased to a similar extent. The volume of distribution calculated by the area (V\textsubscript{d,area}) and the steady-state method (V\textsubscript{d,ss}) was not significantly different between the control and the CCl\textsubscript{4} treated group (Fig.15). Therefore, the prolongation of t\textsubscript{1/2} was due to the reduction in the total body clearance.
Table IV

Biochemical Information after Carbon Tetrachloride Treatment

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma creatinine (mg/dL)</td>
<td>0.5±.2(6)*</td>
<td>0.71±.16(7)</td>
</tr>
<tr>
<td>Creatinine Clearance (ml/min)</td>
<td>2.0±.5(6)</td>
<td>0.77±.26(6)</td>
</tr>
<tr>
<td>PUN (mg/dL)</td>
<td>16±2 (6)</td>
<td>18±2 (7)</td>
</tr>
<tr>
<td>SGOT (IU/L)</td>
<td>65±30 (6)</td>
<td>2170±1640 (7)</td>
</tr>
</tbody>
</table>

* ( ) number of animals
Fig. 14. Representative plasma MCP concentration vs. time curve after carbon tetrachloride pretreatment (◇, control and ◇, CCl₄).
Fig. 15. Kinetic parameters obtained for MCP after CCl₄ pretreatment (c, control and t, test). The bars represent the mean ± standard deviation.
Urine Study. The 24 hr cumulative urinary excretion of intact drug as the percentage of dose increased two-fold after \( \text{CCl}_4 \) treatment when compared to the saline treated animals. (Fig. 16a). The 24 hr cumulative excretion of the de-ethylated metabolite was constant between the two groups. (Fig. 16b).

RENAL FAILURE -

Body Weight - Table V shows that the body weight of the rats pretreated with uranyl nitrate decreased with time during the period of experimentation whereas the weight of the controls was slightly increased. This was partly due to the loss of appetite and wasting of muscle mass resulting from renal insufficiency (Voegtlin and Hodge, 1949a and Chamutin and Ferris, 1932). The weight of the animals after TSN was slightly, but not significantly decreased on day 1. On subsequent days, the weight of the TSN group was observed to be constant (Table V). The weight of the sham operated rats fluctuated between 260-270 g during experimentation indicating insignificant change \((p < 0.05)\) in body weight (Table V). The BUL treated group showed no significant weight loss \((p < 0.05)\) (Table V).

Biochemistry - The PUN and plasma creatinine levels steadily increased to about 20 and 6 times, respectively, above normal, 6 days after uranyl nitrate treatment (Table VI and VII). The creatinine clearance was significantly decreased in the test animals (From \(1.7 \pm 0.4(n=6)\) to \(0.38 \pm 0.11(n=6)\) ml/min for TSN and \(2.0 \pm 0.5(n=6)\) to \(0.07 \pm 0.03 (n=6)\) ml/min for UN). There was no significant differences in the PGOT levels between the control
Fig. 16. Percent of dose recovered as a) intact drug and b) de-ethylated metabolite after CCl₄ pretreatment (c, control and t, test). The bars represent the mean ± standard deviation.
### TABLE V

**Body Weight Pattern**

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUL control</td>
<td>247±15</td>
<td>249±7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(6)*</td>
<td>(6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUL</td>
<td>254±17</td>
<td>253±17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSN control</td>
<td>270±14</td>
<td>270±12</td>
<td>260±15</td>
<td>270±18</td>
<td>260±14</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td>TSN</td>
<td>270±2</td>
<td>255±7</td>
<td>245±3</td>
<td>244±8</td>
<td>243±3</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td>UN control</td>
<td>241±11</td>
<td>244±12</td>
<td>-</td>
<td>243±24</td>
<td>258±21</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(8)</td>
<td></td>
<td>(8)</td>
<td>(8)</td>
</tr>
<tr>
<td>UN</td>
<td>236±21</td>
<td>212±20</td>
<td>211±20</td>
<td>199±21</td>
<td>190±26</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(8)</td>
<td>(8)</td>
<td>(8)</td>
<td>(8)</td>
</tr>
</tbody>
</table>

( )* number of animals
<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUL control</td>
<td>-</td>
<td>16±6*</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BUL</td>
<td>-</td>
<td>117±11</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TSN Control</td>
<td>18±2</td>
<td>18±2</td>
<td>20±2</td>
<td>18±2</td>
<td>16±2</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
<td>(3)</td>
<td>(5)</td>
</tr>
<tr>
<td>TSN</td>
<td>24±1</td>
<td>60±2</td>
<td>87±17</td>
<td>40±2</td>
<td>78±20</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td>UN Control</td>
<td>15±4</td>
<td>17±4</td>
<td>14±2</td>
<td>16±3</td>
<td>16±2</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(7)</td>
<td>(3)</td>
<td>(6)</td>
<td>(7)</td>
</tr>
<tr>
<td>UN</td>
<td>18±4</td>
<td>51±27</td>
<td>92±40</td>
<td>130±107</td>
<td>300±89</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(5)</td>
<td>(4)</td>
<td>(6)</td>
<td>(6)</td>
</tr>
</tbody>
</table>

(* number of animals)
TABLE VII

Plasma Creatinine Levels (mg/dL)

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUL-control</td>
<td>-</td>
<td>0.54±.23</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUL</td>
<td>-</td>
<td>2.8±.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSN Control</td>
<td>0.53±.08</td>
<td>0.44±.13</td>
<td>0.43±.07</td>
<td>0.6±.3</td>
<td>0.5±.3</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
<td>(3)</td>
<td>(5)</td>
</tr>
<tr>
<td>TSN</td>
<td>0.65±.06</td>
<td>1.4±.4</td>
<td>1.6±.4</td>
<td>1.6±.6</td>
<td>1.5±.3</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td>UN-control</td>
<td>0.5±.2</td>
<td>0.38±.1</td>
<td>0.53±.06</td>
<td>0.74±.3</td>
<td>0.51±.15</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
<td>(3)</td>
<td>(6)</td>
<td>(6)</td>
</tr>
<tr>
<td>UN</td>
<td>0.64±.2</td>
<td>1.2±.7</td>
<td>1.8±2.0</td>
<td>3.3±1.8</td>
<td>5.4±2.5</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(5)</td>
<td>(4)</td>
<td>(6)</td>
<td>(6)</td>
</tr>
</tbody>
</table>

(  )* number of animals
and the renal impaired rats on the day of drug administration (Table VIII). This suggests that significant hepatic cell necrosis was unlikely as a result of UN treatment.

The plasma creatinine and PUN levels were significantly elevated in the BUL group as compared to the sham operated controls (Table VI and VII). None of the BUL rats lived more than 48 hrs. Similar to the UN group the PGOT levels were not significantly different from each other (Table VIII). Plasma creatinine and PUN increased the least in the TSN group as compared to the two step sham operated rats (Table VI and VII). In addition, creatinine clearance was significantly reduced for the TSN and UN rats further substantiating the loss of renal function. No apparent difference in cell necrosis was observed between the TSN and the control group as indicated by the PGOT levels (Table VIII).

**Plasma Study and Urine Study** - Fig. 17 clearly shows that there is a significant decrease in the slope of a log plasma concentration vs. time curve indicating that the elimination half-life of MCP is prolonged in all the renal failure models studied. All of the plasma MCP concentration vs. time curves can be described by a bi-exponential equation with the following general equation:

\[ C_p = Ae^{-\alpha t} + Be^{-\beta t} \]  

(12)

where \( C \) is the plasma concentration, \( \alpha \) the distribution rate constant, \( \beta \) the rate constant of the terminal elimination phase and \( A \) and \( B \) are the intercept values for the \( \alpha \) and \( \beta \) phases,
<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUL control</td>
<td>-</td>
<td>73±20 (5)*</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BUL</td>
<td>-</td>
<td>86±11 (7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSN control</td>
<td>61±4 (5)</td>
<td>69±11 (5)</td>
<td>66±14 (5)</td>
<td>52±9 (3)</td>
<td>70±25 (5)</td>
</tr>
<tr>
<td>TSN</td>
<td>51±10 (4)</td>
<td>67±20 (5)</td>
<td>67±28 (5)</td>
<td>40±2 (4)</td>
<td>45±6 (5)</td>
</tr>
<tr>
<td>UN control</td>
<td>68±14 (7)</td>
<td>46±14 (7)</td>
<td>51±7 (3)</td>
<td>51±18 (6)</td>
<td>65±26 (7)</td>
</tr>
<tr>
<td>UN</td>
<td>63±20 (7)</td>
<td>56±18 (5)</td>
<td>90±18 (4)</td>
<td>104±36 (6)</td>
<td>58±10 (6)</td>
</tr>
</tbody>
</table>

( )* number of animals
Fig. 17. Representative log plasma MCP concentration vs. time curves obtained after a 15 mg/kg dose of MCP was given to the bilateral ureteral ligated (BUL), 5/6 two step nephrectomized (TSN) and uranyl nitrate (UN) rats (---, control and ----, test).
respectively. Table IX, X and XI summarize the pharmacokinetic parameters obtained from the plasma and urine studies. The area under the plasma MCP concentration vs. time curves (AUC) was significantly increased when the renal impaired rats were compared to the control rats (Fig.17). The UN pretreated group had the highest increase in AUC (> 3 times) whereas the AUC increased approximately 2 times in the TSN and the BUL groups when compared to the control animals (Fig.18). This reflects the extent of renal failure induced by the uranium compound. Similarly, the total body clearance was significantly reduced in surgically or chemically induced renal impaired rats (Fig.19) with the half-life showing a corresponding increase (Fig.20). The volume of distribution calculated by the area method (Vd_area) and steady state method (Vd_ss) was only slightly reduced when the renal impaired rats were compared to the controls (Fig.21). It has been observed in our laboratory that the 48 hr cumulative urine levels decreased more than 4 times in the UN rats when compared to control (Fig.22a) while the TSN group shows a slight reduction in total cumulative MCP excretion (Fig.23a). A similar pattern has been reported in the 48 hr cumulative excretion of DE-MCP in both the UN and TSN groups (Fig.22b and 23b). The reduction in the urinary excretion of MCP and its de-ethylated metabolite (DE-MCP) does not reflect the extent of change in the pharmacokinetic parameters obtained from plasma and studies in renal impaired rats as compared to the controls.
<table>
<thead>
<tr>
<th></th>
<th>Controls (Sham)</th>
<th>BUL</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta (\text{min}^{-1})$</td>
<td>$0.0138 \pm 0.0022(6)^*$</td>
<td>$0.00609 \pm 0.0023(6)$</td>
</tr>
<tr>
<td>$t_\frac{1}{2} (\text{min})$</td>
<td>$50 \pm 8(6)$</td>
<td>$128 \pm 46(6)$</td>
</tr>
<tr>
<td>$\text{AUC}_0 \to \infty$</td>
<td>$250 \pm 60(6)$</td>
<td>$600 \pm 190(6)$</td>
</tr>
<tr>
<td>$\text{AUC}_{\text{TB}} (\text{mL/min})$</td>
<td>$16 \pm 5(6)$</td>
<td>$7 \pm 3(6)$</td>
</tr>
<tr>
<td>$V_{d_{\text{area}}}$ (L/kg)</td>
<td>$4.3 \pm 0.7(6)$</td>
<td>$4.4 \pm 1.4(6)$</td>
</tr>
<tr>
<td>$V_{d_{SS}}$ (L/kg)</td>
<td>$3.9 \pm 0.8(6)$</td>
<td>$4.1 \pm 1.3(6)$</td>
</tr>
</tbody>
</table>

( )* number of animals
<table>
<thead>
<tr>
<th></th>
<th>Controls (Sham)</th>
<th>TSN</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$ (min$^{-1}$)</td>
<td>0.00981±.001(6)</td>
<td>0.0058±.0013(7)</td>
</tr>
<tr>
<td>$t_{1/2}$ (min)</td>
<td>70±8(6)</td>
<td>130±36(7)</td>
</tr>
<tr>
<td>AUC $^{0 \rightarrow \infty}$ (mcg-min/ml)</td>
<td>350±40(6)</td>
<td>670±100(7)</td>
</tr>
<tr>
<td>$C_{\text{T}}$ (ml/min)</td>
<td>11±2(6)</td>
<td>5.50±8(7)</td>
</tr>
<tr>
<td>$C_{\text{R}}$ (ml/min)</td>
<td>2.0</td>
<td>0.72</td>
</tr>
<tr>
<td>$V_d$ (area) (L/kg)</td>
<td>4.5±1.0(6)</td>
<td>4±1(7)</td>
</tr>
<tr>
<td>$V_{d_{ss}}$ (L/kg)</td>
<td>3.9±0.6(6)</td>
<td>3.8±0.7(7)</td>
</tr>
</tbody>
</table>

( )* number of animals
### TABLE XI

**Uranyl Nitrate**

<table>
<thead>
<tr>
<th></th>
<th>Controls (Sham)</th>
<th>UN</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta (\text{min}^{-1})$</td>
<td>0.0116±0.003(6)*</td>
<td>0.00484±0.0014(6)</td>
</tr>
<tr>
<td>$t_{1/2} (\text{min})$</td>
<td>60±10(6)</td>
<td>160±50(6)</td>
</tr>
<tr>
<td>AUC $\int_0^\infty (\text{mcg-min/ml})$</td>
<td>330±50(6)</td>
<td>1100±500(6)</td>
</tr>
<tr>
<td>$C_{1_{TB}} (\text{ml/min})$</td>
<td>12±2(6)</td>
<td>3.4±1.6(6)</td>
</tr>
<tr>
<td>$C_{1_R} (\text{ml/min})$</td>
<td>2.9</td>
<td>0.2</td>
</tr>
<tr>
<td>$V_{d_{(area)}} (\text{L/kg})$</td>
<td>4.0±.8(6)</td>
<td>3.2±0.7(6)</td>
</tr>
<tr>
<td>$V_{d_{SS}} (\text{L/kg})$</td>
<td>3.6±0.5(6)</td>
<td>3.0±0.7(6)</td>
</tr>
</tbody>
</table>

( )* number of animals
Fig. 18. Area under the plasma versus time curves after BUL, TSN and UN treatments (c, control and t, test). The bars include plus and minus one standard deviation.
Fig. 19. Total body clearance after BUL, TSN and UN (c, control and t, test). The bars include plus and minus one standard deviation.
Fig. 20. Half life obtained after BUL, TSN and UN (c, control and t, test). The bars include plus and minus one standard deviation.
Fig. 21. Volume of distribution calculated by the area method for BUL, TSN and UN treated animals (c, control and t, test). The bars include plus and minus one standard deviation.
Fig. 22. Percent of dose excreted as a) intact drug and b) metabolite after UN pretreatment (c, control and t, test). The bars include plus and minus one deviation.
Fig. 23. Percent of dose excreted as a) intact drug and b) metabolite after TSN pretreatment (c, control and t, test). The bars include plus and minus one standard deviation.
DISCUSSION
Inspection of the chromatogram for the derivatives of MCP and DE-MCP shows that base-line resolution between the derivatives of the MCP and DE-MCP peaks was not attained when a 3% OV-225 column was used. However, the electronic-integration method provided consistent results which permitted simultaneous quantitation of both the MCP and DE-MCP peaks. The minimal detectable amount of the HFB derivatives is the low picogram range. It has been observed that the sensitivity of the HFB derivative of DE-MCP was about twice as sensitive as that of the MCP.

Structural Confirmation of Derivatives

The fragmentation patterns of the derivatized free base and urine extracted from metoclopramide dosed animals were found to be identical by GLC-mass spectrometry, indicating that the derivatives of MCP and DE-MCP were being analyzed from the urine samples. Like its procaine analogs (Cowan et al., 1976) the derivative of MCP cleaved at the amine bond (m/e 423), as well as the carbonyl-amide bond (m/e 99 and 380). The base peak, m/e 86, was a result of the cleavage at the carbon-carbon bond beta to the amine nitrogen (Scheme II). The mass spectrum of the derivative of DE-MCP is similar to that of MCP except the base peak is at m/e 380 and the most abundant peaks occurred at 396, 409 and 422. The postulated fragmentation pattern is shown in Scheme III. Since the molecular ions of both of the derivatives are not readily identified, CI-MS was employed to monitor the molecular ion.
Scheme II. The postulated fragmentation pattern of the HFB-derivative of MCP.
Scheme III. The postulated fragmentation pattern of the HFB-derivative of DE-MCP.
Since electron impact mass spectrometry was not conclusive, chemical-ionization mass spectrometry was employed to reveal the molecular ion. From the chemical-ionization mass spectrum (Fig 7), a very intense m/e 496 peak, which corresponded to the (MH)$^+$ peak, for the derivative of MCP was observed. The other two peaks, m/e 478 and 446, were postulated to be (MH - water)$^+$ and (MH - water - methyl alcohol)$^+$, respectively (Scheme II). The base peak was observed to be at m/e 636 which corresponds to the removal of the ethylene group at the terminal nitrogen. The [MH]$^+$ peak at m/e 664 suggested a disubstituted HFB derivative of MCP was formed (Scheme III).

Removal of Excess of Derivatizing Agent

The presence of trace quantities of HFBA in the derivative solution has been reported to cause spurious peaks and broad solvent fronts. Therefore, development of a method which would remove the excess reagent without diminishing the response of the derivative was necessary. Two methods were suggested by Walle and Ehrsson (1970): one involved drying of the reaction mixture by a gentle stream of nitrogen after incubation, and the other involved hydrolysis of excess of HFBA with water and neutralization with aqueous ammonia. Tam and Axelson (1978) have recently reported that the former method decreased the response of the derivative of MCP by at least 67%. Similar results were observed for the derivative of DE-MCP; this
was probably due to the volatility of the derivative when the former method was employed.

Applicability of the Assay Method

A highly sensitive GLC-ECD assay has recently been developed to quantitate minute amount of MCP (≈ 20 pg) in a small volume of rat plasma (Tam and Axelson, 1978). This assay has been modified to simultaneously quantitate the HFB derivatives of MCP and DE-MCP in rat urine (Tam and Axelson, 1979). This later method is presently reported in this thesis. Both methods have been respectively shown to suitably quantitate MCP in plasma and MCP and DE-MCP in urine. Therefore, they were adopted for the subsequent pharmacokinetic studies.

THE PHARMACOKINETICS OF MCP IN NORMAL RATS

The pharmacokinetics of MCP were characterized by studying a wide range of single intravenous doses (1-35 mg/kg). The plasma data revealed that at low doses (< 15 mg/kg) the kinetics of MCP could adequately be described by a first order process (Fig. 8). However, at higher dose levels (> 25 mg/kg), MCP exhibits distinct non-linear characteristics. When the dose was increased from 5 to 15 mg/kg, the average AUC values increased proportionately with dose and the half-lives were insignificantly different from each other (Table I). The total
body and renal clearance values were constant over the 5-15 mg/kg
dose range (Table I). These are typical characteristics of a
linear system. After a 25 mg/kg dose of MCP, a slight decrease in the
slope of the terminal \( \beta \) phase was observed (Fig. 8). This was
accompanied by a dose-dependent increase in half-life and a dis propor tionate increase in AUC. Both the total body and renal clearance
decreased when compared to the low dose levels (5 and 15 mg/kg) (Table I).
Evaluation of the 35 mg/kg plasma curves revealed that the non linear nature of MCP kinetics became more prominent (Fig. 9). The tri phasic plasma curves exhibited an intermediate phase with an
associated half-life of 104 ± 14 minutes (Fig. 9, insert).
Approximately 400 minutes after MCP administration, the plasma
MCP curve after a 35 mg/kg dose demonstrated a decline which was
parallel to that seen after 5 and 15 mg/kg of MCP. The nonlinear
kinetics of MCP are, perhaps, a result of a transient alteration in
drug disposition due to saturation of intrinsic mechanisms involved
in either absorption, distribution or elimination. In this instance,
nonlinear absorption could be ruled out since MCP was given i.v. for
the dose dependency study.

Inspection of the urinary excretion products should reveal
whether or not the mechanisms of MCP dose dependency are caused by
changes in the intrinsic mechanisms responsible for drug elimination.
Saturation of any major metabolic pathways would normally result in
an increase in the percentage of intact drug recovered in the urine.
Similarly, saturation of any active renal excretory pathways for
intact drug would be expected to increase the metabolite(s) levels accumulated in urine. Since the renal clearance of MCP (2.9 ml/min) is higher than the creatinine clearance in rat (2.0 ml/min, measured in our laboratory) active tubular excretion of MCP is suggested. However, the percentage of dose excreted as intact drug is independent of dose suggesting that saturation of an active excretory process is unlikely (Table II). Similar observation was made with one of the many metabolites (Table III). This suggests that the non-linear nature of MCP kinetics may not be explained by conventional means using Michaelis-Menten kinetics. Since only 30-40% of the dose (comprising intact drug and the de-ethylated metabolite) could be accounted for in the urine, changes in the kinetics of the other metabolic pathways might also explain the observed nonlinearity of MCP at or above 25 mg/kg. Unfortunately the present study lacks sufficient information to prove or disprove this hypothesis.

Since the total plasma concentration (free and bound MCP) has been measured one might speculate that, at high doses, the protein binding or the distribution equilibrium between the red blood cells and plasma was changed, therefore causing the apparent conflict between the plasma and urine data. The blood/plasma ratios (B/P) observed after 15 and 35 mg/kg doses are insignificantly different from each other (p < 0.05) (Figure 24). This rules out the possibility of a distributional change between the plasma and red cells.

The non-linear kinetics of MCP absorption, distribution and elimination are not adequately described by conventional Michaelis-Menten
Fig. 24. Blood/Plasma ratios after 15 and 35 mg/kg MCP. (5-150 minutes after MCP administration). The bars represent the mean ± standard deviation.
theory, since none of these processes showed clear perturbation at high dose levels of MCP. Therefore, a more rigorous assessment of plasma and urine data was required to explain the anomalous observations. Since MCP is extensively metabolized (Teng, et al., 1977) it is reasonable to assume that the non-renal clearance of this drug approximates its metabolic clearance. After a low dose of MCP (15 mg/kg) the non-renal plasma clearance of MCP approaches the hepatic blood flow ($Q_H$) of 10-15 ml/min in a 200-300 g rat (Roth and Rubin, 1976). Physiologically, the blood clearance rather than the plasma clearance should be used since distribution between red blood cells and plasma is very rapid (Rowland, 1972) and the drug within the red cells is available for elimination. The mean blood clearance of MCP calculated from the plasma data is 9.3 ml/min. If one assumes that the non-renal clearance is the hepatic clearance then the extraction ratio ($E$) calculated using the equation developed by Rowland (1973) (equation 1)

$$C_{1H} = Q.E.$$  

is rather high (0.52-0.80). Interestingly, the renal clearance of MCP (2.9 ml/min) approaches renal blood flow (4-9 ml/min) (Sophasan et al., 1979). Thus, it could be postulated that the hepatic and renal elimination of MCP should be blood flow-rate dependent. After high doses (>25 mg/kg), MCP may transiently reduce the cardiac output which, in turn, reduces the blood flow to the eliminating organ(s) thereby, reducing its own clearance. Once
the blood level of MCP falls to the range where it may no longer
elicit haemodynamic changes, the blood flow should return to normal.
It is clearly shown (Fig. 9) that the plasma MCP half-life returns to
normal, approximately 7 hrs. after drug administration. This is
further substantiated by the initial reduction and then a subsequent
return to control levels of ICG clearance after a high dose of
MCP (Fig.13).

The effect of diminished blood flow on the elimination
of MCP can be more readily understood after examination of equation 14.

Equation 14 shows that the total body clearance \( C_{TB} \) is

\[ C_{TB} = Q_K E_K + Q_H E_H \] (14)

equal to the renal \( Q_K E_K \) and the non-renal (hepatic), \( Q_H E_H \) clearances where \( Q_K \) and \( Q_H \) are kidney and hepatic blood flow,
respectively. \( E_K \) and \( E_H \) denote the extraction ratios of kidney
and the liver, respectively. From equation 6, it can be inferred
that if the blood flow to the eliminating organs is affected to
a similar extent and if the extraction ratios are high in all the
eliminating organs, a reduction of the average clearance after a
high dose of MCP will proportionally reduce the metabolic and
excretory pathways. Therefore, the cumulative amount of intact
MCP and metabolite excreted into urine should remain unaltered at
time infinity. Examination of the cumulative excretion of intact
drug and de-ethylated metabolite (Tables II and III) shows identical
percentage excretion at all dose levels, or no dose-dependency,
further supporting this hypothesis. This hypothesis is also supported by the decline of the average total body and renal clearance after a high dose (35 mg/kg) of MCP \[\text{Cl}_{\text{TB}}\]: from 12.0 ± 3.5 ml/min at 5 mg/kg to 6.73 ± 2.1 ml/min at 35 mg/kg, \[\text{Cl}_{\text{R}}\]: from 2.9 ml/min at 5 mg/kg to 1.64 min at 35 mg/kg. According to the perfusion model (Rowland, 1973) a drug having a high extraction ratio will undergo first-pass metabolism. However, this does not appear to be the case for MCP. The AUC after a 15 mg/kg oral dose of MCP is not significantly different from the AUC obtained after an equal i.v. dose (Fig. 12). The urine data supports the plasma data on this point since there is no apparent difference in the percentage excretion of intact drug and measured metabolite after the hepatic and systemic routes of administration (Table II and III). This clearly shows that MCP does not undergo first-pass metabolism at this dose level.

What remains untested and therefore unproven is whether or not MCP undergoes first-pass metabolism in rat at doses less than 1 mg/kg. In view of the inferred extraction ratio (0.52-0.80) one might hypothesize that MCP could exhibit a threshold dose for saturation of first-pass metabolism at or lower than 1 mg/kg, similar to that seen for propranolol (Suzuki et al., 1972, 1974; Evans et al., 1973; Routledge, 1979). Unfortunately, this hypothesis cannot be confirmed or denied by the present assay capabilities, in the existing animal model, due to analytical detection limits. Another untested hypothesis would be that extrahepatic metabolism may play an important role in MCP elimination. This also could possibly
explain why MCP does not undergo first-pass metabolism.

Cowan et al., (1976) have identified a number of metabolites of MCP using rat liver slices, *in vitro*. Ingrand et al., (1970) reported that the total radioactivity was concentrated in the liver, gastro-intestinal tract and the brain shortly after an intramuscular and intragastric dose of $^{14}$C-MCP. These observations suggest that the liver may be one of the major eliminating (or binding) organs for this drug. The hypothesis that extra-hepatic metabolism may also play a significant role in MCP elimination cannot be readily ruled out with presently available information. Preliminary studies in our laboratory indicate that the rat kidney may eliminate MCP by metabolism as well as excretion. However, further study of this observation is required.

**PHARMACOKINETIC STUDIES IN HEPATIC AND RENAL IMPAIRED RATS**

**Carbon Tetrachloride.** It is well known that the administration of carbon tetrachloride leads to impairment of liver function, including necrosis (Gallagher, 1962), fatty infiltration (Slater, 1966) and decreased activity of microsomal enzymes that catalyze the oxidation of drugs (Kato et al., 1962 and Dingell and Heimberg, 1968). The toxic effects of CCl$_4$ are not unique to hepatic injury; in fact, mortality can depend on the fate of organs other than the liver (Drill, 1952, 1958). Gyorgy et al., (1946) revealed that CCl$_4$ caused
more serious renal lesions in male rats than in females.

Although the toxic effects of CCl₄ are not specific to the liver, the onset of extensive lesions in other organs such as the kidney do not occur until several days after the exposure to this toxin (Smetana, 1939; Moon, 1950). As shown in Table IV, the PUN and plasma creatinine remained unchanged after CCl₄ treatment, however, creatinine clearance was significantly reduced implying renal injury.

The plasma MCP data after CCl₄ treatment suggest that the decrease in metabolic activities due to hepatic injury does decrease the elimination rate of MCP. This is indicated by a greater than two-fold increase in AUC and half-life, and a similar decrease in the total body clearance (Fig. 15). The percentage of dose excreted in 24 hours has significantly increased, indicating a reduction in metabolic activity after CCl₄ (Fig. 16a). It has been postulated that MCP removal by the excretory mechanisms of the kidney involves tubular excretion since renal clearance of MCP (2.9 ml/min) was higher than the glomerular filtration rate (GFR) as measured by creatinine clearance (2.0 ml/min). The renal clearance of MCP after CCl₄ was slightly decreased (2.9 ml/min for saline vs. 2.25 ml/min for CCl₄ treated animals). This implies that the reduction of GFR did not markedly reduce the overall elimination of MCP by either GFR or tubular excretion, or both. We were unable to account for all the administered dose as intact drug in the urine despite extensive damage of the liver. This observation may be due to the incomplete
destruction of the microsomal enzymes in this organ. Very interestingly, the percentage of dose excreted as the de-ethylated metabolite of MCP was not altered after CCl₄ treatment (Fig. 16b). If the liver were to be solely responsible for this oxidative metabolic pathway and if the amount of cytochrome P-450 were reduced in the liver by this treatment (Sasame et al., 1968), one would expect the level of the de-ethylated metabolite produced (DE-MCP) to decrease. Therefore, this suggests that extra-hepatic metabolism may, in part, be responsible for the formation of this metabolite.

Renal Failure - The three renal failure models employed have been shown to produce renal insufficiency as indicated by the significant increase in the PUN and plasma creatinine levels (Tables VI and VII). The decrease in creatinine clearance in the animals also signified the loss of renal function. As indicated by the PGOT levels, liver cell necrosis was apparently minimal in the BUL and TSN models, while the UN animals showed a progressive increase in PGOT up to day 4 and a return to control levels on the day of the experiment. The body weight of the UN treated animals decreased gradually whereas a significant weight loss has been observed for TSN rats in the first two days, (Table V). This is probably due to the loss of appetite and muscle wasting resulting from renal insufficiency (Chanutin and Ferris, 1932; Voegtlin and Hodge, 1949a). The weight of the BUL rats did not change significantly
on the first day. However, these animals did not live long enough to show any significant changes in body weight. The degree of renal impairment as measured by creatinine clearance, PUN and plasma creatinine was the highest with the UN treatment followed by the BUL and TSN.

As previously reported (Voegtlin and Hodge, 1949b and Giacomini, 1979), the time course to achieve the highest degree of renal impairment after a single dose of UN was around 4-6 days. This observation has been confirmed by using PUN, plasma creatinine and creatinine clearance as the renal function indicators (Table VI and VII). The PUN, plasma creatinine and creatinine clearance have indicated that the condition of renal dysfunction has been stable during the six day test period for the 5/6 TSN group (Table VI and VII). Significant reduction of the renal function after TSN was achieved within the first day. With the BUL rats, the degree of renal impairment developed rapidly and no animal was observed to live more than 48 hrs after the surgery confirming the results reported by Giacomini (1979). Based on the biochemical information collected, it was decided to administer MCP on the day when renal impairment was observed to be significant. Therefore, the drug administration time for the UN rats was on the 6th day whereas MCP was administered to the BUL and TSN animals on the first day after the surgery was performed.

The plasma levels achieved were significantly higher in the
renal impaired rats produced by all the treatment when compared to
the controls (Fig. 17). Calculation of the pharmacokinetic parameters
for MCP in renal failure shows that the half-life and AUC values were
increased by at least 2-fold while the total body clearance and renal
clearance were reduced by the same order of magnitude (Tables
IX, X and XI). Although there was a 4-fold reduction in the urinary
excretion of the intact drug into the urine after UN treatment
(Fig. 22) and to a lesser extent after TSN (Fig. 23) treatment, this
reduction could not account for such a dramatic alteration in the
plasma kinetics of MCP since the percentage of intact drug recovered
in the urine of the control rats ranged from 20-25%. A complete
shutdown of the excretory function of the kidney, assuming the
function of the other eliminating organs such as liver is unchanged,
would be expected to cause no more than 20-30% change in the plasma
pharmacokinetic parameters. It is now known that the hepatic
elimination of some drugs by acetylation, reduction and, in some
instances, ester hydrolysis is affected by the state of renal
insufficiency (Reidenburg, 1978). Hogan et al., (1979) have
shown that the important oxidative pathways for drug transformation
in the liver, e.g. the hepatic microsomal N-demethylation of
aminopyrine and ethylmorphine, were significantly reduced in rats
with renal failure. This may be one of the causes for the
significant reduction of the de-ethylated metabolite (DE-MCP)
levels in the urine. However, this hypothesis seems unlikely because
results of the present hepatic failure study using carbon tetrachloride
as an hepatotoxin showed that the level of DE-MCP in urine did not
drop significantly. This would seem to indicate that reduction of hepatic function does not have an effect on the DE-MCP metabolite levels in urine. The reduction of this metabolite level (DE-MCP) may result from the decrease in the excretory function of the kidney tissues and/or reduction of the formation of the metabolite in the kidney during renal insufficiency. The observation of unexpectedly large alteration in the kinetic parameters in renal failure and the insignificant change in the urine level of DE-MCP in hepatic injury leads us to postulate that extrahepatic metabolism may play an important role in MCP removal in the rat. A negative correlation was observed when the $C_{\text{TB}}$ was plotted against the plasma creatinine and PUN levels ($r^2 = 0.76$, $r^2=0.71$) (Fig. 25 and 26). A positive correlation ($r^2>0.97$) was observed when the average total body, renal and nonrenal clearance were individually plotted against the creatinine clearance (Fig. 27). These findings clearly indicate that the metabolic removal of MCP is dependent upon the renal function. Together with the observations in hepatic dysfunction these finding further substantiate the hypotheses of extrahepatic metabolism for MCP. Further experimentation, such as isolated kidney and liver perfusion studies, are required to confirm the present hypotheses. If these observations are further confirmed, one may explain in part why MCP does not undergo first-pass metabolism after a hepato-portal route of administration (oral or i.p.) in the rat. Since the same order of magnitude of changes in the kinetic parameters of MCP was observed both in man (Bateman, 1980b) and in the rat (the present study), the underlying mechanism of this
Fig. 25. A Plot of plasma creatinine vs. the total body clearance of MCP (■=UN, ▼=TSN and ◊=BUL) (r² = 0.76).
Fig. 26. A plot of PUN levels vs. the total body clearance of MCP (■ = UN, ▼ = TSN and ◇ = BUL) \((r^2 = 0.71)\).
Fig. 27. A plot of the average total body (♦, $r^2=0.99$), non-renal (■,$r^2=.98$) and renal (▼,$r^2=.97$) clearance of MCP vs. the average creatinine clearance.
unexpected observation in man may prove to be somewhat similar. Investigation of MCP pharmacokinetics in humans is currently underway.

Conclusions

The GLC assay has been shown to be highly sensitive and suitable for the analysis of MCP and DE-MCP in rat urine. The disposition of MCP in normal rats has been found to be sensitive to perfusion changes and dose dependent. This unique characteristic was proposed to be due to a self reduction in the perfusion to the eliminating organs after a high dose of MCP (≥25 mg/kg). Although the extraction ratio of MCP was postulated to be high, presystemic clearance after hepato-portal administration (i.p. and oral) was not significant. Studies using rats with renal and hepatic dysfunction has indicated delay of MCP elimination. The degree of renal impairment was positively related to the changes of metabolic clearance of MCP. With the evidence that the percentage of dose excreted as the de-ethylated metabolite was unchanged in hepatic dysfunction, extra-hepatic metabolism was postulated to play an important role in MCP disposition. This could, in part, explain why MCP does not undergo first pass metabolism.
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