The Disposition of Morphine and its 3- and 6-Glucuronide Metabolites in Humans and Sheep

A Thesis submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Science at the University of Adelaide

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Corrigenda

Page 4	The paragraph at the top of the page should be relocated to immediately follow the section heading "1.2 Chemistry" on page 3.
Page 42 (lines 2, 5 and 7)	"a_1-acid glycoprotein" should read "α_1-acid glycoprotein".
Page 90	In the footnote "Amoxicillin" and "Haemacell" should read "Amoxycillin" and "Haemaccel", respectively.
Page 113 (line -11)	"supernate" should read "supernatant".
Page 115 (line -9)	"supernate" should read "supernatant".
Page 135	To the unfinished sentence at the bottom of the page should be added "appears to be responsible for the formation of M3G, but its relative importance remains unknown. It was also proposed that morphine is involved in an enterohepatic cycle."
Page 144	Add "Both infusions ceased at 6 hr" to the legend to Figure 6.3.
Page 195 (line 2)	"was" should read "were".
Page 198 (line 13)	"ro re-examine" should read "to re-examine".
Pages 245 to 291	Bibliography: For the references by Aitkenhead *et al.* (1984), Atweh & Kumar (1983), Bodenham *et al.* (1989), Osborne *et al.* (1986), Osborne *et al.* (1992), Pacifici & Rane (1982), Poulain *et al.* (1988), Prescott *et al.* (1993), Regnard & Twycross (1984), Runciman *et al.* (1984), Säwe *et al.* (1983b), Shelly *et al.* (1986), Woolner *et al.* (1985), "Br.." should read "Br.". For the reference by Bhargava *et al.* (1991), "J. Pharmacol. Exp. Ther." should read "*J. Pharmacol. Exp. Ther.*" For the reference by Chapman *et al.* (1984), a full-stop should appear after "*Biomed*" . For the reference by Murphey *et al.* (1994), a full-stop should appear after "pig".

* negative line numbers should be read from the bottom of the page

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Abstract

- Morphine is an opiate alkaloid used for the relief of severe pain. In man, it is eliminated predominantly by metabolism to morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G), which are excreted mainly in urine. There is considerable evidence that M6G has analgesic activity in humans and animals, and that M3G is a functional antagonist of some of the pharmacological actions of morphine and M6G.

- To examine the disposition of morphine and its 3- and 6-glucuronide metabolites in humans and sheep, an improved HPLC method was developed to measure the concentrations of the three compounds in plasma and urine. The stability of the compounds during storage in plasma was also established.

- The influence of renal function on the renal clearance of morphine, M3G and M6G was examined in patients receiving morphine by continuous i.v. infusion while under intensive-care. There were significant linear relationships between renal function, measured by the renal excretory clearance of creatinine, and the renal clearances of morphine, M3G and M6G. There was evidence for the net secretion of morphine by the kidney, but there was no net secretion or reabsorption of M3G and M6G. In ten of the patients who had received a constant infusion for at least 6 hr, there were significant associations between the dose-normalized concentrations of M3G and M6G in plasma and the reciprocal of the clearance of creatinine predicted from its concentration measured routinely in plasma. The results demonstrated the importance of renal function in determining the renal clearances from, and concentrations of M3G and M6G in, plasma during infusion with morphine in these patients.

- The regional elimination of morphine and the net formation of M3G and M6G were examined in chronically-catheterized healthy sheep administered constant infusions of morphine at four separate rates. The dose-normalized concentrations of morphine and M3G in plasma were independent of the rate of infusion. There was significant net extraction of morphine by the liver and kidney, net extraction of M3G and M6G by the kidney, and net formation of M3G

by the gut. There was net uptake of summed morphine, M3G and M6G by the liver. Clearance of morphine from blood by the kidney exceeded the renal excretory clearance of morphine. The liver and kidney were the important organs for the elimination of morphine, with evidence for its metabolism by the kidney in addition to excretion, while the kidney was important for the elimination of M3G and M6G. There was also evidence for the enterohepatic cycling of morphine.

- The disposition of M3G in chronically-catheterized normal sheep was compared during separate constant infusions of morphine and M3G, each at a single rate of infusion. During infusion with M3G, there was net extraction by the kidney only, and the value was not different from its extraction during infusion with morphine. The renal excretory clearance of M3G was greater during infusion with morphine than with M3G, suggesting that morphine was metabolized to M3G by the kidney. No morphine or M6G was detected in plasma and urine while infusing with M3G. While either morphine or M3G was being infused, there was a lack of mass-balance between the summed morphine, M3G and M6G or of M3G, respectively, taken up by the kidney and the respective amounts excreted in urine, indicating that the predominant metabolite, M3G, whether from arterial blood or formed *in situ*, was still accumulating within the kidney or was metabolized further. Upon ceasing the infusions, the elimination of M3G formed from morphine was prolonged relative to preformed M3G, which may be due to any combination of its continued formation from body-stores of morphine, enterohepatic cycling and a diffusional barrier into venous blood from its site of formation within the liver.

- The disposition of morphine, M3G and M6G during infusion with morphine, and of M3G during infusion with M3G, was examined in chronically-catheterized sheep with renal failure. There was no effect of renal failure on the total clearance of morphine, or on hepatic clearance and extraction, but clearance and extraction by the kidney were reduced. The concentrations of M3G and M6G in plasma during infusion with morphine were elevated, as were the concentrations of M3G during infusion with M3G, because of the greatly reduced renal clearances for M3G and M6G. The evidence indicated that the failed kidney still metabolized

morphine to M3G. Lesser fractions of the doses of morphine and M3G were excreted in urine, and it was proposed that biliary excretion of formed M3G became relatively more important during renal failure

Declaration

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any University and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where reference is made in the text.

I consent to this copy of my thesis, when deposited in the library of the University of Adelaide, being made available for loan and photocopying.

Robert William Milne

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Publications in Support of this Thesis

- Milne, R.W., Nation, R.L., Reynolds, G.D., Somogyi, A.A. & Van Crugten, J.T. (1991) High-performance liquid chromatographic determination of morphine and its 3- and 6-glucuronide metabolites: improvements to the method and application to stability studies. *J. Chromatogr.* **565**, 457-464.

- Milne, R.W., Nation, R.L., Somogyi, A.A., Bochner, F. & Griggs, W.M. (1992) The influence of renal function on the renal clearance of morphine and its glucuronide metabolites in intensive-care patients. *Brit. J. Clin. Pharmacol.* **34**, 53-59.

- Milne, R.W., Sloan, P.A., McLean, C.F., Mather, L.E., Nation, R.L., Runciman, W.B., Rutten, A.J., & Somogyi, A.A. (1993) Disposition of morphine and its 3- and 6-glucuronide metabolites during morphine infusion in the sheep. *Drug. Dispos. Metab.* **21**, 1151-1156.

- Milne, R.W., McLean, C.F., Mather, L.E., Nation, R.L., Runciman, W.B., Rutten, A.J., & Somogyi, A.A. Comparative disposition of morphine-3-glucuronide (M3G) during separate i.v. infusions of morphine and M3G in sheep: the importance of the kidney (submitted)

- Milne, R.W., McLean, C.F., Mather, L.E., Nation, R.L., Runciman, W.B., Rutten, A.J., & Somogyi, A.A. The disposition of morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) during i.v. infusion of morphine in sheep with renal failure, and comparison of the disposition of M3G during separate i.v. infusions of morphine and M3G. (in preparation)

Abbreviations, Prefixes and Symbols

The abbreviations and prefixes of the International System of Units have been used in this thesis, except for the alternatives listed below. Also listed are additional abbreviations and terminology, and pharmacokinetic symbols. The latter comply closely with the guidelines established by the American College of Clinical Pharmacology (Aronson et al., 1988).

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
Arterial	For the experiments with the sheep refers to blood (or plasma) obtained from the aorta, as distinct from pulmonary arterial (from the pulmonary artery)
$AUC(t_1\text{-}t_2)$	Area under the concentration in plasma-time curve from time t_1 (hr) to time t_2 (hr)
BSA	Bovine serum albumin
cAMP	Cyclic 3',5'-adenosine monophosphate
C_b/C	Ratio of the concentration in blood to that in plasma
$CL_{Cr,meas}$	Renal clearance of creatinine from plasma calculated from the concentrations in plasma and urine measured by HPLC
$CL_{Cr,pred}$	Clearance of creatinine predicted from the concentrations in plasma determine by the method of Jaffé
^{51}Cr-EDTA	Complex of 51Cr and ethylenediaminetetraacetic acid
CSF	Cerebrospinal fluid
C.V.	Coefficient of variation
df	Degrees of freedom
ED_{50}	Dose producing 50% of maximal response
EDTA	Ethylene diamine tetra-acetic acid
GABA	γ-aminobutyric acid
GFR	Glomerular filtration rate
GLC	Gas-liquid chromatography
GLC-MS	Gas-liquid chromatography-mass spectrometry

GTP	Guanosine triphosphate
hr	Hour(s)
HPLC	High-performance liquid chromatography(-ic)
I_{50}	Concentration of substrate required to displace 50% of a ligand which binds specifically to a receptor
i.c.v.	Intracerebroventricular
IUPAC	International Union of Pure and Applied Chemistry
i.p.	Intraperitoneal(ly)
i.t.	Intrathecal(ly)
i.v.	Intravenous(ly)
K_i	Equilibrium dissociation constant for the interaction of a competitive inhibitor with the receptor
K_m	Michaelis-Menten dissociation constant
M3G	Morphine-3-glucuronide
M6G	Morphine-6-glucuronide
M3S	Morphine-3-sulphate
M6S	Morphine-6-sulphate
min	Minute(s)
n	Number within a sample
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NM3G	Normorphine-3-glucuronide
NM6G	Normorphine-6-glucuronide
NMDA	N-methyl-D-aspartic acid
p	Probability
P	Organic solvent/water (or buffer) partition coefficient
pK_a	Acid dissociation constant
RIA	Radioimmunoassay
s.c.	Subcutaneous(ly)
S.D.*	Standard deviation

sec	Second
TLC	Thin layer chromatography
UDPGT	Uridinediphosphate glucuronosyltransferase
UV	Ultraviolet
V_m	Maximum velocity
V_{ss}	The apparent volume of distribution at steady-state
V_z	The apparent volume of distribution during the terminal phase

* Unless unavoidable, values of standard error from other sources are not given, but if so are clearly indicated.

Chapter 1

Morphine: A Review of the Literature

The purpose of this review is firstly, to introduce the reader to the historical and current uses for morphine and to give a brief outline of the relevant physicochemical properties for morphine and its important metabolites; secondly, to outline the developments in the understanding, and the current knowledge, of its mode of action; thirdly, to review comprehensively the pharmacokinetics of morphine, the evidence for the pharmacological activity of some of the more important metabolites formed in humans and their pharmacokinetics; and fourthly, to review the methods that have been used for the determination of morphine, and two of the more important metabolites, M3G and M6G, in plasma and urine.

1.1. History of morphine

Opium is a hard, darkened, air-dried sticky mass derived from the milky exudate collected from incisions made on the unripe seed capsule of the poppy plant, *Papaver somniferum.* The first recorded use of opium as an anodyne was by Heraclides in 230 B.C. He also used it for cholera and cough. Before this time, Hippocrates (420 B.C.) had used it in the form of a wine as a narcotic, nutritive and purgative agent (Grier, 1937). Opium was included in many theriacs and appears to have contributed to the considerable popularity of these complex antidotes to all imaginable poisons and ills for many centuries. It was usually taken orally or smoked. An English physician, Thomas Sydenham, devised an alcoholic extract, Tincture of Laudanum, which was more convenient for oral administration (LaWall, 1927). Laudanum was a solid preparation made into the form of a pill from a tincture containing opium, saffron, musk, castor and oil of nutmeg and from which the liquor had been evaporated.

Robert Boyle (1626-1691) experimented with opium and attempted to form a more concentrated liquid by extraction of the active principle into spirit of wine. This, and other liquid preparations of stronger effect, were often preferred to Tincture of Laudanum. It was not until 1817 that the German apothecary, Friedrich Sertürner, crystallized morphine (which he named

morphium, from Morpheus, the Greek god of dreams) from opium and recognized its basic properties (LaWall, 1927). However, morphine was used less frequently than the opium preparations until the development of the hypodermic syringe, when it could then be administered by the s.c. route. The latter route generated a more rapid relief from pain, but also contributed to the rapid euphoric effects and more severe abuse of morphine (Melzack, 1990). Nevertheless, opium was still given orally because its action was considered to be different from that of s.c. morphine (Grier, 1937). Until the end of the 19th century, laudanum and morphine were readily available and used as analgesics for pain of neuralgic or abdominal origin, or due to pleurisy, gastric ulcer, cancer, labour or peritonitis; for insomnia, excitement and delirium; with ammonia as an expectorant; for aortic regurgitation, angina and cardiac dyspnoea it increased the peripheral supply of blood; and for diarrhoea (Squire's Companion to the British Pharmacopœia., 1916). Of interest is the recommendation by this publication that the use of opium was contraindicated in patients with kidney diseases. Following the isolation of morphine from opium, a further one hundred years passed before a structure was proposed (Gulland & Robinson, 1923), later to be confirmed by a full chemical synthesis (Gates & Tschudi, 1956).

The World Health Organization (1986) regards morphine as the preferred opioid for the relief of moderate to severe pain. The oral route of administration is preferred for the relief of chronic pain while parenteral routes are more common for short-term use (Foley, 1985). In the ten countries with the highest consumption, expressed as doses per million of population, that consumption has increased four-fold between 1979 and 1987 (World Health Organization, 1990) and probably reflects a changing philosophy in the treatment of chronic pain (Melzack, 1990). Examples of its use for acute pain are after surgery, myocardial infarction, trauma, burns and for orthopaedic pain; its chronic use is most commonly for pain associated with cancer and in some cases of neurogenic pain (Bushnell & Justins, 1993; Foley, 1985; Schug *et al.*, 1991; Twycross, 1975). It is most effective in the relief of nociceptive pain (McQuay, 1988), while some patients obtain partial or full relief from neurogenic pain (Portenoy *et al.*, 1990). In contrast to the previous "as needed" use of morphine, being given only after pain returns, there is now a greater acceptance for its more regular administration according to a schedule which

prevents the reappearance of pain (Bushnell & Justins, 1993; Foley, 1985; Melzack, 1990). The effective analgesic dose given orally for chronic pain varies considerably and may range from 5 to 200 mg, but usually pain is controlled with doses of 5 to 30 mg given every 4 hr (World Health Organization, 1986). There are a number of factors which contribute to the variability and these will be reviewed below.

Morphine is also given to patients receiving mechanical ventilation (Marx *et al.*, 1993) and to patients with dyspnoea associated with left ventricular failure (Timmis et al., 1980). It has also been found that intermittent injections (Bruera, et al., 1993) or additional oral administration of morphine (Twycross & Lack, 1990), supplementary to analgesic doses, is of value for the management of dyspnoea in patients with lung cancer.

The most frequent adverse reactions from morphine reported to the Adverse Drug Reactions Advisory Sub-Committee of the Australian Drug Evaluation Committee between 1972 and 1993 were hypotension, confusion, nausea, vomiting, pruritus, rash, urticaria, respiratory depression and increased sweating (Australian Department of Human Services and Health, 1994). Schug *et al.* (1992) reported constipation, nausea and vomiting as the most frequent side-effects in cancer patients relieved of pain with relatively low doses of morphine, while the review of Walsh (1990) described constipation, nausea and vomiting, and sedation as side-effects in patients with cancer, but respiratory depression was considered a minor problem.

1.2. Chemistry

1.2.1. Structure

Morphine [(7,8-didehydro-4,5,-epoxy-17-methyl-(5α,6α)-morphinan-3,6-diol] (Chemical Abstracts Service or systematic name) or (4aR,5S,7aR,8R,9cS)-4a,5,7a,8,9,9c-hexahydro-12-methyl-8,9c-iminoethanophenanthro[4,5-bcd]furan-3,5-diol (IUPAC name) has the structure shown in Figure 1.1.

Several recent monographs have reviewed the chemistry of morphine and its biosynthesis by plants (Casy & Parfitt, 1986; Lenz et al., 1986; Archer, 1993). Briefly, a substituted benzylisoquinoline, reticuline, is synthesized from two molecules of tyrosine. Intramolecular coupling of reticuline forms salutaridine (Kirby, 1967), the first morphinan intermediate in the

As an introduction to the following sections on the chemistry and pharmacology of morphine and some of its metabolites, the scheme in Figure 1.1 shows the predominant metabolites of morphine in humans along with the approximate percentages of an i.v. dose excreted in urine (section 1.5.3.2).

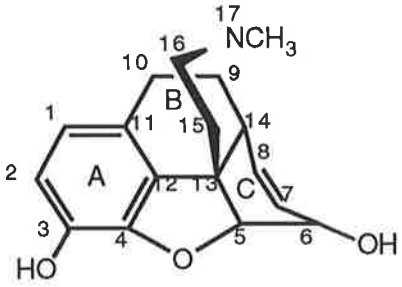
biosynthetic pathway. Further modification of functional groups results in the formation of codeine and then morphine. From the structure, it is evident that there are five asymmetric carbon centres at C-5(R), C-6(S), C-9(R), C-13(S) and C-14(R). The configuration and conformation of morphine have been determined using X-ray crystallography, chemical degradation and proton nuclear magnetic resonance spectroscopy, from which it was deduced that rings A and B of the phenanthrene nucleus are in a plane perpendicular to the plane formed by ring C and the piperidine ring (Figure 1.1). Ring C is arranged in a near-boat conformation with the hydroxyl group in the α (or bowsprit) position (Lenz et al., 1986). When the C ring is saturated, as in dihydromorphine, it exists in the chair form. The (-) isomer of morphine is the naturally occurring form. There is strong evidence to suggest that morphine is also synthesized by mammals via a pathway similar to that in *P. somniferum* (Amann & Zenk, 1991; Donnerer *et al.*, 1986; Matsubara *et al.*, 1992; Weitz *et al.*, 1987).

As described later (section 1.3.3), M6G is an important metabolite and preliminary studies have suggested it is a possible alternative to morphine for use as an analgesic (Osborne *et al.*, 1992). The major metabolite, M3G, also appears to be important because of the evidence demonstrating its possible role as a functional antagonist of morphine and M6G (section 1.3.4).

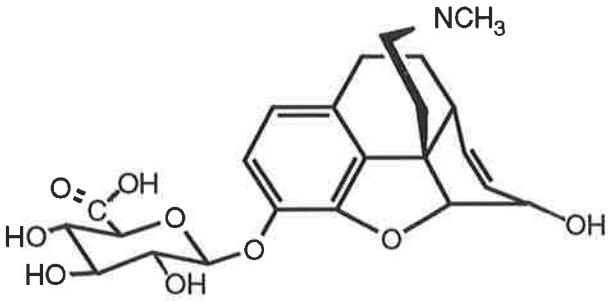
1.2.2. Physical and chemical properties of morphine, M3G and M6G

1.2.2.1. Morphine

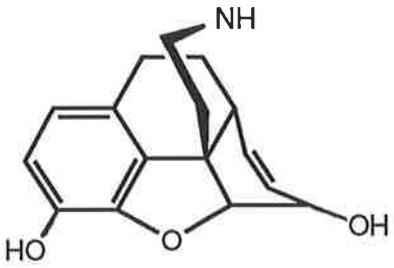
The molecular weights of morphine, morphine hydrochloride (trihydrate) and morphine sulphate (pentahydrate) are 285.3, 375.9 and 758.8, respectively. Schill & Gustavii (1964) measured the pKa values of the amino and phenolic groups as 8.30 and 9.50, respectively, at 20°C; those measured by Kaufman *et al.* (1975) at 37°C were 7.93 and 9.63, respectively,



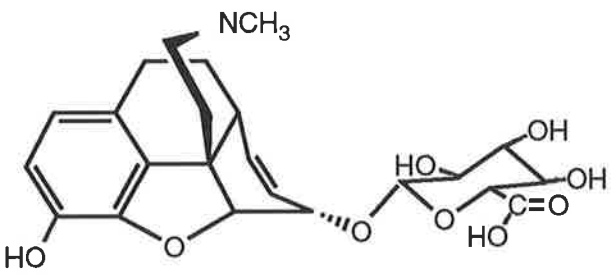
Morphine (5 to 10%)



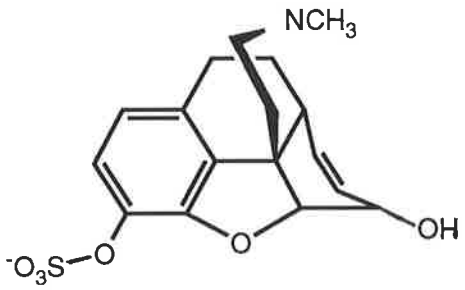
M3G (50 to 60%)



Normorphine plus conjugates (1 to 6%)



M6G (10%)



M3S (5 to 10%)

Figure 1.1 *Structures of morphine and some of its more important metabolites. Shown in parentheses are the approximate percentages of an i.v. dose of morphine administered to humans recovered as these compounds in urine.*

while Roy & Flynn (1989) determined the pKa for the amine to be 8.08 at 35°C. Kaufman *et al.* (1975) found that the pKa values at 20°C were approximately 0.1 units higher than those measured at 37°C.

From the calculations of Schill & Gustavii (1964), it is evident that, at 20°C in an aqueous medium of pH 7.4 and ionic strength 0.1, approximately 90% of morphine exists in the cationic form, 8% as the uncharged form and 2% as the zwitterion.

The apparent partition coefficients (organic/aqueous, pH 7.4) reported for morphine between different solvent systems are given in Table 1.1:

Table 1.1. *Apparent partition coefficients (P) of morphine*

Apparent P	Solvent system[1]	Temp. (°C)	Reference
1.0	octanol/0.067 mol/l phosphate buffer	25	Misra *et al.* (1974)
1.4	octanol/1 mmol/l tris-HCl	-	Mulé *et al.* (1974)
0.5	octanol/0.01 mol/l tris-HCl[2]	37	Pöyhiä & Seppälä (1994)
0.61	octanol/phosphate buffer	37	Van Crugten *et al.* (1991)
1.27	octanol/phosphate buffer[3]	37	Plummer *et al.* (1990)
0.87	octanol/0.02 mol/l phosphate buffer	21	Drustrup *et al.* (1991)
1.17	octanol/water[4]	20	Kaufman *et al.* (1975)
0.70	octanol/water	37	Roy & Flynn (1988)
1.42	octanol/water[4]	37	Kaufman *et al.* (1975)
<0.00001	heptane/0.2 mol/l phosphate buffer	-	von Cube *et al.* (1970)
0.042	dichloroethane/0.2 mol/l phosphate buffer	-	von Cube *et al.* (1970)

[1] pH of aqueous phase adjusted to 7.4.; ionic strengths of 0.168 [2], 0.1[3], and less than 0.01[4]

Kaufman *et al.* (1975) observed that the apparent partition coefficient at 37°C increased from 0.80 to 2.30 over the pH range of 7.10 to 7.70.

1.2.2.2 M3G and M6G

Woods (1954), when characterizing a metabolite of morphine which, from the tests performed, may be assumed to be M3G, observed that it was very soluble in water but not

soluble in methanol, ethanol or acetone. Carrupt *et al.* (1991) measured the pK_a of the carboxylic acid of M3G as 2.83 and also that for M6G as 3.23.

Carrupt *et al.* (1991) used reversed-phase HPLC to observe the influence of the pH of the mobile phase on the capacity factor (k_i) of morphine, M3G and M6G. Capacity factors were used as an indicator of the partition between octanol and water, and therefore lipophilicity. Near pH 7.4, the lipophilicity of the M6G was only 0.5 log k_i units below that of morphine while that for M3G was only a further 0.5 units lower. The experimental values for k_i were approximately two orders of magnitude higher than the calculated values derived from the k_i values for morphine and glucuronic acid. From a theoretical analysis (Carrupt *et al.*, 1991; Gaillard *et al.*, 1994), these authors proposed that the unexpectedly higher lipophilicity of M3G and M6G was due to intramolecular folding so that the area of the polar surface of the molecules was reduced when in lipophilic media such as membranes. In an aqueous medium, the extended form of the molecules was more stable.

Van Crugten *et al.* (1991) found no significant difference in the concentrations of M3G or M6G in phosphate buffer (pH 7.4) before and after equilibration with octanol for 24 hr, indicating negligible partitioning of M3G and M6G into the organic phase.

The molecular weights of M3G and M6G are 461.5.

1.2.2.3. Other Metabolites

From solubility data in water and TLC analysis, M3S appeared to be intermediate in polarity between morphine and M6G (Yeh *et al.*, 1979).

1.3 Pharmacology of Morphine and its Metabolites

Terms used in this section, such as hyperalgesia, opioid, pain, nociceptive pain, neurogenic pain and analgesia, are as defined or explained by Jaffe & Martin (1991), Lindblom *et al.* (1986), Millan (1993) and Twycross & Lack (1990).

1.3.1 Historical Development Of Opioid Pharmacology

Prior to the 1970s, the view generally held was that morphine and other opioids mediated their pain-relieving properties by binding to elements in macromolecular tissue called receptors. The concept of a receptor for drug action developed from studies performed in the early part of the 20th century by Paul Ehrlich on chemotherapeutic agents and by J.N. Langley on curare. Beckett & Casy (1954) proposed a three-dimensional model of the opioid receptor from structure-activity studies with morphine congeners. On the basis of the differences in subjective effects elicited by different opioids and the biphasic dose-response curves obtained for agonist-partial agonist/antagonist mixtures, Martin (1967) suggested that there may be more than one class of opioid receptor. The existence of an opioid receptor was purely speculative until the early 1970s when stereospecific and saturable binding of opioid analgesics and antagonists to homogenates (Pert & Snyder, 1973; Simon *et al.*, 1973) or fractions containing plasma membranes (Terenius, 1973) from brain was observed. It is noteworthy that Pert & Snyder (1973) observed differences in the displacement of naloxone by various agonists when homogenates prepared from the brain of the rat and small intestine of the guinea pig were compared, and they suggested that the opioid receptor may differ in the two tissues. This suggestion was borne out by Martin *et al.* (1976) and Gilbert & Martin (1976) who postulated the existence of at least three opioid receptors (μ, κ and σ) from their work examining the effect of opioids on the response of the spinal reflex to a mechanical stimulus in the spinally-transected dog (Martin *et al.*, 1964). Subsequently, Lord *et al.* (1977) proposed the existence of the δ receptor, but whether the σ receptor is a true opioid receptor remains controversial (Simon & Gioannini, 1993). There are differences in the distribution of the different types (and putative subtypes) of opioid receptor throughout the central and peripheral nervous system, both within and between species (Atweh & Kuhar, 1983; Mansour & Watson, 1993; Millan, 1986), and this has added to the complexity in interpreting their relative importance in the perception and response to pain. Rothman *et al.* (1993) reviewed the evidence for the proposed existence of μ-δ opioid receptor complexes, with allosteric coupling between the two types, in addition to the independent μ, δ and κ receptors. This evidence is from non-competitive binding interactions observed *in vitro* between μ and δ agonists and modulation *in vivo* of the efficacy of agonists

of the μ receptor by sub-effective doses of δ agonists. Pasternak & Wood (1986) have questioned the existence of the classic μ and δ opioid binding sites and postulated the existence of receptor sub-types of the μ receptor, namely μ_1 and μ_2. It was proposed that morphine and some enkephalins (which selectively bound to the δ receptor) were bound to a third site, the μ_1 receptor, with relatively high affinity while the μ_2 subtype was highly selective but had a lower affinity for morphine and other μ selective opioids and was synonymous with the originally proposed μ receptor. However, both postulates remain controversial (Przewlocki, 1991) and, as remarked by this author, it is interesting that anatomical localization of the subtypes (Goodman & Pasternak, 1985) revealed a relative distribution between the μ_1 and μ_2 subtypes which was similar to that between the μ_1 and δ receptors. Nevertheless, it has been proposed that supraspinal antinociception is mediated primarily, but not exclusively, via the μ_1 receptor whereas respiratory depression is mainly via the μ_2 subtype (Pasternak & Wood, 1986). The relative contribution of the subtypes to spinal and supraspinal analgesia remains unclear (Porreca & Burks, 1993; Przewlocki, 1991).

The sequence of events between the interaction of an agonist with the opioid receptor and the electrophysiological response of the neurone remain speculative. The reviews of Crain & Shen (1990), Di Chiara & North (1992) and North (1993) may be summarized briefly. Both μ and δ receptors are coupled to potassium channels which, when opened, cause hyperpolarization of the membrane and inhibition of neuronal conductance. The μ, δ and κ receptors are also coupled to a type of calcium channel. Stimulation closes the channel, inhibiting the release of neurotransmitter(s). Coupling between the receptor and the channels is believed to be via one or more GTP-binding proteins closely associated with the receptor. Combination of morphine with all three receptor types also inhibits the activity of adenylyl cyclase which, possibly via additional messenger systems, may also control ion conductance.

Notwithstanding the proposals for linkage between the different types of opioid receptor, and for the subtypes within each class, the existence of μ, δ and κ receptors is well accepted (Watson & Girdlestone, 1994). Complementary DNA has been isolated and used to express the individual μ, δ and κ receptors. Similarities in sequences appear to be located in regions

believed to be coupled to GTP-binding proteins, while differences in the sequence are thought to be associated with extracellular domains that bind opioid ligands (Reisine & Bell, 1993).

Reports of endogenous compounds from brain extracts that bound to the opioid receptor (Terenius & Wahlström, 1975) or had similar pharmacological properties to morphine (Hughes, 1975) culminated in the isolation and identification of the peptides, [Met]enkephalin and [Leu]enkephalin, from brain extracts (Hughes *et al.*, 1975). Currently, there are numerous peptide opioids which all appear to be derived from three proteins of approximately 250 amino acids: pro-opiomelanocortin, proenkephalin and prodynorphin. The peptides are released at the synaptic terminals of opioidergic neurones and appear to influence numerous physiological processes including nociception, cardiovascular function, respiration, thermoregulation as well as behaviour, learning and memory (Khachaturian *et al.*, 1993). However, the role of the endogenous opioid systems in the control of nociception is exceedingly complex (Fields, 1993; Millan, 1986), and in part is due to the contrasting roles of the endogenous opioids, the complexity of interpretation at the different levels of receptor and neuraxis, the quality and duration of induced nociception, and the nature of the response.

1.3.2 Pharmacology of Morphine

The mechanism of action of morphine and other opioids in eliciting analgesia and its other effects has been the subject of recent reviews and monographs (Crain & Shen, 1990; Di Chiara & North, 1992; Fields, 1993; Johnson & Fleming, 1989; Lipp, 1991; North, 1993; Porreca & Burks, 1993; Stein, 1993; Yaksh, 1993). The inhibitory activity of morphine on the contraction of a preparation of myenteric plexus from the guinea pig, one relatively rich in μ but also containing κ receptors, is markedly greater than that for the vas deferens from the rabbit, a tissue rich in κ receptors only (Corbett *et al.*, 1993; Kosterlitz *et al.*, 1985). Studies of the displacement by morphine of a range of ligands, thought to be specific for each receptor located in homogenate or membranes prepared from mouse, rat, guinea pig, rabbit and bovine brain components, suggest dissociation constants of the order of 1 to 6 nmol/l (Abbott & Palmour, 1988; Christensen & Reiff, 1991; Frances *et al.*, 1992; Oguri *et al.*, 1987), 106 to 161 nmol/l (Christensen & Reiff, 1991; Frances *et al.*, 1992; Oguri *et al.*, 1987) and 12.5 to 128 nmol/l

(Abbott & Palmour, 1988; Christensen & Reiff, 1991) for the μ, δ and κ receptors, respectively.

Only those aspects of the pharmacology of morphine which are considered important in its therapeutic use, either analgesia or side-effects, or for which comparable effects have been observed for the metabolites, are discussed in this section or in sections 1.3.3, 1.3.4. and 1.3.5.

1.3.2.1 Analgesia

The data above support the view that morphine elicits its analgesic action predominantly via the μ receptor. Furthermore, as noted in section 1.3.1, Pasternak & Wood (1986) have proposed that the μ_1 subtype mediates analgesia. However, the *in vivo* studies of Takemori & Portoghese (1987) in mice do not exclude the possibility that morphine may also elicit analgesia by interaction with δ and κ receptors. In view of the differences in receptor distribution between species, the applicability to man of studies in animals is uncertain. In addition, pain in humans involves both a nociceptive and affective component (Beecher, 1946, Lindblom *et al.*, 1986). The effectiveness of morphine on the former is readily measured in animals subjected to acute, noxious mechanical, thermal or electrical stimuli (Taber, 1973), the more common examples being the hot-plate and tail-flick methods. Tests for the affective component involve moderate, continuous pain generated by injured tissue and, while not as well developed experimentally in animals, include the use of repetitive exposure to acute noxious stimuli, the development of chronic arthritic pain in the rat, the application of pressure to inflamed tissue, behavioural alterations following subdermal injection of irritants (Millan, 1993; Ward *et al.*, 1991) or, in humans, evaluation during acute or chronic clinical pain (Jensen *et al.*, 1986). Experimental pain may also be generated in humans but in many cases lacks the psychological component of clinical pain and raises ethical problems (Brady & Lakas, 1984). Fortunately, however, the results from acute tests in animals appear to be reasonably predictive of analgesic activity, or pain relief, in humans (Taber, 1973).

Considerable evidence from the studies reviewed by Fields (1993), Lipp (1991), Porreca & Burks (1993) and Yaksh (1993) suggests that morphine elicits analgesia both spinally and

supraspinally. The transmission of sensory information from nociceptors to motor neurones is depressed as is nociceptive stimulation of neurones projecting into the supraspinal region. A significant component of this depression is mediated via μ receptors located in the dorsal horn of the spinal cord. Morphine also modulates nociception via its effects on interconnecting neurones located in the midbrain and medulla, from which pathways project into the spinal cord. The modulatory network of the midbrain and medulla is also influenced by neuronal input from the cortex and limbic system, both systems being associated with the affective component of pain. While it is clear that morphine modifies the perception or appreciation of pain, the mechanism by which this is achieved is unclear. In part this is because of a lack of understanding of the neurophysiological processes involved in the psychological component of pain. Morphine has been shown to alter neuronal activity in areas likely to be associated with the affective component, including the amygdala, hippocampus, hypothalamus, thalamus and cortex. A proportion of the pathways from the spinal cord (the spinothalamic tract) terminate in the thalamus, the rest passing to the midbrain and medulla.

While the number of controlled clinical studies performed is relatively small, evidence from these and a number of studies with animals suggests that morphine elicits peripheral antinociceptive effects via interaction with opioid receptors located in inflamed tissues (Stein, 1993; Stein *et al.*, 1991).

In essence then, the analgesic activity of morphine is not confined to the perception of pain and the resultant reflex response at the spinal level. Of considerable importance is the ability of morphine to alter the appreciation and tolerance of pain and the associated fear, anxiety or emotional distress.

1.3.2.2 Respiration

Respiratory depression is one of the principal adverse effects limiting the use of morphine, especially with acute administration. The effect of morphine on respiration in humans and animals has been reviewed by Flórez and Hurlé (1993), Martin (1984), Shook *et al.* (1990) and Yeadon & Kitchen (1989). In humans, morphine induces a dose-dependent depression in the ventilatory response to an increase in the partial pressure of carbon dioxide in arterial blood

(pCO_2). There is a reduction in respiratory rate and tidal volume, the partial pressure of oxygen in and pH of arterial blood, and an increase in pCO_2. These effects of morphine are elicited via receptors located in the medulla. Ling *et al.* (1985) have suggested the involvement of the μ_2 receptor which, Pasternak & Wood (1986) argue strongly, is distinct from the purported μ_1 receptor that mediates analgesia. Amongst other workers there is general agreement for the role of both μ and δ receptors in respiratory depression (Flórez and Hurlé, 1993; Freye *et al.*, 1991; Yeoman & Kitchen, 1989). All agree that opioids exhibit differential selectivity for respiratory depression as against analgesia (Freye *et al.*, 1991; Pasternak & Wood, 1986).

1.3.2.3 Gastrointestinal effects

As noted by Maguire *et al.* (1981), constipation is an inevitable complication during the use of opioid analgesics. Kromer (1988, 1993) has reviewed the effects of morphine and other opioids on the gastrointestinal tract of animals and humans. Mittal *et al.* (1986) found that morphine lowered oesophageal sphincter pressure, oesophageal contraction and inhibited gastric emptying of both solids and liquids in humans. In mice, morphine increased transit times within the small and large intestine and prevented castor oil-induced diarrhoea (Shook *et al.*, 1989). The i.c.v. and s.c. doses of morphine required to inhibit transit were 8 and 4 times those necessary for the inhibition of diarrhoea. Studies in animals suggest that these effects are mediated via an interaction with μ and δ receptors located peripherally and supraspinally (Kromer, 1993) and that the effect on transit time is separate from the enhanced absorption of water and electrolytes (Shook *et al.* 1989). Evidence for an effect of morphine on electrolyte and water absorption in humans is lacking. In the small intestine of most animal species and humans, morphine probably inhibits propulsive contractions while stimulating segmental contractions of smooth muscle (Kromer, 1988). Certainly, in humans morphine increases the myoelectric activity associated with mixing and segmentation of the colon but decreases the activity associated with propulsive muscular contractions (Schang *et al.*, 1986).

Morphine is known to cause biliary colic in some patients, especially those with biliary calculi. By increasing basal pressure and the frequency of pressure waves along the Sphincter of Oddi, morphine reduced the flow of bile along the common bile duct (Helm *et al.*, 1988).

Using ultrasonography, Zsigmond *et al.* (1993) found that morphine decreased significantly the diameter of the common bile duct.

1.3.2.4 Nausea and vomiting

As noted in section 1.1., another relatively common problem in the clinical use of morphine is nausea and vomiting. Morphine elicits a dose-dependent emesis in humans, probably by stimulation of opioid receptors in the chemoreceptor trigger zone on the surface of the medulla. Excitation of this zone stimulates a vomiting "centre" deeper within the medulla which coordinates muscles associated with retching and expulsion. Vestibular stimulation may enhance the nausea and vomiting induced by morphine (Flórez & Hurlé, 1993) and indeed, Comroe & Dripps (1948) found a greater incidence of nausea in ambulatory patients given s.c. morphine compared with those in the supine position. However, the possible contribution of postural hypotension to the nausea in the latter study cannot be excluded.

1.3.2.5 Cardiovascular effects

In humans, morphine appears to have dual, opposing effects, mediated by opioid receptors, on vasomotor control within the central nervous system. The overall effect in the unanaesthetized human is a modest decrease in peripheral arteriolar and venous resistance and a decrease in heart rate (Martin, 1984). However, subjects made dependent on 240 mg/day of morphine and maintained for 29 weeks exhibited an elevation of systolic and diastolic blood pressure and heart rate (Jasinski, 1981).

The effects of morphine and general anaesthetics on the regional flow of blood in dogs and humans have implications for the clearance of morphine and will be discussed in section 1.5.4.

1.3.2.6 Tolerance and dependence

Tolerance is described as the need for successive increases in dose, in the absence of the progression of the disease causing pain, to achieve the same effect during chronic treatment. For humans receiving morphine or other opioids for the relief of pain, tolerance is generally not

considered a major problem. In the early stages of pain relief, most reports describe the need for an initial increase in dose, probably due to some tolerance and in an effort to control pain, but thereafter the dose remains relatively stable. Dosage may then increase if pain intensifies, or decrease as discomfort abates. Exceptions appear with patients suffering from nonmalignant chronic pain and given a high concentration of i.t. morphine. A more common reason for an escalation in dose is the progression of the disease causing the pain, notably in patients with cancer (McQuay, 1989; Melzack, 1990; Twycross & Lack, 1990; World Health Organization, 1990; Zenz, 1991).

Tolerance is most likely to develop in animals and humans not suffering pain. Rats given intermittent doses of i.p. morphine in the presence of chemically-induced pain over 3 days did not develop tolerance (Vaccarino *et al.*, 1993). In contrast, tolerance developed in the absence of the pain. Rats made tolerant to twice daily s.c. morphine may remain at the same level of sensitivity for at least a week after administration has stopped (Tiseo & Inturrisi, 1993).

Physical dependence is characterized by the appearance of a typical withdrawal syndrome after the discontinuation of a drug or administration of an antagonist, and by cessation of these symptoms upon resumption of the drug. After 12 hr of continuous administration of morphine, withdrawal symptoms and therefore dependence, may be demonstrated in mice (Way *et al.*, 1969). The symptoms of the withdrawal syndrome are usually exaggerated and opposite to those observed during maintenance with morphine and include hyperalgesia, hyperventilation, diarrhoea, mild hypertension, mydriasis, hypothermia and dysphoria (Way, 1993).

In humans, withdrawal of morphine produces peak symptoms within 24 hr to 48 hr, most of which have disappeared within a week (Way, 1993). Some signs of dependence may persist for longer. From a study of subjects made dependent on 240 mg/day of morphine (Jasinski, 1981), a reduction in the dose to zero over a period of 3 weeks produced a primary abstinence syndrome which continued for between 4 and 10 weeks after the last dose. It was characterized by decreasing blood pressures, heart rate and body temperature, increasing respiratory rate and pupil diameter and a respiratory centre hypersensitive to carbon dioxide. During a secondary phase lasting up to another 20 weeks, systolic and diastolic blood pressure, heart rate, body temperature and pupil diameter remained below the pre-drug control values and the respiratory

centre was hyposensitive to carbon dioxide (Martin & Sloan, 1977; Jasinski, 1981). A similar profile of primary and protracted secondary abstinence syndrome was observed in the rat (Martin *et al.*, 1963).

In humans, physical dependence on morphine usually accompanies the development of tolerance (Way, 1993). Similar findings of co-development have occurred with many *in vitro* studies using tissue culture and isolated organ systems (Johnson & Fleming, 1989; Way, 1993). Development of dependence may be influenced by pre-existing pain. Vaccarino & Couret (1993) found that withdrawal symptoms after i.p. naloxone were significantly greater in rats given chronic morphine in the absence of a nociceptive stimulus compared to those given morphine in the presence of a formalin-induced stimulus.

Events at the cellular level can be summarized from the reviews of Cox (1991, 1993), Johnson & Fleming (1989) and Way (1993). Tolerance represents an adaptation to changes in the net stimulus received by the neurone from additional ligand. Events within the cell during the development of tolerance and upon withdrawal are not clearly understood. Earlier changes may include alterations in receptor affinity and uncoupling of the receptor from the GTP-binding protein. Later stages could include a decreased concentration of opioid receptors (down-regulation) following their internalization, while a common finding is an increase in the activity of adenylyl cyclase. With respect to the latter, it has been found that inhibitors of protein synthesis prevent the development of tolerance and physical dependence in rats (Loh *et al.*, 1969). When morphine is withdrawn or displaced by an antagonist, removal of the inhibitory effect leaves an unbalanced, amplified adenylyl cyclase system that evokes a hyperexcited system and characteristic withdrawal symptoms (Way, 1993).

To add to the complexity, there is growing evidence to suggest the involvement of NMDA and nitric oxide in the development of tolerance (Kolesnikov *et al.*, 1992 and references therein). The noncompetitive and competitive NMDA receptor antagonists, MK-801 (dizocilpine) and NPC17741 (2R,4R,5S-(2-amino-4,5-(1,2-cyclohexyl)-7-phosphonoheptanoic acid), respectively, inhibit the development of tolerance to and dependence on morphine given to rats (Trujillo & Akil, 1991) and mice (Kolesnikov *et al.*, 1993). Also, Tiseo & Inturrisi (1993) showed that the development of tolerance to morphine in the rat could be prevented and

existing tolerance reversed by the competitive antagonist, LY274614 (6-phosphonomethyl-decahydroisoquinolin-3-carboxylic acid). The adaptive changes at the supraspinal level are undoubtedly complicated and may also include the influence of other trophic factors.

Psychological dependence to morphine (and opioids in general) is rarely a problem in the clinical treatment of pain (Porter & Jick, 1980; Portenoy & Foley, 1986). In a retrospective study, Porter & Jick (1980) examined the files of almost 12,000 patients who had received at least one "narcotic" analgesic and, of these, only four had developed well-documented psychological dependence. Dependence was more likely to be a problem when morphine was given irregularly and pain was not adequately controlled (Zenz, 1991) or in patients with a previous history of substance abuse (Melzack, 1990).

1.3.3 Pharmacology of M6G

While it was considered previously that glucuronide metabolites were pharmacologically inactive and were a mechanism for the detoxification and elimination of the parent compound as more polar metabolites via urine and bile, it is now recognized that their formation has important pharmacological and toxicological implications (Kroemer & Klotz, 1992; Mulder, 1992). For these reasons, the formation and disposition of the glucuronide metabolites of a number of drugs, including morphine, has come under more intense study in recent years.

Interest in the possible pharmacological activity of the conjugated metabolites of morphine was generated initially by the findings of Casparis (1950). When examining derivatives of morphine for their possible analgesic action, but without the undesirable side-effects of morphine, it was found that the antinociceptive effect of i.v. morphine-6-glucoside was of a longer duration than it was for i.v. morphine in the rat and the rabbit.

1.3.3.1 Animal studies

Like morphine, M6G has been found to bind to opioid receptors in homogenate and membranes isolated from regions of brains taken from the mouse, rat, guinea pig, rabbit and cow. Studies cited here are those which have compared the abilities of morphine and M6G to displace ligands considered specific for the different types of opioid receptor. From these

studies, either a K_i or an I_{50} has been determined. In general, it has been found that, with respect to the μ receptor, the K_i or I_{50} of M6G is of the order of 0.5 to 13 times greater than the corresponding values for morphine (Abbott & Palmour, 1988; Chen *et al.*, 1991; Christensen & Reiff, 1991; Frances *et al.*, 1992; Hucks *et al.*, 1992; Oguri *et al.*, 1987; Pasternak *et al.*, 1987; Paul *et al.*, 1989). Following on from the proposal of Pasternak & Wood (1986) of two subtypes of the μ receptor, Paul *et al.* (1989) found no differences in the relative binding of morphine and M6G, while Hucks *et al.* (1992) found that morphine was more selective for the μ_2 subtype. From relative K_i or I_{50} values, the affinity of M6G for the δ receptor was 1.2 to 1.9 times that for morphine (Christensen & Reiff, 1991; Frances *et al.*, 1992; Hucks *et al.*, 1992; Oguri *et al.*, 1987). An interesting observation by Abbott & Palmour (1988) was that, at concentrations less than 1% of the I_{50} for M6G, the binding of dihydromorphine, etorphine and naloxone were enhanced. No such effect was observed for morphine. Together, these observations suggest quantitative and qualitative differences in the binding of morphine and M6G to opioid receptors.

Using undifferentiated human neuroblastoma cells (SH-SY5Y) which express predominantly μ opioid receptors, Lambert *et al.* (1993) found that morphine, at a concentration about a quarter that of M6G, displaced 50% of radiolabelled diprenorphine from the receptors. Furthermore, these authors measured the ability of both compounds to inhibit the formation of cAMP, and found that approximately twice the concentration of morphine compared to M6G, was required to achieve 50% of maximal inhibition. However, the authors were unable to explain the lack of effect of morphine and M6G on the release of noradrenaline by cells previously loaded with the neurotransmitter. While the role of adenylyl cyclase in the release of neurotransmitter still remains unclear, the observations with respect to the formation of cAMP suggest that there may be differences in the intrinsic efficacy of the two compounds.

Kataoka *et al.* (1977), when comparing the effect of morphine and M6G on the inhibition of the nicotine-induced contraction of the guinea pig ileum, found that M6G was approximately an order of magnitude more potent. Massi *et al.* (1994) found that M6G was about 50% more potent, while Schmidt *et al.* (1994) found the two compounds to be equipotent, in inhibiting electrically-induced contraction of the isolated ileum from the guinea pig. However, with

membranes prepared from the ileum, the I_{50} for the displacement of naloxone by morphine was less than one quarter the value for M6G (Massi *et al.* 1994). From the use of selective antagonists, Schmidt *et al.* (1994) proposed that morphine and M6G acted reversibly on the μ opioid receptor.

In vivo, morphine and M6G have been compared for their effects on nociception and respiratory function. These effects will be influenced by the animal species, nociceptive stimulus, route of administration of the compounds and their physicochemical properties, assuming that access to the central nervous system is a prerequisite for their action. The influence of the route of administration was clearly demonstrated by comparison of the graded or quantal antinociceptive activity of equal mg/kg s.c. (Paul *et al.*, 1989; Frances *et al.*, 1992) or oral (Frances *et al.*, 1992) doses of morphine and M6G in mice. While the maximum effect for M6G was greater, the time to reach that maximum was less for morphine. However, the effect of M6G was maintained for a markedly longer period. When doses which gave comparable maximum responses were given i.c.v. or i.t., differences in the time course of antinociception were less marked (Abbott & Palmour, 1988; Paul *et al.*, 1989) but probably still reflect differences in transfer to and from their site of action. In most studies, peak antinociceptive effect has been quantified, although in one (Frances *et al.*, 1992), the area under the effect-time curve was also determined. The results of Yoshimura *et al.* (1973) demonstrated that penetration of M6G into the brain after i.p. injection is much slower than for morphine.

In none of the studies employing s.c. and oral dosing was the possibility examined that morphine, a possible product from the hydrolysis of M6G *in vivo*, was contributing to the antinociceptive effect. Schulz & Goldstein (1972) concluded that the analgesic effect of levorphanol-3-glucuronide after i.c.v. injection was due to the free base resulting from hydrolysis *in vivo*. Yoshimura *et al.* (1973) were unable to detect any free morphine in the brain of rats given i.p. injections of ^{14}C-M6G at a dose that would be expected to demonstrate antinociception. However, background radioactivity was 10 to 20% of that due to intact M6G. It would have been preferable to have used a sample of M6G of higher specific activity, allowing an increase in sensitivity for the detection of labelled morphine, and to have analyzed the time course of radioactivity in the brain as M6G and morphine. Therefore, the question of

whether M6G acts partially as a prodrug or in its own right, remains unanswered. Nevertheless, given the far greater potency of M6G compared to morphine after i.c.v. administration (*vide infra*), even if there was any hydrolysis or indeed contamination of the administered M6G with morphine, the conclusions regarding the pharmacological activity of M6G would remain valid.

Comparison of antinociception after M6G and morphine has been performed after oral, s.c., i.t. and i.c.v. injection to rats and mice. On the basis of dose, M6G was more potent than morphine in all studies, irrespective of the route of administration and the test performed (Shimomura *et al.*, 1971; Mori *et al.*, 1972; Abbott & Palmour, 1988; Paul *et al.*, 1989; Gong *et al.*, 1991; Frances *et al.*, 1992). After i.c.v. injection, M6G was 70 to 360 times as potent as morphine when pressure or heat was the nociceptive stimulus, but only 9 to 78 times when evaluated following the injection of chemical irritants, either s.c. formalin into the paw of the rat (Abbott & Palmour, 1988), i.p. acetic acid (Frances *et al.*, 1992) or i.p. ethacrynic acid (Gong *et al.*, 1991). With heat as the stimulus, Paul *et al.* (1989) found that a potency ratio of 112 after i.c.v. injection was increased to 808 after i.t. injection of M6G and morphine. However, the ratio was reduced to between 4 and 9 when they were given via the s.c. route (Abbott & Palmour, 1988; Paul *et al.*, 1989; Shimomura *et al.*, 1971). Differences in spinal and supraspinal involvement by the various nociceptive stimuli and, in addition, regional differences in distribution of morphine and M6G and of the various opioid receptors through which the two compounds primarily express their activity, may account for the differences in their relative potency with the variety of routes and stimuli.

For those studies in which *in vitro* binding was conducted in addition to evaluation of antinociceptive activity when heat was the stimulus, the ratio of the ED_{50} for M6G to that for morphine following i.c.v. administration was approximately 15 to 90 times less than their relative binding affinities (from I_{50} values or dissociation constants) to the μ receptor (Abbott & Palmour, 1988; Paul *et al.*, 1989; Frances *et al.*, 1992). However, given the differences in binding to μ and δ receptors, relative binding to the μ receptor may be an oversimplification. In addition, the assumption that i.c.v. injection permits greater and equal access to the site(s) of antinociceptive action may not be valid. Nevertheless, these findings suggest that M6G possesses a greater intrinsic efficacy than morphine for the opioid receptor. A similar conclusion

may be reached from the studies by Massi *et al.* (1994) mentioned previously with the isolated ileum from the guinea pig.

Sullivan *et al.* (1989) compared the effect of i.t. M6G with that of i.t. morphine, obtained in a previous study (Dickenson & Sullivan, 1986), on the electrical responses of afferent neurones in the dorsal horn to transcutaneous electrical stimulation. Parallel dose-response curves were generated and the ED_{50} (nmol) of M6G was thirteen times less than morphine.

The influence of M6G on respiratory function in animals has been determined in the anaesthetized rat (Gong *et al.*, 1991), the awake dog (Pellegrino *et al.*, 1989a, 1989b) and the neonatal guinea pig (Murphey & Olsen, 1994). Relative to morphine, M6G exerted a greater reduction in nociception (measured by the response of the tail to heat and pressure) than minute volume in the rat, when both compounds were given via the i.c.v. route. However, when antinociceptive activity using the writhing test was compared with ventilatory response, the relative potency between the two compounds was similar (Gong *et al.*, 1991). In the dog, M6G produced a dose-dependent increase in the partial pressure of arterial carbon dioxide and a decrease in ventilatory drive in response to carbon dioxide. Log dose-response curves of M6G and morphine, given by infusion into the fourth ventricle, were parallel but M6G was four- and nine-fold more potent in eliciting the respective responses (Pellegrino *et al.*, 1989a, 1989b). With the neonatal guinea pig, both s.c. morphine and s.c. M6G depressed respiratory minute volume in response to inspired carbon dioxide, but the time to peak effect was greater for M6G (Murphey & Olsen, 1994). The ventilatory response to morphine relative to placebo was relatively consistent from day 3 up to 14 days; M6G was equipotent with morphine at 3 days, but more potent after 7 and 14 days.

The influence of M6G on gastrointestinal motility has also been compared with that of morphine in mice and rats. M6G administered s.c. was nine-fold more potent than morphine in inhibiting the transit of charcoal in mice, at s.c. doses nearly one-hundredth those required to elicit antinociception (Paul *et al.*, 1989). M6G given i.c.v. was approximately fifty-fold more potent in rats (Massi *et al.*, 1994) and mice (Paul *et al.*, 1989) and the reduced transit due to M6G was antagonized by i.c.v. naloxone (Massi *et al.*, 1994).

M6G was also shown to have powerful behavioural reinforcing effects in the rat, indicating that it has rewarding properties similar to morphine (Abbott & Franklin, 1991). The effect was blocked by naltrexone suggesting that it may be mediated via an opioid receptor.

Tolerance to M6G developed rapidly in mice (Frances *et al.*, 1992). These authors observed a higher rate of development for M6G compared with morphine when approximately equimolar s.c. doses (assessed from the peak effect) were given for 2.5 days. The findings of Sosnowski & Yaksh (1990) provide support for the concept that high-efficacy opioid agonists with a larger receptor reserve show a lesser rate of development of tolerance. A similar observation might have been expected for M6G, for which Frances *et al.* (1992) have proposed a greater efficacy. Therefore, their finding of a higher rate for the development of tolerance is unexpected. Taking into account their observation of a more prolonged antinociceptive activity after s.c. M6G compared to s.c. morphine, prolonged continuous stimulation of opioid receptors during repeated s.c. dosing with M6G may have led to greater down-regulation. Less frequent, equipotent s.c. doses of morphine and M6G were given over a longer period by Shimomura *et al.* (1971) and visual observation of their data suggests a slightly lesser rate in the development of tolerance to M6G. Equipotent i.c.v. or i.t. doses may overcome problems of differences in distribution and provide more meaningful data.

In conclusion, from the studies in animals there is overwhelming evidence that M6G, in addition to morphine, binds to opioid receptors, has antinociceptive activity and depresses respiratory function. However, the binding and antinociceptive action appears to be quantitatively and qualitatively different from that of morphine.

1.3.3.2 Human studies

The need for lower doses of morphine in patients with renal failure was noted by Regnard & Twycross (1984). Peterson et al. (1990) reported a significant relationship between renal function and the ratio of the concentrations of M6G to morphine in plasma. It would appear that, in renally impaired patients, M3G and M6G accumulate in plasma to concentrations far higher than are achieved in patients or subjects with normal renal function and, when morphine administration has stopped, remain elevated for a prolonged period while morphine

concentrations decrease to undetectable levels. Additional circumstantial evidence of a pharmacological effect from M6G is available from case reports of patients in renal failure who received morphine. These reports described continued, exaggerated respiratory depression for between 24 hr and 14 days after the last dose of morphine (Bodd et al., 1990; Don et al., 1975; Hasselström et al., 1989; Osborne et al., 1986; Shelly et al., 1986). Intermittent naloxone rapidly reversed the depression suggesting that excessive opioid agonist was present. All of the patients had poor renal function of different severity but none had signs of overt hepatic failure. In three of the studies, high concentrations of M3G and M6G were found in plasma in the absence of measurable morphine (Osborne et al., 1986; Shelly et al., 1986; Bodd et al., 1990). In a fourth study, comparably high concentrations of glucuronide were also found but, in addition, a concentration for morphine of 1 nmol/l was measured at the time when naloxone was discontinued and respiratory function had been restored (Hasselström et al., 1989). Breheny *et al.* (1993) also concluded that prolonged high concentrations of M6G in the plasma of a patient who developed renal failure, following an overdose of slow-release tablets of morphine, may have contributed to the depression of consciousness and respiration observed, but could not exclude the possible contribution from the measurable concentrations of morphine in plasma. These reports provide evidence for an opioid pharmacological effect which may be attributed to M6G. Nevertheless, while morphine was not detectable in plasma during three studies, the concentrations in plasma may not reflect the concentrations at the site of action nor can the possibility be excluded that renal failure may influence the interaction between morphine and the opioid receptor.

Portenoy et al. (1992) divided cancer patients receiving i.v. morphine for the relief of pain into three groups depending on the observed mean ratio for the concentration in plasma of M6G to morphine calculated over a 4 hr period from the start of the infusion. They found that between the groups, the higher the ratio the greater the relief from pain. This suggests that, with respect to the concentration in plasma, M6G is more potent than morphine in relieving pain. The contribution from M6G may have been underestimated because, while it is unlikely that steady-state concentrations of either compound in plasma and at the site of action would have been achieved during the infusion, the shorter half-life for morphine compared to M6G would

produce concentrations of morphine nearer to steady-state (sections 1.5.4.1 and 1.6.1). The concentrations of M3G were not measured but, given the consistency in the ratio of the concentrations for M3G to M6G (section 1.6.1), the corresponding ratios for M3G to morphine probably correlated with those observed for M6G. However, the possible antagonism by M3G of the analgesic effect (section 1.3.4), as a complicating factor in the above observations, is difficult to predict.

Direct evidence for the pharmacological effects of M6G in humans has been obtained from administration of the metabolite to patients and normal subjects. Osborne et al. (1992) gave a single dose (range 0.5 to 4 mg/70 kg, i.v.) to patients with cancer and suffering moderate to severe pain. Of the 19 patients, 17 reported pain relief ranging in duration from 2 to 24 hr with 5 enjoying complete relief for 12 hr. The possibility of a relationship between the concentration of M6G in plasma and relief was not examined. No patients reported sedation, euphoria or nausea and vomiting, there was no change in respiratory frequency and only minor changes in heart rate and blood pressure. Hanna et al. (1990) compared M6G with morphine (0.5 mg of each, i.t.) in 3 cancer patients with chronic pain, by recording their requirements for pethidine that was on demand for 24 hr from a patient-controlled analgesic system. Inspection of their data revealed that, compared to M6G, patients given morphine demanded more pethidine to maintain mild or absent pain, suggesting a lesser potency for morphine. This trend was maintained over the 24 hr of observation.

When healthy subjects were given approximately 2 mg and 4 mg of i.v. M6G on separate occasions, Peat et al. (1991) found that respiratory minute volume was unaffected but the subjects were less sensitive to a challenge with carbon dioxide, although to a lesser degree compared with morphine. Morphine (6.2 mg of base, i.v.) reduced the minute volume as expected. The authors cautiously suggest that M6G may offer advantages over morphine. A similar conclusion may be drawn from the preliminary results of Thompson et al. (1990). However, any claims of a superior therapeutic index for M6G over morphine must await comparative clinical trials with the two compounds.

1.3.4 Pharmacology of M3G

Initially, Woods (1954) evaluated the pharmacological effect(s) of a metabolite of morphine, identified as M3G, which was isolated from the bile of dogs. The author noted that doses of 1 mg/kg i.v. given to dogs or of 150 mg/kg (s.c. or i.p.) given to mice did not produce noticeable changes in behaviour. A dose of 4.5 mg/kg i.v. did not alleviate the severity of the abstinence syndrome in a monkey upon withdrawal of morphine.

Unlike morphine and M6G, M3G is relatively poorly bound to opioid receptors. Affinity for these receptors in bovine or guinea pig brain preparations ranged from 31 to 4,000 times less than that determined for morphine (Chen *et al.*, 1991; Christensen & Jorgensen, 1987; LaBella *et al.*, 1979; Pasternak *et al.*, 1987). Furthermore, Kataoka *et al.* (1977) found that M3G inhibited nicotine-induced contractions of the guinea pig ileum, but at concentrations almost five hundred times the EC_{50} for morphine, implying that, at best, it is a weak agonist. Schulz & Goldstein (1972) found that M3G at concentrations up to 210 nmol/l had no effect on electrically induced contractions of longitudinal muscle from the small intestine of the guinea pig. Meanwhile, concentrations of morphine of approximately 100 nmol/l produced around 50% of the maximum response. In comparison to the observation for M6G (section 1.3.3.1.), Lambert *et al.* (1993) found that concentrations of M3G approximately three hundred-times those of morphine were required to produce 50% displacement of diprenorphine from the opioid receptors of SH-SY5Y human neuroblastoma cells, and that it did not inhibit the formation of cAMP.

In vivo, M3G did not elicit antinociception in mice and rats. Shimomura *et al.* (1971) found that an i.c.v dose of M3G 8.8 times greater than the ED_{50} of morphine and given by the same route produced no antinociception. Hewett *et al.* (1993) and Sullivan *et al.* (1989) found that direct application of 10 to 1000 nmole of M3G to the spinal cord of the rat (analogous to clinical i.t. injection) had no effect on noxious stimuli-evoked activity in neurones located in the dorsal horn of the rat.

Of greater interest is the finding that i.c.v. doses of M3G two orders of magnitude below the i.c.v. ED_{50} values for antinociception induced by morphine (with the tail flick and hot plate tests) produced hyperalgesia, while those an order of magnitude below produced muscular

excitation and convulsions (Gong *et al.*, 1991). LaBella *et al.* (1979) found that grooming, wet dog shakes, frozen stares, body jerks and seizures, excitatory symptoms associated with high doses of morphine given by the i.c.v. route, could be reproduced with doses of M3G approximately two orders of magnitude lower. Woolf (1981) observed hyperaesthesia in rats given i.t. injections of M3G (32.5 nmole) and morphine (526 nmole). Curiously, this effect appeared when morphine congeners containing a sterically unhindered, substituted or unsubstituted 6-hydroxy moiety were given, but did not appear to be stereoselective since (+)-morphine appeared equally effective (Jacquet *et al.*, 1977; Woolf, 1981). In another study with rats, the spontaneous agitation observed after an i.t. dose of M3G (6.5 nmole) was decreased significantly by an i.t. dose of morphine (52 nmole) which gave complete antinociception (hot-plate test) when injected alone (Yaksh *et al.*, 1986). What these authors appear to have missed, however, is that the lack of spontaneous activity when morphine was given alone then became apparent, and more importantly the antinociception by morphine significantly reduced, when M3G was given concurrently. In the same study, a 10 times higher dose of morphine alone, while maintaining full antinociception, also produced significant spontaneous agitation. When an opioid antagonist, naltrexone, was given concurrently the antinociception was significantly reduced but the agitation increased. Naloxone produced similar results (Woolf, 1981). Unfortunately the effect of either opioid antagonist on the agitation noted after M3G alone was not determined, although it might be predicted that the antagonist would have had no effect.

When an i.c.v. dose of M3G comparable to the molar ED_{50} for morphine antinociception was given to rats, in addition to the excitatory effects there was a significant increase in respiratory minute volume (Gong *et al.*, 1991). Neither effect was reversed by naloxone but in some cases was exaggerated. Likewise, Pellegrino *et al.* (1989b) observed a dose-dependent stimulation of ventilation in dogs during i.c.v. perfusion with M3G. It is probable that these effects are not mediated via an opioid receptor.

In addition to the earlier result from the study of Yaksh *et al.* (1986), more recent studies have also examined the influence of M3G on the antinociceptive effects of morphine and M6G. Smith *et al.* (1990) confirmed the result from the study of Yaksh *et al.* (1986) with similar relative doses of M3G and morphine. They also found a similar effect of 4.3 nmole of M3G on

the antinociception produced by 0.54 nmole of i.c.v. M6G. However, as pointed out by Hewett *et al.* (1993), the excitatory effects of M3G may complicate the measurement of antinociceptive activity. This may be one reason why Gong *et al.* (1992) found a significant attenuation of the antinociceptive effect of M6G (0.2 nmole, i.c.v.) by M3G (1.1 nmole, i.c.v.) over 30 min using the hot-plate but not with the tail-flick test. M3G had no attenuating effect when evaluated with the writhing test using ethacrynic acid as an irritant. In contrast, they found significant attenuation using all three tests when the same doses of M3G and M6G were given by the i.t. route. However, visual comparison of i.c.v. against i.t. injection for the tail-flick results shows that, for the former, any effect of M3G may have been prevented while operating in the maximum response region of the log dose-response curve for M6G. This does not appear to have been the case in the study by Suzuki *et al.* (1993), who found that M3G (11 nmole, i.t.) had no antinociceptive effect alone nor did it have any significant effect on the antinociceptive effect of morphine (3.5 nmole, i.t.) or M6G (0.11 nmole, i.t.) when the tail-flick and hot-plate tests were used. Hewett *et al.* (1993) have shown that M3G applied to the surface of the spinal cord had no significant effect on the inhibition by morphine, similarly applied, of convergent dorsal horn activity induced by electrical stimulation of the hind paw of the rat. If the opposing effect of M3G is genuine, the results of Hewett *et al.* (1993) and Suzuki *et al.* (1993) add weight to the proposition that the effects of M3G are mediated by central, non-spinal mechanisms.

It may be concluded that when morphine is given in analgesic doses, it alone probably does not contribute to the hyperalgesia and agitation that is observed in some patients. However, at high doses or in patients with renal failure when the clearance of M3G is markedly reduced, morphine and/or M3G may contribute to these undesirable effects. It is unlikely that they are mediated via a μ opioid receptor. Morley *et al.* (1992) noted that chronic administration of high doses of morphine often induced a "paradoxical pain" whereby with continued use the pain was not relieved or became worse with increasing doses of morphine. They described a case report of a patient with cancer whose low back and pelvic pain worsened and who became agitated while receiving high doses of i.t. morphine and oral heroin. High concentrations of M3G were measured in CSF but M6G was undetectable. This is unexpected since, based on

reports of their relative concentrations in plasma (section 1.6.1.), and assuming equivalent ratios in CSF, an estimated concentration of M6G which was about one sixth that of M3G would have been easily measurable. Furthermore, the authenticity of the chromatographic peak identified as M3G was not questioned. Goucke *et al.* (1994) compared the concentrations of M3G and M6G in the plasma and CSF of 11 patients and confirmed the similarity in the ratios of M3G/M6G. Given the evidence for separate isozymes catalyzing the formation of M3G and M6G in humans (Coughtrie *et al.* 1989), it could be, as Morley *et al.* (1992) proposed, that the isozyme responsible for the formation of M6G is lacking or its activity is markedly reduced in some humans. Substitution by Morley *et al.* (1992) of i.t. M6G (1 mg on alternate days) for the morphine gave full pain relief for 7 hr and no pelvic pain for 2 days. They suggested that M3G may be implicated in opposing the analgesic effect of morphine, contributing to the agitation, thus worsening the pain in response to increasing doses of morphine, although the suggestion has generated considerable controversy (Twycross, and other authors, 1993).

It has been speculated that M3G may elicit its excitatory effects via the NMDA receptor complex (Bartlett et al., 1993, 1994). These workers reported that a competitive inhibitor for the NMDA receptor, LY274614, antagonized the excitatory effects of M3G in a dose-dependent manner. However, from *in vitro* binding studies, Bartlett *et al.* 1994) concluded that the effects were probably not mediated through NMDA, AMPA or kainate sites, nor did M3G appear to prevent the reuptake of glutamate into presynaptic nerve terminals, all of which are involved in facilitating the transmission of excitatory aminoacids. Furthermore, M3G did not bind to $GABA_A$ or glycine binding sites; thought to be involved in the inhibition of neurotransmission by GABA and glycine. Of interest also is the possible role of M3G in the development of tolerance to morphine. Ouellet & Pollack (1993b) presented preliminary findings of a decreased antinociceptive response by rats to morphine following a 12 hr infusion of M3G. Lipkowski *et al.* (1994) found that daily administration of M3G for 3 days, at doses equal to or double those for morphine administered concurrently, significantly reduced the development of tolerance to morphine. Ekblom et al (1993) have shown with rats, however, that while a constant infusion of M3G over 3 days does reduce the antinociceptive effect of morphine to some extent, it does not offer a complete explanation for the development of tolerance to morphine.

There have not been any studies which have reported on the pharmacological effects of M3G when given directly to humans.

To conclude, more studies are necessary to clarify the role of M3G in the analgesic action of and development of tolerance to morphine in humans. From studies in animals the evidence suggests that M3G may modulate the antinociceptive activity of morphine, and M6G, and that it would appear to do so via a centrally-mediated non-opioid receptor(s). However, its mechanism of action remains unknown.

1.3.5 Pharmacology of other Metabolites

Hasselström & Säwe (1993) found detectable concentrations of normorphine (greater than 7 nmol/l) in some samples of plasma from only four of seven healthy subjects given an oral dose of 15.2 mg of morphine and in none of the subjects was it detectable after an i.v. dose of 3.8 mg; Svensson (1986) detected normorphine in the plasma and urine of patients with cancer while receiving high doses of morphine.

Using the same nociceptive method, and compared with the ED_{50} value determined previously for morphine in the same laboratory (Shimomura et al., 1971), Oguri et al. (1989) found that normorphine was about 40% as potent. NM6G was about 125 times as potent as normorphine while NM3G, similar to M3G, was devoid of antinociceptive activity but caused convulsions. Sullivan et al. (1989) found that i.t. normorphine was 40% more potent than i.t. morphine (Dickenson & Sullivan, 1986) in inhibiting electrical transmission of nociceptive neurones located in the dorsal horn of the rat. An isolated preparation of ileum from the guinea pig was used to show that the I_{50} (nmol/l) of normorphine was approximately 250% of that for morphine but that the values were comparable when the vas deferens from the mouse, a tissue more responsive to δ-agonists, was used (Corbett *et al.* 1993).

Lasagna & De Kornfeld (1958) compared s.c. morphine against s.c. normorphine for the relief of post-operative pain in patients and found that 7.5 mg of morphine was comparable to 30 mg of normorphine. Glare *et al.* (1990) measured normorphine in the plasma of two patients, given morphine while in renal failure, and found concentrations of 31 nmol/l and 70 nmol/l. The authors questioned whether the accumulation of normorphine during the renal

failure may have contributed to the symptoms of myoclonus that were observed, and they likened the symptoms to those seen in patients in whom norpethidine has accumulated, causing neurotoxicity (delirium, myoclonus, seizures). However, high concentrations of M3G and M6G measured in the plasma of these patients may also have contributed to these side-effects.

The I_{50} for the displacement of naloxone by M3S from opioid receptors of membranes prepared from the brain of the rat was an order of magnitude higher and lower than the value for morphine and M3G, respectively (LaBella et al., 1979). Chen *et al.* (1991) found that the K_i was an order of magnitude higher than morphine but comparable to that for M3G. Following s.c. injections, the antinociceptive activity of M3S was comparable to that for M3G (Mori et al., 1972) while from the experiment by Knowlton & Gross (1943) with the dog, M3S appears to be about one sixtieth as potent as morphine. However, after i.c.v. injection, the ED_{50} of M3S was 30% and 3.6% of the values for morphine and M3G, respectively (Brown et al., 1985). As pointed out by these authors, the significance of these findings is unclear but the formation of conjugates of endogenous and exogenous compounds as sulphates is an important metabolic pathway for some compounds in the brain and, as noted in section 1.4. endogenous morphine has been isolated from the brain of the rat as a sulphate. The possibility cannot be excluded that the formation of M3S and M6S, the latter approximately 8 times more potent than the former after i.c.v. injection (Brown *et al.*, 1985) but probably also a less important metabolite, may be important contributors to the pharmacology of morphine if they are formed within the brain.

Compared to morphine, M3S was approximately one quarter to one fifth as effective in humans in modifying the abstinence syndrome upon withdrawal of morphine (Himmelsbach & Andrews, 1943). Whether the effect was due to M3S or morphine formed from the partial hydrolysis of M3S, is unknown.

Results from studies of the antinociceptive activity in cats of other identified metabolites of morphine (section 1.5.3.1) have been reported by Small & Eddy (1938). Dihydromorphinone and α- and ß-dihydromorphine were found to be three to four times more potent while the isomorphines were about one-tenth the potency of morphine.

1.4 Determination of Morphine and Metabolites in Biological Fluids

A more extensive review of the metabolism of morphine is to be found in section 1.5.3., but the brief outline of the metabolic pathways for morphine given in section 1.2.1. is sufficient for a discussion of the methods that have been used for the determination of morphine and its metabolites in biological fluids.

In the absence of morphine administration, the concentrations of morphine found in the blood of rats (Donnerer *et al.*, 1986) and CSF of humans (Cardinale *et al.*, 1987) are in the picomolar range. They are unlikely to contribute markedly to the concentrations in biological fluids measured during pharmacokinetic studies when recommended doses of morphine are given. Preliminary evidence suggests that endogenous morphine exists as a conjugate in CSF, predominantly the sulphate (Cardinale *et al.*, 1987), while Donnerer *et al.* (1987) demonstrated its presence as the sulphate, more likely M3S, in spinal cord from the rat. The conjugate is probably formed via a reaction catalysed by sulphotransferase, for which activity has been found in human brain for a number of other compounds (Renskers *et al.*, 1980).

Early methods for the determination of morphine involved the formation of coloured complexes with chemical derivatives of morphine (Woods *et al.*, 1954). Conjugated morphine was determined as the difference between the amount of total morphine following hydrolysis, and the unconjugated molecule. The method lacked sensitivity but was used for studies in animals given relatively large doses of morphine such that concentrations greater than the suggested limit of sensitivity of about 5 μmol/l were measurable. The determination of the conjugates by difference introduces cumulative errors and does not distinguish M3G from M6G, nor either of these from other conjugates. Reliable determination of 0.35 nmole of morphine was achieved by oxidation of morphine, following extraction from biological fluids, to a fluorescent derivative, pseudomorphine (Kupferberg *et al.*, 1964), while a further ten-fold increase in sensitivity was claimed with the improvements of Takemori (1968).

Many workers have achieved considerable improvements in sensitivity by utilizing radiolabelled morphine and a modification of the original solvent extraction method of Yeh & Woods (1970). Unconjugated morphine and total morphine after hydrolysis may be measured, the difference allowing an estimation of the concentration of conjugated morphine. Both codeine

and normorphine are coextracted with morphine. Proper usage requires an assurance of quantitative extraction of morphine and hydrolysis of the conjugates, and of the stability of morphine base if hydrolysis is performed in the presence of acid and heat. Alternatively, ß-glucuronidase may be used to hydrolyse the conjugates. Garrett & Türkan (1978) found no significant differences among methods based on liquid scintillation after solvent extraction, GLC (*vide infra*), and radioisotopic labelling followed by solvent extraction and TLC, for the determination of morphine in plasma and urine from a dog given morphine.

The methods described below, in general, offer improvements in sensitivity and/or selectivity (although, as noted, there are exceptions to the latter) and in many cases are more convenient.

1.4.1 Radioimmunoassay

Intense interest in the search for highly sensitive methods for the determination of morphine in biological fluids led to the development of the first RIA technique by workers from Spector's laboratory (Spector & Parker, 1970; Spector, 1971). The antiserum used however, appeared to lack specificity, having been generated from the 3-carboxymethyl derivative of morphine coupled as hapten to bovine serum albumin (BSA). Catlin (1977), when generating antisera from rabbits against a 2-azidomorphine-BSA conjugate, found that the specificity of the antibody differed between the rabbits in which it was generated and over the period of immunization. It may be concluded from this study that the measurable concentrations of "morphine" observed for up to 48 hr after a s.c. dose of morphine given to rats (Berkowitz *et al.*, 1974; Spector & Vesell, 1971) was probably due to cross reactivity with M3G.

Quinn *et al.* (1988) developed a RIA method with a preliminary solid phase extraction step. They compared the method with one developed by Moore *et al.* (1984), which lacked the extraction step, by measuring the concentrations of morphine in plasma after a 30 mg oral dose. Their method could not detect morphine in plasma 24 hr after the dose, in contrast to the one of Moore *et al.* (1984) which measured a concentration of "morphine" of 70 nmol/l. These workers also found that their RIA method generated an AUC for morphine which was not significantly different from that obtained using a HPLC method developed by Aitkenhead *et al.*

(1984). In comparison, the mean AUC generated using the RIA method of Moore *et al.* (1984) was a factor of ten greater.

The problems occurring with RIA methods lacking specificity are well illustrated by the study of Ball *et al.* (1985) using the method of Moore *et al.* (1984). Ball *et al.* (1985), during administration of a constant infusion of morphine to patients with diverse renal function and in intensive-care, concluded from the concentrations measured in plasma that the systemic clearance of morphine was dependent on renal function. Antiserum used by Moore *et al.* (1984) was reported to have a cross-reactivity with M3G of 1.1%. When measuring morphine and M3G by HPLC, Hasselström & Säwe (1993) and Osborne *et al.* (1990) found that the AUC of M3G was approximately 10 times that for morphine after i.v. morphine was given to healthy subjects. In patients with renal failure, the ratio of the concentrations measured by HPLC of M3G to morphine at 9 hr was about 400 (Osborne *et al.*, 1993) and a cross-reactivity of 1.1% could invalidate the conclusion of Ball *et al.* (1985). Aherne & Littleton (1985) found considerable differences in the cross-reactivity of M3G and M6G to antisera raised against conjugates of 6-succinylmorphine-BSA in the goat and to N-succinylnormorphine-BSA in the sheep. M6G was more effective than morphine in displacing radioligand from antiserum raised in the goat.

Notwithstanding the problems of potential lack of specificity, the sensitivity of RIA for morphine is comparable to chromatographic methods. With careful selection of immunogen structure (Findlay *et al.*, 1981) and/or prior extraction of plasma (or serum) to separate morphine from its metabolites (Grabinski *et al.*, 1983; Lee *et al.*, 1991; Quinn *et al.*, 1988; Sandouk *et al.*, 1984), and proven specificity over a range of concentrations for morphine and potential competitors (including extractable normorphine) for the displacement of radiolabelled ligand, concentrations of morphine comparable with those measured by chromatographic techniques may be achieved with M3G:morphine ratios in plasma of up to 500 (Lee *et al.*, 1991). Therefore, with these provisos, RIA may be used for the determination of morphine in plasma from healthy subjects and patients with normal renal function. However, for the determination of morphine in plasma obtained from patients with renal failure, no direct

comparisons have been made between RIA methods using extraction and specific HPLC methods.

Hand *et al.* (1987) described a radioimmunoassay for the determination of morphine, M3G and M6G in plasma. Calculation of the concentration for each compound relied on the differential binding of the three compounds to three antisera. A method based on a similar technique but with two antisera was described by Chapman *et al.* (1994) for the determination of morphine and M6G. The methods would appear to suffer from additive errors and also assume a consistent cross-reactivity of the compounds with each antiserum, independent of their concentration.

1.4.2 Gas-Liquid Chromatography

Methods based on GLC have been developed which use prior solvent or solid-phase extraction at pH 8.9 to 9.0 and detection by flame-ionization (Wilkinson & Way, 1969), electron-capture (Dahlström & Paalzow, 1975; Dahlström *et al.*, 1977; Edlund, 1981; Garrett & Gürkan, 1978; Lora-Tamayo *et al.*, 1987) or mass spectrometry (Cole *et al.*, 1977; Schuberth & Schuberth, 1989). While some of the methods are highly sensitive, they entail time-consuming extraction and derivatization of morphine (and other unconjugated metabolites). Furthermore, the concentrations of the conjugates of morphine as glucuronides in biological fluids can only be quantified as total glucuronides, after hydrolysis.

1.4.3 High-Performance Liquid Chromatography

The disadvantages encountered with the above methods has led to the development in many laboratories of HPLC methods for morphine and, with the increasing interest in the disposition of M3G and M6G, for methods which isolate and quantify all three compounds simultaneously. For morphine alone, sample preparation generally involved separation from biological fluids by extraction at pH 8.9 to 9.0 with a relatively polar solvent such as chloroform (Turner & Murphy, 1988; Vandenburghe *et al.*, 1982), or combinations of 5 to 15% propan-1-ol, butan-1-ol or butan-2-ol in either chloroform (Owen & Sitar, 1983; Schneider & Ravenscroft, 1989; Tagliaro *et al.*, 1988; Todd *et al.*, 1982; Wallace *et al.*, 1980), benzene

(Derendorf & Kaltenbach, 1986), or ethyl acetate (White, 1979). Some of these methods also entailed back-extraction into an aqueous phase followed by re-extraction into an organic solvent. Alternatively, solid-phase extraction cartridges or columns containing underivatized (Gourlay *et al.*, 1985; Zoer *et al.*, 1986) or hydrocarbon-bonded (C_2, C_8 or C_{18}) silica (Abbott *et al.*, 1987; Barrett *et al.*, 1991) particles have been used. The latter material has also been used to separate morphine, M3G and M6G simultaneously from biological fluids. Typically, all three compounds were adsorbed onto the reversed-phase material at pH 7.5 (Venn & Michalkiewicz, 1990) or pH 9.0 to 9.4 (Glare *et al.*, 1991; Hartley *et al.*, 1993; Joel *et al.*, 1988; Konishi & Hashimoto, 1990; Koopman-Kimenai *et al.*, 1994; Mason *et al.*, 1991; Moore *et al.*, 1984; Murphey & Olsen, 1993; Svensson *et al.*, 1982; Svensson, 1986), impurities removed by washing, and the three compounds subsequently eluted with an acidified mixture of either acetonitrile/water (Glare *et al.*, 1991; Joel *et al.*, 1988; Konishi & Hashimoto, 1990; Koopman-Kimenai *et al.*, 1994; Svensson *et al.*, 1982; Svensson, 1986) or methanol/dichloromethane (Murphey & Olsen, 1993), or ammoniacal methanol (Wielbo *et al.*, 1993), or by elution with methanol alone (Hartley *et al.*, 1993; Mason *et al.*, 1991; Moore *et al.*, 1984; Wright *et al.*, 1994). Most methods, irrespective of whether solvent or solid-phase extraction of morphine (or M3G and M6G) was used, reported recoveries from biological fluids of more than 80%.

Further separation of morphine alone from remaining interferences has been achieved using normal phase (Barrett *et al.*, 1991; Gourlay et al., 1985; White, 1979; Zoer *et al.*, 1986), reversed-phase (Owen & Sitar, 1983; Tagliaro *et al.*, 1988; Todd *et al.*, 1982; Wallace et al., 1980) or paired-ion reversed-phase chromatography (Aitkenhead *et al.*, 1984; Derendorf & Kaltenbach, 1986; Moore *et al.*, 1984; Turner & Murphy, 1988; Vandenburghe *et al.*, 1982). The simultaneous separation of morphine, M3G and M6G has usually employed paired-ion reversed-phase chromatography with sodium dodecyl sulphate (Glare *et al.*, 1991; Hartley *et al.*, 1993; Joel *et al.*, 1988; Konishi & Hashimoto, 1990; Mason *et al.*, 1991; Murphey & Olsen, 1993; Svensson *et al.*, 1982; Svensson, 1986) or heptane sulphonic acid (Koopman-Kimenai *et al.*, 1994) in a mobile phase adjusted to pH 2.1, or cetyltrimethylammonium bromide at a pH of 7.5 (Wright *et al.*, 1994). Under the conditions at pH 2.1, the capacity factors of M3G and M6G relative to morphine were approximately 0.3 and 0.5, respectively,

while Wright *et al.* (1994) found relative capacity factors of about 0.6 and 1.4, respectively. Gradient elution with trifluoroacetic acid as the ion-pairing agent was used in a method developed by Venn & Michalkiewicz (1990). Normal-phase chromatography with a diol-bonded column generated longer retention times for M3G and M6G compared to morphine and normorphine (Wielbo *et al.*, 1993), improving the separation of the glucuronides from interfering compounds eluting near the solvent front of a reversed-phase system.

Given the almost quantitative recovery from biological fluids during extraction, the limiting factor for HPLC has been the sensitivity of the detection methods for monitoring the eluent. Concentrations of morphine in the range of 7 to 35 nmol/l of plasma are reliably quantifiable with amperometric (Aitkenhead *et al.*, 1984; Gourlay *et al.*, 1985; Owen & Sitar, 1983; Tagliaro *et al.*, 1988; Todd *et al.*, 1982; Wallace *et al.*, 1980; White, 1979; Zoer *et al.*, 1986), and fluorescent (Barrett *et al.*, 1991; Schneider & Ravenscroft, 1989) detection methods. However, Osborne *et al.*, (1990) found that the concentrations of morphine in plasma of normal subjects had decreased to 7 nmol/l, 8 hr after an i.v. dose of 3.8 mg of morphine. Reliable measurement of lower concentrations is desirable because, as noted in section 1.5.4.1., there is evidence of a further dispositional phase from plasma beyond this time. The sensitivity of chemiluminescence as an alternative method of detection is not sufficiently developed (Abbott *et al.*, 1987).

The greatest sensitivity and selectivity has been achieved with coulometric methods for the detection of morphine only (Derendorf & Kaltenbach, 1986) or morphine and M6G (Joel *et al.*, 1988; Konishi & Hashimoto, 1990; Koopman-Kimenai *et al.*, 1994; Mason et al., 1991; Svensson, 1986). However, for the detection of M3G, a fluorimetric (Joel *et al.*, 1988) or UV absorbance detector (Konishi & Hashimoto, 1990; Koopman-Kimenai *et al.*, 1994; Svensson, 1986) connected in series is also necessary. Alternatively, fluorescence (Glare *et al.*, 1991; Hartley *et al.*, 1993; Venn & Michalkiewicz,1990) or UV absorption at 214 nm (Murphey & Olsen, 1993) or 230 nm (Wielbo *et al.*, 1993) has been used for the detection of all three compounds. Many of these reports have claimed limits of detection, on the basis of signal to noise ratios of 2 or 3, but lack specific details of accuracy and reproducibility at this level. The data of Konishi & Hashimoto (1990) for morphine, M3G and M6G is superior to that of other

authors, showing acceptable values of accuracy and reproducibility for concentrations in plasma of 7 nmol/l, 92 nmol/l and 8.8 nmol/l, respectively. However, this method requires the use of an additional pump, a column switching valve and both coulometric and UV detection.

1.4.4 Other Methods

The radioreceptor assay developed by Levi *et al.* (1987) suffers from similar problems of specificity as those shown by many RIA methods. During i.v. infusion of morphine to one patient, visual inspection suggests that the plasma concentrations of morphine determined by radioreceptor assay were up to 50% more than those determined by these authors using an HPLC method. As noted previously (section 1.3.3.1), M6G has receptor affinity which is a factor of 0.5 to 13 less than that for morphine. Given that the concentrations of M6G in plasma following a single i.v. injection of morphine to humans with normal renal function are comparable to those of morphine (Hasselström & Säwe, 1993; Osborne *et al.*, 1990), it might be expected that morphine concentrations measured in plasma by the radioreceptor assay would be greater than those measured by the more specific method of HPLC.

Methods using capillary electrophoresis have been developed for the measurement of morphine in samples of hair (Tagliaro *et al.*, 1993) and of M3G in urine (Wernly *et al.*, 1993), mainly for forensic purposes. Their sensitivity and reliability do not appear to be sufficiently advanced for the routine analysis of morphine, M3G and M6G in plasma and urine during disposition studies.

1.5 Pharmacokinetics of Morphine

When reviewing the pharmacokinetics of morphine, M3G and M6G in animals and humans, only those studies which used methods specific for morphine, M3G and M6G (the latter two either individually or combined) have been included. Therefore, the numerous studies which have used an RIA method, but without satisfying the criteria of section 1.4.1, have not been included. In some instances, studies using less specific methods for the measurement of morphine have been included, but only after assuming that minimal interference from

metabolites is likely to have occurred; for example, in biological fluids at times when the predicted concentrations of M3G and M6G would have been relatively low.

1.5.1 Absorption after Oral Administration

Administration of morphine by the oral route has become increasingly popular over the last 15 to 20 years because of its greater convenience during chronic dosing. While the solution is the most common dose form, the use of controlled-release solid dose forms is becoming more frequent.

Numerous studies comparing oral with i.v. morphine have found that the mean availability of oral morphine was low, with considerable intersubject variability. Mean (± S.D.) values of 0.20 ± 0.08 (Osborne *et al.*, 1990), 0.22 ± 0.06 (Westerling *et al.*, 1993) and 0.36 ± 0.07 (Baillie *et al.*, 1989) in healthy subjects, and of 0.26 ± 0.13 (Gourlay *et al.*, 1986), 0.38 ± 0.17 (Säwe *et al.*, 1981) and 0.47 ± 0.14 (Säwe *et al.*, 1985) in patients with cancer, were obtained after giving morphine in solution. Hasselström & Säwe (1993), using concentrations measured in plasma up to 24 hr and data for the rate of urinary excretion up to 72 hr, calculated a rate constant for elimination from a third dispositional phase. They determined a mean (± S.D.) availability of 0.29 ± 0.07, with individual values ranging from 0.20 to 0.41. Furthermore, they noted that, if extrapolation for the AUC had been performed assuming only biphasic elimination, the calculated availability would have been 0.19. The two values from the studies of Säwe *et al.* (1981, 1985) may not be reliable estimates; Säwe *et al.* (1981) used data from plasma collected, in some instances, for only 1.5 hr, 2 hr or 3 hr after i.v. administration, while Säwe *et al.* (1985) may have overestimated the AUC after oral administration. The rate constant of elimination that was used to calculate the extrapolated AUC may have underestimated the true value because of continued absorption. Additionally, if enterohepatic cycling of morphine were to occur (section 1.5.4.1), the expected enhanced availability would not have been detected with such short collection times. When given orally in solution, the peak concentration of morphine in plasma was reached at a median time of 0.65 hr (Gourlay *et al.*, 1986) and 0.5 hr (Poulain *et al.*, 1988), mean (± S.D.) of 0.7± 0.2 hr (Baillie *et al.*, 1989) or 1.1 ± 1.1 hr (Hasselström *et al.* (1991), or between 0.3 and 1 hr (Westerling *et al.*, 1993).

Gourlay *et al.* (1989) found that neither this time nor the peak concentration was influenced by concurrent administration with a high-fat meal. However, these workers did observe an increased AUC after the meal due mainly to a consistently greater fractional AUC between 4 and 10 hr after the dose. Bile flow may have been stimulated by the high fat meal and, via an enterohepatic cycle, hastened the return of morphine to the systemic circulation. This is probably of no clinical importance since there was no difference between the phases in the requirement of the patients for additional methadone. Conventional tablets (Säwe *et al.*, 1983a) and controlled-release oral formulations (Hasselström *et al.*, 1991) were found to have similar extents of availability to an oral solution. The latter study made comparisons after single doses and after 4 days of regular doses in patients with cancer.

While the systemic availability of morphine is low, the comparable recovery, after systemic and oral administration, of radioactivity in the urine of the rat administered radiolabelled morphine (Iwamoto & Klaassen, 1977; Walsh & Levine, 1975), and the comparable and high recovery after both routes of administration of the sum of morphine, M3G and M6G in the urine of humans (Hasselström & Säwe, 1993), would suggest that it is well absorbed from the gut. The low availability is probably due to presystemic metabolism in the gut mucosa and/or the liver.

Studies in animals have determined an oral availability for morphine in the rat of 0.21 ± 0.02 (Iwamoto & Klaassen, 1977; Tan *et al.*, 1990b) and in the dog of 0.12 ± 0.004 (Tan *et al.*, 1990a) when the concentrations in plasma were measured for up to 6 hr. With loop segments *in situ* of the gastrointestinal tract of the rat, the most rapid absorption (greater than 80% in 1 hr) was from the duodenum and jejunum while approximately 70% and 40% from the ileum and rectum, respectively, but less than 10% from the stomach was absorbed in 1 hr (Tan *et al.*, 1989).

In summary, while absorption of morphine in humans is almost complete following oral administration, a large fraction of the absorbed dose is removed before it reaches the systemic circulation. In addition, a more realistic estimate of oral availability would be obtained if sampling of plasma was continued for a time sufficient to consider the possible return to the

systemic circulation of some of that large fraction removed by the liver following absorption. This will be discussed further in section 1.5.4.1.

1.5.2 Distribution

Distribution volumes with respect to plasma have been determined in animals and humans. Mean volumes (V_z) of 10.6 l/kg in rats (Iwamoto & Klaassen, 1977), 3.4 to 5.1 l/kg in dogs (Garrett & Jackson, 1979; Merrell *et al.*, 1990) and from 2.1 to 4.0 l/kg in humans (Hasselström et al., 1990; Osborne *et al.*, 1990; Patwardhan et al., 1981; Säwe *et al.*, 1981; Säwe *et al.*, 1985; Westerling *et al.*, 1993) have been estimated. Säwe *et al.* (1981) estimated the mean V_{ss} to be 15% lower than V_z in humans. For some of these reports, the volumes were calculated from morphine concentrations measured in plasma collected for up to 12 hr, while for others the plasma was collected for a far shorter period. However, when concentrations of morphine in plasma were measured for 24 hr after a dose given to the rat, a V_{ss} of 26.9 ± 2.8 l/kg was determined (Bhargava *et al.*, 1991). Using an AUC calculated with combined data from concentrations measured in plasma up to 24 hr and urine to 72 hr, and a second rate constant (λ_2) reflecting disposition in plasma up to approximately 8 hr, a volume of 2.9 l/kg was estimated by Hasselström & Säwe (1993) in healthy humans. However, using the rate constant from a third, slowly declining phase of elimination, determined by the authors from the urinary excretion data to have a half-life of 15.1 hr, a V_z of 24.3 l/kg may be calculated. The smaller estimate would suggest that about 94% of the total drug in the body is outside the extracellular fluid during the second dispositional phase. From the larger estimate, greater than 99% of the drug would appear to be outside the extracellular volume during the third phase and occupying a space in which it is either tightly held or has limited access to the systemic circulation, and therefore to eliminating organs. An example of the latter would be involvement in an enterohepatic cycle. A recent report (Ouellet & Pollack, 1993a), comparing bile duct-cannulated to normal rats given morphine, showed that the V_{ss} and mean residence time of morphine were decreased by 62% and 58%, respectively, and that the mean residence time of M3G was reduced by 86%, in adding support to the proposition that the larger volume for

morphine (Bhargava *et al.*, 1991; Hasselström & Säwe, 1993) was due to enterohepatic cycling involving M3G.

1.5.2.1 Binding in Plasma

The binding of morphine in plasma has been determined in humans and animals. The fraction unbound in plasma was found to be: 0.85 (Mistry & Houston, 1987) and 0.86 (Baggot & Davis, 1973) in the rat; 0.79 (Kreek *et al.*, 1978) and 0.84 (Baggot & Davis, 1973) in the rabbit; 0.72 (Blaney & Woods, 1956), 0.65 (Garrett & Jackson, 1979), 0.86 (Hug *et al.*, 1981) and 0.88 (Baggot & Davis, 1973) in the dog; greater than 0.95 in the pregnant ewe (Szeto *et al.*, 1982); 0.70 (Rane *et al.*, 1984), 0.72 (Lynn *et al.*, 1991) and 0.81 (Baggot & Davis, 1973) in the monkey; and 0.65 (Leow *et al.*, 1993; Olsen, 1975; Olsen *et al.*, 1975), 0.69 (Pöyhiä & Seppälä, 1994), 0.76 (Judis, 1977), 0.80 (Patwardhan *et al.*, 1981) and 0.88 (Baggot & Davis, 1973) in healthy humans. Binding appeared to increase with age in the monkey (Lynn *et al.*, 1991). The binding was independent of concentration over an approximately one thousand-fold range of concentrations (Olsen, 1975; Garrett & Jackson, 1979), although Judis (1977) found a decrease from 24% bound to 20% bound when the concentration of morphine was increased from the usual therapeutic concentrations by approximately sixty-fold. Most of the binding in human plasma was to albumin (Judis, 1977; Leow *et al.*, 1993; Olsen, 1975) while gammaglobulin (Judis, 1977; Olsen, 1975) and α_1-acid glycoprotein (Leow *et al.*, 1993) contributed approximately 5% each. Binding was dependent on the concentration of albumin (Olsen, 1975), which may account for the marginal increase in the unbound fraction to between 0.70 and 0.75 in uraemic patients with hypoalbuminaemia (Olsen *et al.*, 1975). These authors observed a 10% decrease in binding in two patients with liver failure while Patwardhan *et al.* (1981) observed only a mean decrease of 3% in patients with cirrhosis. Leow *et al.* (1993) found that the binding decreased by 3 to 4% when the pH was adjusted from the range 7.75 to 8.85 (unadjusted plasma) down to pH 7.4, while, as expected, it increased by 7 to 10% when incubation and ultrafiltration was performed at 23°C rather than 37°C. These latter observations may account for some of the variability in binding within and between species in the studies reported above. Nevertheless, morphine is poorly bound in plasma. It might be expected that

binding would be decreased marginally in disease-states with associated hypoalbuminaemia. This may be compensated for during the acute phase of a disease when the concentration of a_1-acid glycoprotein in plasma is increased, or in disorders associated with an increase in gammaglobulin. This was borne out by the findings of Raveedran *et al.* (1992). The binding of morphine in plasma collected from patients in the active phase of cancer, with increased a_1-acid glycoprotein but hypoalbuminaemia, and from patients in remission, with normal albumin concentrations and slightly raised a_1-acid glycoprotein, was not different from that in normal subjects.

1.5.2.2 Distribution into Red Blood-Cells

The value of C_b/C for morphine was found to be: 1.34 (Mistry & Houston, 1987) in the rat, 1.3 (Rane *et al.*, 1984) in the monkey, and 1.21 (Mazoit *et al.*, 1987) and 1.1 (Patwardhan *et al.*, 1981) in healthy humans. The ratio in humans was consistent over the concentration range of 35 to 140 nmol/l of blood (Mazoit *et al.*, 1987). In contrast to the values of C_b/C greater than unity in the above species, a value of 0.91 may be calculated in the dog from the value of 1.11 for the ratio of the concentrations in red blood cells to those in plasma-water (Garrett and Jackson, 1979); a ratio that was consistent over a one thousand-fold range of concentrations in whole blood. The haematocrit in this latter study was similar to that reported by Mazoit *et al.* (1987) and, given the comparable binding of morphine in the plasma of both species, suggests a difference in binding to red blood cells.

1.5.2.3 Distribution into the Central Nervous System

From studies investigating the antinociceptive effects of morphine given via different routes to animals, it is evident that the doses required to elicit antinociception are far less when given i.c.v. than when given systemically (Abbott & Palmour, 1988; Paul *et al.*, 1989; Frances *et al.*, 1992). While some of the difference may be accounted for by metabolism and distribution to tissues other than the central nervous system, where it is assumed that the majority of the opioid receptors are located, a contributing factor could be the relatively hydrophilic property of the molecule, precluding rapid and extensive transfer across the cerebrovascular endothelial cells

of the blood-brain barrier (Oldendorf, 1974). Herz & Teschemacher (1971) demonstrated clearly that as lipophilicity decreased within a series of non-peptide opioids, the ratio of the effective i.v. dose to the i.c.v. dose increased. In addition, the speed of onset of antinociception decreased but the duration of effect increased. As noted in section 1.3.3.1., these effects would be magnified with systemic, compared to i.c.v., administration of the more polar M6G and M3G.

Despite the lack of specificity in the RIA used, the results of Finck *et al.* (1977) following an i.v. dose of morphine given to the dog clearly show a lesser rate of decrease in the concentrations of morphine in brain compared to plasma. The concentrations of M3G and M6G in brain tissue would have been relatively low compared to those for morphine, and also compared to the concentrations in plasma. A more specific assay might have demonstrated an even greater difference between the two tissues. Hug *et al.* (1981a) also observed a greater half-life for the disappearance of morphine from CSF (121 min) compared to plasma (74 min). When Pellegrino *et al.* (1989a) gave an i.v. loading dose followed by a constant infusion of radiolabelled morphine to dogs for 4 hr, the concentrations of morphine measured by HPLC in CSF were approximately 35% of those measured in plasma and remained almost constant between 0.5 hr and 4 hr. It is noteworthy that, while the mean concentrations of total radioactivity in plasma remained relatively constant over the same period, those in CSF at 4 hr had increased by an approximate factor of 2, suggesting continued accumulation, presumably from plasma, of more polar metabolites (section 1.5.3.1).

During i.v. infusion of morphine to five young adults for between 36 and 432 hr, the ratios of the concentrations of morphine in lumbar CSF to those in plasma ranged from 0.52 to 1.00 and there appeared to be no obvious trend with the duration of the infusion (Greene *et al.*, 1987). Sjogren *et al.* (1993), in a report of a patient being infused with i.v. morphine (100 mg/hr), found that the ratios of the concentrations of morphine, M3G and M6G measured in lumbar CSF to those in plasma were 0.53, 0.16 and 0.11, respectively. Unfortunately, the duration of the infusion was not given. Portenoy *et al.* (1991) measured the concentrations of morphine and M6G in the plasma and CSF of patients with cancer and receiving chronic morphine. The mean ratios of the concentrations of morphine and M6G in ventricular CSF to

plasma were 0.71 and 0.077, respectively, while the ratios for lumbar CSF to plasma were 0.75 and 0.12, respectively. However, as measures of relative distribution into CSF the values remain uncertain, since some patients were receiving chronic oral morphine or received supplementary parenteral doses, and the plasma and CSF samples could not be collected concurrently in some cases. Nevertheless these ratios are probably reasonable indicators of the values that might be expected under steady-state conditions. Goucke *et al.* (1994) reported median ratios, between CSF and serum collected 4 hr after a dose, of 1.23, 0.14 and 0.12 for morphine, M3G and M6G, respectively, in patients receiving chronic oral or s.c. morphine. The relatively low values for M3G and M6G from the above studies suggest that the rate constant of removal from, relative to the rate constant of uptake into, CSF is greater for M3G and M6G than for morphine.

While there appears to be facilitated and active mechanisms for the transport of polar endogenous compounds across the blood-brain barrier (Van Bree *et al.*, 1992), whether such mechanisms exist for morphine, M3G and M6G remain unknown. If the theoretical analysis of Carrupt *et al.* (1991) is borne out, then the diffusional permeability of M6G may be greater than that expected on the basis of the partition measured between octanol and buffer of pH 7.4 (Van Crugten *et al.*, 1991). Drugs in blood also have access to the extracellular fluid of the brain via the CSF and their appearance in CSF may be dependent on transport across the choroid plexus (Spector & Johanson, 1989; Van Bree *et al.*, 1992). *In vitro* measurement of the uptake of morphine and M3G into the choroid plexus isolated from the rabbit has shown that morphine is subject to saturable uptake to concentrations 8 times greater than those in the incubating medium (which contained relatively high concentrations ranging from 1 to 8 mmol/l) while the uptake of M3G was relatively low (Muraki, 1971). Takemori & Stenwick (1966) also observed accumulation of morphine within the choroid plexus but, because of limitations with the sensitivity of the assay, the tissue was incubated once again with very high concentrations of morphine (in the range of 0.2 to 8 mmol/l), and were well above the concentrations found in the plasma of dogs given relatively high doses of morphine (Garrett & Jackson, 1979) or in humans given therapeutic doses (Säwe *et al.*, 1983b). While these studies demonstrate the

existence of transport processes for morphine and M3G in the choroid plexus, the direction of net movement *in vivo* remains unknown.

Of interest is the finding of Baba *et al.* (1988) of an enhanced uptake of sodium fluorescein into the brain of mice predosed with morphine either via the s.c. or i.c.v. route. This, and similar effects with other μ agonists, was partially but significantly antagonized by naloxone. For a relatively polar solute such as fluorescein, with a P between octanol and an aqueous medium of 0.62 (Van Bree *et al.*, 1988), permeability through the blood-brain barrier may be more dependent on access through pores between the endothelial cells (Van Bree *et al.*, 1988). A similar limitation may apply to morphine (with a P between 1.0 and 1.42, section 1.2.2.1.), but even more so for M3G and M6G. Whether morphine has any effect on the uptake of its conjugated metabolites remains unknown. Baba *et al.* (1988) proposed that the influence of morphine on the permeability of the blood-brain barrier may be mediated by neurotransmitters.

1.5.2.4 Distribution to Other Tissues

Woods (1954) examined the distribution of morphine and its conjugated metabolites into a range of tissues at selected times after i.v. and s.c. administration of relatively high doses of morphine to the rat (150 mg/kg) and dog (30 mg/kg). Morphine was distinguished from the conjugates by paper chromatography, and morphine and combined morphine and conjugates (following hydrolysis) were quantified by colorimetry (Woods *et al.*, 1954). In rats, the highest concentrations of the sum of morphine and hydrolyzed conjugates in tissue were found in the kidney. At 1.5 hr, the ratio of the concentrations of conjugated to unconjugated morphine was almost one, but at 4 hr had increased to a value of almost four. Similar relative changes, but at lower concentrations, occurred in the liver (Woods, 1954). In a later study with rats, Hahn *et al.* (1976) found that, after 0.5 hr, the uptake of total radioactivity (per mass of tissue) by the kidney was approximately 160% and 300% of that by the liver and lung, respectively. Woods (1954) also found relatively high concentrations of unconjugated morphine in the kidney, gall bladder bile and spleen of the dog at 1.5 hr, which had decreased rapidly by 4 hr and were not measurable at 12 hr. The method of assay used was sensitive only down to 5 μmol/l. In

contrast, very high concentrations of conjugates in the bile and relatively high concentrations in the liver and kidney were found at 1.5 hr. While the concentrations in the liver and kidney had decreased by about half at 4 hr, those in bile continued to increase up to 12 hr. Approximately 80% of the dose could be recovered in urine, and bile from the gall bladder, at 12 hr. At 24 hr and 48 hr, about 25% and 15%, respectively, of the dose remained in bile as total morphine, most of which was conjugated morphine. The distribution of unconjugated and conjugated morphine in the bile, kidney and liver of the monkey (Mellett & Woods, 1956) was similar to that found for the dog. These studies, while involving very high doses of morphine, do suggest that morphine is subject to considerable enterohepatic cycling and show that at least 15% of the dose was still involved in the cycle 48 hr after morphine was administered.

Kreek et al. (1978) found that 27% of a bolus dose of morphine was taken up within 2 min by the isolated perfused liver of the rabbit but, by this stage, less than 0.2% had appeared in the bile. Morphine was taken up by kidney slices from the dog (Hug, 1967) and mouse (Teller *et al.*, 1976) such that the concentrations were of the order of 4 to 8 times, respectively, those in the incubating medium; ratios which were greater than those of approximately 2 after incubation of the slices in the absence of oxygen or after they had been boiled. Both studies concluded that uptake was mediated in part by carrier-mediated mechanisms, and Hug (1967) suggested that the mechanism shared similar characteristics with the choroid plexus.

Uptake of morphine into isolated hepatocytes from the guinea pig was found to be saturated at concentrations in the suspension medium above 200 µmol/l which, from further metabolic inhibition studies, was suggestive of active transport (Iwamoto *et al.*, 1978). Passive diffusion became increasingly important at higher concentrations. In a similar study, Déchelotte *et al.* (1993) observed a profile of uptake at 100 µmol/l morphine which was comparable to that seen by Iwamoto *et al.* (1978) at 200 µmol/l, but also found that at 1 µmol/l and 10 µmol/l morphine the rapid uptake for up to 5 min was followed by a decrease in cellular concentrations of morphine, probably reflecting metabolism to M3G and M6G. The observations at the two lower concentrations of morphine, which are closer to those found in plasma and therefore liver water during most *in vivo* animal and human experiments, probably reflect more closely the situation *in vivo*.

Administration of morphine into the pulmonary artery of patients revealed rapid transient uptake during the initial passage through the lung (Boer *et al.*, 1992). Accumulation in the isolated perfused lung from the rabbit (Davis & Mehendale, 1979) appeared to be by diffusion down an electrochemical gradient producing a five-fold greater concentration in lung compared to perfusate. At the beginning of an i.v. infusion with morphine, Persson *et al.* (1986) observed a marked difference between the concentrations of morphine in blood from the pulmonary and radial arteries, indicating that morphine was taken up by the lungs.

1.5.3 Metabolism and Excretion

1.5.3.1 Metabolites and Excretory Profile

Numerous studies in humans have demonstrated that the majority of a parenteral or oral dose of morphine is excreted as metabolites in urine (Brunk & Delle, 1974; Hasselström & Säwe, 1993; Osborne *et al.*, 1990; Yeh, 1975). Following an i.v. dose of N-methyl-^{14}C-morphine to healthy subjects, 80 to 90% of the total radioactivity given was recovered in urine after 48 hr (Brunk & Delle, 1974; Brunk *et al.*, 1973) and, from inspection of their data, an additional 5 - 10% would have been recovered if urine had been collected beyond 48 hr. These workers also detected radioactivity in expired carbon dioxide, suggestive of metabolism to normorphine. Yeh *et al.* (1977), using TLC and GLC-MS, detected M3G, M6G, morphine-3,6-diglucuronide, M3S, normorphine, NM3G and NM6G in the urine from subjects given s.c. morphine, 240 mg per day, for 4 weeks. These authors estimated that the amount of M6G and M3S excreted was approximately 1% of that of M3G. They were unable to detect any morphine-2,3-quinone, 2-hydroxymorphine or dihydromorphinone. In an earlier report by Yeh (1973), the ratio of M3S to morphine glucuronide was estimated to be about 1:4. After 24 hr, approximately 8%, 44% and 11% of an i.v., and 2%, 42% and 9% of an oral, dose was recovered in the urine from healthy subjects as morphine, M3G and M6G, respectively (Osborne *et al.*, 1990). Hasselström & Säwe (1993) recovered 8%, 57% and 10%, respectively, over 72 hr after administering an i.v. dose to healthy humans. The percentage of a dose of morphine excreted as normorphine and its glucuronide metabolites was 1 to 6% (Yeh, 1975) while Hasselström & Säwe (1993) recovered 0.4% of an oral dose as unconjugated

normorphine. Codeine was identified as a metabolite in urine and constituted 0.7% to 0.9% of an oral or i.v. dose given to subjects and patients who had not recently taken codeine (Boerner & Abbott, 1973), but its formation could not be confirmed by Yeh (1974). Coughtrie *et al.* (1989) concluded that two UDPGT isozymes were responsible for the metabolism of morphine to M3G and M6G in humans. At saturating concentrations of morphine, the rates of formation of M6G by microsomal preparations from the liver and kidney were 18% and 5%, respectively, of those for M3G. Figure 1.1 summarizes the structures of the more important metabolites of morphine, and their recovery, including that of the parent, in the urine of humans given i.v. morphine.

While some of the metabolites identified in humans have also been isolated in animals, there are species differences in the relative importance of these metabolites. Yeh *et al.* (1979) performed a comprehensive analysis of urine collected from guinea pigs, rabbits, rats, monkeys, cats and dogs given morphine in the short- or long-term. Separation was performed on a resin of polystyrene-divinylbenzene followed by TLC and GLC, with identification by GLC-MS using authentic standards for comparison. In the absence of standards, some metabolites were identified tentatively. In urine from the guinea pig these workers identified morphine, normorphine, morphine N-oxide, α- and ß-dihydromorphine, γ- and ß-isomorphine (tentatively) and monohydroxymorphine (tentatively identified as either 1-hydroxy-, 2-hydroxy- or 8-hydroxymorphine). C-3 glucuronides of all of these compounds, C-6 glucuronides of morphine and α-dihydromorphine and the 3,6-diglucuronide of morphine were also identified. From the urine of rabbits they identified M3G, M6G, free and conjugated normorphine, conjugated α- and ß-dihydromorphines, dihydromorphinone, morphine-3,6-diglucuronide and monohydroxymorphine (tentatively); from rats M3G, M6G, normorphine, dihydromorphinone, α-dihydromorphine, mono- and dihydroxymorphine (both tentatively); and from monkeys M3G, M6G, normorphine and dihydromorphinone. Monohydroxymorphine was tentatively identified as a metabolite in the urine of cats and is additional to M3S, the major metabolite, M3G and normorphine identified previously by Yeh *et al.* (1971). Yeh *et al.* (1979) did not identify any metabolites in the urine collected from the dog that were additional to those found by Misra *et al.* (1970). The earlier study identified M3G as the major metabolite, M6G (2 to 4%

of the dose) and M3S (1 to 1.5%). Fujimoto & Haarstad (1969) isolated M3S and M3G in apparently similar quantities from the urine of the chicken and cat. An unknown metabolite from the chicken, intermediate in polarity between M3S and morphine, was also apparent from TLC. Mori *et al.* (1972) recovered 27% of a dose of morphine given to the cat as M3S.

From urine collected for up to 24 hr after a s.c. dose of morphine to the rabbit, guinea pig and rat, Oguri *et al.* (1970) recovered 68%, 41.5% and 23%, respectively, as morphine and its conjugates. In the rat, nearly 85% of the urinary recovery was in the form of unchanged morphine. Walsh & Levine (1975), collecting urine and faeces from the rat up to 89 hr post-dose, recovered 65% of the dose of morphine in urine and 20% in faeces. Approximately 27% of the urinary recovery was unchanged morphine. From Wistar rats given i.p. morphine (25 mg/kg) for 8 days, Klutch (1974) recovered between 35% and 57.5% of the dose in the urine as free and conjugated morphine, with approximately 20% of the total as free morphine; 2.5 and 6.3% of the dose given on each day was recovered as normorphine. Rush *et al.* (1983) found that about 20% of an i.p. dose of morphine was recovered in the urine of male and female Fischer 344 rats as morphine and conjugates of morphine hydrolyzed by ß-glucuronidase. While the excretion of morphine glucuronide was comparable between sexes, females excreted undetectable amounts of morphine but more of a conjugate hydrolyzed by a crude preparation of ß-glucuronidase (reported to contain sulphatase also) which retained catalytic activity in the presence of glucaro-1,4-lactone. The Fischer 344 strain may be unique in eliminating greater amounts as the sulphate. In addition to the rabbit, guinea pig and rat, Oguri *et al.* (1970) also collected urine from humans and mice and, from visual inspection of the TLC results, noted that the ratio of M6G to M3G appeared similar in the urine of humans, rabbits and guinea pigs and greater than that found in rats and mice. *In vitro* studies with hepatic microsomes confirm that rats, in contrast to humans, have a markedly reduced capacity to form M6G relative to M3G (Coughtrie *et al.*, 1989; Kuo *et al.*, 1991). It is noteworthy that codeine-6-glucuronide (C6G) was a minor metabolite in the urine of rats given codeine while M6G was undetectable (Oguri *et al.*, 1990). However, for guinea pigs and rabbits the same study found that a majority of the dose of codeine was excreted as C6G while M6G was detectable in urine, albeit as only 1 to 2% of the dose. It would appear that the rat has a limited capacity to form the 6-glucuronide of either

morphine or codeine. Unconjugated normorphine was also found in the urine of the rabbit, guinea pig, rat, mouse and human (Oguri *et al.*, 1970).

Kuo *et al.* (1991) recovered 68%, 38%, 39% and 62% of a s.c. dose of morphine as summed morphine, M3G and M6G over 24 hr in the urine of mice, rats, guinea pigs and rabbits, respectively. Mice and rats excreted M3G in the urine but M6G was undetectable, although the authors did state that M6G was detected but not quantified following further clean-up of the urinary extracts from rats. Guinea pigs and rabbits excreted both M3G and M6G. The ratios of the mean percentage excretion of M6G to M3G were almost identical with the ratios of their rates of formation by microsomal preparations from the livers of the corresponding species. Comparisons between species of *in vitro* activity are difficult because it is unclear whether saturating concentrations of morphine were used by Kuo *et al.* (1991) although, based on the studies of Coughtrie *et al.* (1989), it is probable that the relative activities observed were proportional to the amount of UDPGT. Of the four species, the guinea pig produced the most M6G relative to M3G, the ratio being about 0.25. This value approximated the ratios estimated from visual inspection of the results of Aasmundstad *et al.* (1993) and Déchelotte et al. (1993) during incubation of morphine with hepatocytes from the guinea pig. Most of the remainder of the dose given to rats by Kuo *et al.* (1991) was probably excreted in the bile and either retained in the gut or excreted in faeces. Roerig *et al.* (1974) recovered 49% of the radiolabelled dose over 1.5 hr via the cannulated bile duct and, after separation by countercurrent distribution, estimated that morphine contributed 10% and morphine glucuronide 39% of the dose. Iwamoto & Klaassen (1977) recovered 48% of an i.v. radiolabelled dose in the bile after 6 hr but found less than 1% as unchanged morphine. This appears to be in keeping with observations from bile collected during single-pass experiments with the isolated perfused liver from the rat (Evans & Shanahan, 1993; O'Brien *et al.*, 1993). About half of the M3G formed within the liver was excreted via the bile. Alternatively, the fraction of the dose given to male rats and unaccounted for by Kuo *et al.* (1991) may have been metabolites not measured in their study, for example, normorphine and NM3G (*vide infra*).

Aasmundstad *et al.* (1993) incubated morphine with hepatocytes isolated from the guinea pig and rat and observed the formation of M3G and M6G by both species, but the amount of

M6G formed by the rat and appearing in the incubation medium was extremely low and measurable only at concentrations for morphine during incubation of 200 µmol/l. However, M6G was measurable within the hepatocytes at concentrations of morphine between 5 and 200 µmol/l. The concentrations of M3G and M6G remained unchanged after all of the morphine had been metabolized, suggesting that they were not metabolized further. Normorphine and NM3G (the latter tentatively identified after hydrolysis) were also formed by hepatocytes from the rat, the sum of the two being greater than the amount of M3G formed at all concentrations of morphine, but normorphine or NM3G were not detectable after incubating morphine with hepatocytes from the guinea pig. During tentative identification of NM3G when normorphine was incubated with hepatocytes from guinea pigs, these authors also noted a small chromatographic peak, with a retention slightly less than that for M6G, which disappeared after hydrolysis with ß-glucuronidase, and concluded that it was NM6G.

Lawrence *et al.* (1992), using hepatic microsomes prepared from the human, sheep, pig, guinea pig, rabbit, rat and mouse, found that M3G was produced by all species, but for M6G the pig, rat and mouse failed to produce measurable amounts, the sheep produced trace amounts and the human, guinea pig and rabbit produced quantifiable amounts. The guinea pig produced the most M6G, at a rate approximately 16% of that for M3G. However, the ratio of the relative rates of formation for M3G and M6G by microsomal enzymes was dependent upon the concentration of selected metal ions in the incubation medium. These findings provide support for the conclusions of Coughtrie *et al.* (1989), who also provided evidence for the involvement of two isozymes of UDPGT. Only the isozyme responsible for the formation of (-)-M3G existed in microsomes prepared from the liver of rats, and neither was present in the kidney. These authors suggested that the UDPGT isolated and purified by Puig & Tephly (1986) from hepatic microsomes of the rat was the form responsible for the formation of (-)-M3G.

When male and female Wistar rats were given s.c. [N-methyl-^{14}C]morphine, approximately 5% of the dose was recovered in the expired air of males as $^{14}CO_2$, but less than 0.5% from females (March & Elliott, 1954). Furthermore, when s.c. [N-methyl-^{14}C]codeine was administered to Sprague-Dawley rats of both sexes, Yeh & Woods (1969) found that 17.7% and 7.6% of the radiolabelled dose was recovered in the expired air of males and

females, respectively. Microsomes prepared from the liver of adult male rats of unspecified (Axelrod, 1956a), Sprague-Dawley (Blanck *et al.*, 1990) or Long-Evans (Elison *et al.*, 1963) strain metabolized morphine to normorphine at about 10, 20 and 8 times, respectively, the rate for adult females. Neonatal castration or post-pubertal hypophysectomy reduced the rate in the adult males to that in the females (Blanck *et al.*, 1990). The rate of formation of normorphine in male rats was 19% of the rate for the formation of M3G (Rane *et al.*, 1985). Confirming the microsomal studies, Shanahan & Evans (1993) used the isolated perfused liver from the Sprague-Dawley rat to demonstrate a sex-difference in the relative formation of normorphine from morphine. There was no difference in the partial clearances of morphine to M3G, a finding in agreement with the *in vitro* results of Rush *et al.* (1983) of comparable formation of glucuronide by hepatic microsomes from both sexes of Fischer 344 strain. Of further interest is the additional observation that the rate of formation of normorphine by microsomes prepared from male Sprague-Dawley rats pretreated with escalating doses of morphine for 14 days was reduced by 80% (Rane *et al.*, 1985). Axelrod (1956b) also found that pretreatment with escalating doses for 35 days reduced the rate of formation of formaldehyde from morphine and hydromorphone by 90% and 80%, respectively. In a further study, Rane & Ask (1992) speculated that the reduction was due to the induction of a female secretory pattern for growth hormone. They also demonstrated an inhibition of enzymic activity by a monoclonal antibody, and concluded that the formation of normorphine was catalyzed by cytochrome P450 2C11. The rate of formation of M3G was not affected by pretreatment with morphine. Therefore, male and female rats differ in the relative formation of the N-demethyl-metabolites of morphine (and codeine), a difference common to the Sprague-Dawley and Wistar strains. In view of these findings, it should be noted that for the metabolic and pharmacokinetic studies reviewed in this Chapter, all have used male Sprague-Dawley (or less commonly Wistar) rats except where mentioned otherwise.

Evidence for the formation of the N-oxide metabolite of morphine catalyzed by a flavin-containing monooxygenase enzyme present in microsomal preparations from the liver of the guinea pig was provided by Yuno *et al.* (1990), although the possible catalytic involvement of a cytochrome P450 enzyme cannot be excluded in this or other species.

When incubating morphine with hepatocytes isolated from the rat, Nagamatsu *et al.* (1986) identified M3G, normorphine, morphinone and a morphinone-glutathione conjugate as metabolites. They found that morphinone, a reactive electrophile, depleted cellular concentrations of glutathione, resulting in cell damage. Although no quantitative data were presented, the authors stated that the glutathione conjugate was one of the major metabolites. The formation of morphinone was catalyzed by cytoplasmic morphine-6-dehydrogenase in the liver (Yamano *et al.*, 1985). Kumagai *et al.* (1990) estimated that 4 hr after a dose of morphine was given to the guinea pig, 10.6% was eliminated in the bile as total morphinone, of which 90% was the glutathione conjugate. Nagamatsu *et al.* (1983) found considerable irreversible binding of a radiolabelled morphinone-cysteine conjugate following proteolytic digestion of protein from mouse liver. They suggested that morphinone binds covalently to cysteine residues of protein within the liver of the mouse.

1.5.3.2 Sites of Metabolism

For all species investigated, the liver appears to be the major organ responsible for the metabolism of morphine. From the data presented in section 1.5.4.1 on the clearance of morphine from plasma, and assuming a value for C_b/C in the range of 1.1 to 1.3 (section 1.5.2.2), it is apparent that the total clearance of morphine from blood exceeds hepato-splanchnic blood flow in almost all species (Davies & Morris, 1993). Evidence is available from *in vitro* and *in vivo* studies confirming the involvement of other organs, but occasionally that evidence is contradictory in some species.

The study by Merrell *et al.* (1990) in the dog demonstrated negligible extraction of morphine across the kidney but that approximately 56% was removed during passage through the liver. Circumstantial evidence for a lack of metabolism of morphine to M3G by the kidney comes from the almost identical values for the renal excretory clearance of M3G after separate doses of morphine and M3G administered to the dog (Garrett & Jackson, 1979). Jacqz *et al.* (1986) demonstrated that approximately half of the total clearance of morphine from plasma observed in control dogs remained after hepatic devascularization. Some of the clearance may be accounted for by metabolism by the intestine but a contribution from the kidney during this

study cannot be excluded. In contrast, a brief report by Hug *et al.* (1981b) showed that, following an i.v. dose of 3 mg/kg, the clearance of morphine from plasma was reduced from 20 and 32 ml/min/kg in two sham-operated dogs to a mean of 1.7 ± 0.3 ml/min/kg in four hepatically devascularized dogs, suggesting that almost all of the total clearance is by the liver.

Horton & Pollack (1991) performed studies with the female Sprague-Dawley rat after ligation of the renal pedicles and cannulation of the bile duct. With the prevention of enterohepatic cycling and the assumption that ligation did not alter hepatic clearance, it was estimated that 57% of systemic clearance was hepatically derived while 43% was due to the kidney. Of the total clearance in the intact rat, clearance by the kidney accounted for 54% and of this, only one third was excretory clearance. These findings appear to conflict with those from the *in vitro* studies (*vide infra*) of Rush & Hook (1984) and Rush *et al.* (1983) with respect to the contribution from the kidney of both sexes of Fischer 344 rats to the metabolism of morphine. The latter authors measured only the activity of UDPGT towards morphine. It is possible that strain differences or the formation of other metabolites, for example normorphine, by the kidney may contribute to the disparity between the *in vitro* and *in vivo* observations.

Bodenham *et al.* (1989) gave a single dose of morphine to patients undergoing transplantation of the liver. The anhepatic stage lasted for 40 min and during this period the concentrations of morphine in plasma decreased rapidly to almost static levels, presumably subsequent to distribution and some renal elimination, while urinary concentrations of the glucuronides were less than 5% of those for morphine. Upon reperfusion of the transplanted liver, loss of morphine from plasma occurred at an increased rate while the concentrations of M3G and M6G, previously less than 15 nmol/l, increased to a mean of 510 nmol/l and 15.4 nmol/l, respectively, after 50 min. While the results from this study provide strong evidence for the almost exclusive metabolism of morphine by the liver in humans, urine output, although maintained with dopamine, and other indicators of kidney function were not reported. From the study of Persson *et al.* (1986), the lung would not appear to be an important organ for the irreversible extraction of morphine, although these authors did observe a net extraction of 0.23 and 0.24 in two diabetic patients, the significance of which remains unclear. The results from

studies examining the involvement of the kidney in humans appear conflicting, but are examined in greater detail in section 1.5.4.3.

Metabolism of morphine to M3G, normorphine and NM3G has been observed with the isolated perfused liver from the male Sprague-Dawley rat (Shanahan & Evans, 1993). Approximately 75% and 17% of the morphine perfusing the liver was metabolized to M3G, and summed normorphine and NM3G, respectively. In contrast, Imamura & Fujimoto (1980) recovered 35% as M3G in bile and perfusate, but less than 3% of the dose of [N-methyl-^{14}C]morphine in perfusate from the isolated liver of the male Sprague-Dawley rat, *in situ*, was recovered as radiolabelled carbon dioxide, a product of the formation of normorphine. However, these authors did not examine the ethyl acetate-extract of the bile or perfusate for the presence of normorphine.

Josting *et al.* (1976), using a closed intestinal loop from the rat *in situ*, found that 40% of the morphine absorbed through the mucosa into the venous blood had been converted to glucuronide metabolites. They also found that 3.6% of the morphine placed originally within the closed loop was present as the same metabolites suggesting that they may have formed in the mucosa and then passed into the gut lumen. Metabolism of morphine to M3G (Ratcliffe *et al.*, 1985), and M3G and M6G (Van Crugten *et al.*, 1991), by the isolated perfused kidney of the rat was not detected. However, while Van Crugten *et al.* (1991) used a HPLC method capable of detecting M3G and M6G, it is not clear how Ratcliffe *et al.* (1985), using a RIA method, distinguished M3G from morphine. Davis & Mehendale (1979) found no evidence for the metabolism of morphine by the isolated perfused lung of the rabbit.

Proximal tubular segments from the rabbit kidney were used by Schäli & Roch-Ramel (1982) to demonstrate the formation of conjugates of morphine which were hydrolyzable by ß-glucuronidase. Teller *et al.* (1976) were unable to demonstrate conclusively that morphine was metabolized after being taken up by slices of kidney from the mouse.

Koster *et al.* (1985) observed saturable metabolism of morphine to M3G by intestinal epithelial cells isolated from the male rat. There was no detectable formation of M6G, M3S or normorphine. Aasmundstad *et al.* (1993) were able to show the formation of M3G, normorphine and NM3G, and Bodd *et al.* (1986) of M3G, by hepatocytes from the male rat. In

addition, Aasmundstad *et al.* (1993) and Déchelotte *et al.* (1993) were able to demonstrate metabolism of morphine to M3G and M6G by isolated hepatocytes from the guinea pig. From the preliminary data of Déchelotte *et al.* (1993), the ratio of V_{max} to K_m for the formation of both M3G and M6G by hepatocytes was of the order of 4 to 20 times greater than for intestinal epithelial cells and far greater than for epithelial cells from the colon and gastric mucosal cells. That Iwamoto *et al.* (1978) were unable to demonstrate the formation of morphine glucuronides by hepatocytes from the rat may reflect either differences in intrinsic metabolic clearance between strains, reduced viability of the hepatocytes or, more likely, a lack of sensitivity of the assay (from the study by Déchelotte *et al.* (1993), the concentrations of the major metabolite, M3G, were less than 1% of morphine).

In vitro studies with hepatic microsomes have shown that morphine is metabolized to M3G and M6G by the liver of humans (Coughtrie *et al.*, 1989; Säwe et al., 1985), monkeys (Rane *et al.*, 1984), rabbits (Kuo *et al.*, 1991) and guinea pigs (Kuo *et al.*, 1991), and from the kidney (Yue *et al.*, 1988) and brain (Wahlström *et al.*, 1988) of humans. The formation of glucuronides of morphine was mediated by microsomes prepared from the liver, intestine and kidney of the adult human (Cappiello *et al.*, 1991) and human foetus (Pacifici & Rane, 1982), but no activity was evident in the lung from the adult human (Cappiello *et al.*, 1991). Microsomes from the liver of the rabbit (Del Villar *et al.*, 1977), and from the liver (Mistry & Houston, 1987) and intestine (Del Villar *et al.*, 1974; Koster *et al.*, 1985; Mistry & Houston, 1987) of the rat, but not from the kidney (Rush & Hook, 1984; Rush *et al*, 1983), also catalyzed the formation of glucuronides. In all species where the formation of glucuronides was demonstrated *in vitro* from more than one organ, activity was greatest in the liver. The finding of Wahlström *et al.* (1988) is interesting in that M6G formed within the brain may contribute partially to the analgesia of exogenous morphine. Furthermore, Ghersi-Egea *et al.* (1988) found that microsomes prepared from endothelial cells of capillaries located in the brain of the rat were able to catalyze the formation of 1-naphthol glucuronide. As yet, no comparable experiments have been performed with morphine as the substrate.

To summarize, the evidence for the involvement of organs other than the liver is inconsistent between species and even comparing studies with the same species. For the male

rat, there is evidence from studies *in vivo* and *in vitro* for the metabolism of morphine by the gut but not by the kidney, while one report has provided evidence from female rats *in vivo* for the clearance of morphine by the kidney. *In vitro* evidence supports the involvement of the kidney in rabbits but there is no clear evidence from any studies for the kidney in the dog. However, *in vivo* the studies would suggest that other non-hepatic organs within the dog contribute to the clearance of morphine. For humans, *in vitro* studies provide evidence for the metabolism of morphine by the liver and kidney, but the gut has not yet been examined. As noted in section 1.5.4.3, the importance of the kidney in humans remains uncertain.

1.5.4 Elimination

1.5.4.1 Normal Hepatic and Renal Function

The pharmacokinetics of morphine has been examined in animals and humans. Most studies have measured the concentrations of morphine in plasma for up to 12 hr after an i.v. dose, some for a much lesser period because of limitations in analytical sensitivity, and have reported a bi-exponential decrease in the concentrations with time.

Studies with animals *in vivo* have established that morphine is highly extracted by the liver, but less so by the gut. After i.v. and intraportal administration to the rat, the extraction ratio of morphine by the liver ranged from 0.45 (Mistry & Houston, 1987) to 0.61 (Iwamoto & Klaassen, 1977). The ratio was consistent over a one hundred-fold range of doses (given over 30 sec). In the dog anaesthetized with pentobarbitone, the hepatic extraction was reported as 0.35 (Merrell *et al.*, 1990). Re-analysis of the mean data presented suggests an hepatic extraction closer to 0.55. Dahlström & Paalzow (1978), excluding the role of enterohepatic cycling, calculated hepatic and intestinal extraction ratios in the rat of 0.72 and 0.46, respectively, following i.v., intraportal and oral doses. Interestingly, from antinociceptive data these workers calculated an oral availability of 0.31, which was identical to the availability of 0.31 determined from the concentrations in plasma, including cycling. On the one hand, this is not surprising since M6G, which possesses antinociceptive activity, is not detectable in the plasma of rats given morphine. Conversely, the ratio of the concentrations of M3G to those of morphine in plasma after the oral dose would have been greater than after the i.v. dose (section

1.6) and, given the evidence for the functional antagonism by M3G of the antinociceptive activity of morphine, a lesser oral availability might have been predicted from the antinociceptive data. Comparable, high extraction was seen by the isolated perfused liver from the rat (Brouwer & Turner, 1989; Shanahan & Evans, 1993).

Mean values for the clearance of morphine from plasma ranged from 37 ml/min/kg to 104 ml/min/kg in the rat (Bhargava *et al.*, 1991; Ekblom *et al.*, 1992; Gårdmark *et al.*, 1993; Horton & Pollack, 1991; Iwamoto & Klaassen, 1977; Mistry & Houston, 1987; Tan *et al.*, 1990b) and 86 ml/min/kg to 118 ml/min/kg in the rabbit (Catlin, 1977; Chast *et al.*, 1991). There were considerable differences within the respective studies. In general, when comparing studies, lower mean values were obtained when female rats were used. Differences in age, reflected in the range of weights across studies, may also have contributed to the wide range in the mean values. The brief report of Horton *et al.* (1993) suggested that the clearance of morphine from the plasma of rats decreased by 58% between 3 and 9 months of age, but increased by 260% between 9 and 12 months. These changes seemed to parallel those for hepatic clearance, and the percentage of the dose of morphine excreted into bile, by the perfused liver isolated from rats of similar ages. There was no evidence of dose-dependence within studies over a hundred-fold (Catlin, 1977; Mistry & Houston, 1987) or four-fold range (Bhargava *et al.*, 1991). In rats given i.v. morphine, the clearance from plasma was increased by 26% when the bile duct was cannulated (Horton & Pollack, 1991), indicating that a significant fraction of the morphine excreted into the bile returns to the systemic circulation.

In contrast to the dose-independence observed in the rat and rabbit, Hug *et al.*, (1981a) and Garrett & Jackson (1979), over seven- and one hundred-fold dose ranges, respectively, provided evidence of dose-dependent clearance in the dog which may have been due to hypotension induced by the rapid injection of morphine at the higher doses of 2 mg/kg (Hug *et al.* 1981a; Schmidt & Livingston, 1933) when the animals were rendered unconscious (Schmidt & Livingston, 1933). Dogs given 7.2 to 7.7 mg/kg morphine i.v. over 10 sec by Garrett & Jackson (1979) remained unconscious for at least 3 hr, exhibited oliguria for almost 2 hr and produced no bile for between 10 and 35 min. Doses near 0.4 mg/kg had a pronounced effect on urine flow for up to 75 min. These observations at the higher doses contrast with those of

Priano & Vatner (1981), who found that dogs remained conscious and mean arterial pressure relatively stable for up to 25 min after an i.v. infusion of morphine (3 mg/kg over 5 min). Comparable clearances from plasma at similar doses were determined by Garrett & Jackson (1979) and Hug *et al.* (1981a) and ranged from 21 to 33 ml/min/kg at doses between 2 mg/kg and 7.6 mg/kg, and from 51 to 59 ml/min/kg at doses between 0.02 mg/kg and 0.3 mg/kg. From the data of Merrell *et al.* (1990) after a dose of 0.5 mg/kg infused over 5 min, a value of about 42 ml/min/kg may be calculated, while after a 1 mg/kg dose infused over an unspecified time, Jacqz *et al.* (1986) determined a value of 52 ml/min/kg. Garrett & Jackson (1979) found that metabolic clearance was compromised with increasing doses and suggested that it was due to a reduction in hepatic blood flow or saturable elimination processes. Priano & Vatner (1981) measured the blood flow through the cranial mesenteric artery, which supplies the small and large intestines, and the renal artery of the dog, with electromagnetic and Doppler flow probes, respectively, for 30 min after starting a 5 min infusion of morphine. Despite the relatively unchanged arterial pressures, they observed a 26 to 55% increase in mesenteric blood flow and a 13% increase in renal blood flow, at a dose of 1 mg/kg of morphine, but a reduction of 29 to 44% and an increase of 21 to 34%, respectively, after a 3 mg/kg dose. Therefore, in the dog at least, the dose-dependent clearance of morphine may in part be due to its dose-dependent effects on hepatic blood flow which, given the intermediate hepatic extraction, will be an important determinant of clearance.

Of further interest is the observation of a terminal third dispositional phase for morphine, apparent from the rates of urinary excretion, with a mean half-life of 16.6 ± 7.5 hr (Garrett & Jackson, 1979). Importantly, this phase was not seen in bile duct-cannulated dogs. Although the percentage increase in the AUC due to the third dispositional phase was not determined, these authors did estimate that about 15 to 27% of the dose of morphine was subjected to enterohepatic cycling. In a study by Yoshimura *et al.* (1993), dogs given constant infusions of morphine at rates of 2.5 and 5 mg/kg/day for 7 days, produced steady-state concentrations in plasma of 83.4 nmol/l and 153 nmol/l, respectively. From these data, clearance may be calculated as 72.9 ml/min/kg and 79.4 ml/min/kg. Blood pressure and heart rate during the infusions did not appear to be different from the respective values prior to the infusions. In the

studies of Garrett & Jackson (1979) and Hug *et al.* (1981a) the concentrations in plasma at the higher doses had not decreased to approximately 150 nmol/l until at least 8 hr and 4 hr, respectively, after the dose was given. It is noteworthy that, in dogs given 2.5 mg/kg of s.c. morphine twice a day for 7 days, the concentrations of morphine in plasma after the thirteenth dose were similar to those determined after the first dose (Yoshimura *et al.*, 1993). It may be that only a small percentage of the dose remains in the body after 12 hr. Alternatively, any expected accumulation of residual morphine may have been compensated for by the development of tolerance to the effect of morphine (2.5 mg/kg) on hepatic blood flow, compared to its predicted effect (Priano & Vatner, 1981) after a single dose.

Only one pharmacokinetic study using an assay specific for morphine has been reported using the rhesus monkey. Following a single i.v. dose (0.27 to 0.28 mg/kg) given to three monkeys, the clearance of morphine from blood ranged from 9.2 ml/min/kg to 21.3 ml/min/kg (Rane *et al.*, 1984). The difference in the concentrations in plasma from the hepatic portal and hepatic veins was used to calculate hepatic extraction. Considering that extraction (range 0.61 to 0.74) was determined during the elimination phase, their values probably underestimate the true values.

Considerable pharmacokinetic data are available from studies in humans. However, after single i.v. doses, most studies have reported concentrations for morphine in plasma up to approximately 12 hr only. As such, relatively short mean half-lives of 1.6 hr (Westerling *et al.*, 1993) to 2.5 hr (Patwardhan *et al.*, 1981) for a second dispositional phase were determined. Hasselström & Säwe (1993) combined the concentrations of morphine in plasma measurable up to 24 hr after the dose with data for the rate of urinary excretion obtained up to 72 hr to record mean half-lives for a third dispositional phase of 15.1 ± 6.5 hr in healthy subjects. They stated that, after the i.v. and oral doses, the additional AUC attributed to the third phase was 30% and 80%, respectively, of the AUC that would have resulted from the assumption of only two dispositional phases. This, together with the findings of Garrett & Jackson (1979) in the dog, is consistent with enterohepatic cycling being the major contributor to the final dispositional phase. Garrett & Jackson (1979) proposed that the persistence of morphine in plasma and urine was due to the time lag in enterohepatic cycling with concomitant gall bladder storage, postponing

the return of biliary secreted M3G as morphine. Further confirmation of a third dispositional phase in humans comes from a study of the comparative availabilities of two oral formulations after single doses and 5 days of dosing (Hasselström *et al.*, 1991). After the single doses the AUC was determined from the concentrations of morphine in plasma collected up to 12 hr with extrapolation beyond, while on the 5th day of dosing the AUC was calculated over a dosing interval. For both formulations, the AUC was approximately 45% greater after 5 days.

Therefore, it is quite likely that, of the studies performed in humans and animals, those that have sampled blood over a relatively short period will have determined values for the clearance of morphine from plasma or blood which are overestimates, and probably representative of total systemic clearance that would have been determined in the absence of a significant return of morphine to the system, more specifically via enterohepatic cycling. Therefore, when comparing studies which used healthy subjects, it is difficult to reconcile the value of 21.1 ± 3.4 ml/min/kg determined by Hasselström & Säwe (1993) for the clearance from plasma, with values of 18.3 ml/min/kg (Westerling *et al.*, 1993), 20.0 ± 1.4 ml/min/kg (Zhou *et al.*, 1993) and 20.5 ± 2.5 ml/min/kg (Baillie *et al.* (1989) from plasma, and of 14.8 ml/min/kg from blood when C_b/C was measured as 1.1 (Patwardhan *et al.*, 1981). However, it is not difficult to reconcile with the value of 32.2 ± 10.6 ml/min/kg from the study of Owen *et al.* (1983), when the concentrations in plasma were measured in samples collected for only 3 hr. Zhou *et al.* (1993) found that normal caucasians cleared morphine at two-thirds the rate of chinese subjects and was associated with significant differences in the partial clearances to M3G and M6G, but not to normorphine. Concomitant with these findings was a lesser ventilatory response of the caucasian group to carbon dioxide when given morphine. There was no difference in the AUC of M6G between the two groups, because the renal clearance of M6G was significantly less in the Caucasian subjects.

From studies with patients, values for the clearance of morphine from plasma of 21.0 ± 7.0 ml/min/kg (Dahlström *et al.*, 1982) and 28.0 ± 5.6 ml/min/kg (Säwe *et al.*, 1985) in patients with cancer, and of 27.7 ± 5.5 ml/min/kg (Sitar *et al.*, 1986) and 18.4 ± 6.0 ml/min/kg (Tanguy *et al.*, 1987) in surgical patients were found after a single dose. Values of 17.3 ± 6.4 ml/min/kg (Gourlay *et al.*, 1986) and 19.1 ± 5.9 ml/min/kg (Persson *et al.*, 1986) from blood after single

doses given to cancer patients and after 5 hr of constant infusion to surgical patients, respectively, have also been reported. These findings suggest that cancer or surgery does not contribute greatly to the variability in clearance among individuals. Tanguy *et al.* (1987) determined the clearance of morphine from plasma in two groups of surgical patients who were given either an i.v. bolus or had been receiving an i.v. infusion for between 15 and 43 hr. Clearance during the infusion (36.8 ± 9.1 ml/min/kg) was greater than that after the bolus dose (18.4 ± 6.0 ml/min/kg). Concentrations in plasma were measured for up to 5 hr only, after the bolus, but the relative magnitude of the two values for clearance is difficult to explain.

In a study of four patients with cancer and receiving chronic oral morphine for severe pain, the concentrations of morphine, M3G and M6G in plasma prior to a dose were proportional to the dose. Within patients, the dose escalated and declined over a sixteen to twenty three-fold range over a period of 5 to 8 months (Säwe *et al.*, 1983b). The observations also suggest that the capacity to metabolize morphine remained unchanged with continued administration. There were considerable differences between patients in the relationship between the dose and the concentrations of morphine in plasma, but these differences were less apparent for M3G and M6G. Since most of the dose of morphine is recovered in urine as M3G and M6G, the latter finding is not unexpected in subjects with normal renal function.

The influence of anaesthetic agents on the clearance of morphine from plasma has been examined in dogs and humans. Merrell *et al.* (1990) found that induction of additional anaesthesia in the dog with halothane and pentobarbitone, compared to control dogs receiving pentobarbitone alone, significantly reduced the clearance of morphine from plasma by 40% after an i.v. dose but had no significant effect on clearance following portal administration. These authors proposed that the reduction in systemic clearance was due to a reduction in hepatic blood flow. Murphy & Hug (1981) determined the clearance of morphine in patients maintained on a mixture of enflurane-nitrous oxide during anaesthesia. The duration of anaesthesia relative to the sampling of blood was not reported and a control group was lacking. However, the mean clearance (23 ± 1 ml/min/kg) was comparable to values reported from studies with healthy subjects (Hasselström & Säwe, 1993; Owen *et al.*, 1983; Westerling *et al.*, 1993).

Säwe (1986) determined the renal excretory clearance of morphine from plasma in patients with cancer who were receiving chronic oral morphine. In those patients with no evidence of renal dysfunction, the renal clearance was 103 ± 40 ml/min, confirming the observations reported in section 1.5.3.1 that renal excretion is a minor route for the elimination of morphine in healthy humans. Therefore, if severe renal impairment were to reduce only the renal excretory clearance of morphine, it would be predicted that impairment would have a marginal effect on the total clearance. The mean renal excretory clearance of morphine from plasma in the dog was 85 ± 9 ml/min (Garrett & Jackson, 1979).

1.5.4.2 Hepatic Dysfunction

Laidlaw *et al.* (1961) reported that patients with a history of impending or overt hepatic coma were more sensitive to changes in electroencephalographic recordings induced by morphine. Patients without liver disease or those with hepatic cirrhosis but without encephalopathy, jaundice or ascites, were more tolerant of therapeutic doses. To date, studies examining the influence of hepatic dysfunction on the pharmacokinetics of morphine have only been performed in humans. Patwardhan *et al.* (1981) found no difference in the total clearance of morphine from plasma when they compared healthy subjects against five patients with alcoholic and one with post-necrotic cirrhosis. In contrast, Hasselström *et al.* (1990) found, on average, complete oral availability in seven patients with cirrhosis compared to an availability of 0.47 in healthy subjects examined in an earlier study (Säwe *et al.*, 1985), and a 59% reduction in total clearance in the patients after an i.v. dose. Furthermore, a 66% reduction in the ratio of the AUC of M3G to that of morphine after the oral dose was in contrast to the nonsignificant difference between the two groups in the same ratio when i.v. morphine was given. Evidence of ascites in six of the seven patients, suggestive of more severe cirrhosis, may have contributed to the different finding in this study compared to that by Patwardhan *et al.* (1981). A possible explanation for the observations of Hasselström *et al.* (1990) is that the 'effective' blood flow to the liver, assuming it to be the major eliminating organ, has been reduced by about one half, either by portocaval shunting or decreased functional perfusion of hepatocytes.

1.5.4.3 Renal Dysfunction

Renal dysfunction is known to increase the likelihood and severity of side-effects such as respiratory depression, sedation and miosis associated with morphine. Using a nonspecific RIA for the determination of the concentrations of morphine in plasma, Ball *et al.* (1985) concluded that the systemic clearance of morphine was dependent on renal function. Later studies, using methods more specific for the measurement of the concentrations of morphine in plasma, have refuted this conclusion. Aitkenhead *et al.* (1984) compared the clearance of morphine from plasma in normal subjects and patients with chronic renal failure and found no significant difference. However, it is difficult to draw valid conclusions when blood was sampled for only 2 hr. Säwe & Odar-Cederlöf (1987) compared seven patients with advanced renal failure against six patients with cancer but having normal renal and hepatic function, while Woolner *et al.* (1986) compared four patients on haemodialysis with three healthy subjects. In both studies the clearance of morphine from plasma was not significantly different between the respective groups. However, the power of either study to detect a difference was probably low, especially that by Woolner *et al.* (1986). Osborne *et al.* (1993), in a study with a larger number of healthy subjects and patients, compared the clearance of morphine from plasma in healthy subjects with that in two groups of patients with renal failure, one of which was receiving peritoneal dialysis, and with another group with renal failure receiving a transplanted kidney. They found a significantly greater total clearance in the healthy subjects (1966 ± 313 ml/min) than in the patients with renal failure and not receiving dialysis (1171 ± 337 ml/min) or those receiving dialysis (971 ± 184 ml/min). Peritoneal dialysis had no significant effect. However, all of the patients were undergoing surgery and, after premedication, had received isoflurane and nitrous oxide for the maintenance of anaesthesia. The influence of this anaesthetic regimen on the clearance of morphine in humans has not been studied. While isoflurane alone has been shown to reduce significantly the mean arterial blood pressure in healthy humans (Dolan *et al.*, 1974; Shimosato *et al.*, 1982), its use in combination with nitrous oxide effected a more modest reduction (Dolan *et al.*, 1974). However, Dolan *et al.* (1974), on noting an increase in the flow of blood to muscle, could not exclude the possibility that the flow to other organs was compromised. Nevertheless, the transplanted patients, who received the same anaesthetic

regimen but also cyclosporin post-operatively, displayed values for clearance (1596 ± 468 ml/min) which, while on average lower, were not significantly different from those of the healthy subjects, but were significantly greater than the group receiving dialysis. The half-life for elimination was consistent across all groups because the calculated volumes of distribution at steady-state were significantly lower in the patients given transplants or with renal failure. This study suggests a role for the kidney in the metabolic clearance of morphine in humans. Even a considerable reduction in the renal excretory clearance of morphine would not account for the decreased total clearance observed in the patients with renal failure.

1.6 Pharmacokinetics of M3G and M6G

As noted in sections 1.3.3 and 1.3.4, there is considerable interest in the pharmacology, and the formation and elimination of M3G and M6G in humans and animals.

1.6.1 Human Studies

Three recorded studies have investigated the pharmacokinetics of M6G following M6G administration. In a preliminary study investigating the analgesic activity of M6G after i.v administration of M6G to two patients, one with normal and the other with impaired renal function, Osborne *et al.* (1988) observed a similarity between the clearance of M6G and the clearance of creatinine. Osborne *et al.* (1992), using patients with cancer who were experiencing moderate to severe pain and had diverse renal function, employed a specific HPLC method to measure M6G in plasma collected for 12 hr and urine for 24 hr after a single i.v. dose ranging from 0.5 to 4 mg/70 kg. Including all twenty patients, mean total and renal clearances from plasma were 96 ± 38 ml/min and 36 ± 25 ml/min, respectively, and appeared to be independent of dose. There was a significant correlation between the total clearance of M6G and the clearance of creatinine, the latter predicted by the method of Cockcroft & Gault (1976). However, further analysis of their data revealed no correlation between the renal clearance of M6G and either the calculated ($r = 0.278$) or measured renal clearance of creatinine ($r = 0.003$). This is somewhat surprising and suggests that the former correlation may have been fortuitous or that M6G was eliminated via alternative routes when renal function was compromised and

that clearance by these pathways decreased as renal function declined. Incomplete urine collection was ruled out. On average, only 38% of the dose was recovered in urine and further analysis revealed that there was no correlation between urinary recovery and the rate constant for the elimination of M6G ($r = 0.155$). The authors proposed that alternative routes of elimination may exist, such as metabolism to morphine-3,6-diglucuronide or biliary excretion. No morphine or M3G was detected in urine. This group has since found that the diglucuronide was not detectable in the urine of any subject given morphine or M6G (Patel *et al.*, 1993) but this does not exclude the possibility of biliary excretion of unchanged M6G or metabolism to the diglucuronide and biliary excretion. In addition, there was no indication in the latter brief report that the lack of diglucuronide identification in urine had been confirmed using an authentic sample, nor was the recovery during sample clean-up reported. The findings of Imamura & Fujimoto (1980) in the rat with M3G may provide some explanation for the fate of the M6G. These authors noted that, while only 1% of the M3G perfusing the isolated liver was excreted in the bile, approximately 30% of the M3G was recovered in the bile when the M3G was recirculated through the liver *in situ* after ligation of the renal pedicles.

Hanna *et al.* (1991) compared the disposition of M6G after separate, single i.v. doses of morphine and M6G and found no significant difference between formed and preformed M6G in the half-life of elimination. Based on relative AUC values, the fraction of the dose of morphine metabolized to M6G was 0.12 ± 0.09. Unfortunately, these workers measured the concentrations of M6G in plasma collected for only 5.3 hr and visual inspection of their data suggests that the half-life of formed M6G may indeed be greater than that after i.v. M6G. Also, they did not collect urine.

The disposition of M3G and M6G has been examined after the administration of morphine to healthy subjects and patients with cancer. In studies with healthy subjects, Osborne *et al.* (1990) and Hasselström & Säwe (1993) compared the values for the AUC of M3G and M6G to that for morphine after single i.v. and oral doses. For M3G, the mean ratios after the i.v. and oral doses were 7.9 and 55.8 (Osborne *et al.*, 1990), respectively, and 7.7 and 29.9 (Hasselström & Säwe, 1993), respectively; for M6G the corresponding ratios were 1.4 and 9.7 (Osborne *et al.*, 1990), respectively, and 0.7 and 3.6 (Hasselström & Säwe, 1993),

respectively. The mean ratios of the AUC values for M3G to M6G following i.v. and oral administration were 5.5 and 5.9 (Osborne *et al.*, 1990), respectively, and 9.7 and 8.6 (Hasselström & Säwe, 1993), respectively, while Westerling *et al.* (1994) found ratios after i.v. and transdermal administration of morphine of 4.7 and 4.6, respectively. In subjects administered i.v. morphine, Osborne *et al.* (1990) reported median half-lives of 3.5 hr and 2.7 hr for M3G and M6G, respectively. The value for M6G compares well with the value after i.v. administration of M6G by the same group in a later study (Osborne *et al.*, 1992). However, a renal clearance of 201 ml/min for M6G, estimated from the mean AUC and amount excreted in urine (Osborne *et al.*, 1990), is markedly higher than any value determined in the later study with patients who were older, suffered from cancer and had reduced renal function. The half-lives of M3G and M6G after oral morphine were more than double those after the i.v. dose (Osborne *et al.*, 1992), suggesting retention of a larger fraction of the dose as these compounds in the body, probably during enterohepatic cycling.

From the study by Säwe (1986) in patients with cancer and receiving oral morphine, those with no evidence of renal dysfunction had mean renal clearances for M3G and M6G of 83 ± 29 ml/min and 109 ± 44 ml/min, respectively. The urinary excretion rates were reported to be independent of the flow of urine, suggesting that tubular reabsorption is unimportant. There was a linear relationship between the concentrations of M3G and M6G in plasma and the dose of morphine.

Koopman-Kimenai *et al.* (1994) presented data on the renal clearances of morphine, M3G and M6G following the i.v. administration of 10 to 30 mg of nicomorphine (3,6-dinicotinoylmorphine, a prodrug of morphine) to surgical patients. Considerable variation in the mean renal clearances between and within the different doses was apparent, especially for morphine, but there was a trend towards greater values for morphine compared to M3G and M6G. However, from the concentrations in the plasma-time profiles shown for morphine and M6G, it is apparent that many of the values for clearance would have been obtained from concentrations measured in plasma that were well below the lowest concentrations that were used in the preparation of calibration curves and for which data on precision were given.

The renal clearances of M3G and M6G were compared in normal caucasian and chinese subjects following an i.v. dose of morphine (Zhou *et al.*, 1993). After being normalized for body weight, clearances in the caucasian group were two-thirds of those in the Chinese subjects. However, while the renal clearance of M3G was lower than the value for morphine in both groups, the values for M6G appeared to be comparable to morphine in the chinese group and greater in the caucasians.

There is a paucity of data on the relationship between renal function and the elimination of M3G and M6G. Säwe & Odar-Cederlöf (1987) observed an accumulation of both M3G and M6G and a significant correlation between the half-life of M3G and the plasma concentration of urea in patients with renal failure. Estimated half-lives of up to 118 hr were made on data from plasma collected for only 12 hr after the dose of morphine was given. Peterson *et al.* (1990) found that, as the clearance of creatinine decreased (estimated using the equation of Cockcroft & Gault, 1976), the trough concentration of either glucuronide in plasma increased relative to the trough concentration of morphine. Wolff *et al.* (1988) have shown a significant correlation between the clearance of total glucuronides from plasma (determined as morphine after hydrolysis of the glucuronide metabolites with ß-glucuronidase) and the clearance of ^{51}Cr-EDTA. However, in doing so, these authors assumed that a constant fraction of the i.v dose of morphine was metabolised to the glucuronides in all patients. In addition, by not measuring the individual glucuronides, it was impossible to assess the effect of varying renal function on the clearance of either compound. Beyond the time when graft arterialization of the renal transplant was completed, which was within 3 hr of the dose of morphine being given, Osborne *et al.* (1993) found a significant relationship between the steadily decreasing concentrations of M3G and M6G in plasma and the decreasing concentrations of creatinine. However, the latter are not reliable indicators of renal function or GFR, being dependent on both the rate of formation and the clearance of creatinine. Following i.v. administration of M6G, the clearance of M6G from plasma was significantly lower in patients with chronic renal impairment compared to a group with good renal function after renal transplantation (Hanna *et al.*, 1993). No statistical comparison was performed between the latter group and the healthy subjects studied previously (Hanna *et al.*, 1991).

1.6.2 Animal Studies

While some studies have followed the disposition of M6G in animals after the administration of morphine, there have been no reports examining the disposition of M6G following the administration of M6G. These studies have been performed with M3G but no direct comparisons have been made following separate doses of morphine and M3G within the one study.

During a constant infusion of M3G given to rats over 69 hr, the clearance of M3G from plasma at steady-state was calculated to be 10.5 ml/min/kg (Ekblom *et al.*, 1993). This value was significantly, but only marginally, lower than the value determined after an i.v. dose given as a bolus. There was evidence for deconjugation to morphine, with steady-state concentrations of morphine in plasma after 30 hr that were 1.4% of those for M3G. Following a bolus dose, a biphasic decline in concentration displayed mean half-lives of 13.2 min and 11.6 hr and the AUC of the slower elimination phase accounted for 8% of the total. A value of 1.68 l/kg was estimated for V_{ss}. Olsen *et al.* (1988) after a single i.v. dose of M3G to one pregnant ewe found that the clearance of M3G from plasma was 2.12 ml/min/kg, V_{ss} was 0.31 l/kg and the half-life was 2.6 hr. Comparable values for clearance were observed in the pregnant rhesus monkey, ranging from 2.0 ml/min/kg to 4.5 ml/min/kg (Gerdin *et al.*, 1990). However, their values for the renal clearance of M3G, approximately one half of the total, are difficult to reconcile with the almost complete recovery of the i.v. dose in urine. Garrett & Jackson (1979) gave an i.v. dose of M3G to one dog and determined a total clearance from plasma of 37.1 ml/min (3.4 ml/min/kg), which was comparable to the renal clearance of 42 ml/min. The renal clearance of M3G after a dose of morphine was 41 ± 4 ml/min. These authors estimated a V_z of about 0.25 l/kg. There was a considerable difference in the V_{ss} estimated by Ekblom *et al.* (1993) from the values determined in the other studies. The differences may be due to differences between species or to the collection of plasma from the rat by Ekblom *et al.* (1993) over a longer period. A half-life markedly greater than that found in the other studies mentioned, and increasing concentrations of morphine in plasma with continued infusion of M3G, is in accord with the proposal that M3G is excreted into bile, probably unchanged, where it undergoes hydrolysis and the aglycone is absorbed from the gastrointestinal tract and subjected to conjugative

metabolism. Continued recycling would account for the elimination phase with a half-life of 11.6 hr.

The concentrations of M3G in plasma relative to those for morphine following systemic administration of the latter exhibits considerable differences between species. During a constant infusion of morphine given to rats for 48 hr, the ratio of the mean concentrations of M3G to morphine in plasma was approximately 1.8 to 1 (Ekblom *et al.*, 1992). This contrasts with a higher ratio found in dogs of about 12.7 during a constant infusion for 7 days (Yoshimura *et al.*, 1993). The ratio in the dog is similar to the ratio of the respective AUC values of about 8 to 1 observed in healthy humans given single iv. doses of morphine (Hasselström & Säwe, 1993; Osborne *et al.*, 1990). The rat excreted almost equivalent amounts of morphine and M3G in urine for 24 hr after a single dose (Kuo *et al.*, 1991) but humans excrete approximately five times as much M3G as morphine into urine (Osborne *et al.*, 1990). Yoshimura *et al.* (1993) observed a mean ratio of approximately 40 in the dog for the concentrations of M3G in plasma to those of M6G.

1.7 Summary

There is considerable variation in the doses of morphine that are required to achieve analgesia in humans. From this review, it is clear that a number of factors can be identified as contributing to this variability and which are not completely understood. While the pharmacokinetics of morphine appears to be relatively consistent between the different studies examining humans or particular animal species, within each study there is evidence for considerable variability between subjects. There is also strong evidence that M3G and M6G contribute to the pharmacological effects of morphine, and that these effects are magnified in patients with renal failure. The contribution of different regions within the body to the formation of these important metabolites remains controversial, while a knowledge of the disposition of these metabolites, once formed, is incomplete. Other important factors contributing to the variability in dose are the differences in the intensity and perception of pain between patients, and the potential for the development of tolerance to the analgesic effects of morphine when used for the relief of chronic pain.

To understand further the pharmacokinetic aspect contributing to the variation in doses noted above, the objectives of the studies described in this thesis were:

(1) to develop an improved HPLC method for the measurement of morphine, M3G and M6G in plasma and urine which could be applied to studies in humans and sheep (chapter 2);

(2) to examine the influence of renal function on the renal elimination of morphine, M3G and M6G in humans with diverse renal function, and to elucidate possible mechanisms for their elimination (chapter 3).

Despite the numerous studies reviewed which have examined the regional elimination of morphine, none have addressed the elimination of morphine, M3G and M6G by the different regions simultaneously, during administration of morphine. Given the experimental difficulties and ethical problems of examining regional elimination in humans, a suitable animal was chosen with which to perform further experiments. Therefore, additional objectives of this thesis were:

(3) using normal sheep as a model (chapter 4), to examine the regional elimination of morphine and the regional formation and elimination of M3G and M6G (chapter 5);

(4) to compare the disposition of the major metabolite, M3G, in normal sheep following the separate administration of morphine and M3G (chapter 6);

(5) to re-examine (3) and (4) above in sheep with renal failure (chapter 7).

Chapter 2

High-performance Liquid Chromatographic Determination of Morphine and its 3- and 6-glucuronide Metabolites: Improvements to the Previous Methods

2.1 Introduction

Methods for the determination of morphine, M3G and M6G in biological fluids have been reviewed in Chapter 1. When this thesis was commenced, a method was required for the determination of all three compounds in plasma and urine, but only two methods described their simultaneous measurement in these fluids (Svensson *et al.*, 1982; Svensson, 1986). Svensson *et al.* (1982) reported an HPLC method with UV detection. Without an authentic sample of M6G, the authors identified it tentatively in the chromatograms from samples of plasma taken from patients. Identification was on the basis of UV absorption spectra, the generation of morphine from acidic and enzymic hydrolysis, and the formation *in vitro* of M3G and M6G by microsomal preparations from the livers of humans in proportions similar to those found in plasma. Improvements to the sensitivity and selectivity for morphine and M6G were achieved with the substitution of coulometric detection (Svensson, 1986). Without authentic M6G, it was assumed that it had the same molar absorptivity as M3G (Svensson *et al.*, 1982) or gave an identical coulometric response to morphine (Svensson, 1986). A later method by Joel *et al.* (1988) included automation of the procedure for the pretreatment of samples, the use of authentic M6G as a reference standard, and measurement of M3G in the eluate from the HPLC column by fluorescence. Although no specific details were given, inspection of their chromatograms suggested that the coulometric response of M6G was about 80% of that for morphine. The original method (Svensson et al., 1982) and its modifications (Svensson, 1986; Joel *et al.*, 1988) used two solid-phase extraction cartridges to isolate morphine, M3G and M6G from plasma. The compounds were collected from the second cartridge in 3 ml of eluate (10% acetonitrile in phosphate buffer) and, to achieve the required sensitivity for morphine and

M6G, up to 1 ml of this eluate was injected into the HPLC. Paired-ion chromatography with an octadecylsilane stationary phase packed in a stainless steel analytical column was used to separate morphine, M3G and M6G.

A coulometric detector was not available for the studies described in this thesis and hence the earlier method (Svensson *et al.*, 1982) formed the basis of an improved procedure using UV detection. This chapter describes improvements to the sample preparation and chromatographic conditions used by Svensson *et al.* (1982) while retaining the use of a single and readily available method, namely UV, for the detection and quantification of all three compounds. Using the improved method, the stability of morphine, M3G and M6G in plasma and urine was also examined; an important consideration in pharmacokinetic studies.

2.2 Methods

2.2.1 Chemicals

Morphine HCl (McFarlane Smith, Edinburgh, U.K.) and M3G (Sigma Chemical Co., St. Louis, MO, U.S.A.) were used as supplied. M6G was synthesized by Dr. G. D. Reynolds (School of Chemical Technology, University of South Australia, Adelaide) using the method of Yoshimura *et al.* (1968). Identity was confirmed using mass spectroscopy and nuclear magnetic resonance. Purity was established by chromatographic comparison with a sample of M6G, which contained 86% morphine-6-ß-D-glucuronide and 14% morphine-6-α-D-glucuronide, and was provided by the National Institute of Drug Abuse (DAC Code 5473-886-4, Bethesda, MD, U.S.A.). Hydromorphone (the internal standard) was obtained from commercial tablets (Dilaudid®, Knoll AG, Ludwigshafen, F.R.G.), by crushing and dissolving in methanol followed by centrifugation and collection of the supernate, or from Sigma Chemical Co. 1-Dodecylsulphate sodium was obtained from Regis Chemical Co. (Morton Grove, IL, U.S.A.). HPLC grade acetonitrile (UV cutoff 190 nm) and methanol were from Waters Associates (Lane Cove, Australia). Ammonium sulphate (Fluka Chemie AG, Buchs, Switzerland), sodium hydrogen carbonate, anhydrous sodium carbonate, ammonia solution 28% w/w (Ajax

Chemicals, Auburn, Australia), and sodium dihydrogen phosphate monohydrate (E. Merck, Darmstadt, F.R.G.) were of analytical grade and were used as received.

2.2.2 Extraction of Morphine, M3G and M6G from Plasma and Urine

Two methods were developed for the preparation of plasma and urine, from humans and sheep, for chromatography. The methods were developed using plasma from humans and then applied to plasma from sheep, and to urine. The first method was similar to that of Svensson *et al.* (1982) except that a stronger solvent was employed to elute morphine, M3G, M6G and hydromorphone from the Sep-Pak cartridge. For method II, an improvement on method I, a buffer of sodium hydrogen carbonate/sodium carbonate was substituted for the ammonium sulphate of method I.

For both methods, a Sep-Pak C_{18} cartridge (Waters Associates, Lane Cove, Australia) was washed successively with 10 ml methanol, 5 ml 25% acetonitrile in 10 mmol/l sodium dihydrogen phosphate (pH 2.1) and 10 ml water. These solvents were passed through the Sep-Pak cartridge under positive pressure at an approximate flow rate of 5 ml/min. During application of the sample, and thereafter until elution of the compounds of interest, solvents were passed through the cartridge under positive pressure at an approximate flow rate of 2 ml/min using a syringe pump (Sage Model 351, Orion Research Inc., Boston, MA, USA).

Method I: Plasma (0.5-1.0 ml) and a solution of hydromorphone (0.1 ml, 15.5 µmol/l) were mixed with 3 ml 0.5 mol/l ammonium sulphate (adjusted to pH 9.3 with ammonia, 28% w/w) and applied to the Sep-Pak cartridge. The cartridge was rinsed successively with 20 ml of 5 mmol/l ammonium sulphate (pH 9.3), 0.5 ml water and 0.5 ml 25% acetonitrile in 10 mmol/l sodium dihydrogen phosphate buffer (pH 2.1). Morphine, M3G, M6G and hydromorphone were eluted with a further 0.5 ml of the mixture of acetonitrile and phosphate buffer, of which 0.2 ml was injected onto the HPLC column.

Method II: Plasma (0.5-1.0 ml) or urine (0.02-0.2 ml) was mixed with a solution of hydromorphone (0.1 ml, 15.5 µmol/l) and 3 ml 0.5 mol/l sodium hydrogen carbonate/sodium carbonate buffer (pH 9.3), and applied to the Sep-Pak cartridge. The cartridge was rinsed successively with 20 ml of 5 mmol/l sodium hydrogen carbonate/sodium carbonate buffer (pH

9.3), 0.5 ml water and 0.35 ml 25% acetonitrile in 10 mmol/l sodium dihydrogen phosphate buffer (pH 2.1). Morphine, M3G, M6G and hydromorphone were eluted with a further 0.8 ml of the mixture of acetonitrile and phosphate buffer, and 0.25 ml of this was injected onto the HPLC column.

2.2.3 HPLC Instrumentation and Conditions

Chromatography was performed using a model 6000A pump, a Wisp 710A autosampler, a guard column (23.2 mm x 3.6 mm i.d.) packed with Bondapak C_{18}/Corasil, an RCM-100 Radial Compression Module containing a NOVA-PAK C_{18} 4 µm Radial-PAK cartridge (10 cm x 5 mm i.d) and a model 481 UV detector set at 210 nm (all Waters Associates). Peak areas (expressed as µvolt-sec) were determined using a Chromatopac C-R3A integrator (Shimadzu, Kyoto, Japan) or a Baseline 810 Chromatography Workstation (Waters Associates). Preliminary data as peak heights were also recorded on an SE-120 chart recorder (Goerz-Metrawatt, Vienna, Austria). The mobile phase, consisting of 26.5% acetonitrile and 0.8 mmol/l 1-dodecylsulphate sodium in 10 mmol/l sodium dihydrogen phosphate (pH 2.1), was pumped at a flow rate of 0.8 ml/min. Prior to chromatography of eluates from unknown, standard and control samples, consistency of response was evaluated by injecting 0.05 ml of a test mixture containing morphine (0.133 nmol), M3G (0.108 nmol) and M6G (0.102 nmol). After 5 injections, the mean response (and C.V.) for each compound was 160241 (5.43%), 127652 (4.85%) and 84629 (2.61%) µvolt-sec, respectively.

2.2.4 Preparation of Calibration Standards

2.2.4.1 Plasma

A stock solution of morphine (2.66 mmol/l) was prepared in methanol and diluted 1 in 10 with water, and stock solutions of M3G (0.217 mmol/l) and M6G (0.203 mmol/l) were prepared in water. Working standards containing all three compounds were prepared in water and stored frozen at -20°C. For the preparation of calibration standards, the volume of each working standard added was 10% of the volume of drug-free plasma. The plasma was obtained

from healthy human volunteers, and from sheep prior to an experiment. The relationships between the ratios of the peak areas of morphine, M3G and M6G to the area for hydromorphone, and the respective concentrations for the calibration standards, were analyzed by least-squares linear regression (Statview+^{TM}, Brainpower Inc., Calabasas, CA, USA). The ranges of concentrations prepared were dependent on the experiment and are given in subsequent chapters that describe each experiment.

2.2.4.2 Urine

Working standards of morphine, M3G and M6G were prepared in water and stored at -20°C. Calibration standards of the three compounds in drug-free urine from humans by mixing 0.5 ml of urine, diluted 1 in 50 with water, and 0.2 ml of appropriate working standard; from sheep they were prepared by mixing aliquots of urine (0.1 ml) with 0.2 ml of working standard.

2.2.4.3 Accuracy and Precision

Control samples containing morphine, M3G and M6G in drug-free plasma from sheep were prepared from independent stock solutions at concentrations between 13.3 and 1060 nmol/l, 108 and 8670 nmol/l, and 20.4 and 1020 nmol/l, respectively. Control samples in drug-free urine from sheep were prepared at concentrations of 0.532 and 10.6 nmol/l, 4.33 and 86.7 nmol/l, and 0.611 and 1.63 nmol/l, for morphine, M3G and M6G, respectively. The control plasma (0.5 ml) or urine (0.1 ml) was assayed together with the appropriate standards for calibration and batch of unknown samples of either plasma or urine for a particular day. Accuracy was calculated as the measured concentration in the control samples divided by the nominal concentration, and expressed as a percentage. Precision was calculated as the standard deviation of the measured concentrations for the controls divided by the mean values.

2.2.5 Evaluation of Stability in Plasma and Urine

The stability of morphine, M3G and M6G in plasma was evaluated after each had been added to separate samples of drug-free plasma from humans at concentrations of 133 nmol/l,

10.8 µmol/l and 2.04 µmol/l, respectively, and stored at -20°C. The concentration of morphine in the stored samples was determined at 0, 1, 2, 5, 12 and 36 weeks, using method I, and at 101 weeks using method II, by reference to a calibration curve for morphine constructed from freshly prepared standard solutions of morphine. Limited amounts of authentic M3G and M6G precluded the preparation of fresh standards at each time point.

The stability of morphine, M3G and M6G in urine was established in urine collected from a sheep infused for 6 hr with morphine sulphate at the rate of 10 mg/hr, as described in Chapter 5. Urine was collected via an indwelling catheter between 0 and 6 hr and stored at -20°C. Ten days later the urine was thawed to room temperature and the concentrations of morphine, M3G and M6G determined by HPLC. A repeat determination was performed after 16 months of storage at -20°C.

2.3 Results and Discussion

Triplicate injections of 1.127 nmole of M6G synthesized by Dr. G. D. Reynolds gave a mean peak height of 137 mm (0.1 AUFS). This compared with mean peak heights of 128 mm (0.1 AUFS) for morphine-6-ß-D-glucuronide and of 118 mm (0.02 AUFS) for morphine-6-α-D-glucuronide for a 1.148 nmole sample of M6G supplied by the National Institute of Drug Abuse. The chromatogram of the latter sample of M6G contained a peak that was resolved from and had a retention time that was 88% of that for the ß-diastereoisomer. This was similar to the relative retention of 0.83 for morphine-6-α-D-glucuronide found by Murphey & Olsen (1993), and therefore it was taken to be the α-diastereoisomer. No chromatographic peak was observed in the synthesized sample at the assumed retention time for the α-isomer. Assuming the α-diastereoisomer to have an identical molar absorptivity to the ß-isomer, the relative proportions of the α- and ß-isomers in the M6G supplied by the National Institute of Drug Abuse were calculated to be 16% and 84% on the basis of peak height, values comparable to those specified (Section 2.2.1). From the proportions specified by the National Institute of Drug Abuse, the purity of the synthesized M6G was calculated to be 94% and, by comparison with a solution of morphine of known concentration, it was estimated to contain less than 0.1% morphine.

The previous methods had used 10% acetonitrile in 10 mmol/l phosphate buffer to elute morphine, M3G and M6G from the Sep-Pak cartridge (Joel *et al.*, 1988; Svensson, 1986; Svensson *et al.*, 1982). To provide the required sensitivity, up to 1 ml of the eluate was injected onto the HPLC column (Joel *et al.*, 1988). During development of method I, recoveries of morphine, M3G and M6G were greater than 90% with the use of 10% acetonitrile in phosphate buffer, but a minimum of 1.5 ml of eluent was required. However, it was found that the injection of 1 ml volumes of 10% acetonitrile in 10 mmol/l phosphate buffer resulted in poor peak shape for M3G and M6G. Peak height/peak area ratios of 0.76 min^{-1} and 0.91 min^{-1} for M3G and M6G, respectively, after a 1 ml injection were increased to 1.59 min^{-1} and 1.54 min^{-1} after the same amount of each compound was injected in 0.25 ml of 25% acetonitrile in 10 mmol/l phosphate buffer. The peak height/peak area ratios for morphine and hydromorphone remained unchanged at 0.90 min^{-1} and 0.70 min^{-1}, respectively. With method I, progressively increasing percentages of acetonitrile in phosphate buffer (15%, 20%, 25% and 40%) were tried in an attempt to reduce the volume of eluent from the Sep-Pak cartridge and maintain good recovery from plasma. The percentage and volume was established by treating a sample of plasma containing morphine (2660 nmol/l), M3G (2170 nmol/l), M6G (2040 nmol/l) and hydromorphone (3110 nmol/l), as described above, and collecting approximately 0.1 ml fractions of the mixture of acetonitrile in phosphate buffer. For methods I and II, the recoveries of morphine, M3G, M6G and hydromorphone with 25% acetonitrile, determined after HPLC of each fraction and comparison with an aqueous solution of the four compounds, are shown in Figure 2.1.

All four compounds of interest were collected from the Sep-Pak cartridge in 0.5 ml of eluate using method I or 0.8 ml with method II, when plasma and urine from both humans and sheep were used. The use of 40% acetonitrile offered no improvement because of the considerable tailing of the percentage recovery versus volume of elution peaks. Chromatographic peak shape and resolution from endogenous compounds were improved when a reduced volume of eluate from the Sep-Pak cartridge was injected onto the HPLC column. Elution from the cartridge in a stronger eluent allowed a fraction of the eluate similar to that for Joel *et al.* (1988) and Svensson (1986), but greater than that for Svensson *et al.* (1982),

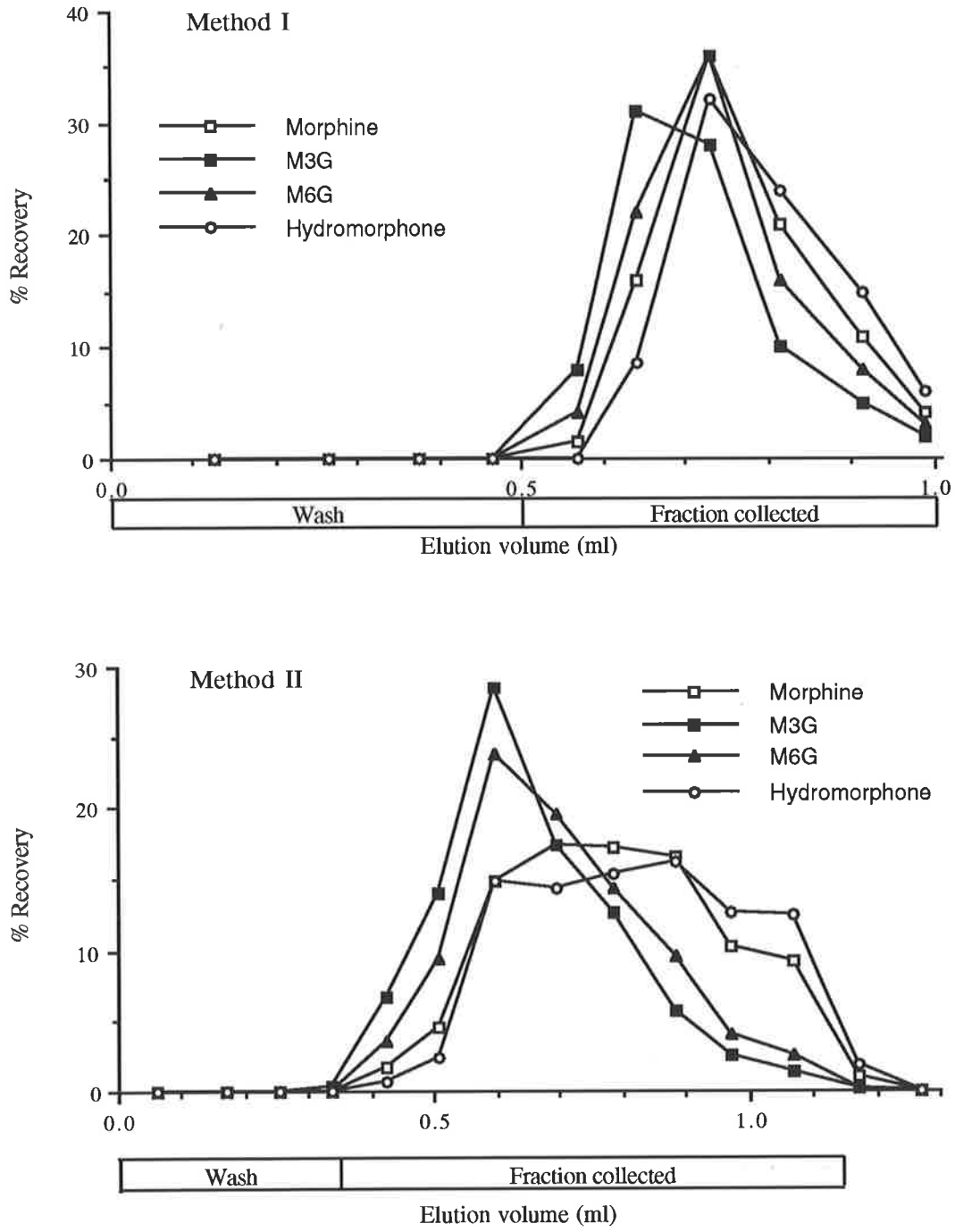


Figure 2.1. *Percentage recovery of morphine (2660 nmol/l), M3G (2170 nmol/l), M6G (2040 nmol/l) and hydromorphone (3110 nmol/l), as a function of the volume of 25% acetonitrile in 10 mmol/l sodium dihydrogen phosphate buffer (pH 2.1) as eluate, from a sample of human plasma treated with a Sep-Pak cartridge using method I (top) and method II (bottom).*

to be injected onto the HPLC column. Later reports have described similar procedures for sample application and elution of interfering compounds from the cartridge, but with the use of predominantly methanolic eluents to collect morphine, M3G and M6G followed by evaporation to dryness and reconstitution in HPLC mobile phase (Hartley *et al.*, 1993; Mason *et al.*, 1991; Murphey & Olsen, 1993). In the present study, unacceptable interferences from endogenous compounds were found when methanol was used.

During the use of either method, the recovery for all four compounds was calculated by comparison of the peak area obtained by direct injection to that obtained after injection of the eluate prepared from each of the calibration standards. Using plasma obtained from humans, the approximate total recoveries for morphine, M3G, M6G and hydromorphone with method I were 90%, 84%, 89% and 86%, respectively. Each cartridge could be used for at least five 1 ml plasma samples without significant losses in recovery or qualitative changes in the HPLC chromatograms. With method II the approximate total recoveries for morphine, M3G, M6G and hydromorphone were 88%, 99%, 85% and 93%, respectively, from plasma of both humans and sheep. When one Sep-Pak cartridge was used to treat fourteen replicate samples of human plasma to which was added morphine (266 nmol/l), M3G (2170 nmol/l), M6G (509 nmol/l) and hydromorphone (1040 nmol/l), the mean ± S.D recoveries with method II were 86 ± 4%, 98 ± 3 %, 89 ± 4 % and 91 ± 5 %, respectively, there being no observable trend with successive samples. There were no qualitative differences in the HPLC chromatograms for the fourteen samples. Re-use of the cartridges for up to fourteen samples (method II) allowed a considerable cost-saving and had not been addressed in previous reports (Joel *et al.*, 1988; Svensson, 1986; Svensson *et al.*, 1982). Prior to application of the next sample, the cartridges were washed with 5 ml water, followed by 10 ml methanol, 5 ml acetonitrile/phosphate buffer and 10 ml water.

A problem encountered with the use of method I for the extraction from plasma was that, after fifteen to twenty injections of eluate into the HPLC, the back-pressure generated at the guard column was approximately doubled and there was a decrease in chromatographic efficiency. When this occurred it was necessary to repack the guard column. Cleaning of the 2 μm filter inserts in the guard column was frequently unsuccessful and they were usually

replaced. The problem was reduced considerably by substituting ammonium sulphate buffer with the sodium bicarbonate/carbonate buffer of method II, so that up to fifty injections of eluate from the Sep-Pak could be performed before replacement of the guard column became necessary. In addition, the 2 μm filter inserts were more easily cleaned, thereby allowing them to be re-used with a considerable cost saving. It is interesting that replacement of the ammonium sulphate buffer with hydrogen carbonate/carbonate buffer effected a change in the elution profile of the four compounds from the Sep-Pak cartridge (Fig 2.1). The reasons for the change in profile and the considerable reduction in back-pressure generated at the HPLC guard column remain unknown.

Using the sample preparation of method II and chromatographic conditions described, typical chromatograms obtained from samples of plasma from sheep which were drug-free or contained morphine (133 nmol/l), M3G (850 nmol/l) and M6G (40 nmol/l) are shown in figure 2.2. Figure 2.3 shows chromatograms obtained from drug-free urine and urine collected during infusion of a sheep with morphine. M3G, M6G, morphine and hydromorphone were eluted after approximately 9, 13, 23 and 33 min, respectively. There were no interferences from endogenous compounds present in drug-free plasma (figure 2.2) or urine (figure 2.3) and the chromatograms obtained from plasma, despite the different methods of preparation (I and II), were similar in appearance. Only minor differences were noticed between humans and sheep in the chromatograms obtained from either plasma or urine. There were no important differences in the appearance of the chromatograms from the plasma of humans when those resulting from the use of two Sep-Pak cartridges, as in the original method of Svensson et al. (1982), were compared with those from one cartridge as in the present method. Thus, sample processing time was reduced considerably without compromising the qualitative appearance of the chromatograms. A chromatographic peak due to endogenous material in plasma, which may interfere with the quantification of morphine, especially with UV detection, is apparent from the reports of Svensson *et al.* (1982) and Svensson (1986). To overcome this problem, the methods of Svensson (1986) and Joel *et al.* (1988) employed coulometric detection while maintaining the original chromatographic conditions (Svensson *et al.*, 1982). However, both methods still required UV (Svensson, 1986) or fluorescence (Joel *et al.*, 1988) monitoring for

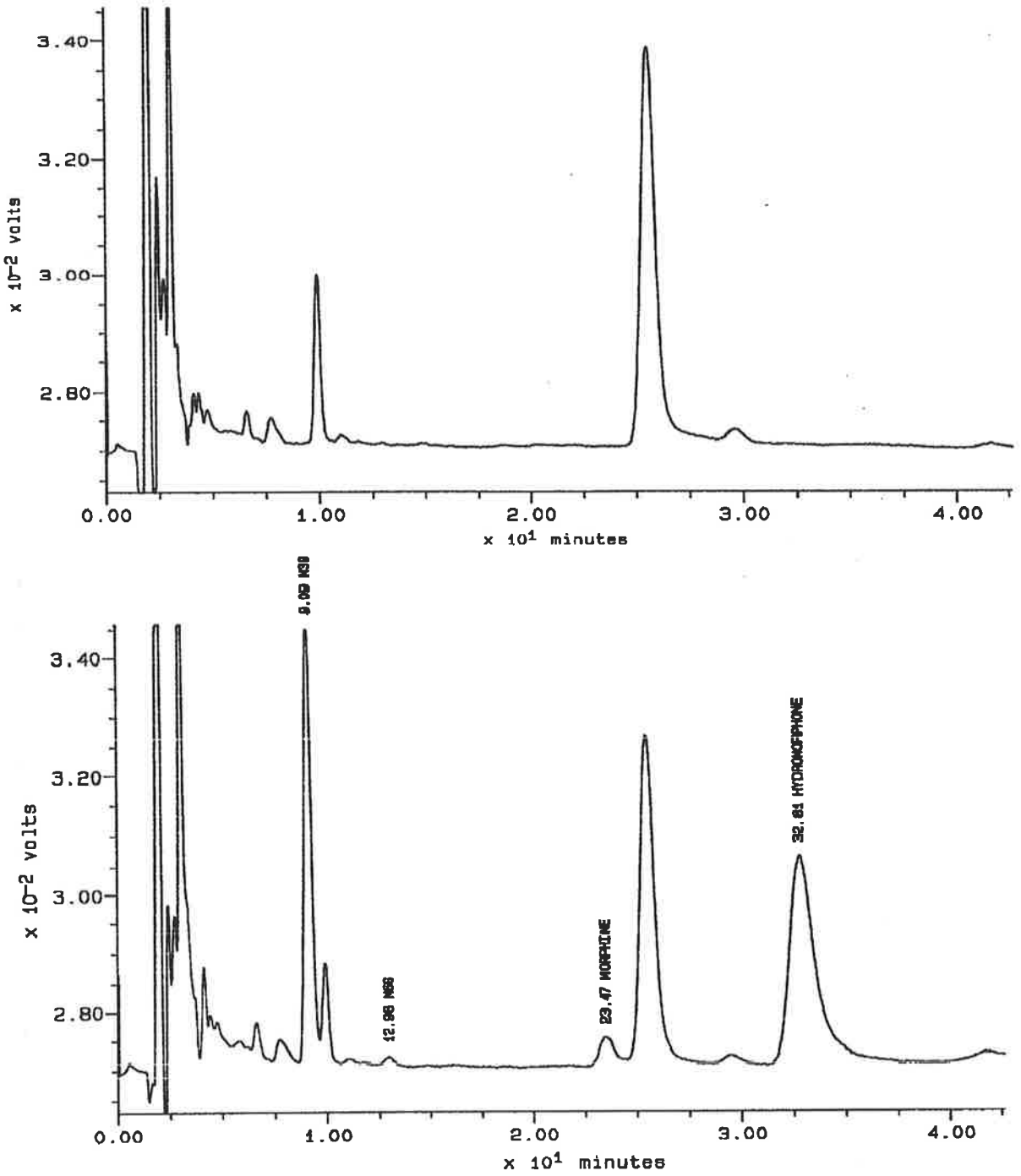


Fig. 2.2. *Chromatograms of: blank plasma (0.5 ml) from sheep (above) and plasma (0.5 ml) from sheep containing morphine (133 nmol/l), M3G (850 nmol/l) and M6G (40 nmol/l) to which hydromorphone (1.55 nmole) was added as internal standard (below). Both samples were prepared for chromatography using method II.*

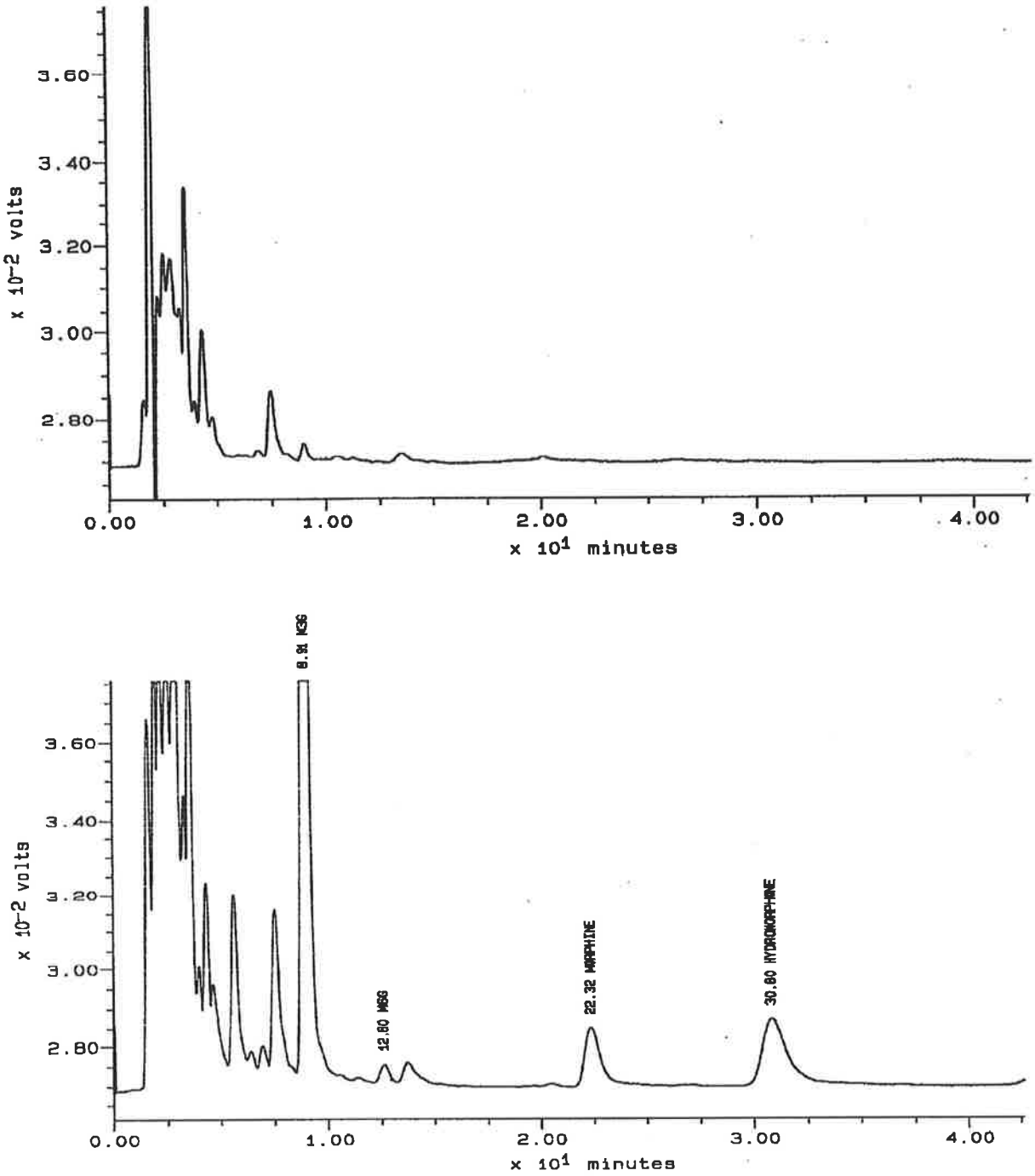


Fig. 2.3. *Chromatograms of: blank urine (0.05 ml) from sheep (above); and urine (0.05 ml) from sheep containing morphine (11 µmol/l), M3G (79 µmol/l) and M6G (1.5 µmol/l) to which hydromorphone (0.78 nmole) was added as internal standard (below). Both samples were prepared for chromatography using method II.*

the detection and quantification of M3G. The reversed-phase column used in the present method gave baseline-separation of morphine from the endogenous compound.

Other solid-phase extraction devices such as the Chrom-Prep PRP-1 320 µl cartridge (Hamilton Co., Reno, NV, U.S.A.) and Baker Octadecyl C_{18} Disposable Extraction Cartridges (J.T. Baker, Phillipsburg, NJ, U.S.A.) were compared with the Sep-Pak cartridges. However, the first generated unsatisfactorily high back-pressures and lost adsorbent with repeated use, while the second displayed poor recovery for M3G and M6G.

Standard curves for morphine, M3G and M6G in plasma from humans and sheep were linear with correlation coefficients greater than 0.990. Within-day precision determined in plasma from humans to which known amounts of morphine, M3G and M6G were added is given in table 2.1.

The concentrations of the three compounds were determined in only four batches of human plasma using method II and, on the four occasions, the concentrations measured in the controls were within 20% of the nominal concentrations.

Between-day accuracy and precision for morphine, M3G and M6G in 0.5 ml plasma from sheep are given in table 2.2. The limits of quantification were set at 20% below the values of 13.3 nmol/l, 108 nmol/l and 20.4 nmol/l, for morphine, M3G and M6G, respectively.

Table 2.1. *Within-day precision for the determination of morphine, M3G and M6G in plasma (1 ml) from humans using methods I and II (n = 5).*

	Morphine		M3G		M6G	
	Conc. (nmol/l)	Precision	Conc. (nmol/l)	Precision	Conc. (nmol/l)	Precision
Method I	26.6	6.2	108	4.5	41	7.4
	266	2.3	4330	4.4	1020	2.9
Method II	13.3	9.3	108	6.6	41	6.7
	266	2.4	4330	4.0	1020	5.9

Table 2.2. *Between-day accuracy (± precision) for morphine, M3G and M6G over a range of concentrations prepared in 0.5 ml of plasma from sheep using method II*

Morphine		M3G		M6G	
Concentration (nmol/l)	Accuracy ± Precision	Concentration (nmol/l)	Accuracy ± Precision	Concentration (nmol/l)	Accuracy ± Precision
13.3	103 ± 15	108	102 ± 15	20.4	98 ± 16
66.5	100 ± 13	217	112 ± 20	40.7	101 ± 12
133	104 ± 5	867	102 ± 7	102	95 ± 10
266	94 ± 7	2170	104 ± 6	204	94 ± 7
532	102 ± 6	4330	98 ± 8	1020	97 ± 5
1060	99 ± 3	8670	96 ± 8	-	-

Table 2.3. *Comparison of between-day precision for the present method (II), using plasma from sheep, with other methods using HPLC for the determination of morphine, M3G and M6G in plasma (0.2 to 1.0 ml) from humans*

Morphine#	M3G#	M6G#	Volume of plasma (ml)	Reference
13 (140)	14 (173)	13 (693)	1.0	Glare *et al.* (1991).
7.5 (175)	7.6 (220)	7.9 (113)	0.2	Hartley *et al.* (1993).
< 10 (70)	< 10 (433)	< 10 (43)	1.0	Joel *et al.* (1988).
7.9 (7.0)	10.0 (87.5)	8.0 (8.8)	0.5	Konishi & Hashimoto (1990).
5.3 (61)	-	3.4 (44)	0.2	Mason *et al.* (1991).
7.6 (77)	1.3 (433)	-	1.0	Svensson *et al.* (1982).*
5.9 (5)	-	-	1.0	Svensson (1986).*
14 (13.3)	15 (108)	16 (20.4)	0.5	Present method.

\# values in parentheses are the lowest concentrations (nmol/l) for which precision was reported.

* within-day, rather than between-day, precision reported.

The data for the lowest concentrations are compared in table 2.3 with the between-day, when given, or with the within-day precision reported by other investigators at the lowest concentrations at which precision was examined.

Table 2.4. *Between-day accuracy (± precision, n = 8), at two nominal concentrations for prepared controls in urine obtained from sheep, when method II was used to extract morphine, M3G and M6G from 0.1 ml of the samples.*

Morphine		M3G		M6G	
Concentration (µmol/l)	Accuracy ± Precision	Concentration (µmol/l)	Accuracy ± Precision	Concentration (µmol/l)	Accuracy ± Precision
0.532	96 ± 10	4.33	99 ± 11	0.611	109 ± 8
10.6	103 ± 6.9	86.7	102 ± 8	1.63	102 ± 6

Standard curves prepared for morphine, M3G and M6G in urine from humans were linear (correlation coefficients > 0.990) over the ranges from 1.33 to 26.6 µmol/l, 10.8 to 217 µmol/l and 2.04 to 50.9 µmol/l for morphine, M3G and M6G, respectively; when urine from sheep was used, curves were linear over the ranges from 0.532 to 10.6 µmol/l for morphine, 4.33 to 86.7 µmol/l for M3G, and 0.41 to 2.04 µmol/l for M6G. Table 2.4 presents the between-day accuracy and precision for the determination of morphine, M3G and M6G in urine from sheep. Analyses of only three batches of human urine were performed and, on the three occasions, the concentrations measured in the controls were within 20% of the nominal concentrations.

The reliability of the method for plasma and urine from sheep was demonstrated further by the lack of any obvious trend in the accuracy of the measurements for the duration of the analyses.

While it is common practice for samples of plasma to be stored for extended periods prior to analysis, there appears to be no information on the stability of morphine and its glucuronide conjugates during storage. Assuming that any instability of the glucuronides during storage would be due to hydrolysis, yielding free morphine, then for some samples of plasma, particularly those from patients receiving morphine when in renal failure, and in whom the

concentrations of the glucuronides in plasma are considerably higher than those of morphine (Osborne *et al.*, 1986; Säwe & Odar-Cederlöf, 1986, Chapter 7), only a small degree of

Table 2.5. *Concentrations of morphine (nmol/l) determined in individual samples of plasma containing morphine, M3G or M6G and stored for a range of times at -20°C.*

Storage Time (Weeks)	Morphine (133 nmol/l*)	M3G (10.8 µmol/l*)	M6G (2.04 µmol/l*)
0	132	< 13.3	< 13.3
1	125	< 13.3	< 13.3
5	138	< 13.3	< 13.3
12	129	< 13.3	< 13.3
36	123	< 13.3	< 13.3
101	126	< 13.3	< 13.3

* nominal concentrations prepared in plasma.

hydrolysis may produce erroneously high concentrations of morphine. It is essential, therefore, to establish the stability of morphine, M3G and M6G in plasma stored frozen after collection from humans or animals administered morphine. The concentrations of M3G and M6G chosen for the study of their stability in plasma approximated those which were achieved in patients with renal failure (Osborne *et al.*, 1986; Säwe & Odar-Cederlöf, 1986).

Morphine, M3G and M6G were stable in human plasma when stored at -20°C for up to 101 weeks. Over this period, the concentration of morphine in samples of plasma spiked with morphine was within 10% of the nominal value (table 2.5). In samples containing M3G (10.8 µmol/l) or M6G (2.04 µmol/l) the concentration of morphine was less than 13.3 nmol/l. Therefore, plasma containing morphine, M3G and M6G can be stored at -20°C for at least this period without any significant change in the concentration of morphine or hydrolysis of the glucuronides to morphine.

With regard to urine, the majority of a dose given to humans was excreted in urine as M3G and M6G (Hasselström & Säwe, 1993; Osborne *et al.*, 1990; chapter 3) and, as found in

Chapters 5, 6 and 7, as M3G by sheep, but urinary excretion was a minor route for the elimination of morphine by both species (figure 1.1 and Chapters 5, 6 and 7). Therefore, with the markedly higher concentrations of M3G, especially, produced in urine, hydrolysis of this compound to morphine could result in overestimated and underestimated renal clearances for morphine and M3G, respectively. The concentrations of morphine, M3G and M6G determined in urine collected from sheep and stored at -20°C for 16 months were 100%, 105% and 104% of the concentrations determined after storage for 10 days. No samples of urine from humans or sheep were stored for a period longer than 16 months.

In conclusion, this chapter describes improvements to the HPLC method for the simultaneous determination of morphine, M3G and M6G in plasma (Joel *et al.*, 1988; Svensson, 1986; Svensson *et al.*, 1982). Most of the methods published subsequently involve modifications to the original method of Svensson *et al.* (1982). The method of Konishi & Hashimoto (1990) is the superior method, but it was not available when studies for this thesis had begun and it requires additional equipment (a column-switching valve and coulometric detector) which was not available for the present studies. Together with changes to the method for sample pretreatment, which allows greater economy in the use of Sep-Pak cartridges and a smaller elution volume, the present method adopts chromatographic modifications which permit the determination of lower concentrations of morphine than was possible previously with UV detection alone. The improved procedure was used to determine the stability of morphine, M3G and M6G, during storage in human plasma and in urine from sheep. Unless otherwise stated, method II will be used throughout the remainder this thesis.

Chapter 3

The Influence of Renal Function on the Renal Clearance of Morphine and its Glucuronide Metabolites in Intensive Care Patients

3.1 Introduction

The major route of elimination for M3G and M6G in subjects with normal renal function is via renal excretion (Osborne *et al*, 1990; Säwe, 1986). Limited data from the literature (Säwe & Odar-Cederlöf,1987; Wolff *et al.*, 1988; Osborne *et al.*, 1988; Peterson *et al.*, 1990) suggest that the elimination of M3G and M6G is influenced by renal function. In patients given morphine, the concentrations of M3G and M6G in plasma increased relative to those of morphine as renal function decreased (Peterson *et al.*, 1990; Säwe, 1986). No studies to date have fully investigated the relationship between renal function and the renal clearance of the individual glucuronide metabolites. In view of the possible influence of these metabolites on the analgesic and adverse effects of morphine, a greater knowledge of this relationship may be of value in predicting the concentrations of the glucuronides that are likely to be achieved in plasma when morphine is given to patients with diverse renal function.

Therefore, the aims were to assess the influence of renal function on the renal clearance of morphine, M3G and M6G; to gain an insight into their possible mechanisms of renal excretion; and to determine if clinical assessment of renal function allows prediction of the concentrations of M3G and M6G likely to be achieved in plasma during constant infusion with morphine.

3.2 Methods

3.2.1 Patients

Fifteen patients in an intensive care unit, with varying degrees of renal function but without any clear evidence of hepatic dysfunction, participated in the study. Details for each

Table 3.1. *Demographic and clinical details of patients*

No	Sex	Age (y)	Wt (kg)	Duration of infusion[a]	Infusion rate (mg/hr)[b]	Diagnosis	Concomitant medication*
1	F	19	56	16 d	2.26	Motor vehicle accident	1,2
2	F	20	60	29 d	3.76	Viral pneumonia, pregnant 24/52	1,2,29
3	M	78	55	12.5 hr	1.50	Gastrointestinal bleeding, pulmonary oedema	3,4
4	F	61	55	3 d	1.50	Pneumothorax, peritonitis	3,5,6,7,29
5	M	72	64	6 hr	1.50	Axillo-bifemoral bypass	8,9,10,11,12,13
6	F	67	58	12 hr	2.26	Oesophago-gastrectomy	3,12,14
7	M	75	65	0 hr[c]	-	Ruptured aortic aneurysm	12,14,15,16,17,18,19,20
8	M	26	75	1 hr	1.50	Motor vehicle accident	21,22,23
9	M	74	70	6 hr	1.50	Partial cholecystectomy, pneumonia, alcoholic dementia, sub-dural haematoma	3,5,6,7,12,16,24
10	F	62	85	0.5 hr	3.01	Acute respiratory failure, pneumonia, breast cancer	7,12,25
11	M	57	70	4 d	1.50	Acute respiratory distress, oesophageal stricture	3,7,12,18,25
12	M	23	65	2 d	3.01	Metastatic aplastic carcinoma	7,8,11,20,24,26
13	M	62	70	9 hr	1.50	Aortic aneurysm	10,18,32
14	F	17	44	3.5 hr	3.01	Mesoatrial graft	1,7,12,16,20,27,28
15	F	69	62	0 hr[d]	-	Septicaemia, pneumonia, acute renal failure	7,12,16,18,30,31

* Key to concomitant medication: 1 = diazepam, 2 = vecuronium, 3 = midazolam, 4 = cefoxitin, 5 = gentamicin, 6 = metronidazole, 7 = salbutamol, 8 = paracetamol, 9 = temazepam, 10 = cephalothin, 11 = ranitidine, 12 = B group vitamins, 13 = Haemacell®, 14 = digoxin, 15 = folic acid, 16 = vitamin K, 17 = ascorbic acid, 18 = pancuronium, 19 = calcium chloride, 20 = frusemide, 21 = tetanus toxoid, 22 = metoclopramide, 23 = oxygen, 24 = amoxicillin, 25 = imipenem, 26 = hydrocortisone, 27 = nystatin, 28 = oxycodone, 29 = total parenteral nutrition, 30 = betamethasone cream, 31 = penicillin G, 32 = chlorpromazine.

a duration of constant infusion prior to study

b as morphine base (1 mg is equivalent to 3500 nmole)

c morphine infusion rate altered during the urine collection.

d morphine infusion ceased at the beginning of the urine collection.

patient are given in table 3.1. The study was approved by the Human Ethics Committee of the Royal Adelaide Hospital and the committee on the Ethics of Human Experimentation of the University of Adelaide.

3.2.2 Experimental Design

Morphine sulphate B.P. was administered by i.v. infusion in doses ranging from 2 to 5 mg/hr (0.020 to 0.122 mg/kg/hr morphine base), the dose having been determined on clinical grounds.

Two arterial blood samples (5 ml) were collected from each patient approximately four hours apart, and the plasma separated by centrifugation. The bladder was emptied prior to collection of the first blood sample and then all urine produced was collected via a catheter up to the time of the next blood sample. The urine volume and pH (measured by pH meter) were recorded. Plasma and aliquots of urine were stored at -20^{o}C prior to analysis.

3.2.3 Analytical Techniques

The HPLC method described in chapter 2 was used to determine the concentrations of morphine, M3G and M6G in 1 ml of plasma and diluted urine (diluted 1 in 50 to 1 in 200 with water). Calibration standards were prepared in drug-free plasma from healthy humans at concentrations ranging from 13.3 to 266 nmol/l, 108 to 2167 nmol/l and 20.4 to 1020 nmol/l for morphine, M3G and M6G, respectively. Standard curves were linear with correlation coefficients greater than 0.990. When the concentrations in plasma from patients were found to be higher than the maximum calibration standard, then the unknown samples were diluted appropriately with drug-free plasma from humans and reanalyzed. Standards were prepared in 1 ml of drug-free urine from healthy humans (diluted 1 in 50 with water) over the range 1.33 to 26.6 µmol/l, 10.8 to 217 µmol/l and 2.04 to 50.9 µmol/l for morphine, M3G and M6G, respectively; the values representing concentrations in undiluted urine. Concentrations greater than the highest standard were diluted further with water and reanalyzed.

Creatinine in plasma and urine was determined by a modification of the HPLC method of Huang & Chiou (1983). Briefly, 0.1 ml of plasma was mixed with 0.25 ml acetonitrile,

centrifuged, and 30 µl of the supernate was injected onto the column; 0.05 ml urine was mixed with 2 ml of 10% acetonitrile in water and 25 µl was injected (Wisp Model 710A Autosampler, Waters Assoc., Lane Cove, Australia) onto a column packed with Partisil-10 SCX (Whatman Chemical Separation, Inc., Clifton, NJ, U.S.A.). The mobile phase, 10% acetonitrile in 0.02 mol/l ammonium dihydrogen phosphate buffer (pH 4.8), was pumped at 1.5 ml/min (model 6000A pump, Waters Assoc.), and monitored at 250 nm (model 440 detector, Waters Assoc.). Standards were prepared by adding known amounts of creatinine to 'blank' plasma and urine to include the ranges 22.1 to 177 µmol/l and 0.884 to 17.7 mmol/l, respectively. Within-day accuracy (± precision, n = 10) for plasma was 103 (± 5)% and 106 (± 2.3)% of nominal values in five control samples containing prepared concentrations of 44.2 and 177 µmol/l, respectively; in urine (n = 10) it was 104 (± 4.4)% and 99.3 (±4.8)% at prepared concentrations of 4.42 and 15.5 mmol/l, respectively.

The concentration of creatinine in plasma on the day of the study was also obtained from the patients' records, as part of the routine biochemical analyses. The concentration was determined by the Jaffé method, as modified for automated analysis (SMAC II, Technicon Corporation, Tarrytown, NJ, U.S.A.).

3.2.4 Studies of the Binding in Plasma

Blood was collected from five healthy drug-free volunteers (age 25 to 39 years) into heparinized tubes and centrifuged immediately. Plasma was removed, spiked with morphine (266 nmol/l), M3G (2170 nmol/l) and M6G (1020 nmol/l), and subjected to ultrafiltration at 37°C using an MPS-1 apparatus fitted with a YMT membrane (Amicon, Danvers, MA, U.S.A.). The concentrations of morphine, M3G and M6G in aliquots of filtrate (0.5 ml) and plasma (1.0 ml), determined by HPLC, were used to calculate the fraction unbound in plasma. Comparison of their concentrations in a solution of isotonic phosphate buffer before and after ultrafiltration indicated that there was no binding of morphine, M3G or M6G to the apparatus used for the ultrafiltration.

Plasma from healthy volunteers was used because an insufficient volume remained from most of the patients after the total concentrations of morphine, M3G and M6G had been measured.

3.2.5 Data Analysis

The data in tables A1.1 and A1.2 were used to calculate the renal clearances of morphine, M3G and M6G as the respective rates of urinary excretion divided by the means of the two concentrations in plasma spanning the interval over which urine was collected. The renal clearances of unbound morphine, M3G and M6G were calculated by dividing the respective renal clearances by the respective unbound fractions determined in healthy subjects.

For the ten patients with a rate of infusion that was constant for at least 6 hr before the first blood sample, the total body clearance of morphine from plasma was calculated as rate of infusion divided by the mean concentration in plasma. In addition, for the same ten patients, the recovery of the dose in urine as morphine, M3G and M6G was calculated from the amount excreted as each compound divided by the amount of morphine infused during the time of urinary collection.

The ratios of the concentrations for M3G/M, M6G/M and M3G/M6G in plasma for each patient were calculated from the means of the concentrations of the glucuronides and morphine determined in the two samples of plasma.

The renal clearance of creatinine from plasma ($CL_{Cr,meas}$), as a marker of renal function, was calculated as the rate of urinary excretion divided by the concentration of creatinine in the first sample of plasma, the concentrations of creatinine in urine and plasma having been determined by HPLC. In addition, the predicted clearance of creatinine ($CL_{Cr,pred}$),was calculated from the formula below:

$$CL_{Cr,pred} = \frac{(140 - age) \times B.Wt.}{0.814 \times S_{Cr}(\mu mol/l)}$$

(Cockcroft & Gault, 1976) based on age, sex (value obtained above multiplied by 0.85 for females), body weight (B.Wt.) and the concentration of creatinine in plasma (S_{Cr}) obtained from the patients' records (table A1.3).

Possible relationships between variables were examined by simple or multiple linear regression, and comparison of means between two or more variables was performed using Student's paired t-test or one-way analysis of variance (Statview+^{TM}, Brainpower Inc., Calabasas, CA, USA). A *posteriori* comparisons were performed using the Scheffé F-test. Values of p less than 0.05 were considered significant. Where appropriate, means ± standard deviations are presented.

3.3 Results

The times over which the rate of infusion with morphine had been maintained prior to the beginning of the study are listed in table 3.1, as are the rates of infusion. Individual data for the concentrations of morphine, M3G and M6G, for creatinine in plasma and urine, and the volumes of urine are presented in tables A1.1, A1.2 and A1.3.

The means of the two concentrations of morphine, M3G and M6G in plasma achieved in the individual patients ranged from 47.9 to 332 nmol/l, 309 to 37,500 nmol/l and 93.0 to 11,500 nmol/l, respectively. During the urinary collection interval, the change in the concentrations of morphine in plasma (calculated for each patient as the difference divided by the mean of the two values and multiplied by 100) in the ten patients receiving morphine at a constant rate of infusion for at least 6 hr ranged from -31.2% to +45.8%; in the four patients in whom the rate of infusion had been altered within 6 hr prior to the first blood sample, the change in the concentrations of morphine in plasma ranged from -59.1% to +35.9%. For these fourteen patients, the ratios of the concentrations in plasma of M3G/morphine and M6G/morphine ranged from 3.95 to 167 and 0.79 to 51.3, respectively (table 3.2). The concentrations of morphine in the plasma from patient 7 were excluded because of a change in the rate of infusion of morphine between collection of the two samples. For each of the fifteen patients, the change in the concentrations of M3G and M6G in plasma ranged from -23.1% to +27.3%, and -24.4% to +27.6%, respectively. The mean (n = 15) ratio of the concentrations in plasma of M3G/M6G (table 3.2) was 5.01 ± 1.12. For patient 7, the concentrations of M3G and M6G in plasma changed by only 22% and 12%, respectively, over the urinary collection period and hence the data for the glucuronides were included in the study.

Table 3.2 *Ratios of the concentrations of morphine, M3G and M6G in plasma, their renal clearances, and those measured and predicted for creatinine, and the total clearance of morphine in the patients*

	Ratio of the concentrations in plasma				Renal clearance (ml/min)				Total clearance (ml/min)
No.	M3G/M	M6G/M	M3G/M6G	Morphine	M3G	M6G	Creatinine[a]	Creatinine[b]	Morphine
1	6.77	1.06	6.36	259	159	207	172	104	1510
2	6.33	1.06	5.95	135	67.4	87.0	113	188	1670
3	11.4	1.94	5.87	60.7	51.3	78.6	44.9	46.6	1120
4	17.8	2.72	6.56	192	93.7	115	130	75.7	1670
5	26.6	5.5	4.84	35.5	33.7	27.0	37.7	35.7	1250
6	4.63	0.98	4.74	47.8	29.6	38.6	52.6	88.5	553
7	-	-	5.05	-	1.69	2.11	2.53	27.4	-
8	3.95	1.45	2.72	110	121	75.1	108	117	-
9	13.6	2.52	5.41	51.4	18.5	16.5	48.7	81.2	1230
10	48.6	8.57	5.67	23.7	7.23	13.2	13.5	21.7	677
11	13.0	2.27	5.72	115	69.2	75.4	96.6	57.8	-
12	46.0	11.5	4.00	22.7	7.87	6.22	10.1	29.2	431
13	11.3	2.93	3.87	119	96.8	107	100	84.0	1840
14	4.09	0.79	5.20	64.0	73.5	58.2	108	113	-
15	67.2	51.3	3.26	17.1	2.72	2.93	3.59	9.79	-

a measured clearance of creatinine

b predicted clearance for creatinine

The renal clearance of creatinine ($CL_{Cr,meas}$) ranged from 2.53 to 172 ml/min table 3.2). There were significant linear relationships between the concentrations of creatinine in plasma (mmol/l) determined by the HPLC and Jaffé methods (Jaffé = 1.13 HPLC + 4.33, r = 0.983, 14 df, $p < 0.001$), and between $CL_{Cr,pred}$ and $CL_{Cr,meas}$ ($CL_{Cr,pred} = 0.666\ CL_{Cr,meas} + 25.8$, r = 0.740, 14 df, $p < 0.002$). Exclusion of the three patients receiving cephalosporins (which

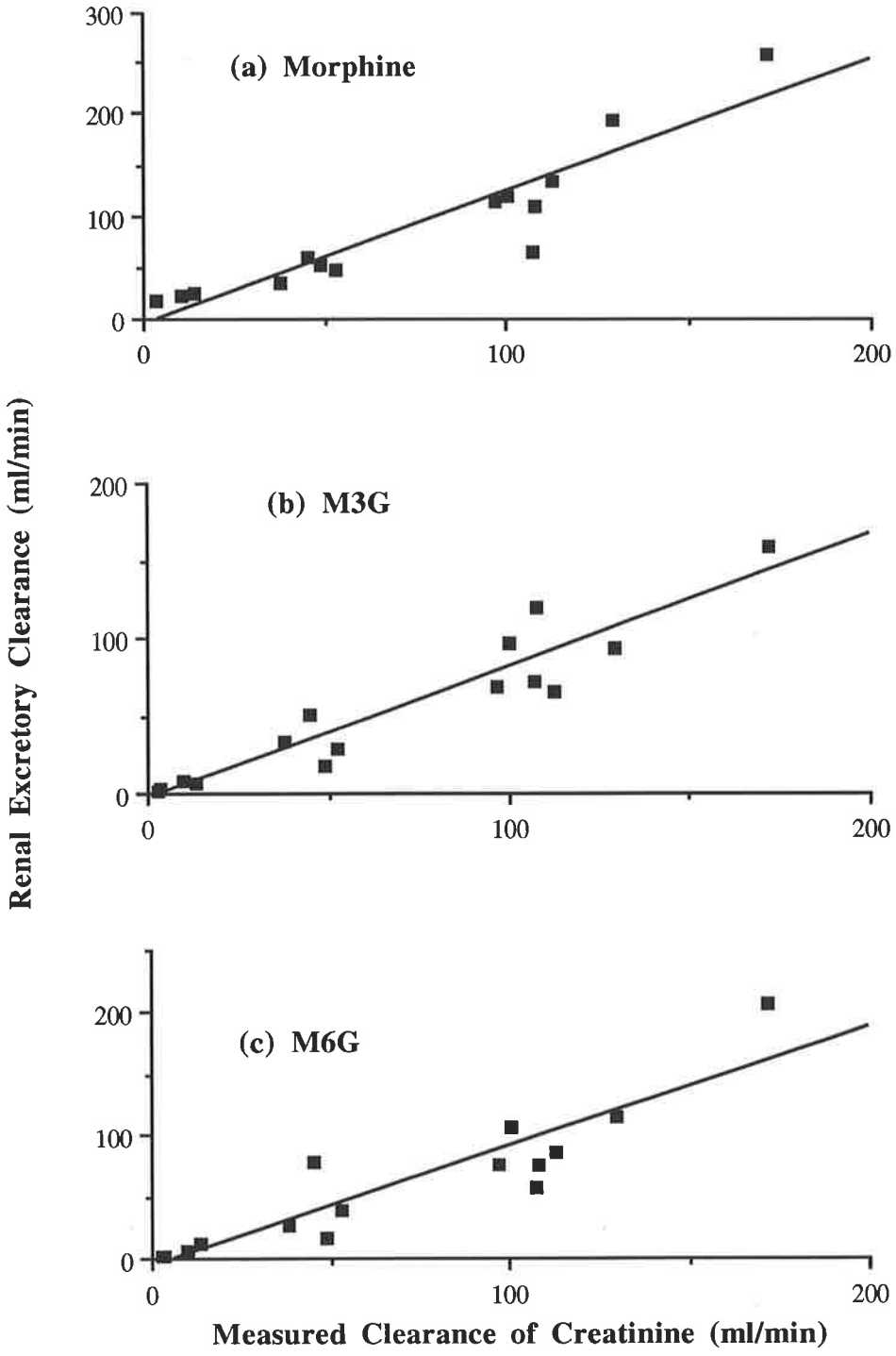


Figure 3.1. *The relationship between renal function, assessed by the renal clearance of creatinine, and the renal clearance of (a) morphine (y = 1.28x - 5.77, r = 0.926, 13 df, p < 0.001), (b) M3G (y = 0.86x - 3.75, r = 0.943, 14 df, p < 0.001) and (c) M6G (y = 0.97x - 6.47, r = 0.910, 14 df, p < 0.001).*

may interfere with the Jaffé method) did not alter substantially the regression between the concentrations of creatinine determined in plasma by the two methods.

There were significant linear relationships between the renal clearances of either morphine, M3G or M6G and $CL_{Cr,meas}$ ($r > 0.910$, $p < 0.001$, figure 3.1). The 95% confidence interval for the slope of the regression line was 0.95-1.61, 0.67-1.04 and 0.70-1.23 for morphine, M3G and M6G, respectively. There was no improvement when, using multiple regression analysis, the additional influence of urine flow or pH was considered for each compound. The renal clearance of morphine was significantly ($p < 0.001$) greater than the renal clearance for either glucuronide. There was no significant difference ($p > 0.50$) in renal clearance between the glucuronides.

The mean fraction of morphine, M3G and M6G unbound in the plasma from five healthy volunteers was 0.74 ± 0.02, 0.85 ± 0.04 and 0.89 ± 0.05, respectively. When these values were used to calculate the renal clearances of unbound morphine, M3G and M6G in plasma, the slope of the regression line for the renal clearance of unbound morphine against $CL_{Cr,meas}$ was significantly greater than unity ($p < 0.004$, 95% confidence interval of 1.29 to 2.18); similar regression analyses for M3G and M6G revealed slopes not different from unity ($p > 0.50$, 95% confidence interval of 0.79 to 1.22 and 0.79 to 1.38, respectively).

Ten of the patients were maintained on a fixed rate of infusion for at least 6 hr, and for the two who received a constant infusion for 6 hr only, the change in the concentration of morphine in plasma over the period of study was +17% and +35%. In this group of ten patients (table 3.2), the clearance of morphine with reference to plasma ranged from 431 to 1840 ml/min (mean: 1190 ± 500 ml/min) or 6.6 to 30.3 ml/min/kg (19.4 ± 8.4 ml/min/kg). There was no significant difference ($p > 0.98$) in the mean clearance of the five patients infused at a constant rate for between 6 and 24 hr, and the five infused for longer than 24 hr. The total recovery of the dose of morphine as morphine, M3G and M6G in urine ranged from 26% to 130% and was not related to $CL_{Cr,meas}$ ($p = 0.085$, 9 df). Individual recoveries of morphine, M3G and M6G ranged from 1.30% to 17.2%, 20.5% to 100% and 3.40% to 18.7%, respectively.

For the ten patients who received a constant infusion for at least 6 hr, figure 3.2 shows the association between the concentrations of M3G and M6G in plasma, normalized for the

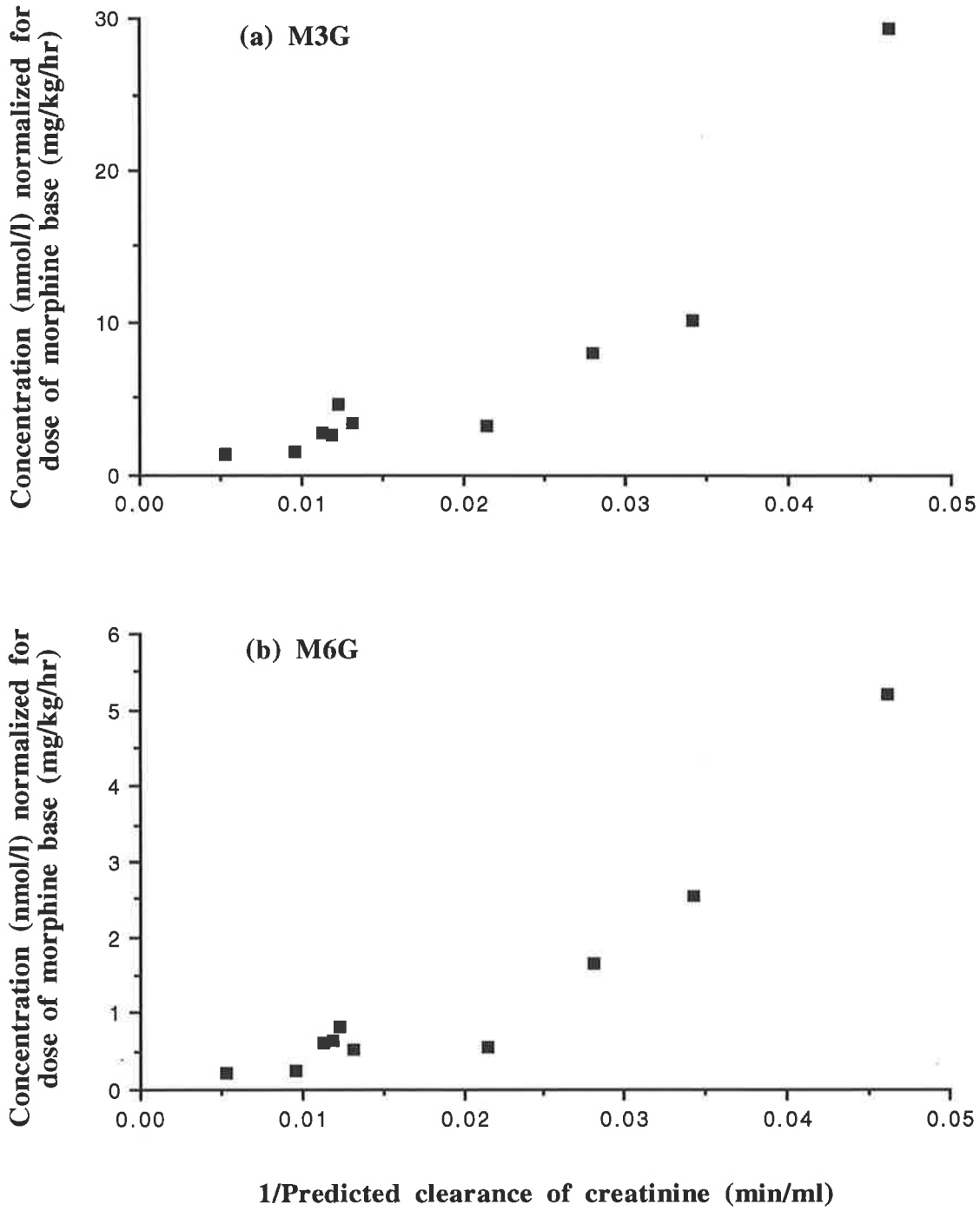


Figure 3.2. *Spearman rank analysis of the association between the dose-rate/kg-normalized concentration of (a) M3G (r_s = 0.976, n = 10, p < 0.001) and (b) M6G (r_s = 0.927, n = 10, p < 0.001) in plasma, and the reciprocal of the clearance of creatinine predicted from the Cockcroft-Gault formula.*

molar dose-rate of morphine per kg body weight, and $1/CL_{Cr,pred}$. Spearman rank-order analysis revealed these to be significant associations ($p < 0.001$).

3.4 Discussion

Previous pharmacokinetic studies have been performed during continuous i.v. infusion with morphine in postoperative patients with apparently normal hepatic and renal functions (Persson *et al.*, 1986; Tanguy *et al.*, 1987). After correcting for the ratio for C_b/C of 1.1 determined for morphine in humans (Patwardhan *et al.*, 1981), the values for the clearance from plasma found in the present study are similar in distribution to the values for the clearance from blood reported by Persson *et al.* (1986). However, they are approximately half the values with reference to plasma reported by Tanguy *et al.* (1987). The patients included in the present study had varying degrees of renal function but no overt hepatic dysfunction. Those included in the calculation for the clearance of morphine from plasma had been infused at a constant rate for between 6 hr and 29 days and it is possible that steady-state concentrations of morphine may not have been achieved in the patients infused for the shorter periods. However, there was no difference between the patients infused from 6 hr to 24 hr and those infused for greater than 24 hr in the estimated values for clearance.

There have been no previous reports on the concentrations of M3G and M6G achieved in plasma during continuous i.v. infusion with morphine. The ratios of the concentrations of M3G/morphine and M6G/morphine in plasma approximated those found previously in patients with cancer and infused with s.c. morphine (Peterson *et al.*, 1990). The wide range in ratios reflects the extremes of renal function in the patients and was not unexpected, since the glucuronides are cleared primarily by urinary excretion, while morphine is cleared principally by metabolism (section 1.5.3.1). The consistency in the ratio of the concentrations of M3G/M6G in plasma between patients and the similarity in renal clearances of the glucuronides within patients suggests a consistency between patients in the relative metabolic clearance for the formation of both compounds from the parent morphine.

The mean ratio for the concentrations of M3G/M6G in plasma of 5.01 ± 1.12 was comparable to the value of 5.5 ± 0.9 reported by Osborne *et al.* (1990) after an i.v. dose of

morphine given to healthy volunteers, and of 5.8 (median) (Goucke *et al.*, 1994) and 6.4 ± 1.5 (Somogyi *et al.*, 1993) determined in patients with cancer and given chronic oral doses of morphine. However, it was lower than the value of 9.4 ± 2.7 found by Säwe (1986) during chronic oral dosing of patients with cancer. The latter author employed the HPLC method of Svensson *et al.* (1982) with UV detection and, without an authentic sample of M6G, assumed a similar molar absorptivity to that of M3G. With an authentic sample of M6G in the present study, it was found that M3G exhibited a 42% greater UV response than M6G (see section 2.2.3), which may explain the higher value obtained by Säwe (1986) for the ratio of M3G/M6G.

When morphine was given orally to patients with normal renal function, the mean ratio of the area under the concentration-time curve of M3G in plasma to that of morphine was 24.3 ± 11.4 (range 9.3 to 42.9) while for M6G to morphine the ratio was 2.7 ± 1.4 (range 1.0 to 6.1) (Säwe, 1986). However, when morphine was given orally to patients with renal failure, the plasma concentrations of M3G and M6G were markedly increased relative to morphine (Säwe, 1986; Peterson *et al.*, 1990). This suggests that the elimination of the glucuronides was decreased since Säwe & Odar-Cederlöf (1987) and Woolner *et al.* (1986) found that the total clearance of morphine was not significantly impaired by renal dysfunction. Osborne *et al.* (1993) found that the total clearance was reduced in patients with renal failure, but only by 40%, while the values of AUC for M3G and M6G appeared to be increased by more than a factor of ten. Osborne *et al.* (1986) and Don *et al.* (1975) have reported cases of morphine intoxication in patients with renal failure and Osborne *et al.* (1986), finding markedly elevated concentrations of M3G and M6G in plasma in the absence of measurable concentrations of morphine, attributed the effect to the accumulation of M6G. Regnard & Twycross (1984) found that pain in patients with impaired renal function was controlled with below average doses of morphine.

Earlier studies of the effect of renal function on the elimination of M3G and M6G have provided limited data and have been reviewed in Chapter 1. The results of the present study demonstrate a significant relationship between the renal clearance of morphine, M3G and M6G, and renal clearance of creatinine, and underlines the importance of renal function in the

clearance of M3G and M6G and their possible contribution to toxicity when morphine is administered to patients with impaired renal function.

Renal function in the clinical setting is usually assessed after the determination of the concentration of creatinine in plasma by automated methods. In the present study, an automated colorimetric method and a more specific HPLC method were used. While there was a significant relationship between the two methods, values obtained by the colorimetric procedure were approximately 13% greater than those determined by HPLC and may reflect the lack of specificity with the Jaffé method. However, the concentration of creatinine, as such, is not a reliable indicator of renal function since it is dependent not only on renal clearance, but also on the rate of appearance of creatinine in plasma, the latter dependent on the muscular mass of the patient and on the intake of dietary protein. In the clinical setting and without a urinary collection, it was found previously that a suitable indicator of renal function was the renal clearance of creatinine predicted from a nomogram which includes the concentration of creatinine in plasma, and the age, sex and body weight of the patient (Cockcroft & Gault, 1976). The clinical details of the patients studied by Cockcroft & Gault (1976) were not given, although those patients with a urinary output of creatinine less than 10 mg/kg per 24 hr and with successive daily concentrations of creatinine in plasma differing by more than 20% were excluded. The nomogram developed by the above authors, however, may be inappropriate for severely ill patients under intensive-care. In the present study, three of the patients showed evidence of decreasing renal function prior to the study and eight had a urinary output of creatinine which was less than 10 mg/kg per 24 hr, findings common to severely ill patients. The regression between $CL_{Cr,pred}$ and $CL_{Cr,meas}$ in the present study, revealed a slope (0.666) and intercept (25.8) not significantly different ($p > 0.4$) from the respective values of 0.81 and 14.0 reported by Cockcroft & Gault (1976). However, when studying a large group of intensive-care patients, Martin *et al.* (1990), using the Jaffé method, found no significant relationship between the clearance of creatinine predicted from the Cockcroft-Gault formula and the measured clearance of creatinine. A modified nomogram derived from a larger group of severely ill patients maybe more appropriate. Nevertheless, in the absence of a more suitable nomogram and with an insufficient number of patients included in the present study to allow

one to be formulated, the formula of Cockcroft-Gault was chosen because of its previously demonstrated reliability in subjects with varying degrees of renal function (Luke *et al.*, 1990) and its widespread use.

Given that the major route of elimination for the glucuronide metabolites is by renal excretion, it would be expected that, for a given dose of morphine, as renal function decreased the concentrations of both M3G and M6G in plasma would increase. Indeed, with decreasing $CL_{Cr,pred}$, there was an increase in the dose-normalised concentrations of both glucuronides in plasma. Moreover, Spearman rank-analysis revealed a significant association between the dose-normalised concentrations of M3G and M6G and the reciprocal of $CL_{Cr,pred}$ (figure 3.2). This may provide a means of predicting the concentrations of M6G likely to be achieved in plasma during i.v. infusion with a prescribed rate of morphine in intensive-care patients. However, in addition to the comments regarding the prediction of the clearance of creatinine from its concentration in the plasma of these patients, the above prediction concerning M6G should be tempered further; firstly by the difficulty in measuring the body-weight accurately in patients who further deteriorate during a prolonged stay in the intensive-care unit and, secondly, by the probability that, in some of the patients included in the present study, steady-state plasma concentrations of M3G and M6G will almost certainly not have been fully achieved. Furthermore, there is insufficient information at present on the relationship between the concentrations of M6G in plasma and analgesia or possible side-effects.

The mean fraction unbound in plasma for morphine of 0.74 was comparable to the values ranging from 0.65 to 0.88 determined previously in healthy volunteers (Baggot & Davis, 1973; Judis, 1977; Leow *et al.*, 1993; Olsen, 1975; Olsen *et al.*, 1975; Patwardhan *et al.*, 1981; Pöyhiä & Seppälä, 1994). The fraction was marginally greater (0.69 versus 0.65) in patients with renal failure (Olsen *et al.*,1975). The value found in the present study was similar to that determined by Raveedran *et al.* (1992) in cancer patients with raised α_1-acid glycoprotein and hypoalbuminaemia. In view of the comments made in section 1.5.2.1, it may be assumed that in the present study the mean fraction unbound of 0.74 determined in healthy subjects would approximate that in the fifteen patients. Similar assumptions may be made for the influence of renal impairment on the binding of M3G and M6G in plasma, both compounds having a lower

binding compared to morphine. The mean values for the fraction of M3G and M6G unbound in plasma from humans, 0.85 and 0.89, respectively, have not been reported previously .

Shemesh *et al.* (1985) and Rapoport & Husdan (1968) showed that the renal clearance of creatinine overestimated GFR, notably at lower values of true GFR (inulin clearance), and attributed the overestimate to an increasing fractional contribution by tubular secretion to the renal clearance. Nevertheless, for the type of patients included in this study, the renal clearance of creatinine remains the most convenient method for estimating renal function.

The renal clearance of unbound morphine was found to be significantly greater than GFR, suggesting net secretion of morphine into urine during passage through the kidney. The renal clearances of unbound M3G and M6G were not significantly different from GFR suggesting that negligible net secretion or reabsorption after filtration at the glomerulus. The lack of effect of urine pH and flow further indicated that reabsorption of M3G and M6G from the tubular lumen of the human kidney was minimal. These conclusions were not altered if approximate corrections, from the data of Shemesh *et al.* (1985), were made to the measured clearances of creatinine to achieve more realistic estimates of GFR. However, the conclusions with respect to M3G and M6G may be influenced by the possible formation of these two compounds by the kidney and their subsequent excretion in urine. Later chapters in this thesis (Chapters 5, 6 and 7) propose that morphine is metabolized by the kidney of the sheep, mainly to M3G. Furthermore, *in vitro* studies have reported the formation of M3G and M6G by microsomes prepared from the kidney of humans (Yue *et al.*, 1988), and of morphine glucuronides by proximal tubular segments from the kidney of the rabbit (Schäli & Roch-Ramel, 1982) and microsomes from the kidney of humans (Cappiello *et al.*, 1991).

The results of the present study are supported by the findings of Garrett & Jackson (1979) in the dog. These authors found that the renal clearance of unbound morphine was greater than GFR while the renal clearance of unbound M3G was similar to GFR. Studies in the chicken (May *et al.*, 1967; Hakim & Fujimoto, 1971) suggest that a cationic system of transport is responsible for the active secretion of morphine. Further studies are needed to elucidate the mechanisms by which M3G and M6G are excreted by the human kidney. This

may be important in predicting the likely effects of concomitant drugs (or their metabolites) which may be excreted by similar mechanisms.

In conclusion, both the relationships between renal function and the renal clearances of M3G and M6G, and the predicted concentrations of the glucuronides likely to be achieved in the plasma of patients of diverse renal function, may be useful clinically. However, a greater understanding of the pharmacodynamics of morphine, M3G and M6G in humans will be required before the findings of this study can be fully utilised.

Chapter 4

Description of the Preparation of the Chronically-catheterized Sheep, and the Measurement of Physiological Parameters

4.1 Introduction

The design of the experiments described in chapters 5, 6 and 7, along with the placement of catheters and measurement of regional blood-flows described in this chapter, allowed examination of the regional elimination of morphine and the formation and elimination of M3G and M6G. Their design offered several advantages over previous studies that have been conducted in humans and animals. Firstly, in humans, ethical limitations on the regional sampling of blood permit the investigation of whole-body pharmacokinetics only, based on peripheral sampling in normal subjects and in those with regional dysfunction; direct examination of the importance of the different regions is virtually impossible. Secondly, the influence of renal dysfunction, for example, on the regional elimination by the impaired organ can be examined, as well as on other eliminating organs and the overall elimination by the body. Sheep were chosen because their relatively large size enabled multiple samples of blood, of adequate volume for analytical purposes, to be collected concurrently from vessels supplying and draining various regions. Multiple sampling was performed during a constant rate of infusion with the compound under investigation, as well as from selected vessels at other times during and after infusion. Thus, in contrast to studies with smaller animals, elimination by different organs in the sheep could be investigated concurrently. Their docile nature meant that they were easily handled and furthermore, that relatively stable physiological measurements were obtained during the period of an experiment which required intensive sampling. Runciman *et al.* (1984) have demonstrated previously that the sheep is a useful animal for studying the regional disposition of drugs *in vivo*. Approval for the studies in sheep was received from the Animal Ethics Review Committee of the Flinders University of South Australia, the institution at which the experiments were conducted.

4.2 Description of Sheep, Source and Maintenance

Merino ewes, aged 1.5 to 2 yr and weighing 40 to 50 kg, were obtained from a constant breeding stock maintained by Mortlock Farm, University of Adelaide. They were acclimatized in metabolic crates for at least 1 week in an animal house (Flinders Medical Centre) maintained at a controlled temperature between 22° to 24°C and relative humidity of 50%. The room was ventilated, producing seventeen changes of air per hr. They had free access to lucerne hay and water at all times except when fasted overnight prior to surgery. Designated numbers for the sheep studied in chapters 5, 6 and 7 represent the order in which they were subjected to the first surgical procedure.

4.3 Induction of Anaesthesia

Prior to the placement of catheters, thoracotomy or removal of a kidney, sheep were anaesthetized with 20 mg/kg sodium thiopentone (Abbott Australasia, Kurnell, Australia) into the left jugular vein and intubated with a cuffed endotracheal tube (Size 10, Portex Ltd., Hythe, U.K.). Anaesthesia was maintained with 1.5 to 2.0% halothane in a mixture of 40% oxygen in nitrogen. Expired carbon dioxide was monitored with an infrared carbon dioxide analyzer (Normocap®, Datex Instrumentation Corp., Finland) and kept between 4 and 5% by adjustment of tidal and minute volume. Hydration was maintained with an i.v. infusion of 0.9% sodium chloride.

4.4 Placement of Catheters

The placement of catheters was performed using the general method of Runciman *et al.* (1984). Under anaesthesia, the right side of the neck was shorn, bathed in a solution of 10% povidone-iodine (Betadine®, Faulding Pharmaceuticals, Adelaide, Australia) and the carotid artery and jugular vein exposed by a longitudinal incision. Using the Seldinger technique, catheters were inserted, via the carotid artery, into the ascending aorta until their tips were approximately 2 cm above the aortic valve. One catheter was used for the sampling of blood, while the other was connected to an aneroid gauge for the measurement of mean arterial pressure. A similar technique was used to insert catheters into the jugular vein: a flow-directed

thermodilution catheter (Swan-Ganz, Edwards Laboratories, Irvine, U.S.A.) was guided into the pulmonary artery for the collection of blood and for the measurement of temperature by the thermistor; single-lumen 7-French polyethylene catheters were guided into the coronary sinus, hepatic vein, renal vein and posterior vena cava for the collection of blood. Two single-lumen 7-French catheters, one for the bolus injection of cold 0.9% sodium chloride, the other for infusing morphine (chapter 5), were placed into the right atrium; for the experiments described in chapters 6 and 7, a 5-French quad-lumen catheter (Edwards Laboratories, Irvine, U.S.A.) was used, instead of the two with single lumens, for infusing morphine (or M3G) and p-aminohippuric acid, the two remaining lumens being available in the event of a blockage. The tip of the catheter in the posterior vena cava was located between the junction of the iliac veins and the entry of the renal veins; braided catheters into the hepatic vein and left renal vein were located 3 to 5 cm upstream from their junction with the posterior vena cava. The location of the tips of all catheters in the desired sites was confirmed by X-ray image intensification and injection of sodium iothalamate, 70% w/v (Conray 420®, May & Baker, Dagenham, U.K.), down the catheter. After the catheters were tied into the carotid artery and jugular vein, the vessels were ligated and cut cephalad to the ties. The catheters were then encased in a rubber sleeve, mounted on a clamp sewn to muscles of the neck, and the wound closed with absorbable sutures.

For the experiments described in chapter 5, blood was to be collected from the coronary sinus. One week before surgery for the placement of catheters, a left-sided thoracotomy was performed to ligate the hemi-azygous vein which, in the sheep, contributes systemic venous blood to the coronary sinus. Without ligation, blood from the coronary sinus would not be representative of myocardial effluent. Following anaesthesia, sheep were placed on their right side, the wool on the left side of the thorax clipped and the skin soaked in povidone-iodine. A 20 cm incision was made in the third intercostal space, commencing 10 cm from the tip of the transverse processes of the vertebrae. To facilitate surgery, manual ventilation was substituted for mechanical ventilation. The ribcage was exposed and, following incision of intercostal tissue, the third and fourth ribs were parted with a rib spreader. A 3 cm portion of the hemiazygous vein proximal to the coronary sinus was separated from surrounding tissue,

ligated at 2 points and cut in-between. The spreader was removed and the ribs returned and held in position with sutures. The lungs were inflated, mechanical ventilation restored and the overlying muscle and skin sutured.

The right subcostal area was shorn and disinfected, and an incision made. The hepatic portal vein and a mesenteric vein were exposed and a single-lumen 7-French catheter inserted into each. After a left paravertebral incision, the descending aorta was exposed and a 7-French catheter inserted with its tip located distally to the renal arteries and proximal to the iliac bifurcation. All three catheters were tied to plastic base-plates which were attached to muscle, and then passed through their respective incisions before suturing. The catheter was inserted into the mesenteric vein only for the studies described in chapter 5, and was used for the infusion of p-aminohippuric acid.

In those sheep in which renal failure was to be induced, a left lumbar incision was made. A 4-French polyethylene catheter was inserted into the left renal artery in an opposite direction to the flow of blood, distal to the Doppler probe (section 4.5.3.2), and fastened to a plastic plate that was tied to adjacent muscle.

After trephination of the parietal bone, a catheter was placed into the sagittal sinus based on the method described by Lindsay & Setchell (1972). The bone was replaced and the skin sutured. This was performed for the experiments described in chapter 5.

After surgery the animal was allowed to recover before being placed into a mobile metabolic crate. The animal was then given 10 - 20 mg of methadone i.v. for the relief of pain. Each catheter was attached to an extension line and continuously flushed with saline (containing heparin, 5000 Units/l) under positive pressure (300 mm Hg) as described by Runciman *et al.* (1984). The catheters were checked daily for patency by flushing with a bolus of the same solution. The sheep was allowed to recover from surgery for at least a week before the first experiment was performed. Renal function was monitored daily by drawing arterial blood in the morning and measuring the concentration of creatinine in plasma by the Jaffé rate-method (Hitachi 717 Random Access Analyzer, Clinical Biochemistry, Flinders Medical Centre, Bedford Park, Australia). Blood was taken from animals in obvious distress (lethargic, body-temperature above 40°C, drop in the intake of food and output of urine), and usually

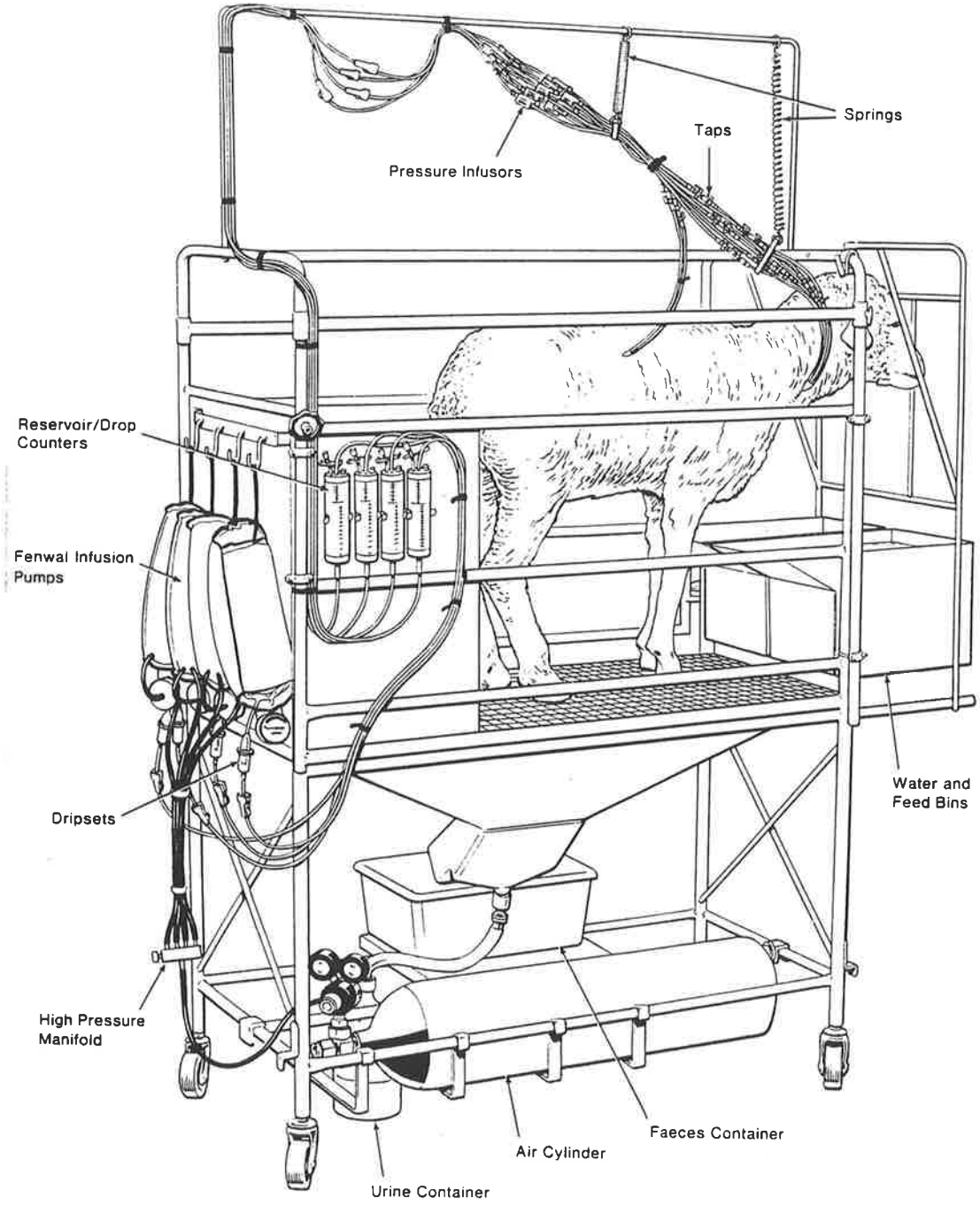


Figure 4.1 *Diagram of a chronically-catheterized sheep in a metabolic crate equipped with a system for the continuous flushing of catheters, containers for feed and water, and collectors for urine and faeces.*

bacteraemia was found (Department of Microbiology, Flinders Medical Centre). These sheep were given a lethal injection of pentobarbitone (Lethabarb®, Arnolds of Reading, Boronia, Australia). Figure 4.1 shows a diagram of a sheep following insertion of the catheters and placement in the mobile crate equipped with a system for flushing of the catheters. Containers for feed and water, as well as for the collection of urine and faeces, are also shown.

On an experimental day, the sheep were moved in the mobile crate to the laboratory at 0630 hr. While they were acclimatizing, a sample of drug-free blood was taken (40 ml) for routine biochemical analysis (for the concentrations of creatinine, total protein, albumin, alkaline phosphatase, alanine aminotransferase and total bilirubin in serum), and for the determination of Cb/C (*vide infra*). After suspending the sheep in a sling, a Foley catheter was inserted into the bladder with the aid of a flexible guide, and a sample of drug-free urine obtained. The catheter was then attached to a bag for the collection of urine, over the period of 6 hr while drug was being infused, and removed at the end of the infusion.

4.5 Induction of Renal Failure

Numerous methods for the induction of chronic renal failure in animals have been developed (Strauch & Gretz, 1988). One of the most common is a reduction in the mass of functioning renal tissue by a combination of ligation of arterial vessels supplying one kidney and resection of the other. Generally, however, renal hypertrophy of the remaining kidney in sheep (English *et al.*, 1977) and other animals (Strauch & Gretz, 1988) requires further ligation, although Eschbach *et al.* (1980) were able to maintain reasonably stable chronic renal failure, without the need for dialysis or additional ligation, for between 3 and 11 months in about half of the sheep in which failure was induced. The possibility of repeated surgery for further ligation was considered impractical, especially given the greater risk of infection in the sheep examined for this thesis. Chemical induction of renal failure has been achieved by injection of the animal with uranyl nitrate, adenine, puromycin or adriamycin (Strauch & Gretz, 1988) and appears to be suitable for the selective damage of particular regions of the nephron (Maïza & Daley-Yates, 1993). However, the possibility exists that these agents may cause direct damage to other organs that is unrelated to renal dysfunction.

Sheep in which the disposition of morphine and M3G was to be examined after renal failure had been induced (chapter 7) were prepared for surgery as described in section 4.2. Some sheep had been used in experiments in which they had received morphine and M3G when normal, while others had not previously been used. For the former group, anaesthesia was followed by a right lumbar incision, location and ligation of the right renal artery and vein, and removal of the corresponding kidney. Those sheep that had not been used previously as models of normal sheep were anaesthetized as described in section 4.2 and catheters inserted as described in section 4.3. The right kidney was removed and left renal arterial catheter inserted as described above. Sheep were allowed to recover and then returned to their metabolic crates.

Between 4 and 10 days after removal of the right kidney, renal failure was induced in the remaining kidney by daily administration into the renal artery of sterile suspensions of polystyrene beads (25 to 30 μm diameter NEM 003 microspheres, Dupont NEN, Boston, U.S.A.) in 0.9% sodium chloride and/or glass beads (75 to 150 μm diameter, acid-washed type 1-W, Sigma Chemical Co., St. Louis, U.S.A.) in 5% glucose. The development of renal failure was monitored on a daily basis by measurement of the concentration of creatinine in plasma (Jaffé rate-method). Between 120,000 to 360,000 polystyrene beads and/or between 50,000 to 300,000 glass beads were administered until the concentration of creatinine reached approximately 0.5 mmol/l. The mean (± S.D.) concentration measured in the normal sheep that were used in the experiments described in chapter 6 was 0.083 ± 0.005 mmol/l. The quantity and type of beads administered was dependent on the incremental concentration of creatinine induced by previous beads, glass beads producing greater increments, and on the concentration of creatinine on the previous day. The development of renal failure, reflected in the concentrations of creatinine in plasma in the seven sheep in which experiments on the disposition of morphine (and in three sheep for M3G) were conducted, is shown in figure 7.1 (chapter 7).

4.6 Measurement of Flows

4.6.1 Cardiac Output

Cardiac output was measured by thermodilution based on the method described by Runciman *et al.* (1981). Ten ml of an ice-cold solution of 0.9% sodium chloride (Baxter Healthcare, Toongabbie, Australia) was injected within 4 s through the proximal lumen of the Swan-Ganz catheter into the right atrium. The signal from the thermistor in the Swan-Ganz catheter (Edwards Laboratories, Irvine, U.S.A.) located in the pulmonary artery was coupled to a cardiac output computer (Model 9520A, Edwards Laboratories, Irvine, U.S.A.), which integrated the response-time data and calculated the output using the following form of the Stewart-Hamilton equation (Dow, 1956):

$$\text{Cardiac output (l/min)} = \frac{V_i \times (T_b - T_i) \times C_T \times SG_i \times SH_i \times 60}{SG_b \times SG_b \times {}_0\int^{\infty} T_b(t).dt}$$

where V_i is the volume (l) of 0.9% sodium chloride injected,

T_b is the initial temperature of blood prior to injection,

T_i is the temperature monitored by a thermistor placed in the mixture of water and ice in the bath holding the injections of sodium chloride,

C_T is a correction factor for the increase in temperature of the injected volume due to transfer of heat from the walls of the catheter rather than from blood,

SG_i and SG_b are the specific gravities of the injection and blood, 1.045 and 1.005 (Ganz & Swan, 1972), respectively,

SH_i and SH_b are the specific heats of the injection and blood, 0.970 and 0.997 (Ganz & Swan, 1972), respectively,

$T_b(t)$ is the temperature of pulmonary arterial blood at time, t, after injection and 60 converts flow from l/sec to l/min.

Cardiac output was measured between 1430 and 1530 hr, coinciding with the 5th to 6th hour of infusion with either morphine or M3G. Quadruplicate measurements of cardiac output were performed every 15 min, averages calculated and from these the mean output over 1 hr

calculated as the quotient of the area under the curve of the averaged rate of flow and the time interval.

4.6.2 Hepatic Blood Flow

Hepatic blood flow was measured by the Fick method using sulphobromophthalein. Sterile injection of sulphobromophthalein sodium (25 mg/ml, Department of Pharmacy, Flinders Medical Centre, Bedford Park, Australia) was infused into the descending aorta using a constant-rate twin-channel infusion pump (Model 940, Harvard Apparatus, Millis, MA, U.S.A.) with individually calibrated glass syringes. A loading dose of 120 mg was infused over 15 min, beginning 4 hr after starting an infusion of morphine or M3G, and was followed by a constant infusion at 4 mg/min for 1.75 hr. The volume infused was approximately 20 ml. Samples of blood (0.4 ml) from the aorta (above the aortic valve) and hepatic vein were collected during the last hour of infusion into 1.5 ml polypropylene centrifuge tubes (Eppendorf®, Disposable Products, Ingle Farm, Australia) containing heparin (25 Units).

The concentrations of sulphobromophthalein sodium in arterial and hepatic venous blood were measured by HPLC (performed by Mr. C. McLean). Briefly, plasma was separated by centrifugation (7700g, 5 min), 0.2 ml was mixed with 0.5 ml acetonitrile containing bromophenol blue (5 µg) in a 1.5 ml polypropylene centrifuge tube, the mixture centrifuged to precipitate protein, and 0.02 ml of supernate injected onto a stainless steel column (150 mm x 4.6 mm) packed with Spherisorb ODS 2 reversed-phase material (5 µm, Phase Separations Ltd., Deeside, U.K.). A mobile phase of 1.25 mmol/l PIC-A (Waters Assoc.) in a mixture of 50% methanol in water was pumped at a flow rate of 2 ml/min, with the eluent being monitored at 234 nm. Bromophenol blue and sulphobromophthalein sodium had retention times of 4.0 min and 5.6 min, respectively. Unknown concentrations were calculated from calibration curves of the ratio of the peak height of sulphobromophthalein sodium to that of bromophenol blue plotted against the standard concentrations of sulphobromophthalein sodium. These were prepared by adding known amounts from the injection (30 mmol/l) into drug-free blood, to produce concentrations over the range 0.6 to 18 µmol/l, and analyzed in an identical manner to the unknown samples. Correlation coefficients for the standard curves were greater than 0.995.

Reproducibility at 0.6 and 7.2 μmol/l, concentrations approximating those measured in hepatic venous and arterial blood, respectively, was 2.0% and 2.8%, respectively (n = 20). Hepatic blood flow was calculated as the quotient of the amount of sulphobromophthalein infused over the period of sampling, and the difference between the areas under the curves of arterial and venous concentrations. It was assumed that 80% of the flow to the liver was via the portal vein, the remaining 20% coming from the hepatic artery (Runciman *et al.*, 1984).

When using this method, it was assumed that the concentration of sulphobromophthalein in hepatic venous blood was representative of that in well-mixed outgoing blood from the liver. A lower pressure in the portal vein compared to the hepatic artery may contribute in some instances to uneven mixing and therefore variability in flows measured over the hour. Furthermore, use of the Fick method as an accurate estimate of hepatic blood flow assumes that all of the sulphobromophthalein infused was eliminated by the liver. When measuring parent and metabolites with a spectrophotometric method, Tucker *et al.* (1971) and West (1989) recovered approximately 80% of a bolus dose of sulphobromophthalein in the bile of sheep after 1 hr. From the data of West (1989), it appeared that recovery would have been greater if bile had been collected for a longer period. Tucker *et al.* (1971) estimated that two metabolites separated by paper chromatography contributed 86% of the biliary recovery, consistent with reports in other species (Klaassen & Plaa, 1967), and believed one to be a glutathione conjugate. Tucker *et al.* (1971) and West (1989) assumed a similar molar absorptivity for the parent and metabolites but for the metabolites it may, in fact, be lower (Krebs & Brauer, 1958) and they may, therefore, have underestimated the true recovery in bile of the dose of sulphobromophthalein. Furthermore, Tucker *et al.* (1971) recovered only 0.8% of the dose in urine collected for 24 hr. Therefore, for the purposes of estimating blood flow to the liver, it may be assumed that the amount of sulphobromophthalein infused over the hour was equivalent to that eliminated by the liver.

4.6.3 Renal Blood Flow

4.6.3.1 Fick Method

Renal blood flow was measured by the Fick method using p-aminohippuric acid. An injection (Aminohippurate sodium 20% w/v, Merck, Sharp and Dohme, West Point, U.S.A.) was diluted with an equal volume of sterile water and infused into the mesenteric vein (for the experiments described in chapter 5) or into the right atrium (chapters 6 and 7) using a constant-rate twin-channel infusion pump (Model 940, Harvard Apparatus, Millis, MA, U.S.A.) with individually calibrated glass syringes. For healthy sheep, a loading infusion of 450 mg given over 15 min was followed by an infusion maintained at 15 mg/min for 1.75 hr. The volume infused was approximately 20 ml. For sheep in renal failure, the loading and maintenance rates were reduced by 50% by diluting the original injection with three volumes of sterile water, and infusing at the same flow. The infusion was begun 4 hr after starting an infusion of morphine or M3G. Samples of blood (0.4 ml) from the aorta (above the aortic valve) and renal vein were collected during the last hour of infusion into a polypropylene centrifuge tube containing heparin (25 Units).

The concentrations of p-aminohippuric acid in blood were measured by HPLC (performed by Mr. C. McLean). Briefly, plasma was separated by centrifugation (7700g, 5 min), 0.1 ml was mixed with 0.2 ml acetonitrile and 0.025 ml of an aqueous solution of iothalamic acid (as sodium iothalamate, 0.2 mg/ml) in a 1.5 ml polypropylene centrifuge tube, the mixture centrifuged to precipitate protein, and 0.01 ml of supernate injected onto a Goldpak ODS reversed-phase column (5 μm, 4.6 mm x 150 mm, Activon Scientific Products, Thornleigh, Australia). p-Aminohippuric acid and iothalamic acid were eluted with a mobile phase of 5 mmol/l tetrabutylammonium phosphate (Ajax Chemicals, Auburn, Australia) in a mixture of 9% acetonitrile in water pumped at a flow rate of 2 ml/min. The eluent was monitored at 254 nm, and p-aminohippuric acid and iothalamic acid had retention times of 2.6 min and 3.9 min, respectively. Unknown concentrations were calculated from standard curves of the ratios of the peak height of p-aminohippuric acid to that of iothalamic acid, plotted against the concentrations of aminohippuric acid (range 4.7 to 230 μmol/l) prepared by adding

known amounts from the diluted injection into drug-free blood. The standards were analyzed in an identical manner to the unknowns. Correlation coefficients for the standard curves were greater than 0.993. The reproducibility at 4.7 and 116 μmol/l was 1.3% and 0.98%, respectively (n = 20). These concentrations included the range observed *in vivo* in normal sheep and sheep with renal failure. Renal blood flow was calculated as the quotient of the amount of p-aminohippuric acid infused over the period of sampling, and the difference between the areas under the curves of arterial and venous concentrations.

Preliminary studies in three healthy sheep had established that 71.0 ± 2.3% of a bolus dose of p-aminohippuric acid (1.0 g) was recovered as unchanged compound in urine collected for 48 hr. Setchell & Blanch (1961) have shown that slices from the kidney of the sheep form N-acetyl-p-aminohippuric acid from the parent at approximately twice the rate (per g of tissue) for slices from the liver. No studies in healthy sheep or sheep with renal failure have established the urinary recovery of a dose of p-aminohippuric acid as summed parent and the N-acetyl metabolite nor whether all of the dose is cleared by the kidney alone. However, Prescott *et al.* (1993) recovered all of a bolus dose given to healthy humans as the parent and N-acetyl metabolite in urine within 3 hr, with about 18% present as the metabolite; 84% was recovered within 8 hr from patients with chronic renal failure, with the metabolite representing about 43% of the dose. On the basis of the similarity between the total clearance of p-aminohippuric acid and the renal clearance of summed parent and N-acetyl metabolite with respect to the sum of both compounds in plasma, these authors concluded that extrarenal clearance of p-aminohippuric acid was minimal in normal subjects and in patients with chronic renal failure. Therefore, in the studies described in subsequent chapters of this thesis, it was assumed that all of the dose of p-aminohippuric acid given to sheep was eliminated by the kidney, and that the application of the Fick method to this indicator provided an accurate estimate of renal blood flow.

4.6.3.2 Doppler Flowmetry

An attempt was made to use an additional method for the measurement of blood flow to the kidney in the sheep used in the experiments described in chapters 6 and 7. A Doppler probe

(Model 545C, Bioengineering, The University of Iowa, Iowa, USA) was attached to the left renal artery when surgery for the insertion of the catheters was performed as described in section 4.3. The probe was affixed to a patch, and the patch sewn onto the vessel as nearly as possible to its junction with the aorta. The probe was held further by tying the leads from the crystal to adjacent muscle. The ends of the leads for attachment to the flowmeter were passed through the incision. Experiments were not performed until at least two weeks after the probe was attached. This allowed time for the formation of fibrous scar tissue around the probe, minimizing movement relative to the artery. However, of the six sheep fitted with probes, only from two was flow data obtained from the *in vitro* calibration. For the segments of renal artery from the other sheep, poor audible signals were obtained during *in vitro* calibration and therefore *in vivo* estimates of the rates of flow could not be determined. Therefore, the data obtained from the two sheep were not used, preferring instead to use the renal arterial flows estimated using p-aminohippuric acid.

Chapter 5

The Disposition of Morphine, M3G and M6G in the Sheep during Infusion with Morphine: Regional Elimination of Morphine and Regional Elimination and Formation of M3G and M6G

5.1 Introduction

As reviewed in chapter 1, from studies performed *in vitro* and *in vivo* with a range of species, there is considerable evidence for the metabolic elimination of morphine by the liver and from experiments with some species for its elimination by the gut also. However, the contribution from the kidney and other regions remains unclear. With one exception (Horton & Pollack, 1991), *in vivo* studies have provided circumstantial evidence for a lack of involvement of the kidney in the metabolic clearance of morphine.

There have been no reports of studies investigating, simultaneously, the regional elimination of morphine and the formation and elimination of M3G and M6G in the same animal preparation. Therefore, the purpose of this study was to determine, firstly, the influence of dose on the concentrations of morphine, M3G and M6G produced in plasma; secondly, the regional elimination of morphine; and thirdly, the regional formation and extraction of M3G and M6G in the sheep, during constant infusion with morphine at four different rates over an eight-fold range.

5.2 Methods

5.2.1 Preparation of the Sheep

The preparation of the sheep and methods for the measurement of regional blood flows were as described in chapter 4. Catheters were inserted into the descending aorta, pulmonary artery, coronary sinus, sagittal sinus, hepatic vein, hepatic portal and renal veins, and posterior vena cava for the collection of blood. As described in chapter 4, catheters were also inserted for the measurement of cardiac output, for the infusions of morphine into the right atrium, p-

aminohippuric acid into the mesenteric artery, and sulphobromophthalein into the descending aorta.

5.2.2 Experimental Design

Four merino ewes of mean (± SD) weight 48 (± 8) kg were given two-stage infusions of morphine sulphate (as morphine sulphate B.P., David Bull Laboratories, Mulgrave, Australia, in saline) into a right atrial catheter: initially 8 times the maintenance dose-rate for 15 min followed by a maintenance rate of 2.5, 5, 10 or 20 mg/hr for 5.75 hr. Morphine sulphate B.P. (as the pentahydrate), 10 mg, is equivalent to 7.52 mg of morphine. The rate of infusion was controlled using a single-channel infusion pump (Model 902A, Harvard Apparatus, Millis, MA, U.S.A.). The rate of flow of the maintenance infusion was approximately 0.5 ml/min. Each animal was to receive all four dosage regimens on separate occasions and in a randomized manner, with at least 3 days between each experiment.

5.2.3 Collection of Samples

Blood (1 ml), at 15 min intervals between the fifth and sixth hour from the beginning of the infusion, was collected simultaneously through catheters inserted previously (sections 4.3, 5.2.1) into the aorta, pulmonary artery, hepatic vein, hepatic portal and renal veins, posterior vena cava and the coronary and sagittal sinuses. The blood was centrifuged within 30 min and the plasma transferred to 1.5 ml polypropylene centrifuge tubes (Eppendorf®, Disposable Products, Ingle Farm, Australia) for storage at -20°C. Drug-free urine was taken before commencement of the infusion. Urine was then collected from 0-6 hr via an indwelling catheter, with free-flowing urine collected from 6-24 hr and 24-48 hr. Urine volume was measured and aliquots stored at -20°C.

5.2.4 Determination of Morphine, M3G and M6G

The concentrations of morphine, M3G and M6G in plasma (0.35 to 0.5 ml) and urine (0.05 to 0.2 ml) were determined by paired-ion HPLC as described in chapter 2. Drug-free plasma and urine was obtained prior to the start of an infusion with morphine and evaluated for any chromatographic interferences to morphine, M3G and M6G prior to its use for the preparation of

standards for calibration. Standards were prepared in plasma over the ranges of 13.3 nmol/l to 532 nmol/l, 108 nmol/l to 4330 nmol/l, and 20.4 nmol/l to 102 nmol/l, for morphine, M3G and M6G, respectively; in urine they were prepared over the ranges of 0.532 to 10.6 μmol/l, 4.33 to 86.7 μmol/l and 0.41 to 2.04 μmol/l, respectively.

5.2.5 Determination of C_b/C

Drug-free arterial blood was collected prior to commencing the infusion of morphine sulphate. A known amount of morphine, M3G and M6G in saline was added (the volume increment being 5% of the volume of blood) and equilibrated by shaking at 50 oscillations per min at 37°C for 30 min. The concentrations prepared in blood were 66.5, 133 and 665 nmol/l for morphine, 217, 2170 and 5420 nmol/l for M3G and 102, 204 and 509 nmol/l for M6G. After centrifugation, the plasma was removed for the determination of morphine, M3G and M6G by HPLC. The values of C_b/C for morphine, M3G and M6G were calculated by dividing the respective nominal concentrations in blood by the concentrations determined in plasma. The haematocrit of arterial blood was determined by centrifuging at 12,000 g for 3 min using a Gelman Hawksley Microhaematocrit Centrifuge (Lansing, England).

5.2.6 Equilibration of Morphine, M3G and M6G in Samples of Blood collected during Infusion with Morphine

A preliminary experiment was performed to demonstrate that equilibration of morphine, M3G and M6G between blood cells and plasma in blood collected during an experiment was complete within the time taken to separate the plasma. The experiment was performed with hepatic portal and hepatic venous blood since it had been found in a pilot study that extraction of morphine by the liver was high, and considerable re-equilibration would have occurred between red blood cells and plasma in post-hepatic blood. If equilibrium had not been re-established by the time that plasma was separated, then regional extraction of morphine and net regional formation or extraction of M3G and M6G, if present, would have been overestimated.

A sheep weighing 48 kg was infused with morphine sulphate at a rate of 20 mg/hr for 24 hr. Blood (20 ml) was collected from the hepatic portal vein and hepatic vein prior to stopping the

infusion. An aliquot (4 ml) was centrifuged immediately (t = 0) for 3 min. The remaining blood was incubated at 37°C with gentle shaking, and further aliquots taken for centrifugation at 0.5, 1, 1.5 and 2 hr. Plasma was removed immediately and frozen at -20°C. Following HPLC as described in chapter 2, the areas of the respective chromatographic peaks from morphine, M3G and M6G in plasma (0.5 ml) were divided by that obtained for the internal standard, hydromorphone.

5.2.7 Pharmacokinetic Analysis

The mean total clearance of morphine from the body, with respect to blood, was calculated from the quotient of the amount of morphine (as the base) infused during the period of sampling, and the product of C_b/C and the pulmonary arterial AUC(5-6). Regional extraction ratios for morphine, M3G and M6G were calculated as the difference between the respective AUC(5-6) for the plasma entering and leaving divided by that entering a given region. It was assumed that 80% of blood flowing to the liver came via the hepatic portal vein with the remainder via the hepatic artery (Runciman *et al.*, 1984). Regional net clearances were estimated from the product of the respective extraction ratio and the regional flow of blood. The renal excretory clearance of morphine was calculated from the product of the total clearance and the fraction of the dose excreted unchanged in urine over 48 hr. The fraction of the summed morphine and glucuronides in blood entering the liver which did not reappear in the hepatic vein as either morphine or glucuronide metabolites (fractional uptake) was calculated as:

$$\frac{\sum (C_b/C)_x[(0.2AUC_{Artx} + 0.8AUC_{HPVx}) - AUC_{HVx}]}{\sum [(C_b/C)_x(0.2AUC_{Artx} + 0.8AUC_{HPVx})]}$$

where $(C_b/C)_x$ is the ratio of the concentration of x (x being morphine, M3G and M6G) in blood/plasma and AUC_{Artx}, AUC_{HPVx} and AUC_{HVx} are the corresponding molar values for AUC(5-6) from the abdominal aorta, hepatic portal and hepatic veins, respectively. In those instances where the concentrations of M6G in plasma were not quantifiable, only morphine and M3G were included in the calculation.

Urinary recovery was calculated as the percentage of the dose of morphine given over 6 hr that was recovered in urine as morphine, M3G and M6G over 48 hr.

5.2.8 Statistics

Comparisons of the dose-normalized pulmonary arterial concentrations of morphine and M3G in plasma between doses and over the 5 - 6 hr sampling period within sheep were performed by analysis of variance (Genstat 5, Lawes Agricultural Trust, Rothamsted Experimental Station, Hertfordshire, England). The results were examined for the main effects of dose and time, and for a dose x time interaction. Missing values were estimated by the program using an iterative method. Where appropriate, other comparisons of means between two or more variables were performed by Student's t-test or analysis of variance (Statview+^{TM}, Brainpower Inc., Calabasas, CA, USA). Values of $p < 0.05$ were considered significant. The Dixon test for outliers was performed as described by Bolton (1990). Overall means (± S.D., n = 4) were calculated from the individual means calculated for each sheep from the values determined at the different rates of infusion. Error bars on graphs indicate standard deviations about means

5.3 Results

Of the sixteen experiments proposed, fourteen were completed. Failure of catheters resulted in the unsuccessful completion of two experiments. Two sheep received all four rates of infusion while the other two received the two lower and one of the two higher rates. In one experiment (sheep 2, morphine sulphate at 10 mg/hr), blood could not be sampled from the hepatic portal vein while in another (sheep 3 at 2.5 mg/hr) no samples were obtained from the sagittal sinus, preventing calculation of extraction by the gut and liver, and by the brain, respectively. During two experiments (sheep 2 and 3 at 5 mg/hr), no samples of blood were collected at 6 hr and therefore the values for regional AUC between 5 and 5.75 hr were normalized to values of AUC(5-6). In all experiments, the concentrations of M3G in plasma were quantifiable while in one experiment (sheep 2, morphine sulphate at 2.5 mg/hr), due to chromatographic interference, the concentrations of morphine in plasma and urine could not be measured. In all four sheep given the lowest rate of infusion and in two infused at 5 mg/hr (sheep 2 and 4), the concentrations of

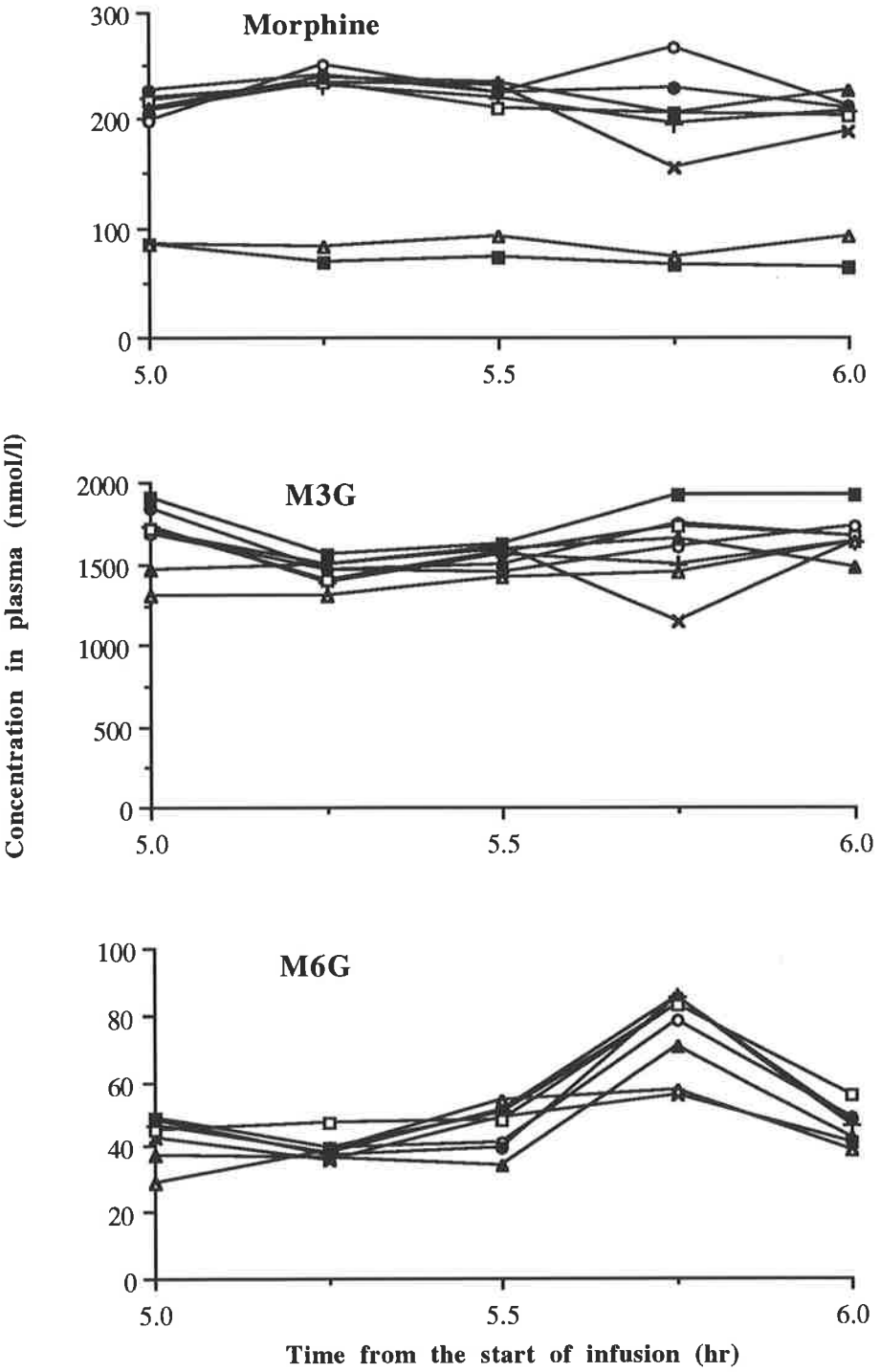


Figure 5.1. *Concentrations of morphine, M3G and M6G in plasma of sheep 4 during the 5th to 6th hour of infusion with morphine sulphate at the rate of 10 mg/hr.*

The concentrations are in samples collected from the abdominal aorta (○), pulmonary artery (●), sagittal (+) and coronary sinuses (x), hepatic vein (■), hepatic portal (□) and renal (Δ) veins, and posterior vena cava (▲)

M6G in plasma were below the limit of quantification. Complete collections of urine were obtained in 8 experiments. Appendix 2 contains the data for the concentrations in plasma and urine from each experiment. Cardiac output and blood flows to the liver and kidney are presented in table A2.16.

Figure 5.1 depicts representative profiles of the concentrations of morphine, M3G and M6G in plasma obtained from sheep 4 when infused at the rate of 10 mg/hr. Within each experiment, there were no significant differences in the dose-normalized pulmonary arterial concentrations of either morphine ($p = 0.314$; 4, 12 df) or M3G ($p = 0.316$; 4, 12 df) in plasma when compared over the 1 hr sampling interval. Moreover, within sheep there were no significant differences between doses in the same dose-normalized concentrations of either morphine ($p = 0.384$; 3, 6 df) or M3G ($p = 0.165$; 3, 7 df). No significant dose x time interaction for either morphine

Table 5.1. *Ratios of the areas of the HPLC peaks of morphine, M3G and M6G to that of hydromorphone, in plasma that was separated from blood at selected times following collection of the blood from the hepatic portal vein and hepatic vein of a sheep infused with morphine sulphate (20 mg/hr) for 24 hr.*

	Ratio of Areas					
	Morphine		M3G		M6G	
Time (hr)	Portal	Venous	Portal	Venous	Portal	Venous
0	0.562	0.111	4.06	4.67	0.0369	0.0377
0.5	0.603	0.105	4.67	4.42	0.0400	0.0360
1.0	0.603	0.107	4.72	4.90	0.0378	0.0351
1.5	0.595	0.117	4.61	4.36	0.0398	0.0428
2.0	0.636	0.115	4.63	4.56	0.0401	0.0417
C.V. (%)	4.40	4.59	5.96	4.69	3.78	8.87

($p = 0.325$; 12, 22 df) or M3G ($p = 0.065$; 12, 26 df) was observed. There were insufficient data to perform a similar analysis for M6G. The overall mean ratios of the pulmonary arterial values of

AUC for M3G/morphine, M6G/morphine and M3G/M6G were 5.52 ± 0.95, 0.17 ± 0.05 and 32.2 ± 9.0, respectively.

The ratios of the areas under the chromatographic peaks from morphine, M3G and M6G in hepatic portal and hepatic venous plasma to that of hydromorphone, following equilibration for up to 2 hr after collection of the two samples of blood, are shown in table 5.1. The coefficients of variation for the ratios over the 2 hr were comparable to the precision of the analytical method for the three compounds and there were no clear trends in the ratios over time, suggesting that equilibration of morphine, M3G and M6G between blood-cells and plasma was complete within the time taken to separate the plasma from the blood.

The overall mean regional extraction ratios of morphine, M3G and M6G are shown in figure 5.2. There was significant extraction of morphine by the liver (0.675 ± 0.015, $p < 0.001$), kidney (0.602 ± 0.039, $p < 0.001$) and brain (0.030 ± 0.017, $p = 0.039$). For M3G there was net

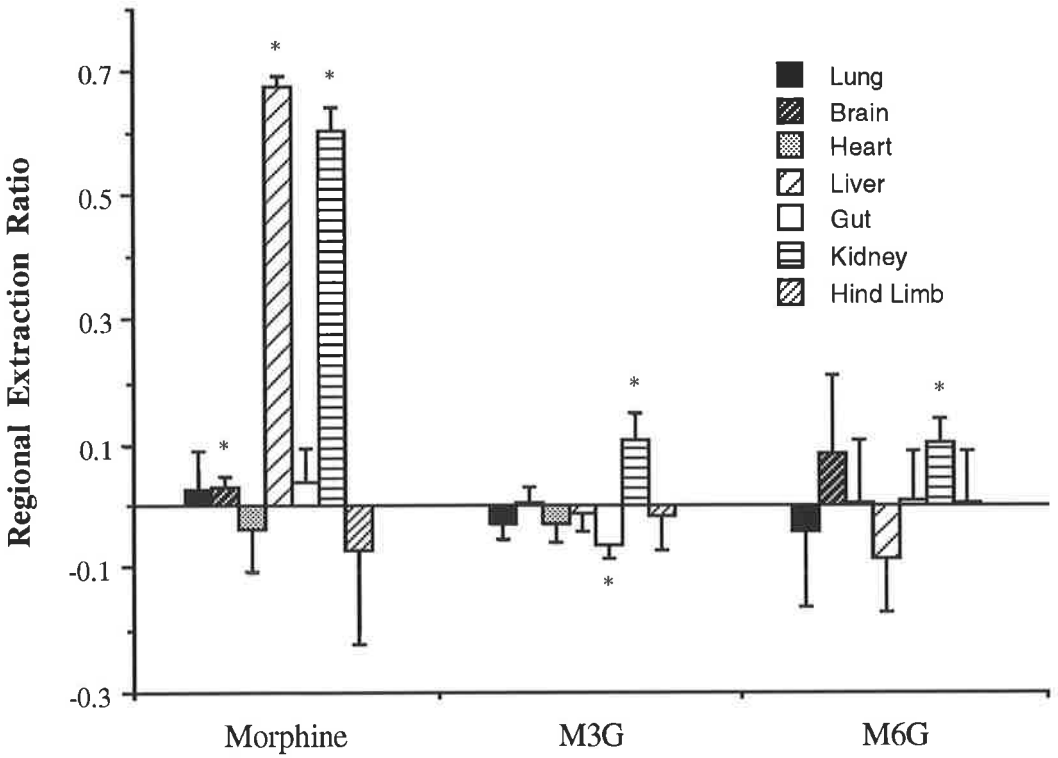


Figure 5.2. *Overall mean regional extraction ratios for morphine, M3G and M6G.*

Ratios are for lung, brain, heart, liver, gut, kidney and hind limb (see legend).

* significantly different from zero ($p < 0.05$).

extraction by the kidney (0.106 ± 0.046, p = 0.019) and net production by the gut (-0.063 ± 0.022, p = 0.011) while for M6G there was net extraction by the kidney (0.103 ± 0.037, p = 0.011). Net clearances of M3G and M6G by the kidney were 0.104 ± 0.045 l/min and 0.092 ± 0.032 l/min, respectively, and were significantly different from zero (p = 0.019 and 0.010, respectively), but not from each other (p = 0.509).

The data for C_b/C is presented in Appendix 2.15. In one sheep, C_b/C was determined for morphine and M3G at the three concentrations on three occasions prior to administration of morphine, the haematocrits being 0.23, 0.24 and 0.33. There was no evidence for the dependence of C_b/C on nominal concentration for morphine (p = 0.094) or M3G (p = 0.217) or that C_b/C

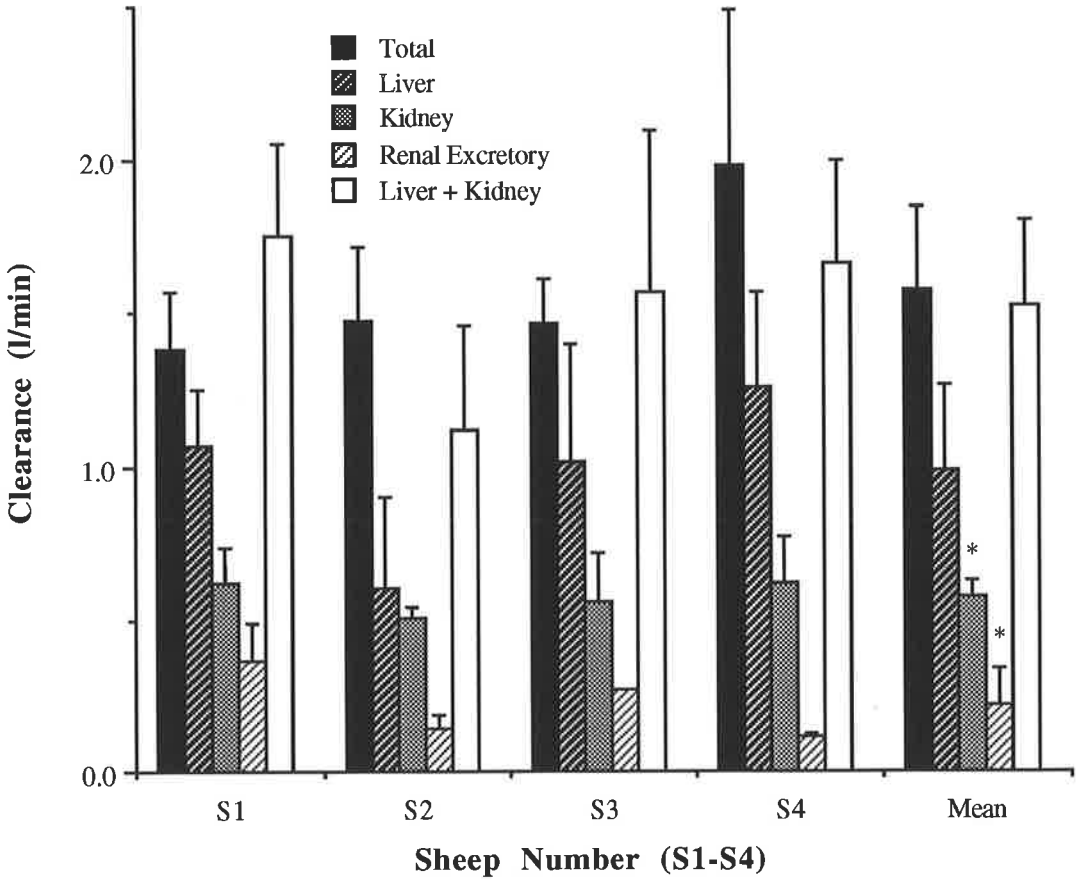


Figure 5.3. *Mean total, regional and renal excretory clearances of morphine in individual sheep and the overall means.*

Clearances are total from the body, by the liver and kidney, renal excretory and the summed liver and kidney (see legend)

* significantly different, p = 0.008

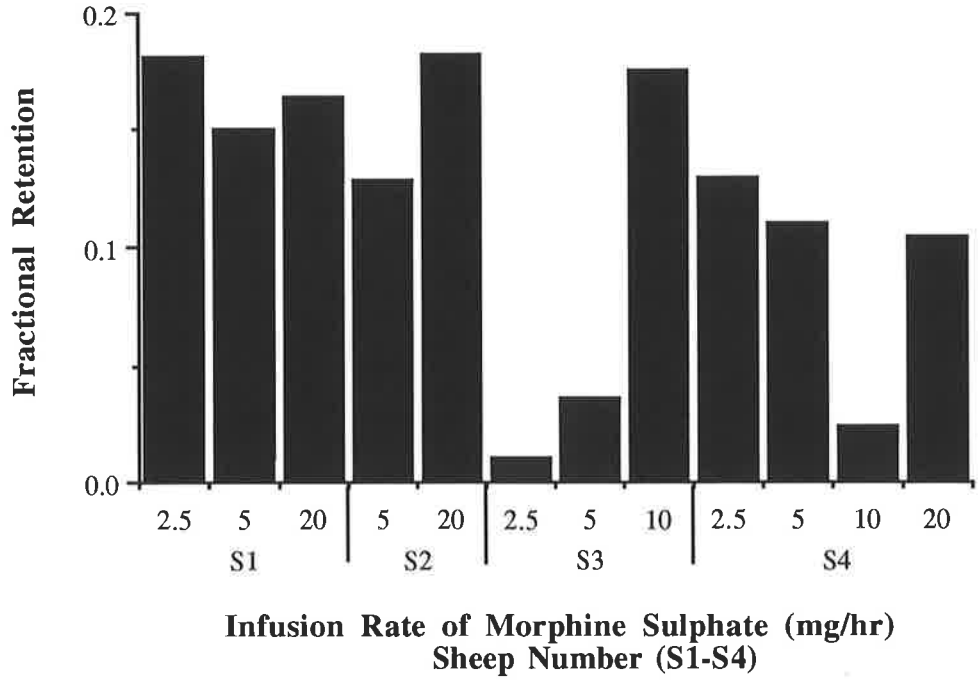


Figure 5.4. *Fractional hepatic retention of summed morphine, M3G and M6G*

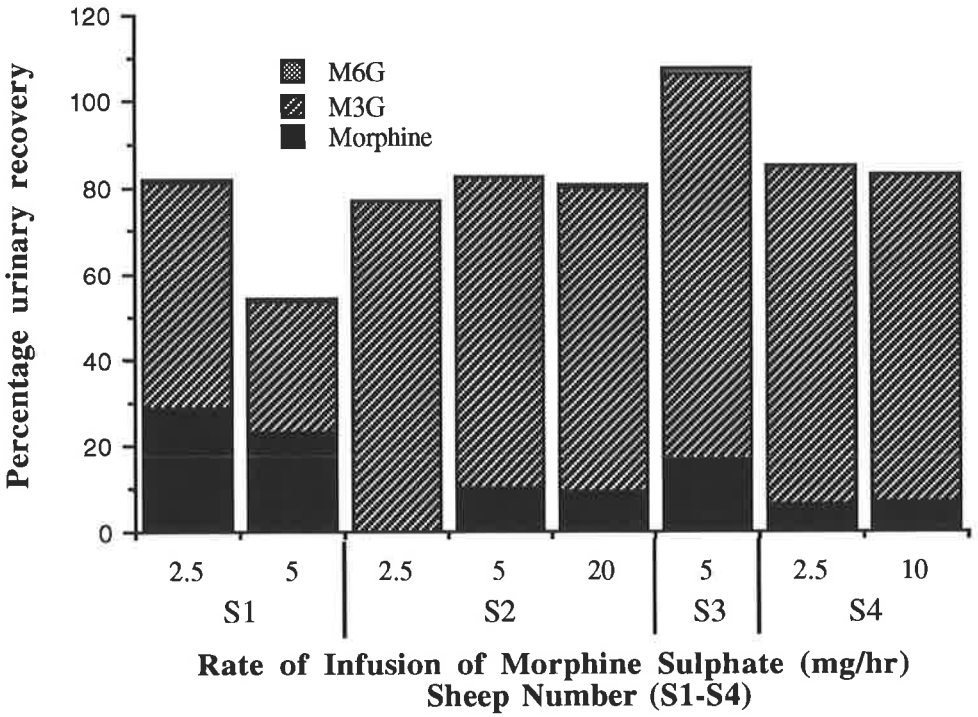


Figure 5.5 *Percentage urinary recovery of the dose of morphine as morphine, M3G and M6G*

differed on each occasion ($p = 0.293$ and $p = 0.367$, respectively). There were insufficient data to allow a similar analysis for M6G and, although the values of C_b/C ranged from 0.65 to 0.99 in the four sheep, there was no obvious trend over the concentrations examined. Therefore, a mean value of C_b/C for morphine, M3G and M6G was calculated for each sheep and used for calculations of the total clearance from blood and for mass-balance across the liver. The overall means of the values of C_b/C were 1.25 ± 0.17, 0.80 ± 0.03 and 0.82 ± 0.09 for morphine, M3G and M6G, respectively. The overall mean haematocrit was 0.27 ± 0.02.

The individual total body clearances, the regional clearances by the liver and kidney and their sum, and the renal excretory clearances of morphine along with the overall means are presented in figure 5.3. Values for cardiac output and blood flow to the liver and kidney are presented in table A2.16. The renal excretory clearance of morphine was significantly less ($p < 0.008$) than the clearance of morphine by the kidney.

The fraction of the summed amounts of morphine, M3G and M6G delivered to the liver and not appearing in the hepatic venous blood is presented in figure 5.4. The overall mean fractional uptake was 0.120 ± 0.044 and was significantly different from zero ($p < 0.025$).

Urinary recovery of the dose of morphine as morphine, M3G and M6G is shown in figure 5.5. The overall mean percentage recoveries as morphine, M3G and M6G were 14.7 ± 8.5, 75.4 ± 11.1 and 0.49 ± 0.39, respectively. While urinary concentrations of morphine could not be determined in sheep 2 infused at 2.5 mg/hr, the concentrations of M3G were measured and the percentage excreted is shown in figure 5.5. M6G could not be detected in the urine from three experiments: sheep 2 at 2.5 and 5 mg/hr and sheep 4 at 2.5 mg/hr of morphine sulphate.

5.4 Discussion

The concentrations of morphine, M3G and M6G in the dog (Yoshimura *et al.*, 1993), and of morphine and M3G in the rat (Ekblom *et al.*, 1992), have been measured in plasma during constant i.v. infusions of morphine. The relative concentrations of M3G to morphine determined in the rat and dog were approximately 1.8 and 12.7, respectively. M6G was not detected in plasma from the rat but in the dog the concentrations were approximately one-third of those for morphine. The three compounds have also been measured in plasma following a single i.v. dose

of morphine to humans and animals. In humans with normal renal function, the mean ratio of the AUC for M3G to morphine was 7.9 (Osborne *et al.*, 1990) and 7.7 (Hasselström & Säwe, 1993) while that for M6G to morphine was 1.4 (Osborne *et al.*, 1990) and 0.7 (Hasselström & Säwe, 1993). Inspection of the data from the rhesus monkey indicates that the concentrations of M3G in plasma were an order of magnitude greater than those of morphine but that those for M6G were about an order of magnitude smaller (Rane *et al.*, 1984). The mean ratio of the AUC for M3G to morphine found in the present study in sheep (5.52 ± 0.95) was about one-half of that determined in the dog and the rhesus monkey and similar to that in humans, while the ratio for M6G to morphine (0.17 ± 0.05) was closer to that observed in the monkey, approximately two-thirds that in the dog and one-fifth the value in humans. No difference in the renal excretory clearances of M3G and M6G was found in humans (chapter 3) and it is unlikely that the renal clearances of the two would differ within other species. Therefore, it may be concluded that the formation clearances are markedly different in different species. *In vitro* studies with microsomal preparations of UDPGT have found marked differences between species in activity toward the 3- and 6-hydroxyl groups of morphine, with the ratios of activities being similar to the ratios of M3G to M6G recovered in urine (Kuo *et al.*, 1991). Applying this finding to the sheep suggests that the activity of UDPGT towards the 3-phenolic group was far greater than for the 6-alcoholic group. Additionally, the net production of M3G, but not M6G, by the gut provides further evidence for the existence of multiple isozymes of glucuronosyltransferase, with different activities toward morphine (Coughtrie *et al.*, 1989) and possibly also different distributions between tissues, that are responsible for the formation of either metabolite.

The lack of a significant change in the arterial concentrations of morphine and M3G in plasma with time over the sampling period implies that steady-state concentrations had been achieved in plasma. Therefore, steady-state concentrations are also likely to have been achieved during this period, in well perfused organs such as heart, lungs, liver, kidney and gut (Davies & Morris, 1993) such that any significant positive or negative regional extraction would suggest net elimination or formation of compound within that region.

Dose-normalized concentrations of both morphine and M3G in plasma were independent of the rate of infusion of morphine over an 8-fold range. Similar observations were made in humans

(Säwe *et al.*, 1983) during chronic oral dosing of cancer patients with an approximately 25-fold escalation in the daily dose-rate of morphine over a prolonged period. Likewise in rats, dose-normalized concentrations of morphine in plasma were independent of two single i.v. doses of morphine differing by 100-fold (Mistry & Houston, 1987). The major metabolite in all three species was M3G and the observations suggest that conversion to and elimination of this metabolite obeyed linear kinetics.

The overall mean C_b/C for morphine of 1.25 was comparable to values reported previously in the rat of 1.34 (Mistry & Houston, 1987), monkey of 1.3 (Rane *et al.*, 1984), and man of 1.21 (Mazoit *et al.*, 1987) and 1.1 (Padwardhan *et al.*, 1981). The values of C_b/C for morphine and M3G were independent of the concentration in blood from sheep over a 10- and 25-fold concentration range, respectively. Even though rigorous statistical analysis of the effect of the concentration of M6G in blood on the values of C_b/C for M6G was not possible, it was probably of no great importance in the calculations for mass balance because of the relatively low concentrations achieved in plasma.

There was considerable extraction of morphine by the liver and the kidney of the sheep. *In vivo* experiments from other studies found that morphine was also highly extracted by the liver of other species such as the rat (Dahlström & Paalzow, 1978; Iwamoto & Klaassen, 1977; Mistry & Houston, 1987), dog (Merrell *et al.*, 1990) and monkey (Rane *et al.*, 1984). Furthermore, the liver was found to account, almost entirely, for the total clearance of morphine from the body of the dog (Hug *et al.*, 1981b; Jacqz *et al.*, 1986) and human (Bodenham *et al.*, 1989). However, until the present study there has been very little direct evidence *in vivo* for a significant involvement by the kidney in the clearance of morphine. This study has found that clearance by the kidney was approximately 37% of the total clearance, and only the study of Horton & Pollack (1991) in the rat has demonstrated a similar contribution by the kidney to the total. However, that contribution, about 54%, was based on the assumption that removal of the influence of the kidney by ligation of the renal artery had no effect on hepatic clearance. In the sheep, the renal excretory clearance of morphine was only 36% of the clearance of morphine by the kidney. Assuming that steady-state concentrations of morphine had been achieved within the kidney between 5 and 6 hr,

the comparison suggests that 64% of morphine taken up by the kidney may be metabolized, most likely to M3G since this was the major metabolite appearing in urine.

The indicator, sulphobromophthalein, was infused concurrently with morphine for the determination of hepatic blood flow. It is an organic anion at physiological pH and probably transported into the hepatocytes by carrier-mediated systems with a broad specificity for the transport of anions (Tiribelli, 1992). There are also analogous systems for the transport of organic cations (Meijer *et al.*, 1990). At physiological pH morphine exists predominantly in the cationic form (Schill & Gustavii, 1964) and, since uptake into isolated hepatocytes appears to be saturable (Iwamoto *et al.*, 1978), hepatic uptake probably occurs via the system for the transport of organic cations. Competition between morphine and sulphobromophthalein for hepatic uptake was unlikely but even if present would not have altered the conclusion that the liver is a major organ for the elimination of morphine. Discussion of the possible influence of the indicator on the hepatic disposition of M3G and M6G will be discussed in chapter 6.

It is interesting that, when the calculated overall mean total body clearance for morphine of 1.58 ± 0.27 l/min was included with approximate mean values of clearance and reported mean body-weights obtained from the individual studies performed in the rat, rabbit, dog, monkey and human (section 1.5.4.1), the relationship between the clearance and body weight (Boxenbaum, 1982), examined using a power function (SPSS for Windows, SPSS Inc., Chicago, IL, U.S.A.), was found to be significant ($r = 0.971$, $p < 0.001$) with a coefficient of 57.1 and exponent of 0.83. The value for the exponent was similar to the values found for a number of other compounds reported to be highly cleared or cleared by metabolic reactions not involving cytochrome P450 (Ings, 1990).

There was a significant positive overall mean net extraction ratio for M3G and M6G by the kidney only, consistent with the finding that 76 % of the dose appeared in the urine as these two metabolites. This indicates that the kidney was the major site for their elimination. The calculated mean net clearances by the kidney of 0.104 l/min and 0.092 l/min for M3G and M6G, respectively, are similar to the GFR reported for sheep (Hecker, 1983) and, given that there was negligible binding of M3G in the plasma of sheep (Olsen *et al.*, 1988; see also chapter 6), suggests that, as with humans (chapter 3), the renal clearances of the unbound M3G and M6G

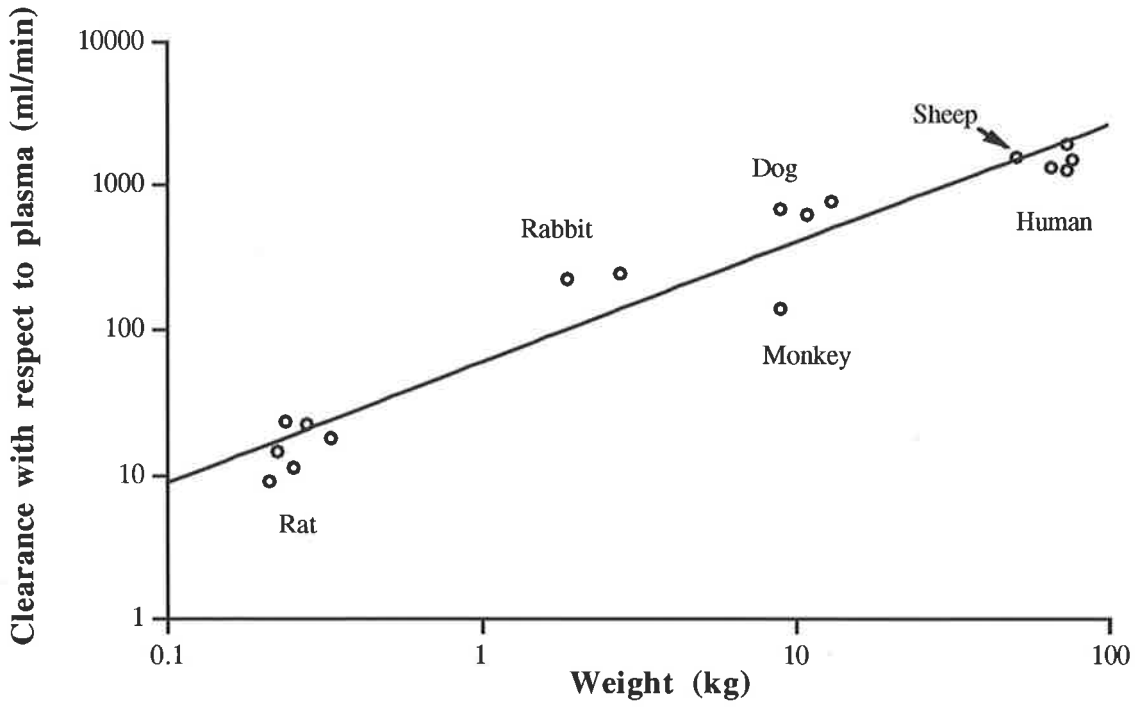


Figure 5.6. *Relationship between the clearance of morphine and body-weight of various species.*

The relationship was described by the equation: $y = 57.3x^{0.83}$, $r = 0.971$, $p < 0.001$.

Points are mean data from individual studies in various species as indicated.

approximate GFR. However, comparison of the calculated net clearances of M3G and M6G by the kidney with GFR to elucidate renal excretory clearance mechanisms may be complicated by the fact that it is not known if any of the glucuronides formed within the kidney appear in the renal venous blood. The experiments described in chapters 6 and 7 provide a greater insight into this aspect.

Determination of the renal clearances of M3G and M6G may be complicated further by the uptake of the highly extracted p-aminohippuric acid by the same renal tubular transport-system, the latter competing successfully with M3G and M6G and thus accounting for the similarity between the unbound renal clearances and GFR. However, evidence for a common path for transport is lacking and there may be a multiplicity of systems for the transport of organic anions in the proximal tubule (Møller & Sheikh, 1983). p-Aminohippuric acid appeared to be transported across the contraluminal and luminal plasma membranes of the S_3 segment of the proximal tubule

by specialized carrier-systems (Ullrich & Rumrich, 1988), and transport by both systems was reduced by probenecid. With regard to glucuronide metabolites, Redegeld & Noordhoek (1986) studied the handling of 1-naphthyl-β-D-glucuronide by the isolated perfused rat kidney and found evidence for active secretion which was sensitive to probenecid. However, the renal clearance of preformed paracetamol glucuronide and p-nitrophenol glucuronide in the dog corresponded to GFR and the clearance of the latter conjugate was insensitive to probenecid (Hekman *et al.*, 1986). The unbound renal clearances of the glucuronide metabolites of 1-hydroxymethylmidazolam (a metabolite of midazolam) (Vree *et al.*, 1989), zidovudine (de Miranda *et al.*, 1989; Singlas *et al.*, 1989), carprofen (Iwakawa *et al.*, 1989) in man were, in contrast to the values for M3G and M6G in man, much greater than GFR. However, the clearances were determined only following administration of the parent compound and, as found in the experiments described in chapter 6, if urinary excretion of the glucuronide were to follow its formation in the kidney, then true renal excretory clearance would be overestimated (Wan & Riegelman, 1972). The possible influence of p-aminohippuric acid on the uptake of M3G and M6G is unknown. However, in the absence of p-aminohippuric acid the renal clearance of unbound M3G and M6G in humans was comparable to GFR, and it was proposed that there was negligible net tubular secretion or reabsorption (chapter 3). The renal clearance of unbound M3G in the dog (Garrett & Jackson, 1979) was about two-thirds of an estimated value of GFR (Davies & Morris, 1993). Assuming that, in the absence of the indicator, the renal clearance of unbound M3G and M6G in the sheep was also similar to GFR and that glomerular filtration was the major path for elimination into the tubular lumen, then one would predict that the indicator would have a minimal influence on the uptake of M3G and M6G by the kidney. Clearly, further studies are needed to elucidate the mechanisms by which M3G and M6G are excreted by the kidney.

There was no significant net extraction or formation of either glucuronide by the liver. Given the markedly greater concentrations of M3G compared to morphine in blood perfusing the liver and the high hepatic extraction of morphine, if all of the M3G formed in the liver were to appear in the hepatic vein, one would expect an approximate 10% increase in the concentrations of M3G in emerging venous blood relative to the inflowing concentration. However, it is possible that there is a relative barrier to transfer of the more polar hepatically-formed glucuronide metabolites into

the blood compared to bile. In addition, any formation of M3G, as above, and subsequent passage into hepatic venous blood may have been compensated for by hepatic uptake of previously formed, circulating M3G. Other workers (Imamura & Fujimoto, 1980) found that approximately 5% of M3G was lost from the portal inflow, of which about one-fifth appeared in bile, during a single pass through an isolated *in situ* perfused liver of the rat. The possible influence of sulphobromophthalein on the hepatic disposition of M3G will be discussed in chapter 6, which examines the comparative disposition of formed and preformed M3G.

The lack of mass-balance between the sum of morphine and the glucuronide metabolites entering and leaving the liver in blood (figure 5.4) suggests a net uptake of morphine and/or the metabolite(s) into the liver, although there was considerable variability in one of the sheep. Previous workers have observed that, when morphine was given parenterally, biliary excretion as morphine-3-glucuronide (Parker *et al.*, 1980) or water-soluble morphine "glucuronide" (Walsh & Levine, 1975) occurred and, with subsequent hydrolysis in the gut, the aglycone became available for absorption (Parker *et al.*, 1980; Walsh & Levine, 1975). In earlier studies with the rat, 32% (Mistry & Houston, 1987) to 46% (Dahlström & Paalzow, 1978) of morphine was extracted by the intestinal mucosa during absorption. In the present study, there was no significant difference between the arterial and portal venous concentrations of morphine in plasma. Any increase in the concentration of morphine in portal venous blood, due to absorption of morphine from the lumen of the gut, may have been offset by a decrease in arterial concentration during perfusion of the gut. This is consistent with the net increase in the concentration of M3G in portal venous relative to arterial blood (figure 5.2). Thus, when considered together, the data suggest the existence of an enterohepatic cycle for morphine and its metabolites in the sheep and provide circumstantial evidence for the metabolism of morphine to M3G by the gut.

Blood flow to the liver and kidney was calculated by the Fick method using the indicators, sulphobromophthalein and p-aminohippuric acid, respectively. As discussed previously, it was assumed that sulphobromophthalein had a minimal effect on the uptake of morphine by the liver. Hurwitz & Fischer (1983) observed that s.c. doses of morphine (10 mg/kg and above) administered 30 min before an i.v. bolus of sulphobromophthalein increased retention of the indicator in the liver and plasma of rats. However, based on data from pharmacokinetic studies of

morphine in rats (Bhargava *et al.*, 1991; Dahlström & Paalzow, 1978; Horton & Pollack, 1991; Mistry & Houston, 1987; Tan *et al.*, 1989), the concentrations of morphine in plasma expected for up to an hour after a dose of 10 mg/kg morphine would have been more than an order of magnitude greater than those achieved during the 5th to 6th hr of infusion at the highest rate in the sheep. In addition, while the rate of biliary excretion of sulphobromophthalein in the rat appears to have been reduced by morphine given at the doses reported (Hurwitz & Fischer, 1983), it is likely that most of the dose of indicator was still eliminated by the liver, therefore providing an accurate measure of hepatic blood flow. The retention of sulphobromophthalein may have been due to reduced biliary flow or competitive inhibition of canalicular transport by morphine or M3G formed from morphine in the liver. With respect to p-aminohippuric acid, Baker & Woods (1957) found that its renal clearance during infusion in the dog was reduced by concurrent infusion with morphine. The effect was probably due to an altered blood flow to the kidney induced by morphine (Priano & Vatner, 1981) infused at a rate approximately 25-times the highest rate administered to the sheep.

In all but one of the present experiments, less than 100% of the dose was recovered in urine. Possible additional metabolites, which were not explored in the present study but have been found in the urine from other species, include normorphine, morphine-3,6-diglucuronide, morphine-3-sulphate, morphine N-oxide, dihydromorphine and various hydroxymorphines (section 1.5.3.1). The possibility of a metabolite(s) other than M3G and M6G being formed in the liver and transferred into hepatic venous blood also may account for the lack of mass-balance across the liver. However, it is also possible that, in some of the experiments, morphine and its metabolites were excreted in faeces.

In summary, this study has found that the concentrations of morphine and M3G produced in plasma were proportional to the rate of infusion with morphine, that the majority of a dose of morphine given to sheep was recovered as M3G in urine and that M6G was only a minor metabolite. Extraction ratios for morphine at both the liver and kidney were high, and it was concluded that the majority of the morphine extracted by the kidney was metabolized. Both organs are probably responsible for the majority of the formation of both metabolites, while the gut also

Chapter 6

Comparative Disposition of M3G during Separate i.v. Infusions of Morphine and M3G in Sheep: the Importance of the Kidney

6.1 Introduction

It was considered previously that glucuronide metabolites of xenobiotics were pharmacologically inactive and their formation afforded a mechanism for the detoxification and elimination of the parent compound as more polar metabolites via urine and bile. It is now recognized that their formation has important pharmacological and toxicological implications (Kroemer & Klotz, 1992; Mulder, 1992). Thus, the formation and disposition of the glucuronide metabolites of a number of drugs, including morphine, has come under more intense study in recent years.

As reviewed in chapter 1, the major metabolite of morphine in all species studied is M3G. Furthermore, the evidence suggestive of a functional antagonism by M3G of the antinociceptive effects of morphine and M6G in animals, and an ability of M3G to stimulate ventilation in dogs and rats and invoke hyperaesthesia and hyperactive motor behaviour in rats was also reviewed (chapter 1). It was postulated that these effects of M3G may result from its interaction with receptors other than the known opioid receptors.

The pharmacokinetics and pharmacodynamics of M3G given alone as either a bolus or a constant infusion have been studied in rats (Ekblom *et al.*, 1993). Other studies have examined the disposition of M3G in the pregnant rhesus monkey following either bolus or infusion doses of M3G (Gerdin *et al.*, 1990), or in one pregnant sheep following a bolus dose of M3G (Olsen *et al.*, 1988). There have been no reports of studies comparing the disposition of M3G during the separate administration of morphine and M3G.

The study described in chapter 5 demonstrated that (i) the liver and kidneys are responsible for the majority of the clearance of morphine; (ii) the kidney both metabolizes and excretes morphine; (iii) M3G is the predominant metabolite, and (iv) there was net extraction of M3G by the kidney and net production by the gut. It was concluded that the kidney was the major site for the elimination of M3G and that morphine was involved in an enterohepatic cycle,

presumably involving biliary excretion of M3G followed by hydrolysis in the gut and reabsorption of morphine. However, it was not possible to conclude whether M3G was both extracted and produced by the liver or to determine the fate of M3G formed from morphine within the kidney. In order to address these questions relating to the disposition of M3G, the aim of this study was to compare, in another group of sheep, the disposition of M3G after separate infusions of morphine and M3G.

6.2 Methods

6.2.1 Preparation of the Sheep

The preparation of the sheep and methods for the measurement of regional blood flows were as described in chapter 4. Catheters were inserted into the descending aorta, pulmonary artery, hepatic vein, hepatic portal and renal veins, and posterior vena cava for the collection of blood. As described in chapter 4, catheters were also inserted for the measurement of cardiac output, for the infusions of morphine, or M3G, and of p-aminohippuric acid into the right atrium, and of sulphobromophthalein into the descending aorta.

6.2.2 Experimental Design

Five merino ewes with a mean (± SD) weight of 48 (± 5) kg and age range of 1.5 to 2 yr, were given two-stage infusions of morphine (as morphine sulphate B.P., David Bull Laboratories, Mulgrave, Australia) or M3G (morphine-3-ß-D-glucuronide, Sigma Chemical Co., St. Louis, MO, U.S.A.), both in saline, through a right atrial catheter. Initially, 8 times the maintenance dose rate was administered for 15 min, followed by a maintenance rate of 10 mg/hr (morphine sulphate) or 4 mg/hr (M3G) for a further 5.75 hr; sheep 6 received M3G at a maintenance rate of 7.5 mg/hr. The rate of infusion was controlled using a single-channel infusion pump (Model 902A, Harvard Apparatus, Millis, MA, U.S.A.). Both infusions were maintained at flows approximating 0.5 ml/min. Each animal received the two compounds on separate occasions, at least 2 days apart. The rate at which M3G was infused to sheep 6 was on the basis of a predicted concentration in plasma of approximately 1000 nmol/l, obtained when morphine was infused at 10 mg/hr (chapter 5), and simulations using the pharmacokinetic data

obtained by Olsen *et al.* (1988). From the concentrations of M3G measured in sheep 6, the infusion rate was adjusted for subsequent experiments with the other sheep.

6.2.3 Collection of Samples

Blood (1 ml), at 15 min intervals from 5 to 6 hr after beginning the infusion, was collected simultaneously through catheters previously inserted into the aorta, pulmonary artery, hepatic vein, hepatic portal vein, renal vein and posterior vena cava. Additional samples were collected from the aorta at 0.25, 0.5, 1, 2, 3, and 4 hr (sheep 6 and 7) or 5, 10, 15, 20, 25 and 30 min, 1, 2, 3 and 4 hr (sheep 8, 10 and 11) and for all sheep at 24 and 48 hr; from sheep 8, further samples were collected at 7, 8, 9, 10, 11 and 12 hr. Samples were centrifuged within 30 min and the plasma transferred to 1.5 ml polypropylene centrifuge tubes for storage at -20°C.

Urine was collected via an indwelling Foley catheter prior to ('blank' urine) and between 0 to 5 hr and 5 to 6 hr from the start of the infusion. At the end of each timed period, the bladder was rinsed with 40 ml of sterile saline and this was combined with the urine. The catheter was removed at the end of the infusion and, thereafter, free-flowing urine was collected from 6-24 hr and 24-48 hr. For each interval, the volume of the combined urine/saline or free-flowing urine was measured and an aliquot stored at -20°C.

6.2.4 Binding of Morphine, M3G and M6G in Plasma

Binding in plasma was determined for samples collected from the aorta and pulmonary artery of individual sheep during the 5th to 6th hour of infusion with morphine sulphate. The plasma remaining after the assay for morphine, M3G and M6G was pooled and, after spiking with additional M6G (81.5 nmol/l) and adjusting the pH to 7.4 with phosphoric acid (1.5 mol/l), duplicate 1 ml samples were subjected to ultrafiltration at 37°C using an MPS-1 apparatus fitted with a YMT membrane (Amicon Corp., Danvers, MA, U.S.A.). The concentrations of morphine, M3G and M6G in aliquots of filtrate (0.5 ml) and pooled plasma (0.5 ml), determined by HPLC, were used to calculate the fraction unbound in plasma. Binding to the ultrafiltration apparatus was negligible (chapter 3).

6.2.5 Determination of C_b/C

The values for C_b/C were determined as described previously (section 5.2.5) in 'blank' blood collected prior to commencing the morphine sulphate and M3G infusions. Duplicate blood samples were spiked with morphine (35.1 and 129 nmol/l), M3G (558 nmol/l) and M6G (26.2 and 98.8 nmol/l) and equilibrated at 37°C for 30 min. The nominal concentration in blood divided by the concentration determined in plasma by HPLC, was taken as C_b/C.

6.2.6 Determination of Morphine, M3G and M6G

Morphine, M3G and M6G in plasma (0.5 ml), plasma ultrafiltrate (0.5 ml) and urine (0.05 to 0.2 ml) were determined by paired-ion HPLC (chapter 2). Drug-free plasma and urine was obtained prior to the start of an infusion with either morphine or M3G and was evaluated for any chromatographic interferences to morphine, M3G and M6G prior to its use for the preparation of standards for calibration. Standards were prepared in plasma over the range of 13.3 nmol/l to 532 nmol/l, 108 nmol/l to 4330 nmol/l, and 20.4 nmol/l to 102 nmol/l, for morphine, M3G and M6G, respectively; in urine they were prepared over the ranges of 0.532 to 10.6 μmol/l, 4.33 to 86.7 μmol/l and 0.407 to 2.04 μmol/l, respectively.

6.2.7 Determination of Creatinine in Plasma and Urine

The concentrations of creatinine, in plasma collected from the aorta at 5 and 6 hr and in urine collected during this interval, were determined by a modification of an HPLC method reported previously (Huang & Chiou, 1983).

Briefly, 0.05 ml of plasma was mixed with 0.1 ml of acetonitrile, centrifuged, and 0.025 ml of the supernate was injected onto a Whatman Partisil 10 SCX strong cation-exchange column, 250 mm x 4.6 mm i.d. (Alltech Associates, Deerfield, IL, U.S.A.). A mobile phase of 2% acetonitrile in 0.1 mol/l ammonium dihydrogen phosphate (pH 4.4) was pumped through the column at 2 ml/min and the eluate monitored at 250 nm. Creatinine had a retention time of approximately 5.5 min. Standards were prepared by adding known amounts of creatinine (range 0.018 to 0.88 mmol/l) to sheep 'blank' plasma. Accuracy and reproducibility, obtained

from the determination of creatinine in five control samples containing prepared concentrations of 0.044, 0.18 and 0.71 mmol/l, were 114 ± 6.3%, 100 ± 1.9% and 108 ± 2.0%, respectively.

For the determination of creatinine in urine, 0.05 ml of a mixture of urine (0.05 ml) and water (2 ml) was injected onto the same column. Creatinine was monitored at 250 nm and, using a mobile phase of 10% acetonitrile in 0.02 mol/l ammonium dihydrogen phosphate (pH 4.6) pumped at a flow rate of 2 ml/min, had a retention time of approximately 8 min. Standards, prepared in water over the range 0.44 to 17.7 mmol/l, were treated similarly to urine. Accuracy and reproducibility, assessed on five replicates from a batch of solutions prepared in water at concentrations of 0.44, 7.07 and 17.7 mmol/l, was 95.8 ± 4.4%, 96.4 ± 2.5% and 98.8 ± 1.8%, respectively.

6.2.8 Pharmacokinetic Analysis

The AUC(0-5) and AUC(5-6) were calculated using the trapezoid rule. The average concentration was calculated as the AUC divided by the time-interval. The total clearance of morphine or M3G from the body with respect to blood was calculated from the quotient of the amount of morphine base or M3G infused during the 5 to 6 hr period and the product of the respective C_b/C and pulmonary arterial AUC(5-6). The term "arterial", as distinct from "pulmonary arterial", refers to samples collected from the aorta. Regional net extraction ratios for morphine, M3G and M6G were calculated as the difference between the respective AUC(5-6) for the plasma entering and leaving divided by that entering a given region. As previously (chapter 5), it was assumed that 80% of blood flowing to the liver came via the hepatic portal vein with the remainder via the hepatic artery. Regional net clearances for morphine and regional net clearances for each glucuronide were estimated from the product of the respective extraction ratio and blood flow. The fraction of the summed morphine and glucuronides in blood entering the liver which did not reappear in the hepatic vein as either morphine or glucuronide metabolites (fractional uptake) was calculated as described previously (Section 5.2.7).

The amount of morphine, M3G or M6G taken up by the kidney during the 5 to 6 hr period was calculated from the product of the respective differences in arterial and venous AUC(5-6) of blood and blood flow to the kidney.

Renal excretory clearance of morphine, M3G and M6G was calculated as the amount excreted during the 0 to 5 hr and 5 to 6 hr intervals of the infusion divided by the respective arterial AUC. Division by the fraction unbound in plasma gave the renal excretory clearance of unbound compound. The renal excretory clearance of M3G with respect to blood during the infusion with M3G was calculated from the quotient of the respective renal excretory clearance and C_b/C. Urinary recovery was calculated as the percentage of the doses of morphine and M3G infused over 6 hr that was recovered in urine over 48 hr as morphine, M3G and M6G, and as M3G and morphine (*vide infra*). Where appropriate, the data for the sheep given M3G at an infusion rate of 7.5 mg/hr were normalized to a rate of 4 mg/hr for comparison with the other sheep.

The renal clearance of creatinine during the 5 to 6 hr period was calculated as the rate of urinary excretion divided by the mean of the concentrations of creatinine in the two samples of plasma collected at 5 and 6 hr.

6.2.9 Statistics

Where appropriate, comparisons of means between two or more variables were performed by Student's t-test or analysis of variance (Statview+^{TM}, Brainpower Inc., Calabasas, CA, USA). Values of $p < 0.05$ were considered significant. Standard methods were used to determine the power of a test (Winer 1971). Variations about means are given as standard deviations (and on graphs are indicated as error bars).

6.3 Results

All five sheep received morphine and M3G. They were apparently healthy, had normal renal function and appeared to suffer no ill-effects from the administration of either morphine or M3G.

6.3.1 Profiles of the Concentrations in Plasma against Time

Figure 6.1 depicts typical profiles of the concentrations in plasma against time for morphine, M3G and M6G during the 5 to 6 hr of infusion with morphine (sheep 7). Concentrations in plasma of M6G during the infusion of morphine to sheep 8 were below the limit of quantification. The data for individual sheep are presented in tables A3.1 to A3.10. As an indicator of the variability in the pulmonary arterial concentrations between 5 hr and 6 hr in each experiment, the coefficients of variation were calculated. These were comparable to the analytical reproducibility for each compound and there were no obvious trends in the concentrations over the time interval. Similar results were found for the concentrations of M3G in plasma during the infusion of M3G, and data from sheep 7 is shown in figure 6.2. There was no detectable morphine or M6G in the plasma of sheep given M3G. For sheep 8, from which additional arterial samples were obtained between 6 and 12 hr, the concentrations of morphine and M3G in plasma during and after the infusion of morphine, and of M3G when M3G was infused, are shown in Figure 6.3.

The mean values of arterial AUC(0-5) for morphine, M3G and M6G during the infusion of morphine were 1180 ± 340 nmol/l.hr, 5020 ± 1130 nmol/l.hr and 170 ± 69 nmol/l.hr, respectively, while the respective arterial AUC(5-6) values were 195 ± 59 nmol/l.hr, 1330 ± 210 nmol/l.hr and 37 ± 12 nmol/l.hr. The respective pulmonary arterial AUC(5-6) values for morphine, M3G and M6G were 197 ± 61 nmol/l.hr, 1340 ± 220 nmol/l.hr and 37 ± 13 nmol/l.hr, respectively. During the M3G infusion, the mean pulmonary arterial AUC(5-6) value was 1040 ± 250 nmol/l.hr. During the 5th to 6th hour of infusion with morphine, the mean pulmonary arterial ratios of the AUC for M3G/morphine, M6G/morphine and M3G/M6G were 7.0 ± 1.4, 0.19 ± 0.05 and 37 ± 8, respectively. The ratio of the pulmonary arterial AUC for M3G during infusion with morphine to that during infusion with M3G, normalized to equimolar doses of morphine and M3G, was 0.44 ± 0.10. After 6 hr of infusion with morphine, the arterial concentration of M3G in plasma averaged 1370 ± 170 nmol/l, and then decreased to 271 ± 134 nmol/l at 24 hr. Normalized to a rate of 4 mg/hr, the average concentration at the end of 6 hr of infusion with M3G was 1060 ± 290 nmol/l but was below the quantifiable limit at 24 hr.

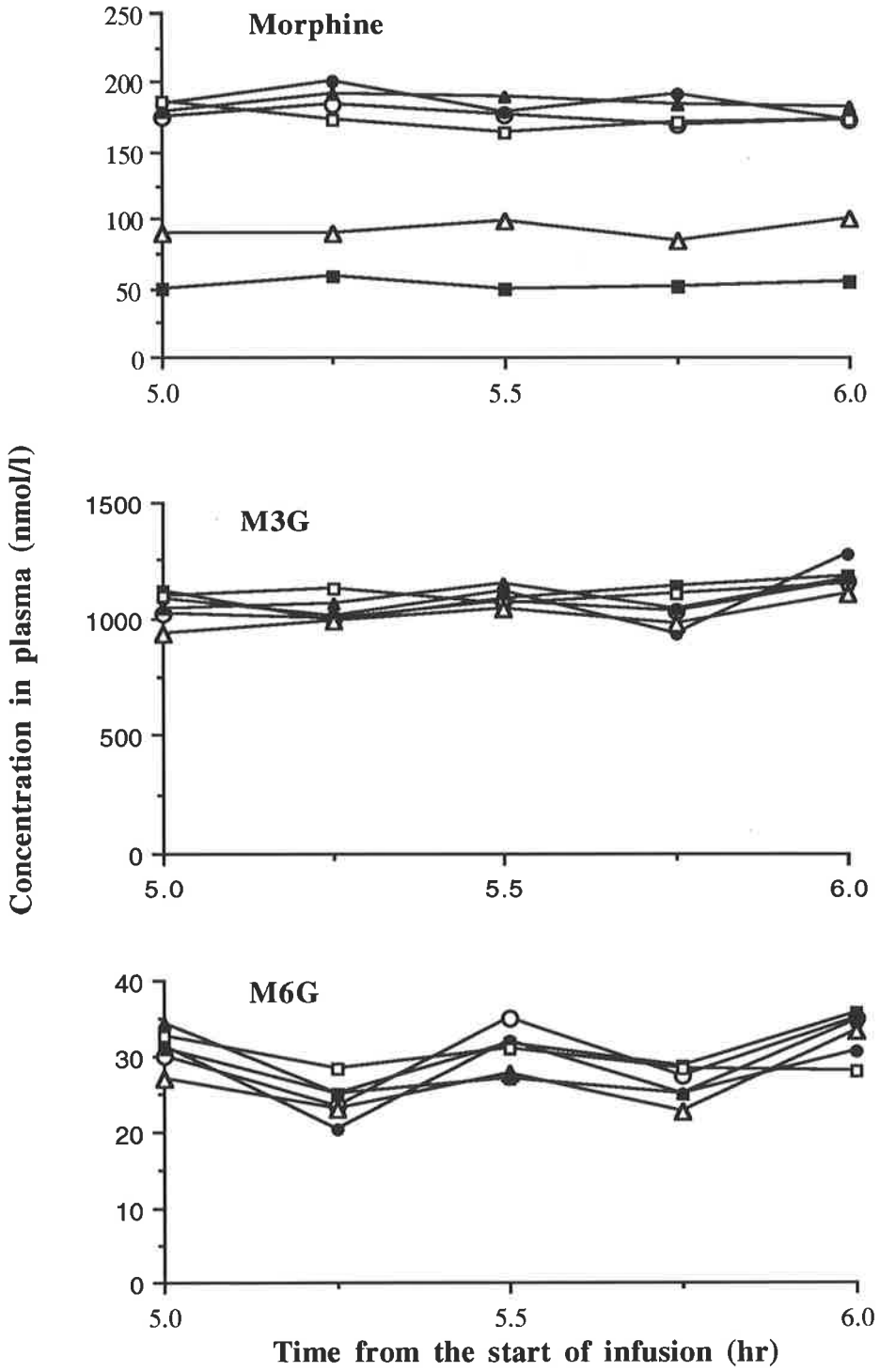


Figure 6.1. *Plasma concentrations of morphine, M3G and M6G in plasma of sheep 7 during the 5th to 6th hour of infusion with morphine sulphate at 10 mg/hr.*

The concentrations are in samples collected from the abdominal aorta (O), pulmonary artery (●), hepatic vein (■), hepatic portal vein (□), renal vein (Δ), and posterior vena cava (▲).

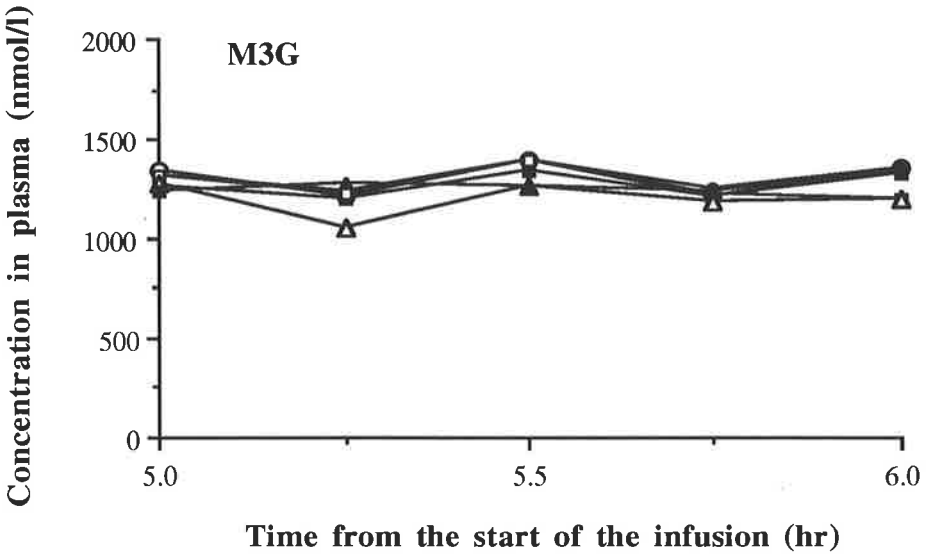


Figure 6.2. *Concentrations of M3G in plasma from sheep 7 during the 5th to 6th hour of infusion with M3G at 4 mg/hr.*

The concentrations are in samples collected from the abdominal aorta (O), pulmonary artery (●), hepatic vein (■), hepatic portal vein (□), renal vein (Δ), and posterior vena cava (▲).

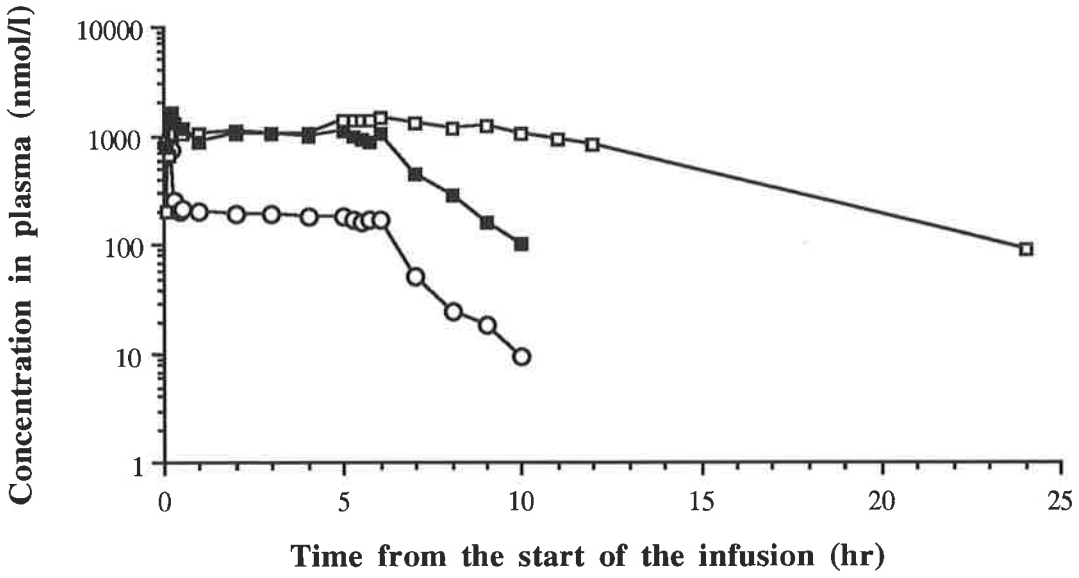


Figure 6.3. *Concentrations of morphine (O) and M3G (□) determined in arterial plasma following infusion of morphine sulphate (10 mg/hr), and of M3G (■) following infusion of M3G (4 mg/hr) in sheep 8.*

6.3.2 Regional Extraction

The mean regional net extraction ratios for morphine, M3G and M6G during administration of morphine and for M3G during administration of M3G are shown in figure 6.4. There was significant extraction of morphine by the liver (0.707 ± 0.030, $p < 0.001$), and kidney (0.593 ± 0.074, $p < 0.001$). There was significant extraction of M3G by the kidney during the administration of morphine (0.109 ± 0.045, $p < 0.006$) and M3G (0.102 ± 0.040, $p < 0.005$) but comparison of the two revealed no significant difference ($p = 0.70$). Significant net production of M3G by the gut was observed during infusion with morphine (-0.047 ± 0.009, $p < 0.001$), but not with M3G (0.024 ± 0.027, $p = 0.113$), and comparison of these two parameters revealed a significant difference ($p < 0.005$). During infusion with M3G there was no significant net formation or extraction of M3G by the liver (-0.005 ± 0.015, $p = 0.500$). There was significant net extraction of M6G by the kidney only (0.122 ± 0.044, $p = 0.012$), during infusion with morphine.

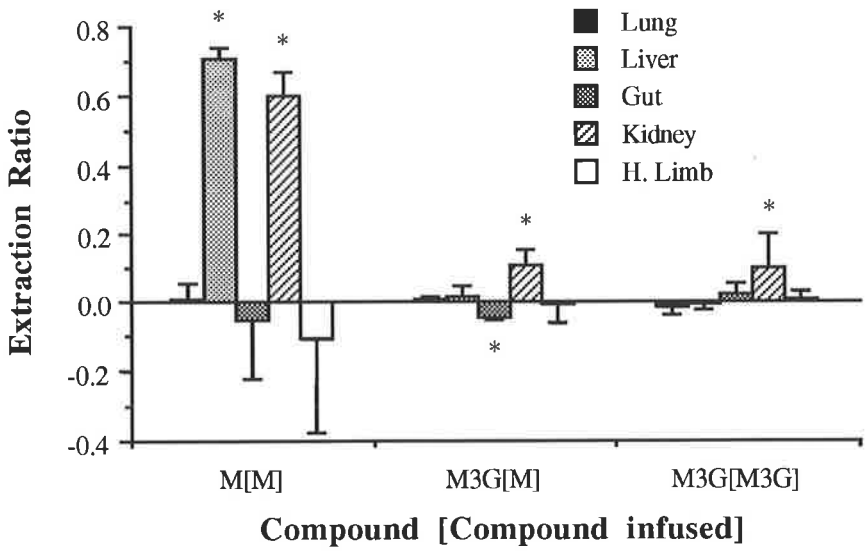


Figure 6.4. *Mean regional extraction ratios of morphine, M3G and M6G during infusion of sheep with morphine sulphate [M], and of M3G during infusion with M3G [M3G].*

* significantly different from zero, $p < 0.050$.

6.3.3 Ratios of the Concentrations in Blood to Plasma (C_b/C)

The values calculated for each sheep are presented in table A3.11. Except for sheep 7 when given M3G, 'blank' blood was obtained from each sheep prior to administration of morphine and M3G. While morphine and M6G were also added to 'blank' blood obtained from the other sheep prior to the M3G infusions, the data were not used for statistical analyses or for calculating the mean values of C_b/C. The values of C_b/C were independent of the nominal concentration for morphine ($p = 0.18$; 1,10 df) and M6G ($p = 0.68$; 1,10 df) and there was no evidence of a sheep x concentration interaction for morphine ($p = 0.24$; 4,10 df) or M6G ($p = 0.16$; 4,10 df). For M3G, there was no significant difference ($p = 0.32$) when comparing the values of C_b/C obtained prior to the morphine and M3G infusions. Therefore, the value used for sheep 7 was common to both experiments. The overall mean ($n = 5$) values of C_b/C for morphine, M3G and M6G determined in blood sampled prior to the experiments with morphine were 1.30 ± 0.12, 0.88 ± 0.11 and 0.84 ± 0.10, respectively, while for the experiments with M3G the mean value ($n = 4$) for M3G was 0.92 ± 0.10. During the experiments with morphine and M3G the mean haematocrits, 0.33 ± 0.02 and 0.32 ± 0.01, respectively, were not significantly different ($p = 0.55$, $n = 4$).

6.3.4 Comparison of Total and Regional Clearances for Morphine and M3G

The total body clearance of morphine with reference to pulmonary arterial blood was 1.86 ± 0.54 l/min. Cardiac output, and blood flows to the liver and kidney of individual sheep are presented in table A3.13. With reference to blood, the mean clearances of morphine by the liver and kidney were 1.65 ± 0.72 l/min and 0.80 ± 0.14 l/min, respectively. The mean of the sum of the two regional clearances for morphine was 2.45 ± 0.80 l/min and, although it was not significantly different ($p = 0.068$) from the total body clearance, the individual sum was consistently greater than the total in all sheep. The individual differences ranged from 0.17 to 1.46 l/min. During infusion with M3G, the total body clearance of M3G with reference to blood, 0.160 ± 0.044 l/min, was not significantly different ($p = 0.76$) from the clearance by the kidney of 0.162 ± 0.062 l/min. In addition, this regional clearance was not significantly

different (p = 0.45) from the corresponding regional clearance of M3G during infusion with morphine (0.147 ± 0.064 l/min).

6.3.5 Binding in Plasma

Individual values are given in table A3.12. The mean fraction of morphine, M3G and M6G unbound in pooled arterial and pulmonary arterial plasma, during the 5th to 6th hr of the infusion with morphine, was 0.80 ± 0.07, 0.86 ± 0.06 and 0.90 ± 0.09, respectively. The values for morphine and M3G were significantly (p = 0.004 and 0.007, respectively) different from 1.0, but the value for M6G was not (p = 0.082).

6.3.6 Renal Clearance and Urinary Recovery

The renal clearance of creatinine during infusion with morphine (0.072 ± 0.020 l/min) was not significantly different (p = 0.51) from that while M3G was infused (0.081 ± 0.005 l/min). The renal excretory clearances of morphine, M3G and M6G during infusion with morphine and of M3G during infusion with M3G are shown in figure 6.5.

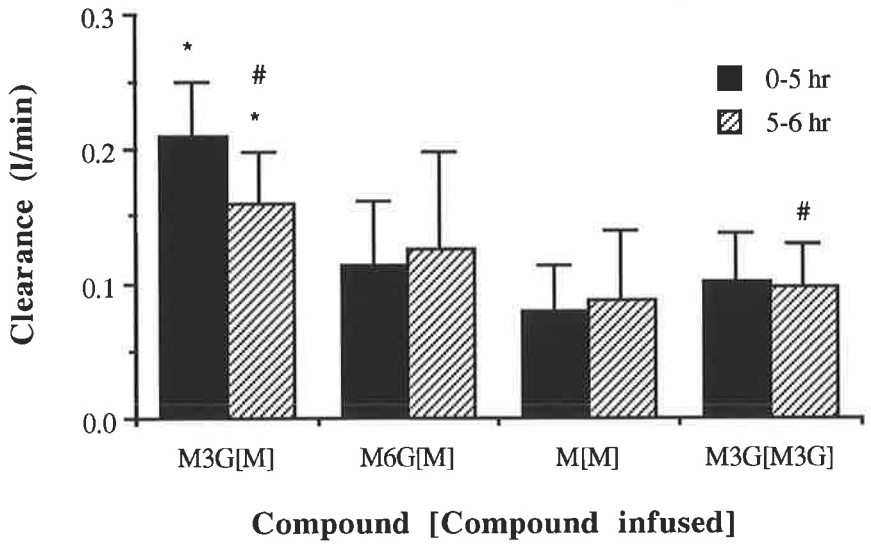


Figure 6.5. *Mean renal excretory clearances (with reference to plasma) for morphine (M), M3G (M3G) and M6G (M6G) during infusion with morphine ([M]), and for M3G (M3G) during infusion with M3G ([M3G]).*

*,# significantly different, p < 0.050.

There was a significant ($p = 0.049$) difference in the renal excretory clearance of M3G during the 0 to 5 hr and 5 to 6 hr periods of infusion with morphine. However, there were no significant differences for the corresponding renal excretory clearances of morphine ($p = 0.45$) and M6G ($p = 0.61$) and for M3G when M3G was infused ($p = 0.76$). Nevertheless, only the data collected during the 5 to 6 hr period of the infusions was used for further comparisons and for the calculation of renal excretory clearances of unbound compound. The renal excretory clearance of M3G during infusion with morphine (0.158 ± 0.040 l/min) was significantly different ($p = 0.009$) from that while M3G was being infused (0.100 ± 0.027 l/min). During infusion with M3G, the renal excretory clearance of M3G with reference to blood (0.110 ± 0.032 l/min), was significantly different ($p = 0.039$) from the regional clearance of M3G by the kidney (0.162 ± 0.062 l/min). The mean ratios of the renal excretory clearances of unbound M3G, M6G and morphine during infusion with morphine, and of unbound M3G during infusion with M3G, to creatinine clearance were 2.6 ± 0.5, 1.9 ± 0.9, 1.5 ± 0.6 and 1.5 ± 0.4, respectively. Only the ratio for M3G during the infusion with morphine was significantly different from unity ($p = 0.002$, 4 df). The ratios for M6G and morphine during infusion with morphine and for M3G during infusion with M3G ranged from 0.96 to 3.0 ($n = 4$), 0.82 to 2.3 and from 1.1 to 2.1, respectively, but were not significantly different from unity ($p = 0.14$, 0.13 and 0.071, respectively).

Figure 6.6 shows the mean urinary recovery of the dose of morphine as morphine, M3G and M6G and of the dose of M3G as M3G and morphine over 48 hr. The mean total recoveries after the infusions of morphine and M3G were $77 \pm 8\%$ and $84 \pm 13\%$, respectively. The mean percentage of the dose of morphine recovered as M3G and M6G was $62 \pm 18\%$ and $1.2 \pm 0.8\%$, respectively. The fraction as M3G, 0.62, was significantly greater ($p = 0.024$, 4 df) than the fraction of the dose of morphine metabolized to M3G, 0.44 (section 6.3.1), that was calculated from the concentrations of M3G in plasma during infusion with morphine and M3G. While M3G was being infused no morphine was detected in urine collected via the catheter. Between 6 and 48 hr, morphine was found in the urine from sheep 6, 7, 8, and 11, but in no sheep was M6G detected. The mean total recoveries of the morphine and M3G doses during the 6 to 24 hr interval were $32 \pm 5\%$ and $25 \pm 6\%$, respectively, and were significantly different (p

= 0.024). The recoveries during the 24 to 48 hr interval were 5.6 ± 3.4% and 3.6 ± 1.6%, respectively, and, while the former was consistently greater, there was no significant difference (p = 0.11) between the two.

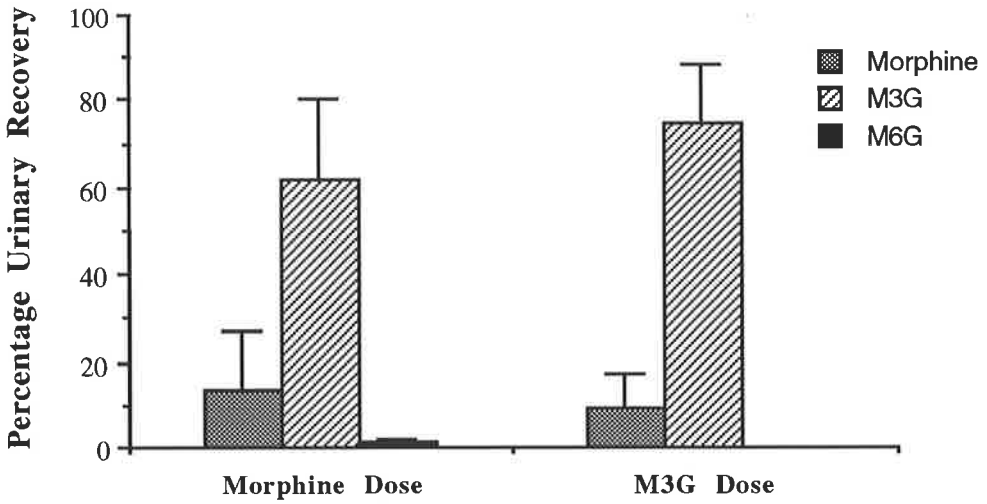


Figure 6.6. *Mean percentage urinary recovery of the morphine dose as morphine, M3G and M6G, and of the dose of M3G as M3G and morphine, over 48 hr.*

6.3.7 Mass-balance across the Kidney and Liver

Figure 6.7 shows, over the 5 to 6 hr period of infusion with morphine, a comparison of the mean amount of morphine, M3G and M6G taken up by the kidney from arterial blood against that excreted in urine. There were significant differences for morphine (p = 0.002) but not for M3G (p = 0.098) and M6G (p = 0.68). For M3G, the difference remained nonsignificant (p = 0.093) even if the amounts were normalized for the AUC(5-6) in blood. There was a significant difference (p = 0.015) when comparing the sum of the amount of morphine, M3G and M6G taken up by the kidney (22 ± 5 μmole) against that excreted in urine (14 ± 3 μmole). A comparison of uptake of M3G against excretion during infusion with M3G (figure 6.7) revealed a significant difference (p = 0.030).

The mean fraction of the sum of morphine, M3G and M6G entering the liver via the hepatic artery and portal vein which did not reappear in the hepatic vein (fractional retention) was 0.13 ± 0.03, and was significantly different from zero (p < 0.001).

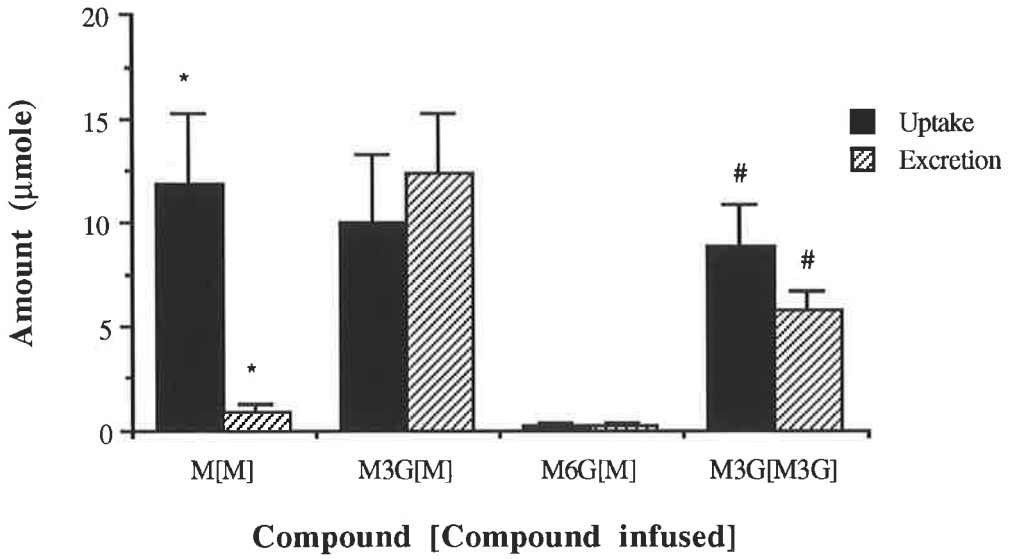


Figure 6.7 *Comparison of the mean amounts of morphine (M[M]), M3G (M3G[M]) and M6G (M6G[M]) during infusion with morphine, and of M3G (M3G[M3G]) during infusion with M3G, taken up by the kidney from arterial blood, with the corresponding mean amounts excreted in urine during the 5th to 6th hour of infusion.*

* significantly different, p = 0.002

\# significantly different, p = 0.030

6.4 Discussion

The concentrations of morphine, M3G and M6G measured in plasma during the 5th to 6th hour of the infusion with morphine sulphate were similar to those found previously (chapter 5) at the same infusion rate of 10 mg/hr. Essentially constant concentrations of morphine, M3G and M6G in plasma were achieved in the present study between the 5th and 6th hr of the infusion. The ratios of the areas under the pulmonary arterial concentration in plasma-time curve for M3G/morphine, M6G/morphine and M3G/M6G in the present study were comparable to the respective ratios obtained over the four-fold range of doses used previously (chapter 5).

In the rat, a constant infusion of M3G (9.4 µmol/hr/kg) achieved nearly constant concentrations of M3G in plasma of 14.4 µmol/l within 4 hr (Ekblom *et al.*, 1993). After 30 hr

of infusion, the concentrations of morphine in plasma reached 200 nmol/l, suggestive of deconjugation of M3G. In the present study there was no systematic change in the pulmonary arterial concentrations of M3G in plasma during the 5th to 6th hour of infusion suggesting that steady-state had been achieved. An infusion rate of 83 μg/hr/kg (0.18 μmol/hr/kg), approximately one fiftieth that given to the rat (Ekblom *et al.*, 1993), achieved a mean concentration for M3G in plasma of 1.04 μmol/l, but without measurable concentrations of morphine during the infusion or at 24 hr or 48 hr.

The mean values of C_b/C for morphine, M3G and M6G were comparable to those determined in the previous study (chapter 5). In addition, the total body clearance of morphine with reference to blood of 1.86 l/min was comparable to the previous value of 1.58 l/min. The slightly larger value may be due to the higher cardiac outputs and blood flows to the liver and kidney (tables A2.16 and A3.13). The sum of the hepatic and kidney clearances for morphine was consistently greater than the total body clearance, but the difference was not statistically significant. Although this consistency was not observed in the previous study (chapter 5), taken together with the significant net uptake of the sum of morphine, M3G and M6G entering the liver and the net production of M3G by the gut, both observations confirming those from the previous study (chapter 5), it does reaffirm the hypothesis of an enterohepatic cycle for morphine and its metabolites. For sheep 8, the majority of the difference between the sum, of the clearances by the liver and kidney, and the total of 1.46 l/min may be accounted for by the net appearance of morphine in venous blood from the hind limb, with the venous concentration being approximately 58% greater than the arterial value. In this sheep, steady-state may not have been achieved in the less well-perfused tissues, including the hind limb, and the higher venous concentration observed may be due to loss from the hind limb of accumulated morphine from the loading infusion. It is unlikely to be due to deconjugation of M3G since no morphine was detected in venous blood from the hind limb during infusion with M3G in this or any of the other sheep. For sheep 11, the concentration of morphine in hepatic portal blood was 35% greater than arterial blood. Given that the estimated portal blood flow was 2.5 l/min (80% of the entire flow to the liver) for this sheep, a difference of 0.73 l/min between the sum of the two

regional clearances and the total clearance may be attributed to the considerable net return of morphine to the systemic circulation from the gut.

During infusion with M3G, the similarity between the total body clearance of M3G with reference to blood and its clearance by the kidney suggests that almost all of the total clearance can be attributed to the kidney. This is consistent with the 84% recovery of the dose in urine, mostly as unchanged M3G. Further support comes from the insignificant extraction of M3G by the liver or any other region and suggests a relative barrier to the uptake of M3G by the liver or other organs. A previous study with the isolated perfused liver from the rat found that only 5% of M3G perfusing the liver was extracted, of which one-fifth was excreted into bile (Imamura & Fujimoto, 1980). The total body clearance of M3G with reference to blood in the present study (3.28 ± 0.64 ml/min/kg) is comparable to the value of 2.12 ml/min/kg determined with reference to plasma in one pregnant ewe following a bolus dose of M3G (Olsen *et al.*, 1988), but is approximately 40% of the value of 10.5 ml/min/kg (also with reference to plasma) determined in rats (Ekblom *et al.*, 1993). No urine was collected during these two studies.

The mean concentration of M3G in plasma, 18 hr after the end of the infusion with morphine, was approximately 20% of the mean concentration found when the infusion was ceased. In contrast, the concentration of M3G in plasma over the corresponding interval had decreased to less than 5% of the mean value when the infusion of M3G was ceased. This difference is shown clearly for sheep 8 (figure 6.3) when more extensive sampling was undertaken. The delayed elimination is consistent with enterohepatic cycling and continuous slow efflux of hepatically-formed M3G into blood. Arterial concentrations of M3G in plasma after 10 min of infusion with morphine were almost half of those ultimately achieved during the 5 to 6 hr period, suggesting a rapid efflux of M3G from the liver, and potentially the kidney, into plasma. A large concentration gradient to plasma water generated by the rapid formation of M3G within the hepatocyte probably ensures the early appearance of M3G in plasma.

From the previous findings that the clearance of morphine by the kidney of the sheep was consistently in excess of its renal excretory clearance, it was proposed that the kidney both excretes and metabolizes morphine (chapter 5). This study provides three additional lines of evidence in support of this proposal. Firstly, during the 5 to 6 hr period of infusion with

morphine, less than 10% of the morphine extracted by the kidney appeared unchanged in urine (*vide infra*). Secondly, the renal excretory clearance of M3G during infusion with morphine, an apparent renal clearance, was significantly greater than its true renal clearance when M3G was infused (figure 6.5). The expression, "true renal clearance," applies to M3G extracted from blood by the kidney and excreted unchanged in urine. It is proposed that a majority of the M3G formed from morphine in the renal tubular cells is excreted into the urine, giving an apparent renal excretory clearance value approximately 61% greater than the true clearance. Finally, the value of 0.44 for the ratio of the AUC of M3G during infusion with morphine to the AUC during an equimolar infusion of M3G was markedly less than the fraction of the dose of morphine excreted as M3G in urine, 0.62, and indicates that a large fraction of M3G formed in the sheep never appeared in plasma; probably the M3G formed within the kidney. This may also apply to any M6G formed within the kidney.

When perfusing the isolated kidney of the rat with 1-naphthol, Redegeld *et al.* (1988) found that 1-naphthol glucuronide formed in the kidney passed into both the perfusate and urine, but Hart *et al.* (1980), performing a similar experiment with paracetamol, found that paracetamol glucuronide appeared in urine but none was detected in perfusate. If half of the M3G formed in the kidney of the sheep during infusion with morphine were to pass into venous blood, then the venous AUC would be approximately 7% less than the arterial AUC and, compared to the 10% lesser venous AUC found during infusion with M3G, the difference between the net extractions of M3G would probably remain non-significant. Therefore, the similarity in net extraction of M3G between infusion with morphine and with M3G is supportive of, but in itself does not provide conclusive evidence that all of the M3G formed in the kidney is excreted directly into urine.

The apparent renal clearance of M3G up to the 5th hr of infusion with morphine was approximately 33% greater than that between 5 and 6 hr (figure 6.5). The mean concentration of M3G in arterial blood between 5 and 6 hr was approximately 32% greater than that during the period up to 5 hr, while the mean arterial concentration of morphine in blood was approximately 21% greater in the period up to 5 hr. Presumably, in the 0 to 5 hr period, M3G formed from morphine within the kidney will contribute more to that component of the apparent

renal excretory clearance which is additional to the "true renal clearance". In contrast, but as expected, the renal excretory clearances of morphine during both intervals were similar (figure 6.5). The similarity in the apparent renal clearances of M6G between the two periods suggests that the kidney may be a relatively minor site for the formation of M6G.

With the possibility of metabolism of the parent compound in the kidney and direct excretion of the metabolites into urine, incorrect conclusions regarding mechanisms of renal excretion of the metabolite (i.e. net secretion or reabsorption) may be drawn if only the parent is administered and an apparent renal clearance of the metabolite is compared with GFR. This is clear from the observations of Duggin & Mudge (1975) in the dog, the authors concluding from a comparison of the renal clearance of paracetamol glucuronide with that of inulin that net secretion occurred when the concentrations of the glucuronide in plasma were low, and net reabsorption occurred at high concentrations. However, from the start of the constant infusion with paracetamol, or when incremental increases in the rate were made, the concentrations of paracetamol glucuronide in plasma would be low relative to those for paracetamol, and would have increased as the infusion continued at a particular rate. As noted earlier, Hart *et al.* (1980) were able to demonstrate the formation and appearance in urine of paracetamol glucuronide from the isolated perfused kidney of the rat. On the basis of the findings in the sheep during infusion with morphine, it might be expected that the apparent renal clearance relative to GFR would be higher when the concentrations of paracetamol glucuronide were low relative to paracetamol. When Hekman *et al.* (1986) administered a bolus of paracetamol glucuronide to the dog, renal clearance was independent of the concentration in plasma and similar to GFR over a wider range of concentrations, but which included the range measured by Duggin & Mudge (1975).

During the 5 to 6 hr period of infusion with morphine only 7.5% of the morphine extracted by the kidney appeared unchanged in urine. It is possible that morphine was still accumulating within the kidney, which may account for some of the discrepancy. But, during infusion with morphine the amount of M3G excreted in urine exceeded by 2.4 μmole that taken up from arterial blood over the same period whereas only 67% of that taken up by the kidney appeared in urine while M3G was infused. Together, these observations suggest that renal

metabolism of morphine to M3G occurred and that transport of the more polar metabolite into the tubular lumen was relatively slow. Previous workers have proposed that accumulation of unmetabolized morphine in proximal tubular segments of the rabbit kidney, to concentrations approximately 15 times those in the medium adjacent to the basolateral membrane, was due to facilitated uptake and intracellular binding (Schali & Roch-Ramel, 1982). These workers also used hydrolysis with ß-glucuronidase and HPLC to confirm the metabolism of morphine by the segments to glucuronide metabolites. Similar tissue/medium relative concentrations were found when slices from the kidney cortex of the rat and mouse were incubated in the presence of morphine (Teller *et al.*, 1976) but TLC analysis could not demonstrate conclusively whether metabolism of morphine had occurred. During the 5 - 6 hr period of infusion with morphine in the present study, only 61% of the summed morphine, M3G and M6G taken up by the kidney could be accounted for in urine. While some of this mass imbalance may be attributed to accumulation of morphine and preformed M3G and M6G taken up from arterial blood plus glucuronides formed within the tubular cells, it is also possible that other routes for the metabolism of morphine exist. However, on average, only 23% of the dose of morphine could not be accounted for in urine as morphine, M3G and M6G and some of this could be due to excretion in the faeces.

During infusion with M3G, the renal excretory clearance of M3G with respect to blood was 67% of the regional kidney clearance. As accumulation continued within the kidney until steady-state was achieved, it is likely that the clearance of M3G by the kidney would decrease to a value approximating the renal excretory clearance.

The fractions of morphine, M3G and M6G unbound in plasma taken from the sheep during the infusions of morphine were similar to those found in the plasma of healthy humans (chapter 3). Previous workers have found negligible binding of M3G in the plasma of pregnant ewes (Olsen *et al.*, 1988), pregnant guinea pigs (Olsen *et al.*, 1989) and dogs (Garrett & Jackson, 1979). Because of the low binding, the fraction of M3G unbound in the plasma of sheep given M3G was assumed to be the same as that during the corresponding infusion with morphine.

Previous workers have found that the clearance of inulin in sheep increases as the intake of protein increases (Rabinowitz *et al.*, 1973; Faix *et al.*, 1988) but that the clearance of creatinine remains unchanged (Faix *et al.*, 1988). For sheep maintained on a low nitrogen diet, creatinine clearance overestimated the clearance of inulin, and presumably glomerular filtration rate (GFR), by approximately 22% but maintenance on a high protein diet produced equivalent clearances of creatinine and inulin (Faix *et al.*, 1988). In the present study, protein intake was not controlled but it was estimated (Hack *et al.*, 1988) to be intermediate between the high and low intake of the previous studies (Rabinowitz *et al.*, 1973; Faix *et al.*, 1988). The mean renal clearances of creatinine determined during the infusions with morphine and M3G in the present study were 0.072 l/min and 0.081 l/min, respectively, which were similar to the previously reported mean values ranging from 0.075 l/min to 0.080 l/min (Faix *et al.*, 1988). Therefore, it may be assumed that creatinine clearance measured in the present study was a reasonable estimate of GFR.

Since M3G, and possibly M6G, was formed within the kidney, only the true renal excretory clearances of unbound morphine during infusion with morphine and of unbound M3G during infusion with M3G may be used to ascertain the mechanisms of renal excretion for the respective compounds in the sheep. For sheep 6, 7, 10 and 11 the ratio of the renal excretory clearance of unbound morphine to creatinine clearance ranged from 1.25 to 2.33, while for sheep 8 it was 0.82. Similarly, the ratio for M3G when M3G was infused ranged from 1.08 to 2.10 in sheep 6, 8, 10 and 11, and was 0.89 in sheep 7. The respective values were not significantly different from unity but, with the variability in the ratios for morphine and M3G, the power of the t-test used to detect significant differences between mean values of 1.5, for both compounds, and unity was only 56% and 30%. In other studies, net secretion of morphine was observed in patients under intensive-care (chapter 3) and with the isolated perfused kidney of the rat (Ratcliffe *et al.*, 1985). For M3G the apparent renal clearance of unbound M3G formed in the patients was similar to GFR (chapter 3). Additional sheep would need to be examined before making comparisons with other studies.

The significant difference between the renal excretory and kidney clearances of M3G, with only two-thirds of the M3G extracted from arterial blood appearing in urine, suggests that

M3G is still accumulating within the kidney or that further metabolism is occurring. An active transport system in the basolateral membrane may concentrate M3G (and M6G) within the tubular cell. It has been proposed that cephaloridine, which, like M3G and M6G, exists as a zwitterion at physiological pH, is transported across the basolateral membrane but not readily across the luminal membrane, creating high intracellular concentrations (Tune *et al.*, 1974). Likewise, the accumulation of the relatively polar M3G (and M6G) is probably due to slower passage through the luminal membrane. From studies in the chicken kidney (Watrous *et al.*, 1970), it was concluded that morphine was secreted via a cation transport system and also metabolized to morphine-3-sulfate (M3S). It was proposed also that preformed M3S was excreted primarily via an anion transport system in the basolateral membrane, although the possible minor involvement of a cation transport system was not excluded. A similar mechanism for the renal excretion of arterial M3G and M6G may exist in the sheep.

Confirming the observations in chapter 5, there was no significant net production or extraction of M3G or M6G by the liver during infusion with morphine. Similar observations for the net extraction of M3G during infusion with M3G suggest that there is a diffusion-limitation, as proposed in the report by Turner & Brouwer (1990), or a system with a low affinity or capacity for the transport of M3G at the sinusoidal membrane. At pH 7.4, M3G would exist as a zwitterion and, despite the predictions of greater than expected lipophilicity by Carrupt *et al.* (1991) and Gaillard *et al.* (1994), diffusion may be limited by its relatively high polarity. When the isolated liver of the rat was perfused with morphine or M3G, Imamura & Fujimoto (1980) found that dehydrocholate sodium inhibited the biliary transport of M3G across the canalicular membrane, but not its efflux into the perfusate when formed in the liver or the uptake of preformed M3G from perfusate across the sinusoidal membrane. This suggests that there are differences in transport across the two membranes. Evidence is accumulating for more than one system for the transport of organic anions across both membranes (Tiribelli, 1992), and whether sulphobromophthalein influences the transport of M3G and M6G is not known. Since, in the absence of competitors, uptake of M3G by the liver from the rat was low (Imamura & Fujimoto, 1980), a similar finding in the sheep probably reflects poor uptake of preformed M3G and slow efflux of hepatically-formed M3G in this species also, irrespective of

the presence of sulphobromophthalein, although the possible modulating influence of the indicator cannot be discounted.

The urinary recovery of the dose of morphine as morphine, M3G and M6G was comparable to that found during the study described in chapter 5. The incomplete recovery of the dose of M3G in urine over 48 hr may have been due to any combination of elimination in faeces, involvement of a small fraction of the dose in an enterohepatic cycle or retention within hepatocytes, slow efflux from renal tubular cells into urine, and further metabolism to, for example, morphine-3,6-diglucuronide. Previous workers (Ekblom *et al.*, 1993) found that, following a bolus dose of M3G given to rats, the kinetics in plasma could be described by a two-compartment model. Without direct evidence, they attributed the second phase, contributing 8 per cent of the AUC and having a half-life of 11.6 hr, to enterohepatic cycling. While a slower elimination phase was not detected in plasma in the present study, an average of 3.6% of the dose of M3G was excreted in urine, as M3G or M3G plus morphine, between 24 hr and 48 hr from the start of the infusion, suggesting retention of a minor fraction of the dose. A greater fractional retention of the dose of morphine in the body, after cessation of the infusion, is evident from the significantly larger mean percentage of the dose, 31.8% compared with 25.1% of the M3G dose, recovered in urine during the 6 hr to 24 hr collection period. Any combination of continuous formation of M3G from body stores of morphine, enterohepatic cycling, barrier(s) to entry into the hepatic venous blood or efflux from renal tubular cells into urine may account for these observations. After oral and intravenous doses of morphine were given to humans, morphine, M3G and M6G were measured in urine collected for up to 72 hr (Hasselström & Säwe, 1993). From the urinary excretion data, these workers determined mean half-lives of 15.1, 11.6 and 12.9 hr for morphine, M3G and M6G, respectively, after intravenous administration and comparable values after oral administration, and proposed that the prolonged elimination was due to enterohepatic cycling.

During the 6 hr period of infusion, urine was collected via an indwelling catheter. While M3G was being infused there was no evidence of morphine in the urine, demonstrating that M3G was not hydrolyzed during collection. However, freely-flowing urine could have been contaminated by faecal material during collection. Enzymatic hydrolysis of M3G in the faeces

(Parker *et al.*, 1980) may account for the presence of morphine in the 6 to 48 hr periods of urinary collection from four of the sheep. Therefore, during infusion with morphine, the fraction of the dose recovered as morphine may be an overestimate of the true fractional excretion. In the study described in chapter 5, the renal excretory clearance of morphine was calculated from the product of the total body clearance and the fraction of the morphine dose excreted unchanged in urine collected in the same manner. Therefore, in that study it is possible that in some sheep the renal clearance of morphine may be an overestimate of the true value. However, the conclusion that the kidneys both excrete and metabolize morphine remains valid.

In summary, this study has found that the disposition of preformed M3G is markedly different from M3G formed at the sites of metabolism for morphine, namely the liver and kidney. Preformed M3G undergoes insignificant hepatic extraction but a large fraction of the dose is eliminated by the kidney. In contrast, M3G formed from morphine in the liver exhibits prolonged elimination from plasma due to any combination of its involvement in an enterohepatic cycle, its possible slow release into plasma because of a relative barrier to transport from the hepatocytes into hepatic venous blood, and its possible continued formation as morphine is released from body stores. M3G formed from morphine in the kidney is excreted in urine rather than being transported into peritubular blood. There is also indirect evidence for the accumulation of M3G within the kidney, whether it be extracted from arterial blood or formed *in situ*.

Chapter 7

The Disposition of Morphine, M3G and M6G during i.v. Infusion of Morphine and Comparison of the Disposition of M3G during Separate i.v. Infusions of Morphine and M3G: Studies in Sheep with Renal Failure

7.1 Introduction

Regnard & Twycross (1984) observed a requirement for lower doses of morphine in patients with renal failure, and it has been noted previously (chapter 1) that the incidence of side-effects was increased when the usual therapeutic doses were administered to patients with renal dysfunction. The pharmacokinetics of morphine in patients with renal failure has been reviewed in section 1.5.4.3. Briefly, two studies (Säwe & Odar-Cederlöf, 1987; Woolner *et al.*, 1986) have found that, compared to humans with normal renal function, the clearance of morphine from patients in renal failure was not significantly altered. In contrast, the results from the study of Osborne *et al.* (1993) suggest that the clearance of morphine may be significantly reduced by renal failure. The importance of the kidney for the elimination of morphine in humans remains unknown but, as found from the studies described in chapters 5 and 6, the kidney was of considerable importance in the clearance of morphine from normal sheep. The kidney of both species was the major organ for the elimination of M3G and M6G, and in humans it has been proposed that the prolonged opioid effects following the administration of morphine to patients with renal failure was due to accumulation of M6G (Bodd et al., 1990; Hasselström *et al.*, 1989; Osborne *et al.*, 1986; Shelly *et al.*, 1986). The previous studies in sheep following administration of morphine have demonstrated that the kidney both metabolizes and excretes morphine, and that M3G is the predominant metabolite.

Following on from the findings of chapters 5 and 6, the aims of the experiments described in this chapter were to examine the influence of experimentally-induced renal failure on (i) the total clearance of morphine from the body and its clearance by the kidney during infusion with morphine; (ii) the elimination of M3G and M6G when morphine was infused; (iii)

the total clearance of M3G and its clearance by the kidney during infusion with M3G, and (iv), with the inclusion of data from normal sheep, the relationships between renal function and the renal excretory clearances of morphine, M3G and M6G.

7.2 Materials and Methods

7.2.1 Animal Preparation

Catheters were placed into the same sites of the sheep as described in chapter 6 using the methods described in chapter 4. With the additional catheter into the renal artery (section 4.3) and the removal of the right kidney, renal failure was induced by the injection of a mixture of glass and polystyrene beads into the renal artery as described in section 4.4. The development of renal failure was monitored by daily measurement of the concentrations of creatinine in plasma (Autoanalyzer, Department of Clinical Biochemistry, Flinders Medical Centre).

7.2.2 Experimental Design

Seven merino ewes with a mean (± SD) weight of 52 (± 4) kg and age range of 1.5 to 2 yr, were given two-stage infusions of morphine (as morphine sulphate B.P., David Bull Laboratories, Mulgrave, Australia) or M3G (morphine-3-ß-D-glucuronide, Sigma Chemical Co., St. Louis, MO, U.S.A.), both in saline, into a right atrial catheter. For the first 15 min, the dose was infused at 8 times the maintained rate, and was followed by a rate maintained at 10 mg/hr (morphine sulphate) or 4 mg/hr (M3G) for a further 5.75 hr. The rate of infusion was controlled using a single-channel infusion pump (Model 902A, Harvard Apparatus, Millis, MA, U.S.A.). The infusion flowed at approximately 0.5 ml/min during the last 5.75 hr. Each animal received the two compounds on separate occasions, at least 7 days apart. For all except sheep 13, the infusion of morphine was given before the M3G.

Four of the sheep in renal failure (7, 8, 10 and 11) had been infused with morphine and M3G in a previous study when normal, and those results presented in chapter 6. The data from an additional normal sheep that had been infused with morphine only but had not been reported previously, were included with the data from the normal sheep (chapter 6) for an unpaired comparison against sheep with renal failure.

7.2.3 Collection of Samples

Blood (1 ml), at 15 min intervals from 5 to 6 hr after beginning the infusion, was collected simultaneously from catheters previously inserted into the aorta, pulmonary artery, hepatic vein, hepatic portal vein, renal vein and posterior vena cava. Additional samples were collected from the aorta at 0.25, 0.5, 1, 2, 3, and 4 hr (sheep 7) or 5, 10, 15, 20, 25 and 30 min, 1, 2, 3 and 4 hr (sheep 8 to 13), at hourly intervals between 6 and 12 hr for sheep 8, and for all sheep at 24, 48, 72, 96, 120 and 144 hr. Samples were centrifuged within 30 min and the plasma transferred to 1.5 ml polypropylene centrifuge tubes for storage at -20°C.

Urine was collected via an indwelling Foley catheter prior to ('blank' urine) and between 0 to 5 hr and 5 to 6 hr from the start of the infusion. At the end of each timed collection period, the bladder was rinsed with 40 ml of sterile saline and this was combined with the urine. The catheter was removed at the end of the infusion and, thereafter, free-flowing urine was collected from 6-24, 24-48, 48-72, 72-96, 96-120 and 120-144 hr. For each interval, the volume of the combined urine/saline or free-flowing urine was measured and an aliquot stored at -20°C.

7.2.4 Binding of Morphine, M3G and M6G in Plasma

The binding of morphine, M3G and M6G was determined in pooled arterial plasma by ultrafiltration as described previously (section 6.2.4), except that there was no additional M6G.

7.2.5 Determination of C_b/C

The values for C_b/C were determined as described previously (section 5.2.5) in 'blank' blood collected prior to commencing the infusions of morphine sulphate and M3G. Duplicate samples of blood were spiked with morphine (70.2 and 257 nmol/l), M3G (1120 nmol/l) and M6G (52.4 and 198 nmol/l) and equilibrated at 37°C for 30 min. The nominal concentration in blood divided by the concentration determined in plasma by HPLC, was taken as C_b/C.

7.2.6 Determination of Morphine, M3G and M6G

Morphine, M3G and M6G in plasma (0.5 ml), plasma ultrafiltrate (0.5 ml) and urine (0.1 to 0.2 ml) were determined by paired-ion HPLC (chapter 2). Plasma and urine was obtained

prior to the start of an infusion with either morphine or M3G and evaluated for any chromatographic interferences to morphine, M3G and M6G prior to its use for the preparation of standards for calibration. Standards were prepared in plasma over the range of 13.3 nmol/l to 1060 nmol/l, 108 nmol/l to 8670 nmol/l, and 20.4 nmol/l to 1020 nmol/l, for morphine, M3G and M6G, respectively; in urine over the range 0.266 to 10.6 μmol/l, 2.17 to 86.7 μmol/l and 0.204 to 2.04 μmol/l for morphine, M3G and M6G, respectively.

7.2.7 Determination of Creatinine in Plasma and Urine

Concentrations of creatinine, in plasma collected from the aorta at 5 and 6 hr and in urine collected during this interval, were determined by reversed-phase HPLC as described previously (section 6.2.7).

7.2.8 Data Analysis

The AUC(0-5) and AUC(5-6) was calculated by the trapezoid rule. The average concentration was calculated as the AUC divided by the time interval. The total clearance of morphine or M3G from the body, with respect to blood, was calculated from the quotient of the amount of morphine base or M3G infused during the 5 to 6 hr period and the product of the respective C_b/C and pulmonary arterial AUC(5-6). The total clearance of M3G from arterial blood when M3G was infused was also calculated as the total amount infused divided by the product of the AUC(0-∞) and C_b/C. The terminal phase rate constants for M3G and M6G, determined from 48 hr and beyond by linear regression analysis of the logarithm of the concentrations in plasma-time data using Multifit (Day Computing, Cambridge, U.K.), was used to calculate the corresponding half-life. The concentrations beyond 144 hr were predicted from the equation of regression. The AUC(0-∞) for M3G during infusion with M3G was calculated from the corrected concentrations (after taking account of the concentrations generated from the dose of morphine, *vide infra)* by the trapezoid rule, with extrapolation beyond the last data value using the line fitted by linear regression. Regional net extraction ratios for morphine, M3G and M6G were calculated as the difference between the respective AUC(5-6) for the plasma entering and leaving divided by that entering a given region. As

previously (chapters 5 and 6), and without evidence to the contrary during renal failure, it was assumed that 80% of the flow of blood to the liver was via the hepatic portal vein with the remainder via the hepatic artery. Regional net clearances for morphine and each glucuronide were estimated from the product of the respective extraction ratio and blood-flow. The fraction of the summed morphine and glucuronides in blood entering the liver which did not reappear in the hepatic vein as either morphine or glucuronide metabolites (fractional uptake) was calculated as described in section 5.2.7.

Renal excretory clearances of morphine, M3G and M6G was calculated as the amounts excreted during the 0 to 5 hr and 5 to 6 hr intervals of the infusion divided by the respective arterial AUC. Division by the fraction unbound in plasma gave the unbound renal excretory clearance. The renal excretory clearances with respect to blood were calculated from the quotient of the respective renal excretory clearance and C_b/C. Urinary recovery was calculated as the percentage of the doses of morphine and M3G infused over 6 hr that was recovered in urine as morphine, M3G and M6G, and as M3G and morphine (*vide infra*), respectively.

Renal clearance of creatinine during the 5 to 6 hr period was calculated as the urinary excretion rate divided by the mean of the concentrations of creatinine in the two samples of plasma collected at 5 and 6 hr.

7.2.9 Statistics.

Comparisons of means between two or more variables were performed by the appropriate paired or unpaired Student's t-test or by analysis of variance (Statview+^{TM}, Brainpower Inc., Calabasas, CA, USA). Values of $p < 0.05$ were considered significant. Standard methods were used to determine the power of a test (Winer, 1971). Variations about means are given as standard deviations, as are error bars on graphs. Where appropriate, the data obtained from this study were also compared with the data reported previously from the study with 5 normal sheep (chapter 6), to which was added the data from 1 further sheep that had received morphine but not M3G (sheep 5). Associations or relationships between parameters were examined by Spearman rank-order correlation or linear regression, respectively (Statview+^{TM}, Brainpower Inc., Calabasas, CA, USA).

7.3 Results

Initially, examination of the influence of varying degrees of renal failure on the disposition of morphine, M3G and M6G was planned. It was thought that the varying degrees might be induced by unilateral nephrectomy followed by progressive infarction induced by the renal arterial injection of occlusive material. Injection of a progressively increasing quantity of polystyrene beads, up to 600,000 per day, induced only mild renal failure, as shown by the approximate doubling of the concentration of creatinine in plasma for sheep 7, 22 days after nephrectomy (figure 7.1). This may have been due to hypertrophy of the kidney (Strauch & Gretz, 1988), compensating for the effect of the polystyrene beads. Injection of glass beads produced a more rapid increase in the concentration of creatinine in plasma, which could then be maintained by periodic injections of polystyrene and/or glass beads (figure 7.1, sheep 9 to 13). Not wishing to subject the animals to prolonged discomfort and the added risk of infection, and having planned to examine the disposition of morphine, M3G and M6G during infusion with morphine, and of M3G during infusion with M3G, and for a further 138 hr after both infusions had ceased, it was decided to perform experiments when the concentrations of creatinine in plasma were within the approximate range of 0.4 to 0.5 mmol/l.The concentrations of creatinine measured routinely in plasma, and the days on which morphine and M3G were infused, are shown for each sheep in figure 7.1. Further details on the quantity of beads administered are given in table A4.17.

All seven sheep in renal failure received morphine but only three (sheep 10, 11 and 13) received M3G. Sheep 7 was given morphine when the concentration of creatinine in plasma had reached 0.16 mmol/l, while for the other sheep the concentrations ranged from 0.40 mmol/l to 0.54 mmol/l on the day when morphine or M3G was infused (figure 7.1). Complete collections of blood and urine were obtained from sheep 7, 8 and 11 given morphine and from sheep 13 given M3G. Blood and urine were collected for 48 hr from sheep 9 and for 72 hr from sheep 12 and 13 when morphine was administered, and for 72 hr and 48 hr from sheep 10 and 11, respectively, after the infusion of M3G. Blood could not be taken from the catheter placed into the hepatic portal vein of sheep 12. The collection of urine from sheep 10 between 5 and 6 hr was incomplete. The data for all sheep are presented in appendix 4: the concentrations of

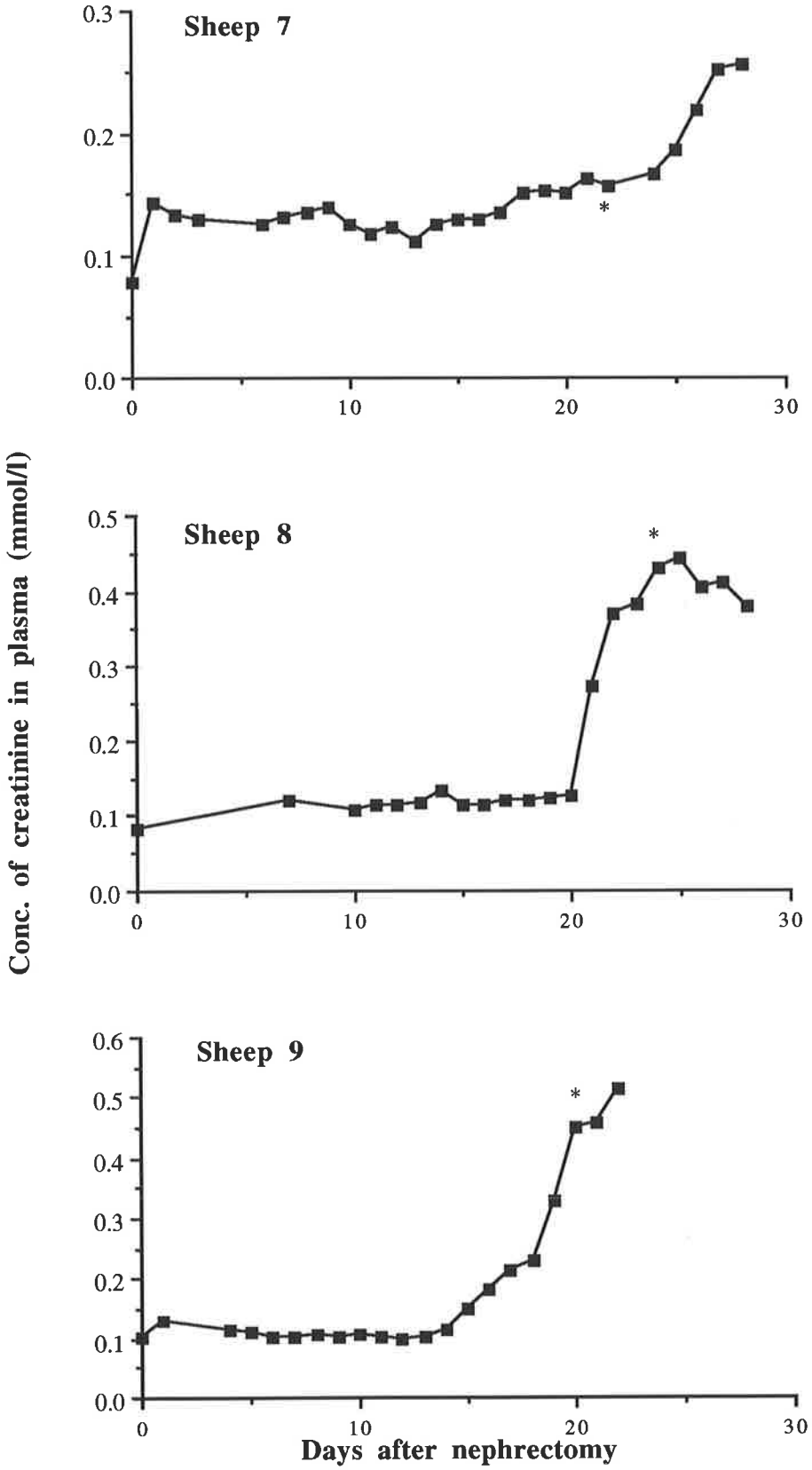


Figure 7.1 *Concentrations of creatinine in plasma of sheep 7, 8, 9, 10, 11, 12 and 13 and the day on which morphine (*) and M3G (#) were infused.*

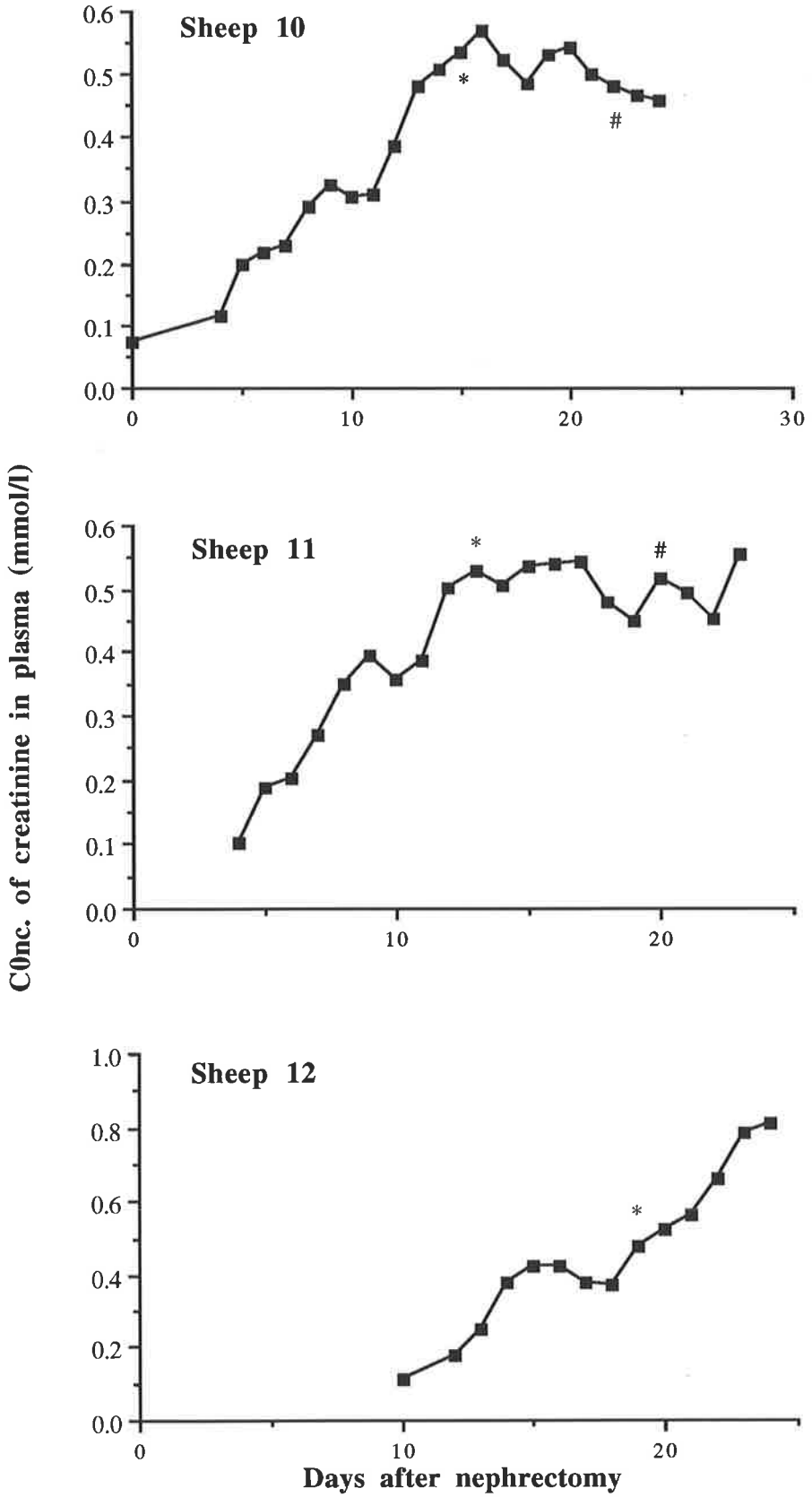


Figure 7.1(cont.) *Concentrations of creatinine in plasma of sheep 7, 8, 9, 10, 11, 12 and 13 and the day on which morphine (*) and M3G (#) were infused.*

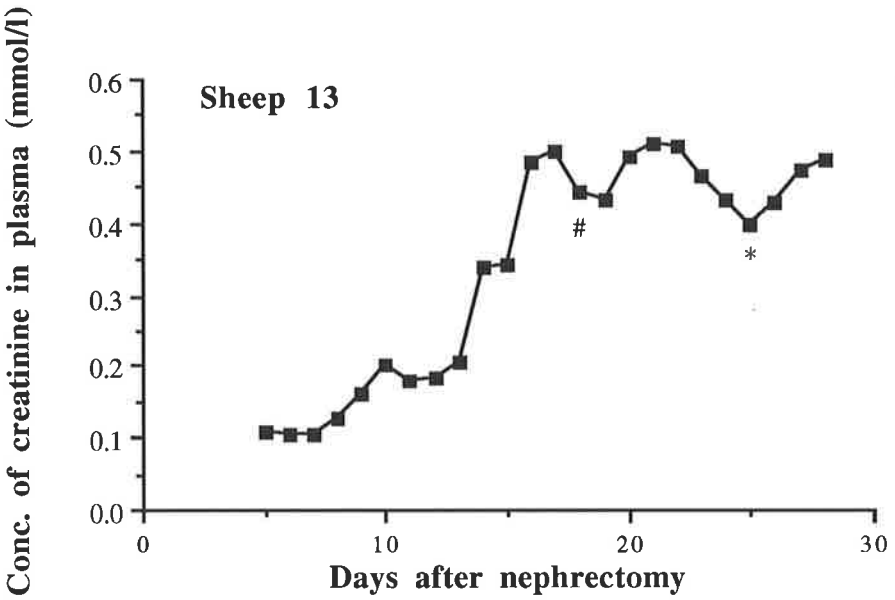


Figure 7.1(cont.) *Concentrations of creatinine in plasma of sheep 7, 8, 9, 10, 11, 12 and 13 and the day on which morphine (*) and M3G (#) were infused.*

morphine, M3G and M6G in plasma in tables A4.1 to A4.7 for sheep infused with morphine, and in tables A4.8 to A4.10 for sheep infused with M3G; tables A4.11 and A4.12 contain the concentrations of morphine, M3G and M6G in urine when morphine and M3G, respectively, were infused. The concentrations of total protein, albumin and total bilirubin, and the activities of alkaline phosphatase and alanine aminotransferase are not presented, but were comparable to the values found in the normal sheep studied in the experiments described in chapter 6. Data for cardiac output and blood-flow to the liver and kidney are presented in table A4.15. The data for sheep 5 infused with morphine, and from which parameters were calculated and combined with the data from the normal sheep described in the experiment in chapter 6, are presented in table A4.18.

7.3.1 Concentrations in plasma-time profiles

Figure 7.2 depicts the profiles from sheep 11 for morphine, M3G and M6G during the 5 to 6 hr of infusion with morphine. As an indicator of the variability in the pulmonary arterial concentrations between 5 hr and 6 hr in each experiment, the coefficients of variation were calculated for morphine, M3G and M6G. These were comparable to the analytical reproducibility for each compound and there were no obvious trends in the concentrations over

the time interval. However, examination of the concentrations in arterial plasma-time profiles for the same sheep between 0 and 6 hr (figure 7.3) showed a gradual increase for M3G and M6G in contrast to those for morphine. Figure 7.4 shows the arterial concentrations in the same sheep over the entire collection period. Concentrations of M3G in plasma over the 5 to 6 hr period in sheep 11 during the infusion with M3G are shown in figure 7.5. There were detectable (i.e. a chromatographic peak was present at the expected time of retention) but not quantifiable concentrations of morphine in all samples of plasma between 5 and 6 hr from the portal vein of sheep 11 given M3G. For sheep 10 the concentrations were quantifiable in three of the samples from the portal vein, ranging from 17 nmol/l to 19 nmol/l, but only detectable in the other two, while for sheep 13 there was no detectable morphine in any of the samples. No M6G was present in the plasma of sheep given M3G. Arterial concentrations of M3G between 0 and 72 hr when M3G was infused into sheep 11 are shown in figure 7.6 and again it is evident that there was a gradual increase in the concentrations of M3G up to 6 hr. The half-lives of M3G and M6G, calculated from 48 hr after starting the infusion of morphine, ranged from 25.9 to 113 hr (n = 4) and 43.1 to 95.1 hr (n = 2), respectively, while the value from sheep 13 after infusion with M3G was 61.6 hr.

For sheep 13, the concentration of M3G remaining at 144 hr after the start of the infusion with M3G, and prior to the start of the infusion with morphine, was below the limit of quantification and, therefore, less than 2% of the mean arterial concentrations of M3G during the 5 to 6 hr period of infusion with morphine. However, for sheep 10 and 11 the concentrations of M3G remaining at 144 hr from the respective infusions with morphine, and prior to starting the infusions of M3G, were 36% and 47% of the respective mean concentrations observed during the 5th to 6th hour of infusion with M3G. The arterial concentrations of M3G in plasma for sheep 11 following the successive infusions of morphine and M3G are shown in figure 7.7. Therefore, before calculation of the total clearances of M3G from the respective values of AUC for these two sheep, the arterial concentrations of M3G during infusion with M3G were corrected for residual concentrations from the infusion with morphine. The latter were predicted beyond 144 hr from the beginning of the infusion with morphine by extrapolation, and the corrected arterial concentrations from the infusion with

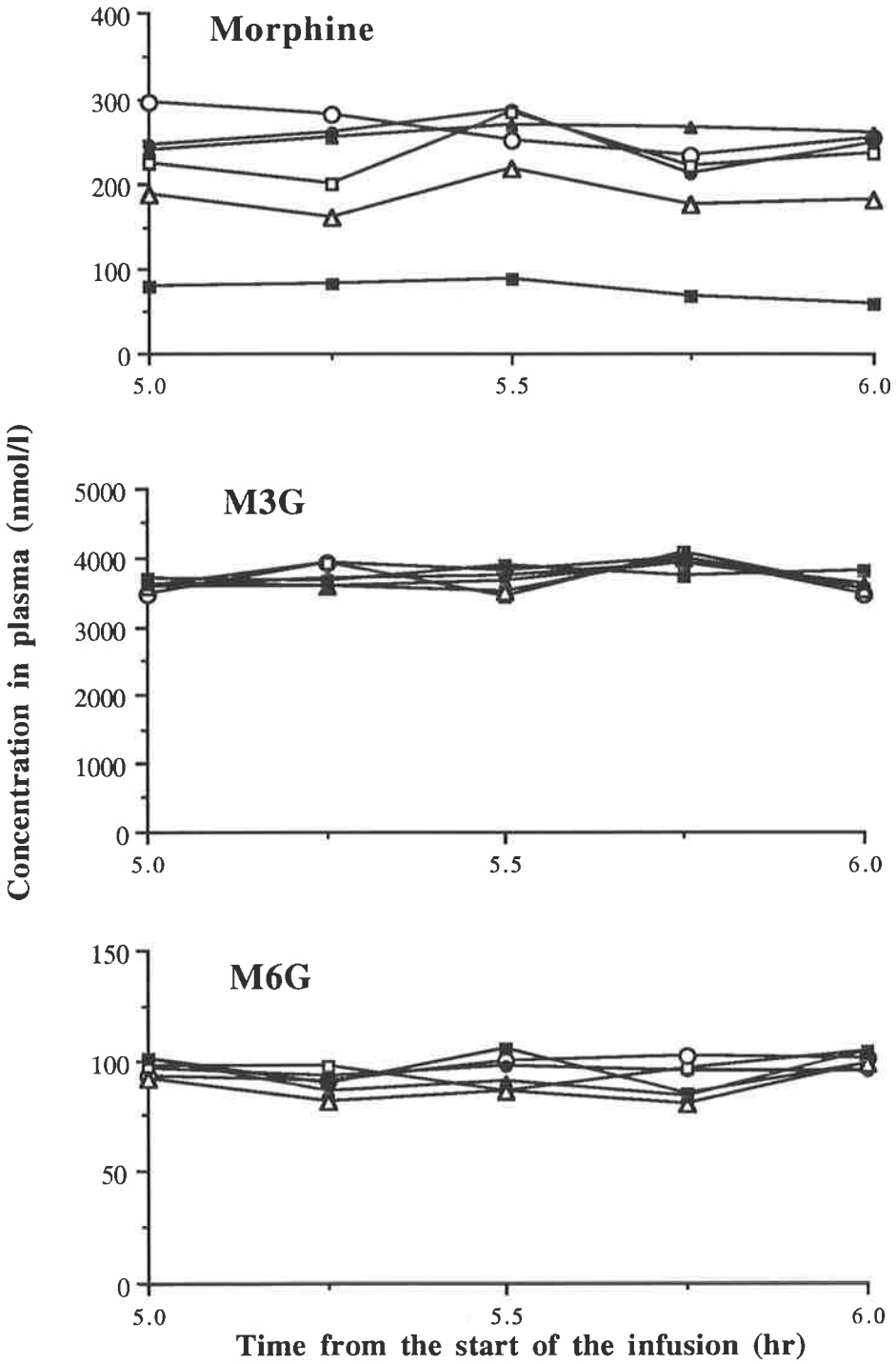


Figure 7.2 *Concentrations of morphine, M3G and M6G in plasma of sheep 11 during the 5th to 6th hour of infusion with morphine sulphate at 10 mg/hr.*

The concentrations are in samples collected from the abdominal aorta (O), pulmonary artery (●), hepatic vein (■), hepatic portal vein (□), renal vein (Δ), and posterior vena cava (▲).

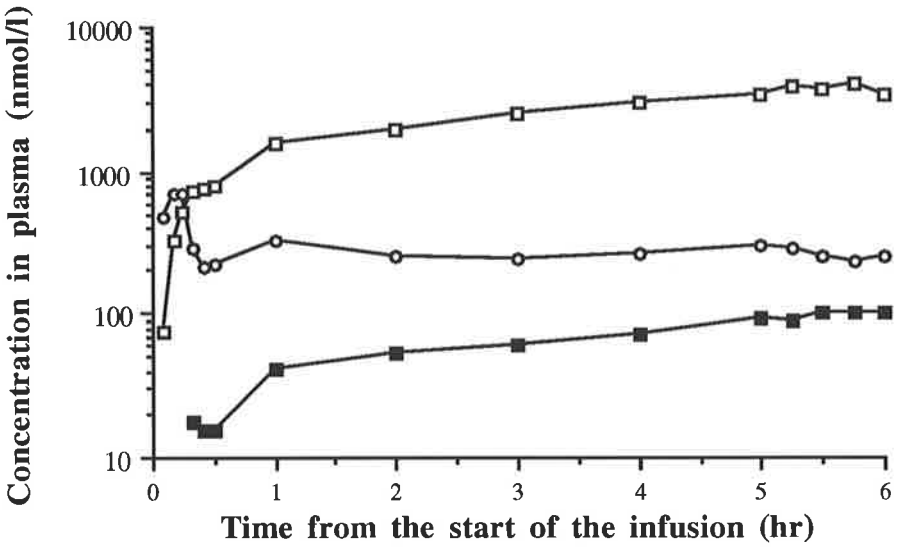


Figure 7.3 *Concentrations of morphine, M3G and M6G in arterial plasma of sheep 11 between 0 and 6 hr of infusion with morphine sulphate at 10 mg/hr.*

Concentrations are for morphine (O), M3G (□) and M6G (■).

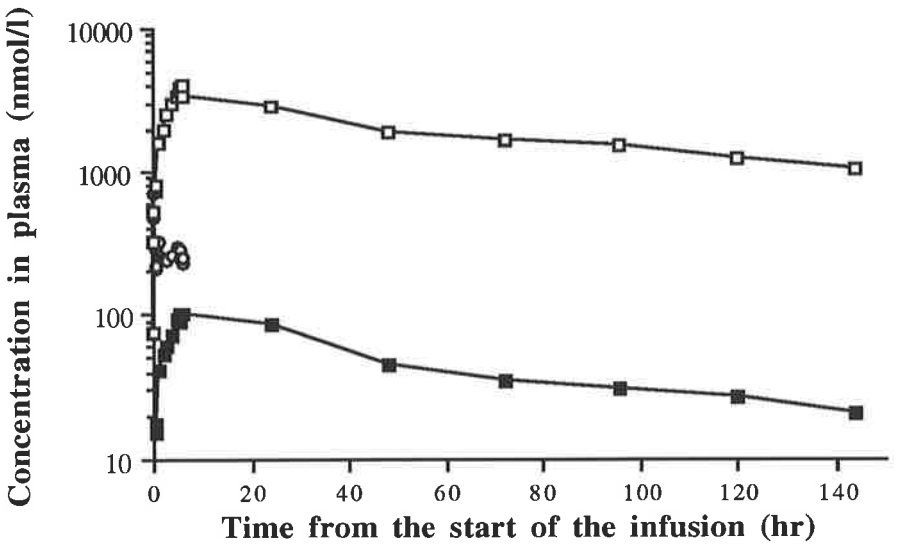


Figure 7.4 *Concentrations of morphine, M3G and M6G in arterial plasma of sheep 11 between 0 and 144 hr from the start of a 6 hr infusion with morphine sulphate at 10 mg/hr.*

Concentrations are for morphine (O), M3G (□) and M6G (■).

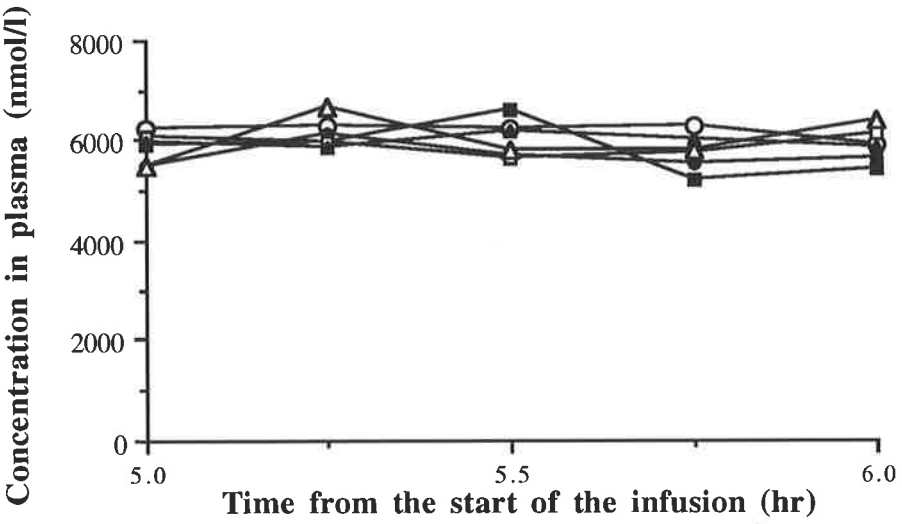


Figure 7.5 *Concentrations of M3G in plasma of sheep 11 during the 5th to 6th hour of infusion with M3G at 4 mg/hr.*

The concentrations are in samples collected from the abdominal aorta (O), pulmonary artery (●), hepatic vein (■), hepatic portal vein (□), renal vein (Δ), and posterior vena cava (▲).

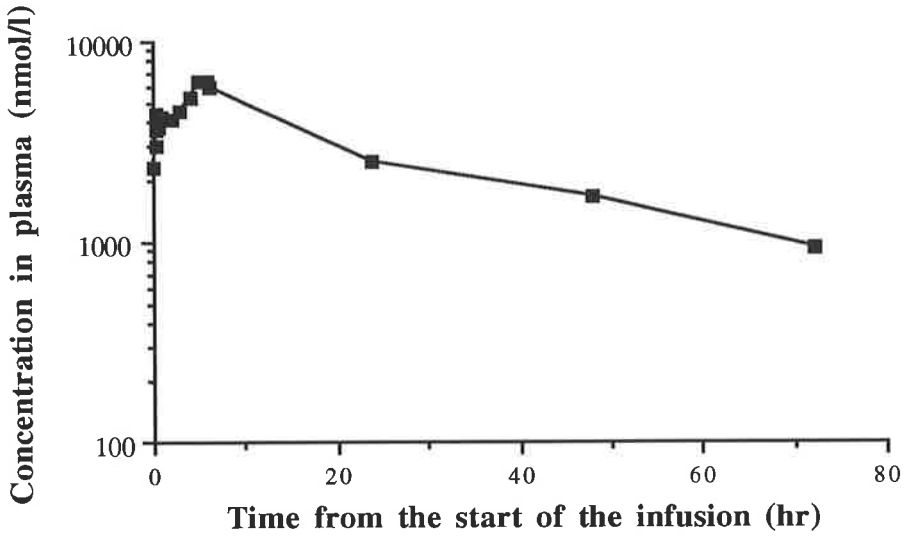


Figure 7.6 *Concentrations of M3G in arterial plasma of sheep 11 between 0 and 72 hr from the start of a 6 hr infusion with M3G at 4 mg/hr.*

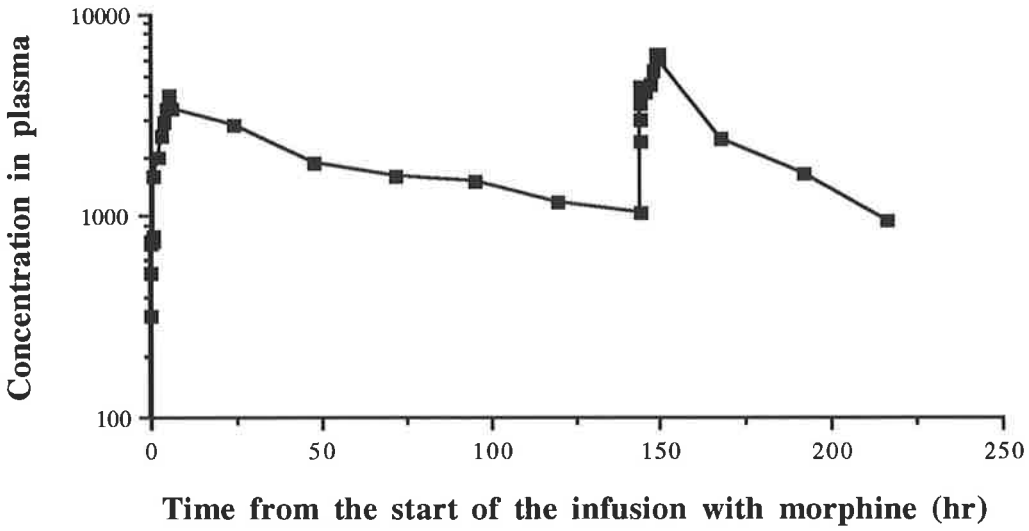


Figure 7.7 *Concentrations of M3G in plasma of sheep 11 during and after successive infusions over 6 hr of morphine sulphate (10 mg/hr), commencing at 0 hr, and M3G (4 mg/hr), commencing at 144 hr.*

M3G calculated by subtraction of the predicted concentrations from the arterial concentrations that were measured during the infusions with M3G. The corrected pulmonary arterial concentrations from infusion with M3G were then calculated from the corrected arterial concentrations to be in the same ratio as the uncorrected (or measured) values. During infusion with morphine the mean pulmonary arterial AUC(5-6) values for morphine, M3G and M6G were 237 ± 39 nmol/l.hr, 4290 ± 890 nmol/l.hr and 112 ± 42 nmol/l.hr, respectively. When M3G was infused, the mean corrected pulmonary arterial AUC(5-6) value was 3880 ± 840 nmol/l.hr. During the 5th to 6th hour of infusion with morphine, the mean pulmonary arterial ratios of the AUC for M3G/morphine and M6G/morphine were 18.3 ± 3.8 and 0.48 ± 0.15, respectively, and were significantly different ($p < 0.001$, 11 df and $p = 0.002$, 10 df, respectively) from the values of 6.8 ± 1.3 and 0.18 ± 0.05 observed in normal sheep (chapter 6). However, the ratio M3G/M6G of 41 ± 13 in the present study was not different ($p = 0.499$, 10 df) from the value in normal sheep (36 ± 7). Furthermore, the ratio of the pulmonary arterial AUC for M3G during infusion with morphine to that during infusion with M3G (corrected for sheep 10 and 11, and normalized for the respective doses of morphine and M3G in all sheep)

was 0.38 ± 0.12 and was not different (p = 0.472, 6 df) from the value of 0.44 ± 0.11 in normal sheep.

Mean arterial AUC(0-5) values for morphine, M3G and M6G during the infusion with morphine were 1380 ± 200 nmol/l.hr, 14000 ± 3000 nmol/l.hr and 361 ± 134 nmol/l.hr, respectively; the respective AUC(5-6) values were 237 ± 42 nmol/l.hr, 4450 ± 830 nmol/l.hr and 116 ± 36 nmol/l.hr.

7.3.2 Regional Extraction

The mean regional net extraction ratios for morphine and M3G during administration of morphine and for M3G during administration of M3G are shown in figure 7.8.

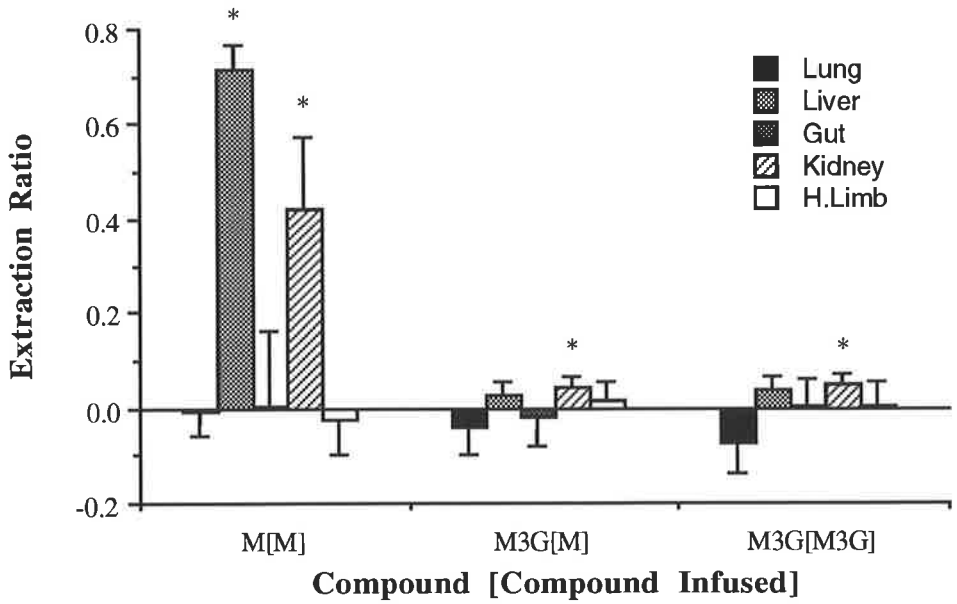


Figure 7.8. *Mean regional extraction ratios of morphine and M3G during infusion of sheep in renal failure with morphine sulphate [M], and of M3G during infusion with M3G [M3G].*

* significantly different from zero, p < 0.050.

There was significant extraction of morphine by the liver (0.723 ± 0.051, p < 0.001, 5 df), and kidney (0.423 ± 0.154, p < 0.001, 6 df). There was significant extraction of M3G by the kidney during the administration of morphine (0.044 ± 0.025, p = 0.004, 6 df) and M3G

(0.042 ± 0.014, p = 0.030, 2 df) but comparison of the two revealed no significant difference (p = 0.901, 8 df). There was no significant net production of M3G by the gut during infusion with morphine (-0.017 ± 0.062, p = 0.054, 5 df) or M3G (0.004 ± 0.060, p = 0.926, 2 df), or extraction of M3G by the liver during infusion with either morphine (0.027 ± 0.030, p = 0.054, 6 df) or M3G (0.037 ± 0.031, p = 0.181, 2 df). There was significant net extraction of M6G by the kidney only (0.051 ± 0.043, p = 0.020, 6 df), when morphine was being infused.

Table 7.1 compares, between sheep with renal failure and normal sheep, the extractions of morphine, M3G and M6G during infusion with morphine, and of M3G during infusion with M3G.

Table 7.1. *Comparison of the regional extractions of morphine, M3G and M6G in sheep with renal failure against the respective values in normal sheep.*

Region	Compound [Compound infused]	Extraction in Renal Failure (n)	Extraction in Normal Sheep (n)	p value
Liver	Morphine [M]	0.723 ± 0.051 (6)	0.708 ± 0.028 (6)	0.534
Kidney	Morphine [M]	0.423 ± 0.154 (7)	0.589 ± 0.067 (6)	0.033
Kidney	M3G [M]	0.044 ± 0.025 (7)	0.109 ± 0.040 (6)	0.004
Kidney	M6G [M]	0.051 ± 0.043 (7)	0.111 ± 0.045 (6)	0.041
Gut	M3G [M]	-0.017 ± 0.062 (6)*	-0.039 ± 0.020 (6)	0.427
Kidney	M3G [M3G]	0.042 ± 0.014 (3)	0.102 ± 0.040 (5)	0.0497

* not significantly different from zero, all other values being significantly different from zero

When the data for the extraction of morphine in normal sheep (chapter 6) and those in renal failure were combined, there was no significant association (p = 0.070, r_s = 0.545, n = 12) between the extraction of morphine by the kidney and the renal excretory clearance of creatinine, but there was a significant association (p = 0.011, r_s = 0.732, n = 13) between the former and the extraction of p-aminohippuric acid by the kidney (figure 7.9).

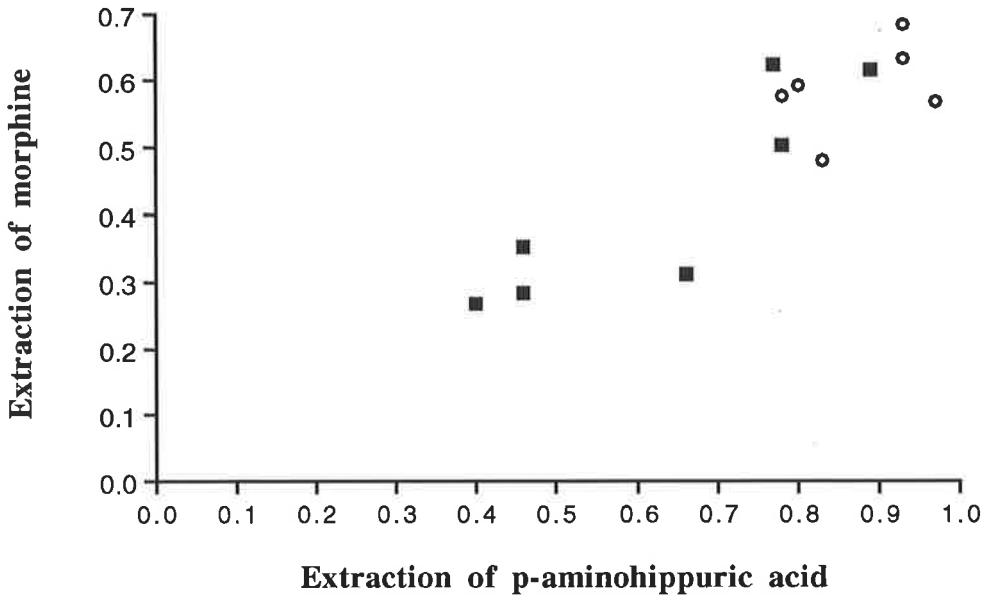


Figure 7.9. *Association between the extraction ratios of morphine and p-aminohippuric acid across the kidney using combined data from normal sheep (O) and sheep in renal failure (■); p = 0.011, r_s = 0.732, n = 13.*

7.3.3 Ratios of the Concentrations in Blood to Plasma (C_b/C)

Values of C_b/C for morphine, M3G and M6G were measured in 'blank' blood obtained from 6 sheep prior to administration of morphine. However, because of the residual M3G from the preceding experiments with morphine in sheep 10 and 11, a value for M3G prior to infusion with M3G could be obtained from sheep 13 only. The individual values are presented in table A4.13. The overall mean (± S.D., n = 6) values of C_b/C for morphine, M3G and M6G determined in blood sampled prior to the experiments with morphine were 1.32 ± 0.17, 0.81 ± 0.16 and 0.79 ± 0.10, respectively. In the absence of individual values for sheep 8, the overall means were used. The value for M3G from sheep 13 prior to the experiment with M3G was 0.90. For sheep 10 and 11 given M3G, the values of C_b/C for M3G from the experiments with morphine were used. The mean haematocrits during the experiments with morphine and M3G were 0.34 ± 0.07 and 0.31 ± 0.08, respectively.

A comparison of data from 3 sheep in which C_b/C had been determined previously when normal and in the present study when in renal failure, revealed no significant differences

between the 2 groups in the values of C_b/C for morphine ($p = 0.478$), M3G ($p = 0.742$) and M6G ($p = 0.114$).

7.3.4 Comparison of Total and Regional Clearances for Morphine and M3G

The total body clearance of morphine with reference to blood was 1.46 ± 0.28 l/min. The mean clearances of morphine by the liver and kidney were 1.52 ± 0.47 l/min and 0.22 ± 0.08 l/min, respectively. The mean of the sum of the two regional clearances for morphine was 1.75 ± 0.49 l/min but was not significantly different ($p = 0.292$, 5 df) from the total. Total body clearance, clearance by the kidney and renal excretory clearance (between 5 and 6 hr) of M3G during infusion with M3G are presented in table 7.2. There were insufficient data for statistical comparisons.

A comparison between normal sheep and those with renal failure, of the total and regional clearances for morphine, is presented in table 7.3. While there was no difference in the total clearance of morphine between the two groups, there was a significant difference in the clearance by the kidney. The power of the test to detect a 20% reduction in total clearance in normal sheep to a value comparable to that determined when in renal failure was 35%.

Table 7.2 *Total clearance, clearance by the kidney and renal excretory clearance by the kidney, all with reference to blood, of M3G during infusion of M3G in sheep with renal failure#.*

Sheep	Clearance with respect to blood (l/min)			
Number	Total [AUC(5-6)]	AUC(0-∞)	Kidney	Renal Excretory$
10	0.123	0.0191*	0.0366	0.0121
11	0.0424	0.0126*	0.0156	0.00572
13	0.0507	0.0131	0.0608	0.00771

* AUC from 0 to 72 hr only

\# no statistical comparisons were performed

$ calculated between 5 and 6 hr

Table 7.3 *Comparison between sheep with renal failure and normal sheep of total and regional clearances of morphine with respect to blood.*

	Clearance with respect to blood (l/min)		
Region	Renal Failure (n)	Normal Sheep (n)	p
Total body	1.46 ± 0.28 (7)	1.84 ± 0.49 (6)	0.106
Liver	1.52 ± 0.47 (6)	1.61 ± 0.65 (6)	0.774
Kidney	0.216 ± 0.075 (7)	0.827 ± 0.139 (6)	< 0.001

In the sheep with renal failure, there was a significant association ($p = 0.048$, $r_s = 0.886$, $n = 6$) between the clearance of morphine by the kidney and the renal excretory clearance of creatinine. There was an even stronger association ($p = 0.001$, $r_s = 0.979$, $n = 12$) when the data from the normal sheep were also included (figure 7.10).

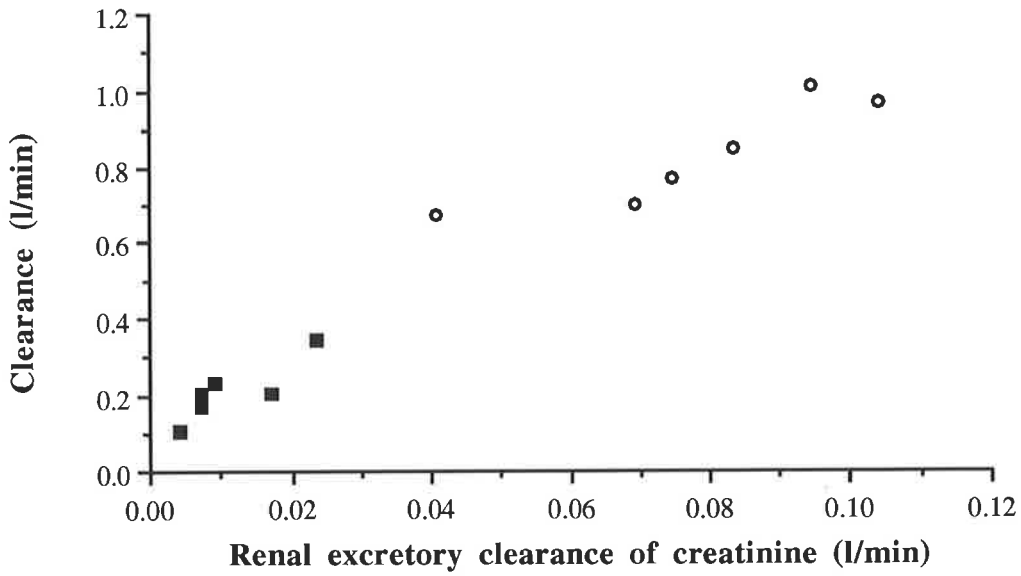


Figure 7.10 *Association between the clearance of morphine by the kidney and the renal excretory clearance of creatinine, using combined data from normal sheep (O) and those in renal failure (■).*

($p = 0.001$ $r_s = 0.979$, $n = 12$)

7.3.5 Binding in Plasma

The mean fractions of morphine, M3G and M6G unbound in pooled arterial and pulmonary arterial plasma collected during the 5th to 6th hr of the infusion with morphine were 0.75 ± 0.03, 0.94 ± 0.06 and 0.83 ± 0.05, respectively. The individual values are listed in table A4.14. There was insufficient pooled plasma from sheep 7 to determine the individual values, and therefore the mean values were used. The means for morphine and M6G were significantly different ($p < 0.001$, 5 df) from 1.0, but not the value for M3G ($p = 0.061$, 5 df). There were no significant differences between normal sheep and those with renal failure in the fractions unbound for morphine ($p = 0.090$, 10 df), M3G ($p = 0.137$, 10 df) and M6G ($p = 0.114$, 10 df).

7.3.6 Renal Clearance and Urinary Recovery

The concentrations of creatinine determined in plasma and urine from each sheep are presented in table A4.16. The mean renal clearances of creatinine during infusion with morphine and M3G were 0.011 ± 0.007 l/min and 0.0077 ± 0.0066 l/min, respectively. During infusion with morphine, they ranged from 0.0045 l/min in sheep 12 to 0.024 l/min in sheep 7, while during infusion with M3G the range was from 0.0033 l/min in sheep 11 to 0.015 l/min in sheep 10. The mean clearance of p-aminohippuric acid by the kidney was 0.33 ± 0.11 l/min. The percentage of the clearance of p-aminohippuric acid due to filtration (which was assumed to be equivalent to the renal clearance of creatinine) was 3.34 ± 1.05%, and was significantly different ($p = 0.004$, 10 df) from the corresponding value of 6.62 ± 1.15% determined in the normal sheep (data not presented in chapter 6). On comparing the renal excretory clearances over the 0-5 hr and 5-6 hr intervals, there were no significant differences for morphine ($p = 0.100$, 5 df), M3G ($p = 0.842$, 5 df) and M6G ($p = 0.134$, 4 df) during infusion with morphine. There were insufficient data to enable a similar comparison for M3G during infusion with M3G. Nevertheless, only the data collected during the 5 to 6 hr period were used for further comparisons and for the calculation of renal excretory clearances of the unbound compounds. The mean renal excretory clearances of morphine, M3G and M6G from plasma during infusion with morphine, and of M3G during infusion with M3G, were 0.011 ± 0.007

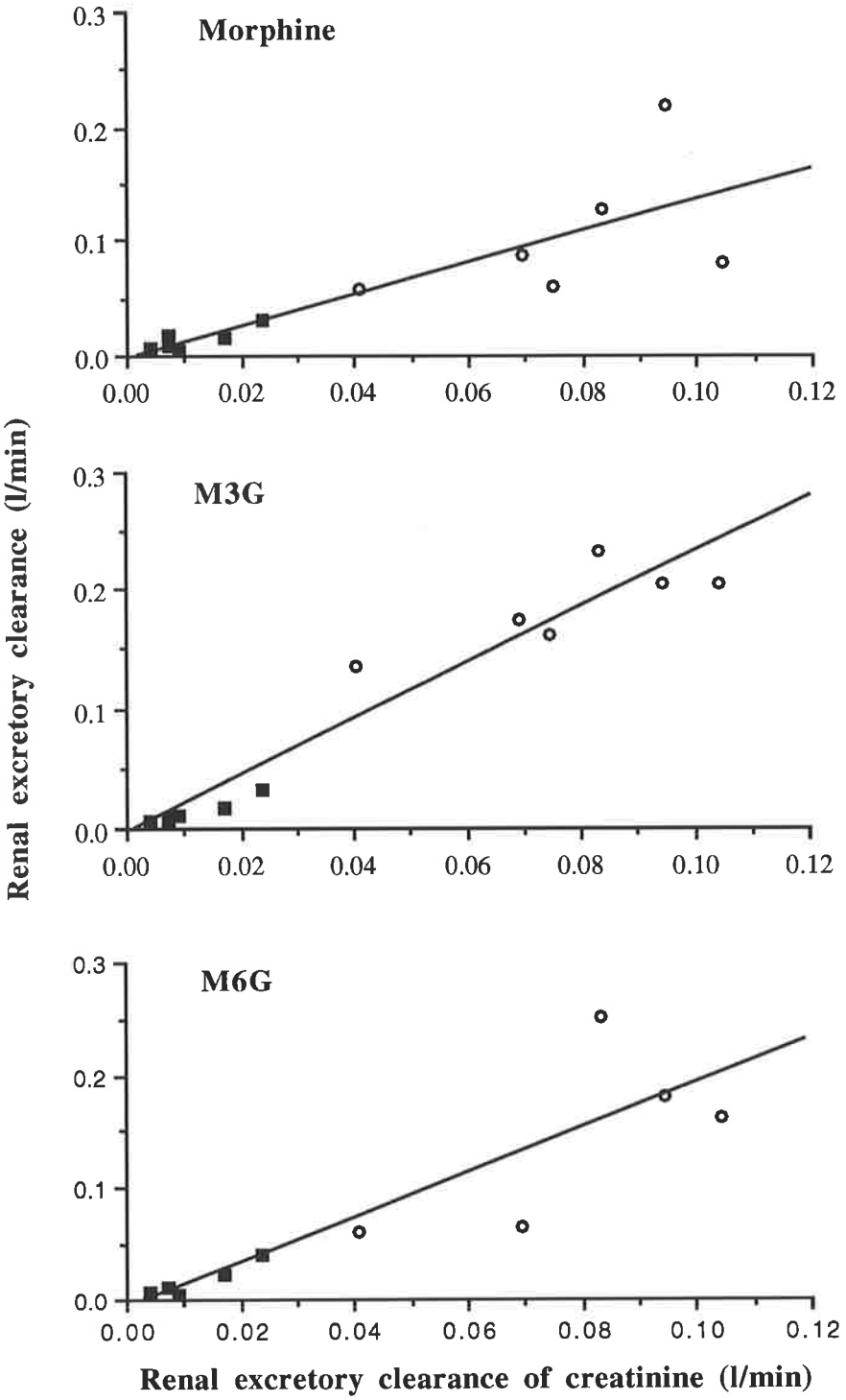


Figure 7.11 *Relationships between the renal excretory clearances of creatinine from plasma and the corresponding clearances of unbound morphine (y = -0.0012 + 1.37x, r = 0.823, p = 0.001, 11 df), M3G (y = -0.0050 + 2.36x, r = 0.965, p < 0.001, 11 df) and M6G (y = -0.0078 + 1.97x, r = 0.886, p = 0.001, 9 df) during infusion of normal sheep (O) and those in renal failure (■)with morphine.*

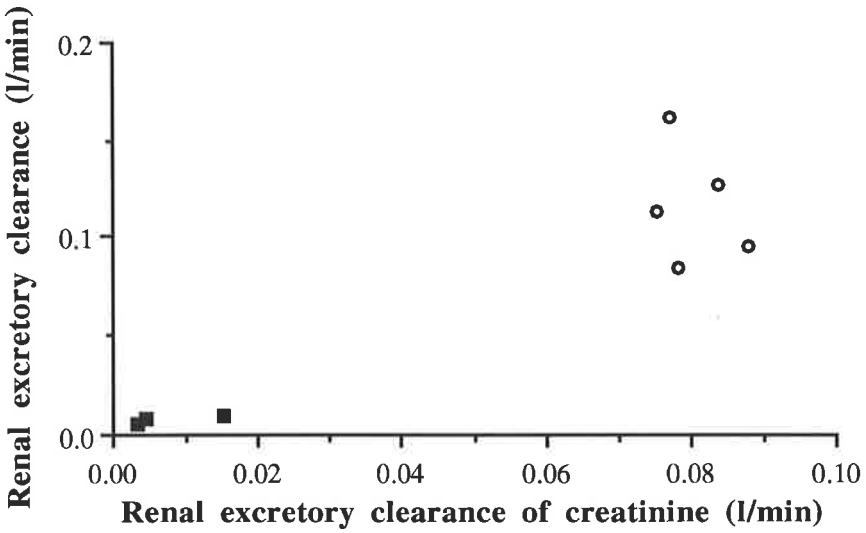


Figure 7.12 *Relationship between the renal excretory clearance of creatinine from plasma and the corresponding clearance of unbound M3G during infusion of normal sheep (O) and those in renal failure (■)with M3G.*

Regression analysis was not performed.

l/min, 0.013 ± 0.009 l/min, 0.014 ± 0.012 l/min and 0.0065 ± 0.0022 l/min, respectively. There was no significant difference ($p = 0.291$, 7 df) in the renal excretory clearance of M3G when compared during the infusions with morphine and M3G. There was a significant relationship between the renal excretory clearances of creatinine and unbound morphine ($p = 0.039$, 5 df), but the significance was increased ($p < 0.001$, 11 df) when data from normal sheep (5, 6, 7, 8, 10 and 11) were also included (figure 7.11). Figure 7.11 also shows the corresponding significant relationships for unbound M3G and M6G during infusion with morphine, when the data from both groups of sheep were combined. The combined data for M3G during infusion with M3G (from sheep 6, 7, 8, 10 and 11 when normal, and 10, 11 and 13 in renal failure) is shown in figure 7.12, but with the clustered data it was considered inappropriate to perform regression analysis. Only the slopes of the relationships for M3G and M6G during infusion with morphine were significantly greater than unity. The 95% confidence intervals were 0.70 to 2.03, 1.91 to 2.80 and 1.13 to 2.81 for morphine, M3G and M6G, respectively, during infusion with morphine. From combined data during infusion with M3G,

the ratio of the renal excretory clearance of unbound M3G to the clearance of creatinine was 1.4 ± 0.5 (range 0.6 to 2.1), and was not significantly different from unity (p = 0.054, 7 df).

A comparison of the net clearances of morphine, M3G and M6G by the kidney against the respective renal excretory clearances with reference to blood during infusion with morphine, and the corresponding net clearances of M3G during infusion with M3G, is presented in table 7.4. Also included are the corresponding clearances in normal sheep, including that from sheep 5 infused with morphine.

Table 7.4. *Comparison between sheep in renal failure and normal sheep of the individual net clearances of morphine, M3G, M6G by the kidney, their renal excretory clearances with reference to blood, and of the respective clearances of M3G during infusion with M3G.*

Clearance [Compound Infused]	Normal Sheep (l/min)	Sheep in Renal Failure (l/min)	p
Kidney - morphine [M]	0.828 ± 0.139 (n = 6)[a]	0.216 ± 0.075 (n = 7)[d]	< 0.001
Excretory - morphine [M]	0.067 ± 0.041 (n = 6)[a]	0.0079 ± 0.0051 (n = 6)[d]	0.006
Kidney - M3G [M]	0.154 ± 0.059 (n = 6)[b]	0.023 ± 0.016 (n = 7)	< 0.001
Excretory - M3G [M]	0.191 ± 0.063 (n = 6)[b]	0.015 ± 0.008 (n = 6)	< 0.001
Kidney - M6G [M]	0.157 ± 0.049 (n = 5)	0.027 ± 0.024 (n = 7)	< 0.001
Excretory - M6G [M]	0.161 ± 0.097 (n = 6)	0.017 ± 0.014 (n = 5)	0.011
Kidney - M3G [M3G]	0.162 ± 0.063 (n = 5)[c]	0.038 ± 0.023 (n = 3)	0.018
Excretory - M3G [M3G]	0.110 ± 0.032 (n = 5)[c]	0.0085 ± 0.0033 (n = 3)	0.002

a,b,c,d significantly different, values for p of < 0.001, 0.045, 0.001, 0.001, respectively

Figure 7.13 shows the mean urinary recovery of the dose of morphine as morphine, M3G and M6G after 48 hr, 72 hr and 144 hr. The data for sheep 10 was included, despite the incomplete collection between 5 - 6 hr, because the percentage of the dose collected over this period was relatively small for the other sheep, ranging from 1.08% in sheep 11 to 4.61% in sheep 7. The mean total recoveries after the infusion of morphine were 39 ± 8%, 50 ± 8% and

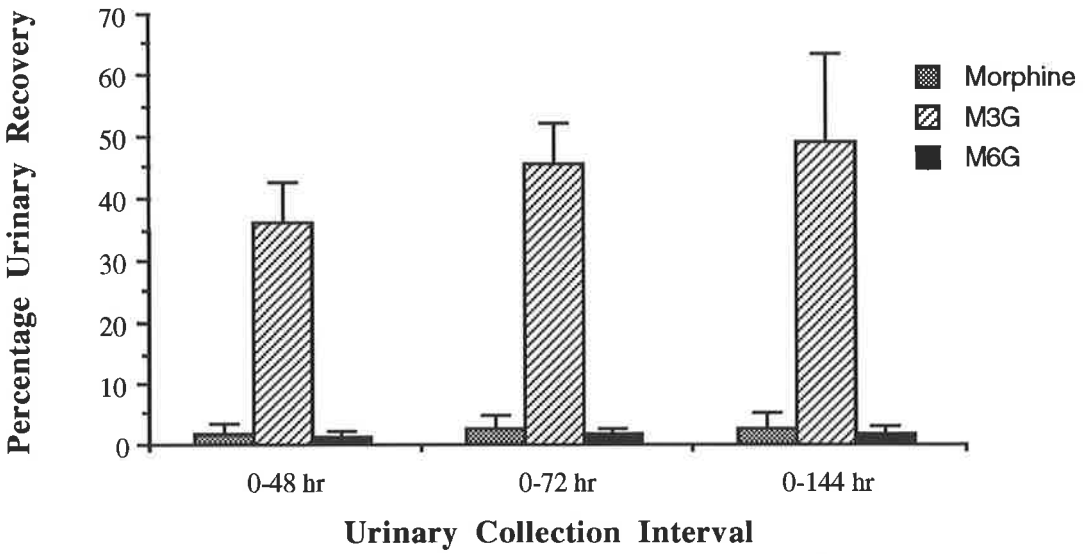


Figure 7.13 *Mean recovery of the dose of morphine administered to sheep with renal failure as morphine, M3G and M6G from the start of the infusion up to 48, 72 and 144 hr.*

54 ± 16% after 48, 72 and 144 hr, respectively. The recovery of the dose of M3G given to sheep 13 was 45% after 144 hr, with none of the dose detected in urine collected between 120 and 144 hr. For sheep 10 after 72 and sheep 11 after 48 hr, 114% and 54%, respectively, of the dose of M3G was recovered in urine. However, 5.0% and 5.9% of the dose of morphine was recovered as M3G from sheep 10 and 11, respectively, in the 24 hr period prior to starting the infusion of M3G. While M3G was being infused, no morphine was detected in urine collected via the catheter. Beyond 6 hr, morphine was found in the urine of all 3 sheep, but no M6G was detected.

7.3.7 Mass Balance across the Liver

The mean fraction of the summed morphine, M3G and M6G entering the liver via the hepatic artery and portal vein that did not reappear in the hepatic vein (fractional retention) was 0.082 ± 0.024, and was significantly different from zero ($p < 0.001$, 5 df). However, the value was not significantly different ($p = 0.070$, 10 df) from the value of 0.121 ± 0.040 determined in sheep with normal renal function.

7.4 Discussion

Following removal of the right kidney, renal failure was successfully induced in all 7 sheep by the introduction of a mixture of polystyrene and/or glass beads into the renal artery of the left kidney. The degree of renal dysfunction was monitored by daily measurement of the concentrations of creatinine in plasma. Renal failure was induced more rapidly by the glass beads, and the concentrations of creatinine were maintained by repeated administration of both types of beads. English *et al.* (1977) employed uninephrectomy and 50% infarction of the remaining kidney to induce renal failure in sheep, but adaptive increases in renal function of the remaining renal tissue ensued only a 64% reduction in GFR and a 79% increase in the concentration of creatinine in plasma. Eschbach *et al.* (1980) induced renal failure in sheep by ligation of branches of the renal artery to one kidney, producing about 90% infarction, followed 2 weeks later by contralateral nephrectomy. The concentrations of creatinine were raised to values comparable to those produced by the administration of the polystyrene and glass beads, and GFR was reduced to below 15 ml/min. The method described in chapter 4 for the induction of renal failure, and used for the experiments described in this chapter, required less surgery but it appeared that Eschbach *et al.* (1980) were able to maintain survival for a longer period, albeit with the aid of haemodialysis for some sheep.

Evaluation of the pathological changes induced by the polystyrene and glass beads was not possible because the presence of beads within the tissue precluded histological examination. However, it is likely that occlusion of the smaller branches of the renal artery was followed by infarction and necrosis. Assuming that the renal clearance of creatinine is a reasonable approximation of GFR in sheep with normal renal function or renal failure, the percentage of the clearance of p-aminohippuric acid due to filtration was significantly reduced from a mean of 6.62 ± 1.15% in normal sheep to a mean of 3.34 ± 1.05% during renal failure. The relative values suggest that filtration was reduced to a greater degree than tubular uptake. However, there are a number of factors that may influence the relative magnitude of these values. Firstly, the difference noted above would be greater if a significant component of the clearance of creatinine during renal failure was due also to tubular secretion. In chapter 6, it was reasoned that the renal clearance of creatinine in healthy sheep was a reliable estimate of GFR. English *et*

al. (1977) found that the ratio of the renal clearances of inulin to creatinine in sheep was unchanged by a 64% reduction in GFR induced by three-quarters nephrectomy. However, in humans it was found that, as renal function decreased, a greater fraction of the clearance of creatinine was due to tubular secretion (Shemesh *et al.*, 1985). Secondly, while the failed kidney remains relatively well-perfused with blood despite the average reduction in total flow of 61% (tables A3.13 and A4.15), whether steady-state conditions for p-aminohippuric acid was achieved within the tubular cells is unclear. The relatively stable concentrations in renal venous blood (data not presented) suggest that somewhat stable concentrations may have been reached within the kidney, but it is possible that the clearance of p-aminohippuric acid by the kidney might have decreased marginally as "true" steady-state was approached. Thirdly, it has been proposed that competition from accumulated anions of small molecular weight (McNay *et al.*, 1976) during renal failure suppresses the renal clearance of p-aminohippuric acid. Nevertheless, overall it would appear that, with the method used for the induction of renal failure, both glomerular filtration and tubular secretion were reduced.

When sheep with renal failure were infused at a constant rate with morphine for 6 hr, essentially constant concentrations of morphine in plasma were achieved between 5 and 6 hr, which was similar to the observations with normal sheep infused with the same dose-regimen (chapters 5 and 6). In contrast, it was apparent from the example depicted in figure 7.3 that the concentrations of M3G and M6G after 6 hr of infusion appeared to be below the concentrations that might have been achieved if the infusions had continued for a longer period. Given that the kidney was the major route for the elimination of M3G and M6G in normal sheep (chapters 5 and 6), it would be expected that the clearance of both compounds would be reduced during renal failure and, assuming an unchanged volume of distribution, that the half-lives of elimination would be increased. Compared to normal sheep, higher concentrations of M3G and M6G were produced during the infusions and maintained for a longer period after the infusions had ceased. While the half-lives of elimination from plasma could not be determined in normal sheep, obviously the values were markedly increased in the sheep with renal failure. Previous investigators (Osborne *et al.*, 1993; Säwe & Odar-Cederlöf, 1987) have reported similar findings for M3G and M6G when morphine was administered to humans with renal failure.

Interestingly, the mean ratio of the AUC(5-6) for M3G to that for M6G during renal failure was not significantly different from the corresponding ratio in normal sheep. Given that the clearances of both compounds were reduced to a similar degree by renal failure (table 7.4), their formation and subsequent appearance in plasma was either unaffected or equally modulated by renal failure.

When M3G was administered to the sheep with renal failure, once again markedly elevated concentrations of M3G in plasma were found compared to normal sheep and, although only calculable for sheep 13, the half-lives of elimination from plasma were increased. In addition, the concentrations reached at the end of the infusion appeared to be well below those that might have been achieved under steady-state conditions (figure 7.6). From inspection of figure 7.7, it may be concluded that after the successive infusions of morphine and M3G have ceased, the disposition of M3G in plasma could be described by at least two rate constants. The higher constant, apparent following the infusion of M3G, probably reflected elimination of M3G from plasma while the lower value, apparent following the infusion of morphine, more likely reflected the continued formation of M3G by the liver and gut during the enterohepatic cycling of morphine that was proposed in normal sheep (chapters 5 and 6). The net appearance of M3G in the portal blood of normal sheep, adding support to the proposal, was probably not detected during renal failure because of the relatively larger concentrations of M3G in blood. However, the net retention of summed morphine, M3G and M6G by the liver, as found in normal sheep, does add support for the existence of the cycle in renal failure. Compared to normal sheep, it is possible also that a greater fraction of the dose of morphine was subject to cycling in sheep with renal failure, since the kidney contributed a lesser fraction to the total clearance from the body. In healthy sheep, it was proposed that the majority of the morphine taken up by the kidney was metabolized to M3G rather than excreted unchanged (chapters 5 and 6), and that the majority of the M3G formed was excreted. Therefore, in sheep with renal failure, it is possible that a greater fraction of the overall M3G formed by the body was formed in the liver, excreted into bile, and thus involved in an enterohepatic cycle.

Given that the mean haematocrits in sheep with renal failure were almost identical to the values determined in blood from normal sheep, the comparable values of C_b/C for morphine,

M3G and M6G between both groups suggest that renal failure has no measurable effect on the binding of the three compounds to red blood cells. Eschbach *et al.* (1980) observed a progressive decrease in the mean haematocrit of uraemic sheep over a period of 4 to 20 weeks. However, simultaneous splenectomy would have hastened the development of the intended anaemia. English *et al.* (1977) observed an 18% decrease in haematocrit, which they attributed to reduced haematopoiesis.

On the basis of the significantly greater clearance of M3G by the kidney compared to the renal excretory clearance with reference to blood when M3G was infused into normal sheep (chapter 6), it was proposed that steady-state concentrations for M3G were not achieved within the tubular cells of the kidney. Only 3 sheep in renal failure were infused with M3G, precluding statistical comparison of the two parameters. That clearance by the kidney was, on average, 4.5-times greater than the renal excretory clearance suggests that conditions at the end of the infusion with M3G were even further removed from steady-state, when compared to sheep with normal renal function. With continued infusion of M3G, the clearance of M3G by the kidney would have decreased to a value approximating the excretory clearance from blood, as steady-state was approached within the kidney.

The total body clearance of morphine with reference to blood of 1.46 l/min was comparable to the value of 1.84 l/min determined in 6 healthy sheep. Clearance by the kidney was only 14% of the combined clearances by the liver and kidney, and contrasts with the 36% contribution to the sum in normal sheep. Osborne *et al.* (1993) compared the total clearance of morphine from the body of normal humans with the value obtained in three groups of patients with failed kidneys that had all undergone surgery: the first for an arteriovenous fistula but without dialysis; the second for the placement of a catheter for peritoneal dialysis with or without additional surgery for a fistula, followed by peritoneal dialysis; and the third for a kidney transplant. While the clearance of morphine in normal subjects was greater than the value in both groups receiving dialysis, it was not different from the clearance determined in the transplanted patients. The findings may have been influenced by the anaesthetic regimen used during surgery (for a further discussion, see section 1.5.4.3). Between the groups of patients, those given the transplant had a significantly greater mean clearance than the group receiving

dialysis, but not the group which did not receive dialysis. Thus, while the study suggests a role for the kidney in the metabolic clearance of morphine, as found in the sheep, a greater number of patients may have provided an unequivocal conclusion. Despite the evidence from regional sampling of normal sheep for a definite contribution by the kidney to the total clearance of morphine, induction of renal failure did not significantly diminish the total clearance. When compared with the study by Säwe & Odar-Cederlöf (1987) in humans, the reduction in the respective means was approximately 21% and 25%. With the sheep, there was a significant association between the clearance by the kidney and the renal clearance of creatinine. All except one of the patients studied by Säwe & Odar-Cederlöf (1987) appeared to be in severe renal failure, while those studied by Woolner *et al.* (1986) were on haemodialysis. Once again, investigation of more patients by these two groups may have provided more conclusive evidence of a role for the kidney in the total clearance of morphine.

From the comparable clearances of morphine by the liver in normal sheep and sheep in renal failure, it can be concluded that renal failure had no detectable effect on the capacity of the liver to eliminate morphine. The concentration of total bilirubin and the activity of alanine aminotransferase in serum were similar to the values in normal sheep, and suggest that there was no overt hepatic failure in the presence of renal failure. Comparing the studies in humans by Padwardhan *et al.* (1981) and Hasselström *et al.* (1990) on the effect of cirrhosis on the total clearance of morphine, it would appear that only with considerable damage to hepatic tissue was a reduction in clearance detectable. In contrast, clearance by the kidney in sheep was significantly reduced by renal failure, due probably to a lesser blood flow and/or intrinsic clearance to M3G (and M6G). As shown in figure 7.9, those decreases in the extraction ratio for morphine due to renal failure were almost identical with the decreases in the extraction for p-aminohippuric acid. Both compounds were highly extracted by the tubular cells in normal sheep, but their extraction may have been reduced in renal failure by damage to, or reduced functional perfusion of tubular cells, and by competition from accumulated endogenous compounds of small molecular weight (McNay *et al.*, 1976). Since steady-state with respect to morphine was almost certainly achieved in these two organs, the regional clearances were probably representative of the true values.

When M3G was infused, the total body clearance of M3G with reference to blood was calculated from both the AUC(5-6) and the AUC(0-∞). The latter generated a value far less than the former for all 3 sheep (table 7.2) and bears out the lack of achievement of steady-state by 6 hr. From figure 7.7, most of the AUC(0-∞) due to infused M3G appeared to have been accounted for by 72 hr in sheep 11; and a similar observation was made for sheep 10 (data not shown). Assuming that the total clearance (from the AUC(0-∞)) was a reasonable estimate of the true value, comparison of the total with the renal excretory clearance (table 7.2) suggests that a significant proportion of M3G given to sheep in renal failure was eliminated by routes other than excretion by the kidney; only in sheep 13 was this able to be confirmed by the similarity between the ratio of the renal excretory clearance to total clearance and the fraction of the dose of M3G excreted in urine. In chapters 5 and 6 a relative barrier to the uptake of M3G from arterial-portal blood by the liver was proposed, and in normal sheep 84% of the M3G infused was eliminated by the kidney. During renal failure, the "true" renal excretory clearance of M3G was reduced by over 90% (table 7.4) so that alternative routes of elimination would have become relatively more important. Therefore the appearance of morphine in the portal vein of 2 sheep was not unexpected. Roerig *et al.* (1974) found that 50% of an i.v. dose of radiolabelled M3G was excreted into the bile of renally-ligated rats as unchanged M3G. While maintaining concentrations of M3G in the normal rat of approximately 14.4 μmol/l by constant infusion with M3G for 69 hr (Ekblom *et al.*, 1993), the concentrations of morphine in plasma reached 200 nmol/l after 30 hr. These authors proposed enterohepatic circulation as an explanation for the appearance of morphine in plasma. The concentrations of M3G between 5 and 6 hr in sheep with renal failure were about 3.3-times those achieved in normal sheep infused at the same rate with M3G. Enterohepatic cycling is the most likely explanation for the appearance of morphine in the portal vein of the 2 sheep, and it is probable that morphine would have been quantifiable in all 3 sheep at a greater number of sites if the infusions of M3G had been continued for a longer period.

As found in normal sheep, the clearance of morphine by the kidney during renal failure was significantly greater than its renal excretory clearance. The ratio of the former to the latter was larger than for normal sheep, indicating that the failed kidney still excreted and metabolized

morphine, but that a greater proportion of the extracted morphine was taken up by the tubular cells and then metabolized, rather than being filtered. This is a likely outcome if, as proposed from the relative clearances of creatinine and p-aminohippuric acid, filtration was reduced to a greater extent than tubular uptake by the induced renal failure. The mean renal excretory clearance of M3G during infusion with morphine, an apparent renal clearance, was almost double its "true" renal clearance while M3G was infused (table 7.4), providing evidence for the renal metabolism of morphine to M3G during renal failure. However, as distinct from the observation in normal sheep where, with the inclusion of sheep 5 infused with morphine, the renal excretory clearance of M3G was now significantly greater than its clearance by the kidney, there was no difference in the two clearances in renal failure. A difference may have become apparent if steady-state with respect to M3G had been achieved within the kidney with continued infusion of morphine, resulting in an expected decrease in the clearance of M3G from blood by the kidney. An additional explanation for the greater ratio of the clearance of morphine by the kidney to its renal excretory clearance in sheep with renal failure is that the concentrations of morphine in the kidney are further from steady-state compared to those that were achieved in normal sheep.

The value of 0.38 for the ratio of the AUC of M3G during infusion with morphine to the AUC during an equimolar infusion of M3G was comparable to the value of 0.44 observed in normal sheep (chapter 6). The ratio remained essentially unchanged irrespective of the capacity of the kidney to clear morphine to M3G, and reinforces the proposition in chapter 6 that most of the M3G (and any M6G) formed in the kidney does not appear in plasma but instead is excreted into urine. It should be recalled, however, that this ratio was generated from fewer sheep infused with M3G while in renal failure (compared to the ratio from normal sheep), and in 2 of the sheep from concentrations for M3G in plasma that were superimposed on those from an earlier infusion with morphine. Although collection of urine from the sheep infused with morphine was over a period shorter than designed, even after 72 hr of collection the fraction of the dose excreted as M3G was greater than 0.38. As indicated in figure 7.13, the calculated fraction would have been increased further if collection of urine from all sheep had continued until 144 hr, or until no more M3G was quantifiable in urine. The similarity between the

extraction ratios of M3G across the kidney during infusion with either morphine or M3G is unlikely to be supportive of the proposition since the concentrations of M3G during infusion with morphine are about 18-times those for morphine, and the increase in the concentration of renal venous M3G resulting from its formation in the kidney and appearance in renal venous blood would be imperceptible.

With normal sheep, the apparent renal clearance of M3G up to 5 hr was greater than the value determined between 5 and 6 hr of infusion with morphine, and it was reasoned that the difference was supportive of metabolism of the morphine to M3G by the kidney (chapter 6). However, the apparent clearances during renal failure were similar for both intervals. While the mean concentrations of morphine in arterial blood over the two intervals were comparable to the concentrations over the corresponding intervals in normal sheep, the mean concentrations of M3G were about 3-times the mean concentrations of M3G observed in normal sheep and, in combination with the 74% reduction in the clearance of morphine by the kidney, would account for the similarity.

The fractions of morphine, M3G and M6G unbound in plasma taken from the sheep during the infusions of morphine were comparable to those found in the plasma from normal sheep (chapter 6). Olsen *et al.* (1975) observed only a minor but significant decrease in the binding of morphine in uraemic patients with hypoalbuminaemia. Morphine was found to be bound predominantly to albumin in plasma from humans (Leow *et al.*, 1993; Olsen, 1975), and the concentrations of albumin in the serum of sheep with renal failure, determined by routine methods, were comparable to those in normal sheep. The fraction of M3G unbound in the plasma of sheep given M3G was assumed to be the same as that during the corresponding infusion with morphine. As expected, renal failure did not alter the binding of M3G and M6G in plasma.

During infusion with morphine, significant relationships between the renal clearance of creatinine and the renal clearances of unbound morphine, M3G and M6G were observed when, to encompass a diversity of renal function, the data from normal sheep were included with those from the sheep with renal failure (figure 7.11). Significant corresponding relationships were also obtained in patients during constant infusion with morphine while in intensive-care (chapter

3). However, while the slope of the relationship for morphine determined in the patients was within the 95% confidence interval of the slope found in sheep, the slopes for M3G and M6G in the patients were below the lower 95% confidence limits for the corresponding compounds determined in sheep. The differences for M3G and M6G provide evidence that the metabolism of morphine to M3G (and M6G) by the kidney is more important in sheep than humans. Before comparing the renal clearance of M3G in humans to that in sheep infused with M3G, more data are needed from sheep. From the discussion in chapter 6, it may be assumed that the clearance for creatinine measured in the normal sheep was a reasonable estimate of GFR. As discussed in chapter 3, the renal clearance of creatinine overestimates GFR in humans, particularly when the GFR is low. While its accuracy as a measure of GFR in sheep with reduced renal function has not been fully established, a similar renal handling of creatinine as for humans would explain the negative intercept on the ordinate, as observed for humans (figure 3.1).

The mean recovery of the dose of morphine as morphine, M3G and M6G in the urine of sheep with renal failure (figure 7.13) was well below that from normal sheep. Recovery up to 48 hr was only 39%, compared with means of 85% (chapter 5) and 79% (chapter 6) over the same period in normal sheep. In both studies with normal sheep, most of the dose was recovered as M3G. It was proposed that a large proportion of the M3G was formed in the liver and subsequently excreted by the kidney; a lesser but significant amount was formed in the kidney and excreted directly into urine. Therefore, with formation and excretion by the kidney diminished by renal failure, alternative routes of elimination would have become more important. Most of the dose that appeared in urine was still as M3G, suggesting extensive metabolism of morphine, but it is probable that, in consequence of a greater direct biliary excretion of M3G from plasma and an increased fraction of the dose of morphine being metabolized by the liver, that elimination of M3G in the faeces has become more important. From sheep 13, the incomplete recovery of the dose of M3G in urine over 144 hr, with none recovered in the interval between 120 and 144 hr, suggests the existence of alternative routes for the elimination of M3G from plasma, such as elimination in faeces. However, involvement of a small fraction of the dose in prolonged enterohepatic cycling, and slow efflux from renal tubular cells, could generate concentrations of M3G (or morphine) in urine that were too low

for quantification. Further metabolism to, for example, morphine-3,6-diglucuronide, may be significant during renal failure. The recovery of the dose of M3G in urine from sheep 10 and 11 would be biased by the continued elimination of the dose of morphine infused 7 days prior to the M3G. Approximately 5% and 6% of the dose of morphine was eliminated in urine in the 24 hr period prior to beginning the infusion of M3G to sheep 10 and 11, respectively.

In summary, this study has found that, compared to normal sheep, the induction of renal failure markedly reduced the importance of the kidney as an organ for the elimination of morphine from the body. Furthermore, from being the major organ for the elimination of M3G and M6G in normal sheep, renal failure greatly reduced the clearance of these two compounds by the kidney and caused considerable retention of M3G and M6G in plasma. The total clearance of morphine from the body was not significantly reduced, probably because an insufficient number of sheep were studied. However, the relative difference in mean values between normal sheep and those in renal failure was small, and the use of further sheep would have been inappropriate. The high concentrations and prolonged elimination from plasma of M3G derived from morphine, mainly in the liver, was due to a combination of its involvement in an enterohepatic cycle, as in normal sheep, and a considerable reduction in its renal clearance compared to normal sheep. Compared to normal sheep, a lesser fraction of a dose of M3G was eliminated by the kidney and nonsignificant hepatic extraction occurred. However, continued recirculation through the liver of the higher concentrations found in plasma during renal failure ensured the involvement of M3G in an enterohepatic cycle and the probability of a greater fraction being eliminated in faeces. That the infusions of morphine and M3G were not continued until steady-state concentrations of M3G and M6G, and of M3G, respectively, were achieved is a distinct disadvantage, and limits interpretation of the data with regard to the disposition of these compounds in the kidney. The study would also have benefited from an examination of the disposition of M3G in a greater number of sheep in renal failure. However, both shortcomings were unavoidable in experiments which required a relatively stable model of renal failure in a chronically-catheterized sheep over an extended period.

Chapter 8

General Conclusion

Morphine, obtained from opium, is one of the oldest analgesics used in medicine, and remains the most common analgesic for the relief of severe pain. It binds selectively to μ opioid receptors located in the peripheral and central nervous system and appears to relieve pain by virtue of an antinociceptive action and a modification of the perception of pain. When administered to humans and animals, it is almost entirely metabolized, with the major metabolite being M3G. There is considerable evidence suggesting that M3G exhibits excitatory effects and may oppose the antinociceptive action of morphine, but it does not bind to known opioid receptors. Comparatively less M6G is formed from morphine. However, it may be an important metabolite given the overwhelming evidence for its binding to the μ receptor, and its greater analgesic and antinociceptive activity in humans and animals, respectively, compared to morphine administered by the same route. M3G and M6G are eliminated primarily by renal excretion, and the lower than usual doses of morphine necessary in patients with renal failure, along with the opioid side-effects often reported when usual doses are given, have been attributed to a reduced elimination of M6G. The precise role of accumulated M3G in contributing to these side-effects remains unknown. The liver is considered to be the major organ for the metabolism of morphine. However, in most species examined, the clearance of morphine from blood exceeds hepatosplanchnic blood-flow and evidence from studies performed *in vitro* and *in vivo* confirm that other organs may also be involved, but often the evidence is contradictory.

As a brief introduction, the aims of the studies performed for this thesis were to examine firstly, the disposition of morphine, M3G and M6G, during constant infusion of morphine into patients having diverse renal function while in intensive-care and secondly, the regional elimination of morphine, M3G and M6G in normal sheep and sheep with renal failure. Therefore, a reliable method for the determination of the 3 compounds in plasma and urine from both species was required. From a method published by other investigators, an improved method was developed for the simultaneous extraction of morphine, M3G and M6G from

plasma and urine followed by paired-ion HPLC and quantification with UV detection. Accuracy and precision was superior to the published method which used UV detection and superior to or comparable to methods published while this thesis was in progress. Furthermore, it was shown that morphine, M3G and M6G were stable when stored in plasma from humans for up to 101 weeks. Since the concentrations of M3G and M6G in the plasma of patients with renal failure are far higher than for morphine, the stability of M3G and M6G was an important consideration that had been previously neglected by other workers.

The influence of renal function on the renal clearances of morphine, M3G and M6G was examined in patients with diverse renal function and under intensive-care. While the patients were receiving a constant i.v. infusion of morphine, two samples of plasma approximately 4 hr apart were collected, along with all urine voided over the interval. There were significant linear relationships between the renal clearance of endogenous creatinine, used as a measure of GFR, and the renal clearances of morphine, M3G and M6G. With limited plasma available from the patients, the fractions of morphine, M3G and M6G unbound in plasma were estimated in plasma obtained from normal subjects and, given the relatively low binding observed for all 3 compounds, it was assumed that the values measured were a reasonable estimate of those in the patients. From the relationships between the renal clearance of creatinine and unbound clearance from plasma, it was concluded that there was net renal secretion of morphine, but that there was no net secretion or reabsorption of M3G or M6G. Furthermore, there being no effect of urinary flow on the renal clearance of the three compounds, it was concluded that there was minimal reabsorption. Of the 3 compounds in urine, M3G was in the majority.

In view of the contradictory conclusions in the literature regarding the role of the kidney and other non-hepatic organs in clearing morphine from the body, the sheep was used to examine the regional elimination of morphine, as well as M3G and M6G. Sheep were chosen because their blood volume was sufficient to allow the collection of multiple samples via catheters placed into vessels supplying and draining selected regions. Furthermore, its docile nature ensured relatively stable physiological conditions throughout an experiment.

The first study, described in chapter 5, examined the regional elimination of morphine, M3G and M6G during constant infusion of morphine, and the relationship between the rate of

infusion and the arterial concentrations of morphine and M3G. When morphine was infused for 6 hr, dose-normalized concentrations of morphine and M3G between the 5th and 6th hr were independent of the rate of infusion. There was significant extraction of morphine by the liver and of M3G and M6G by the kidney, not unexpected given the findings of other investigators with other animals. However, there was also considerable extraction of morphine by the kidney, with the latter contributing 37% to the overall clearance from the body. From comparison of the clearance by the kidney and the renal excretory clearance of morphine, it was concluded that the kidney both excretes and metabolizes morphine. The majority of the dose of morphine was eliminated in the urine as M3G, with lesser amounts as unchanged morphine. As distinct from humans, M6G was a relatively minor metabolite in plasma and urine compared to M3G. From the net retention of summed morphine, M3G and M6G by the liver and net production of M3G by the gut, it was concluded that morphine is also involved in an enterohepatic cycle in the sheep, in accord with the findings in other species. A further study incorporating cannulation of the bile duct would confirm these conclusions.

A second study in sheep was performed to compare the disposition of M3G during separate infusions of morphine and M3G, each at a single rate of infusion. With the differences in polarity of the 2 compounds, it was possible that the disposition of preformed M3G may differ from that formed subsequent to the uptake into and metabolism of morphine within an organ. From the first study with sheep, it could not be determined whether there was significant uptake of M3G by the liver, or whether M3G formed within the liver was transferred into hepatic venous blood. Furthermore, after concluding from that study that the kidney metabolized morphine, the renal clearance of M3G may be overestimated because of its production and excretion by the kidney during infusion with morphine. From the second study, it was found that the disposition of preformed M3G differed markedly from that for M3G formed from morphine. There was nonsignificant hepatic extraction of preformed M3G, and it was concluded that, for M3G formed in the liver from morphine and for M3G in plasma, there was a permeability barrier to its transfer across the sinusoidal membrane. A carrier-mediated system may exist in the canalicular membrane. Of further interest was the confirmation of the formation of M3G by the kidney. It is likely that most of the M3G formed within tubular cells is

eliminated directly into urine rather than passing into renal venous blood. Most of the M3G infused into sheep was excreted in urine, and there was no net secretion or reabsorption by tubular cells. However, steady-state concentrations of M3G had not been achieved within the kidney after 6 hr of infusion with M3G. Retention within the kidney may account for the prolonged elimination of the dose of M3G in urine. That the elimination from plasma of M3G and the urinary excretion of the dose of morphine were even more prolonged may be due to a similar retention of M3G within the kidney and hepatocytes, plus continued enterohepatic cycling.

The final study examined the influence of renal failure, induced by the occlusion of branches of the renal arteries, on the disposition of morphine, M3G and M6G during infusion with morphine, and of M3G during infusion with M3G. Studies performed by previous investigators had examined the influence of renal failure on the elimination of morphine in humans, but the results remain equivocal. This study had a significant advantage over those previous studies, because the effect of renal failure on the clearance of morphine, M3G and M6G by the kidney and other organs could be evaluated directly by sampling of arterial and venous blood. During infusion with morphine, it was clear that the degree to which steady-state concentrations of morphine had been achieved in plasma was similar to that observed in normal sheep. The total body clearance of morphine was marginally but not significantly reduced by renal failure, but the clearance by the kidney was markedly decreased. Clearance by the liver was unaffected. In contrast, but not unexpectedly, the clearance of M3G and M6G was reduced, contributing to the increased half-lives of elimination from plasma. Clearance of morphine, M3G and M6G by the kidney was reduced and, with the higher concentrations of M3G and M6G in plasma and recirculated through the liver, elimination into bile was probably more important. For the M3G formed in the kidney, further evidence was provided for its direct excretion rather than appearance in renal venous blood.

When extrapolating the observations in the sheep to humans, a similar lack of effect of renal failure on the total clearance of morphine in both species neither proves nor disproves the involvement of the kidney in humans. However, during infusion with morphine, the significantly greater renal excretory clearance of M3G compared to GFR in sheep contrasts with

the relative values in humans, and provides more convincing evidence that the formation of M3G from morphine occurs only to a limited extent, if at all, in humans. Supportive evidence is from the minimal reabsorption of M3G from the tubular urine of humans, and a renal clearance for M3G during infusion of sheep with M3G that was comparable to GFR.

The sheep has proved a useful model for an analysis of the regional elimination of morphine, and the elimination and formation of M3G and M6G. They have not been widely used for studies into the metabolism and elimination of compounds, and there is less information available compared to humans and smaller animals. Further studies comparing the activities of UDPGT towards morphine in preparations from the liver and kidney of sheep with the corresponding activities in humans may prove helpful in applying the *in vivo* findings in the sheep to events in humans.

Compared to the considerable data demonstrating metabolism of compounds by the liver, renal metabolism is a relatively neglected area. Clearly, it may be necessary ro re-examine the role of the kidney in the disposition of compounds, and the mechanisms involved in the excretion of renally-generated metabolites.

An interesting finding from this thesis is the difference in disposition between preformed and generated metabolite. In the past it has been assumed that the disposition of the metabolite was independent of whether it was formed from the parent-drug, or administered directly. The difference is not widely appreciated for conjugated metabolites of drugs. These conjugates were considered previously to be inactive products and a means for elimination of the parent drug. With the renewed interest in their pharmacological activity and disposition, this lack of appreciation may be rectified. The differences in disposition probably arise from dissimilar physicochemical properties which influence their transfer across membranes. Thus, in contrast to when M3G is administered, administration of morphine and subsequent metabolism may generate relatively high concentrations of M3G in hepatocytes and renal tubular cells. The consequences from these higher intracellular concentrations await further investigation.

Appendix 1: Data from Studies in Patients described in Chapter 3

Table A1.1 Concentrations of morphine, M3G and M6G in plasma at the beginning and end of the study period during infusion of intensive-care patients with morphine.

Patient number	Concentrations in plasma at the beginning (1) and end (2) of the urinary collection interval					
	Morphine (nmol/l)		M3G (nmol/l)		M6G (nmol/l)	
	1	2	1	2	1	2
1	97.8	77.1	611	572	104	82.0
2	128	134	782	880	132	147
3	68.0	89.4	878	910	160	145
4	61.0	44.5	1010	869	150	137
5	64.1	76.1	1950	1780	427	345
6	184	293	951	1250	200	264
7	-	-	2670	3330	557	631
8	64.1	92.2	267	351	108	119
9	58.9	84.1	1090	862	187	173
10	123	137	6370	6240	1030	1090
11	127	149	1610	1970	284	342
12	109	95.3	4710	4680	1210	1140
13	47.0	48.7	509	574	134	146
14	417	247	1380	1340	258	264
15	290	158	38800	36200	11900	11200

Table A1.2 Concentrations of morphine, M3G and M6G in urine, the volume and pH of urine collected, and the timed collection interval for patients in intensive-care given morphine

Patient number	Concentration in urine (μmol/l)			Urine volume (ml)	pH	Collection interval (min)
	Morphine	M3G	M6G			
1	23.4	97.5	19.9	232	5.8	240
2	33.2	105	22.8	128	6.1	240
3	7.78	74.8	19.5	143	6.9	233
4	4.94	42.9	8.02	554	6.4	270
5	9.32	236	39.0	64	5.3	240
6	5.61	16.1	4.42	487	5.4	240
7	-	74.7	18.4	16.3	5.3	240
8	12.5	54.1	12.4	207	5.1	300
9	6.94	34.0	5.63	127	6.8	240
10	3.79	56.1	18.0	195	4.6	240
11	15.0	117	22.3	254	5.4	240
12	3.22	51.6	10.2	172	5.0	240
13	6.31	58.3	16.7	216	5.4	240
14	78.5	368	56.1	65.0	5.6	240
15	3.01	80.0	26.4	306	4.9	240

Table A1.3 Concentrations of creatinine in plasma determined by HPLC and Jaffé methods, and in urine by HPLC.

	Concentration in plasma		Concentration in urine (mmol/l)
Patient number	HPLC (µmol/l)	Jaffé* (µmol/l)	
1	42	70	7.51
2	20	40	4.33
3	64	90	4.68
4	55	60	3.45
5	121	150	17.1
6	41	50	1.06
7	228	190	8.49
8	78	90	12.2
9	68	70	6.28
10	299	330	4.95
11	74	120	6.72
12	269	320	3.80
13	83	80	9.28
14	34	50	13.4
15	377	470	1.06

* automated analysis by the method of Jaffé

Appendix 2: Data from Studies in Sheep described in Chapter 5

Tables A2.1 to A 2.14 contain the concentrations of morphine, M3G and M6G measured in the plasma and urine collected from each experiment.

Abbreviations used in the tables denote the following:

Art	Arterial (aorta)
PA	Pulmonary arterial
SS	Sagittal sinus
CS	Coronary sinus
Hep	Hepatic vein
Port	Hepatic portal vein
Ren	Renal vein
PVC	Posterior vena cava
N.S.	No sample collected
N.Q.	Not quantifiable
C.I.	Chromatographic interference
I.E.	Integration error of chromatographic peak, data not recoverable

Table 2.15 contains data for the ratio of the concentrations of morphine, M3G and M6G in blood to those in plasma.

Table 2.16 contains the mean values for cardiac output, and blood flow to the liver and kidney

Table A2.1: Sheep 1 - Morphine sulphate at 2.5 mg/hr

Concentrations of morphine, M3G and M6G in plasma

Morphine (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	54.3	50.1	49.1	44.9	58.2
PA	52.6	48.0	49.1	48.7	40.3
SS	48.0	44.5	56.8	47.3	49.1
CS	69.0	59.2	59.2	64.8	71.5
Hep	25.6	20.3	22.4	21.4	22.4
Port	61.7	56.8	46.3	71.5	76.8
Ren	31.2	24.2	22.8	23.1	17.5
PVC	63.1	75.7	84.8	64.1	58.5

M3G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	358	325	336	392	388
PA	353	321	329	390	414
SS	340	327	360	412	446
CS	319	334	321	414	438
Hep	362	347	325	429	423
Port	371	416	366	440	436
Ren	297	258	280	345	342
PVC	321	280	323	364	403

M6G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
PA	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
SS	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
CS	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
Hep	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
Port	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
Ren	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
PVC	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.

Concentrations of morphine, M3G and M6G in urine (μmol/l)

Time(hr)	Morphine	M3G	M6G	Volume (ml)
0 to 6	16.5	39.7	0.715	260
6 to 24	11.6	7.15	N.Q.	580
24 to 48	5.96	3.68	N.Q.	600

Table A2.2: Sheep 1 - Morphine sulphate at 5 mg/hr

Concentrations of morphine, M3G and M6G in plasma

Morphine (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	114	134	114	84.5	120
PA	126	115	115	128	122
SS	103	102	115	90.8	100
CS	127	104	120	98.1	117
Hep	32.9	27.3	35.0	20.3	39.6
Port	75.7	117	117	78.9	110
Ren	40.3	32.6	40.0	30.5	48.0
PVC	121	112	133	113	132

M3G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	503	535	496	537	505
PA	514	559	509	533	466
SS	566	546	531	505	514
CS	657	576	594	485	531
Hep	553	579	533	535	524
Port	353	587	542	511	527
Ren	440	446	427	440	475
PVC	498	546	507	516	483

M6G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	23.8	N.Q.	24.3	N.Q.	27.3
PA	23.2	N.Q.	26.4	N.Q.	28.0
SS	25.6	N.Q.	28.4	N.Q.	24.1
CS	25.4	N.Q.	33.2	N.Q.	22.8
Hep	25.8	N.Q.	22.5	N.Q.	24.3
Port	25.4	N.Q.	23.0	N.Q.	26.2
Ren	26.7	N.Q.	20.6	N.Q.	22.1
PVC	27.7	N.Q.	27.7	N.Q.	20.6

Concentrations of morphine, M3G and M6G in urine (μmol/l)

Time(hr)	Morphine	M3G	M6G	Volume (ml)
0 to 6	17.8	71.5	0.672	200
6 to 24	20.3	11.5	N.Q	700
24 to 48	5.99	8.23	N.Q.	910

Table A2.3: Sheep 1 - Morphine sulphate at 20 mg/hr

Concentrations of morphine, M3G and M6G in plasma

Morphine (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	519	498	536	547	477
PA	449	494	512	473	431
SS	442	508	547	533	494
CS	845	564	564	592	554
Hep	110	154	141	142	137
Port	428	498	484	477	470
Ren	202	204	175	172	206
PVC	512	750	736	659	747

M3G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	1990	2280	2280	2470	2200
PA	2250	2130	2120	2120	1800
SS	2160	2230	2280	2250	1990
CS	2450	2100	2280	2260	2190
Hep	2360	2570	2340	2390	2420
Port	2470	2300	2340	2440	2180
Ren	2020	1830	1670	1860	2030
PVC	2100	2300	1910	2080	2030

M6G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	23.8	N.Q.	24.3	N.Q.	27.3
PA	23.2	N.Q.	26.4	N.Q.	28.0
SS	25.6	N.Q.	28.4	N.Q.	24.1
CS	25.4	N.Q.	33.2	N.Q.	22.8
Hep	25.8	N.Q.	22.5	N.Q.	24.3
Port	25.4	N.Q.	23.0	N.Q.	26.2
Ren	26.7	N.Q.	20.6	N.Q.	22.1
PVC	27.7	N.Q.	27.7	N.Q.	20.6

Concentrations of morphine, M3G and M6G in urine (µmol/l)

Time(hr)	Morphine	M3G	M6G	Volume (ml)
0 to 6				-
6 to 24		No urine collected		-
24 to 48				-

Table A2.4: Sheep 2 - Morphine sulphate at 2.5 mg/hr

Concentrations of morphine, M3G and M6G in plasma

Morphine (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	C.I.	C.I.	C.I.	C.I.	C.I.
PA	C.I.	C.I.	C.I.	C.I.	C.I.
SS	C.I.	C.I.	C.I.	C.I.	C.I.
CS	C.I.	C.I.	C.I.	C.I.	C.I.
Hep	C.I.	C.I.	C.I.	C.I.	C.I.
Port	C.I.	C.I.	C.I.	C.I.	C.I.
Ren	C.I.	C.I.	C.I.	C.I.	C.I.
PVC	C.I.	C.I.	C.I.	C.I.	C.I.

M3G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	308	349	290	327	334
PA	301	312	251	312	358
SS	316	362	306	295	351
CS	312	384	316	373	358
Hep	316	323	280	321	340
Port	295	362	282	303	381
Ren	245	251	316	284	284
PVC	306	310	293	312	321

M6G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
PA	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
SS	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
CS	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
Hep	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
Port	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
Ren	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
PVC	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.

Concentrations of morphine, M3G and M6G in urine (μmol/l)

Time(hr)	Morphine	M3G	M6G	Volume (ml)
0 to 6	C.I	13.8	N.Q.	350
6 to 24	C.I	10.3	N.Q.	2450
24 to 48	C.I	4.55	N.Q.	2000

Table A2.5: Sheep 2 - Morphine sulphate at 5 mg/hr

Concentrations of morphine, M3G and M6G in plasma

Morphine (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	106	75	103	106	N.S.
PA	93.9	79.2	98.1	120	N.S.
SS	97.8	71.8	87.3	136	N.S.
CS	113	83.8	100	133	N.S.
Hep	41.4	27.3	27.3	52.2	N.S.
Port	103	67.3	90.1	98.1	N.S.
Ren	40.7	31.5	43.1	58.5	N.S.
PVC	97.4	64.5	102	114	N.S.

M3G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	561	592	488	550	N.S.
PA	542	626	442	490	N.S.
SS	548	553	507	659	N.S.
CS	542	579	657	639	N.S.
Hep	548	633	533	613	N.S.
Port	830	570	529	537	N.S.
Ren	453	462	498	470	N.S.
PVC	490	429	446	527	N.S.

M6G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	N.Q.	N.Q.	N.Q.	N.Q.	N.S.
PA	N.Q.	N.Q.	N.Q.	N.Q.	N.S.
SS	N.Q.	N.Q.	N.Q.	N.Q.	N.S.
CS	N.Q.	N.Q.	N.Q.	N.Q.	N.S.
Hep	N.Q.	N.Q.	N.Q.	N.Q.	N.S.
Port	N.Q.	N.Q.	N.Q.	N.Q.	N.S.
Ren	N.Q.	N.Q.	N.Q.	N.Q.	N.S.
PVC	N.Q.	N.Q.	N.Q.	N.Q.	N.S.

Concentrations of morphine, M3G and M6G in urine (µmol/l)

Time(hr)	Morphine	M3G	M6G	Volume (ml)
0 to 6	1.56	24.5	N.Q.	700
6 to 24	4.59	21.2	N.Q.	1780
24 to 48	0.368	9.82	N.Q.	1980

Table A2.6: Sheep 2 - Morphine sulphate at 10 mg/hr

Concentrations of morphine, M3G and M6G in plasma

Morphine (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	154	287	232	333	158
PA	176	246	179	295	178
SS	177	246	163	275	188
CS	176	235	209	295	209
Hep	49.1	43.1	33.3	57.5	32.2
Port	N.S.	N.S.	N.S.	N.S.	N.S.
Ren	60.6	79.9	54.7	65.9	83.4
PVC	189	239	139	208	210

M3G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	858	1008	737	914	975
PA	761	880	676	914	628
SS	986	1044	895	771	958
CS	984	1016	1049	1010	891
Hep	899	1077	797	1038	1042
Port	N.S.	N.S.	N.S.	N.S.	N.S.
Ren	1040	917	813	897	865
PVC	1114	1016	845	1164	1068

M6G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	18.6	35.1	35.8	31.2	26.7
PA	18.2	27.7	24.9	24.7	29.9
SS	N.Q	33.6	24.3	19.9	26.2
CS	N.Q	32.5	28.2	27.5	N.Q
Hep	21.9	N.Q	36.4	22.3	N.Q
Port	N.S	N.S	N.S	N.S	N.S
Ren	18.2	35.1	N.Q	27.7	31.6
PVC	N.Q	36.8	N.Q	N.Q	30.1

Concentrations of morphine, M3G and M6G in urine (μmol/l)

Time(hr)	Morphine	M3G	M6G	Volume (ml)
0 to 6				-
6 to 24		No urine collected		-
24 to 48				-

Table A2.7: Sheep 2 - Morphine sulphate at 20 mg/hr

Concentrations of morphine, M3G and M6G in plasma

Morphine (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	554	599	606	480	508
PA	655	547	634	512	512
SS	624	540	739	543	480
CS	589	617	701	547	505
Hep	108	152	159	118	137
Port	459	494	456	477	466
Ren	212	217	235	185	199
PVC	557	599	708	550	564

M3G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	2100	2270	2150	2120	2420
PA	2090	2010	2070	2330	2110
SS	2220	2380	1930	2470	2080
CS	2350	2140	2080	2070	2160
Hep	2330	2630	2720	2580	1610
Port	2330	2500	C.I.	2780	2390
Ren	2000	1970	2090	2180	1670
PVC	2130	2060	3020	2410	2100

M6G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	44.0	48.5	31.9	35.1	66.5
PA	39.9	32.1	41.4	37.9	C.I.
SS	41.2	27.1	22.3	I.E.	28.0
CS	39.9	49.0	40.5	34.9	23.6
Hep	39.2	60.9	42.5	43.3	46.2
Port	31.4	33.6	18.6	50.9	71.1
Ren	42.3	42.5	28.4	49.8	30.8
PVC	58.7	38.1	36.8	56.6	42.5

Concentrations of morphine, M3G and M6G in urine (μmol/l)

Time(hr)	Morphine	M3G	M6G	Volume (ml)
0 to 6	8.03	140	1.79	760
6 to 24	26.8	137	1.76	940
24 to 48	6.34	61.5	N.Q.	900

Table A2.8: Sheep 3 - Morphine sulphate at 2.5 mg/hr

Concentrations of morphine, M3G and M6G in plasma

Morphine (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	58.9	63.4	69.0	59.9	59.6
PA	71.1	70.1	60.3	74.7	79.2
SS	N.S.	N.S.	N.S.	N.S.	N.S.
CS	56.4	81.3	73.2	60.6	70.1
Hep	16.1	49.1	23.8	23.5	24.9
Port	44.9	76.4	66.6	80.6	71.1
Ren	28.4	54.3	24.2	59.6	22.8
PVC	62.4	74.7	76.8	79.2	77.8

M3G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	442	397	472	503	436
PA	509	403	501	540	472
SS	N.S.	N.S.	N.S.	N.S.	N.S.
CS	462	453	535	457	459
Hep	466	507	516	561	583
Port	403	425	505	496	548
Ren	394	349	390	427	405
PVC	464	399	455	537	553

M6G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
PA	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
SS	N.S.	N.S.	N.S.	N.S.	N.S.
CS	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
Hep	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
Port	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
Ren	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
PVC	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.

Concentrations of morphine, M3G and M6G in urine (µmol/l)

Time(hr)	Morphine	M3G	M6G	Volume (ml)
0 to 6				-
6 to 24		No urine collected		-
24 to 48				-

Table A2.9: Sheep 3 - Morphine sulphate at 5 mg/hr

Concentrations of morphine, M3G and M6G in plasma

Morphine (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	140	131	112	119	N.S.
PA	124	142	116	122	N.S.
SS	117	138	119	113	N.S.
CS	127	146	110	127	N.S.
Hep	45.9	46.3	45.6	36.8	N.S.
Port	114	128	C.I.	111	N.S.
Ren	48.0	75.4	47.3	63.1	N.S.
PVC	129	162	104	126	N.S.

M3G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	932	758	843	767	N.S.
PA	899	862	843	637	N.S.
SS	776	810	821	685	N.S.
CS	908	828	784	698	N.S.
Hep	1020	914	828	763	N.S.
Port	776	862	C.I.	769	N.S.
Ren	735	711	724	622	N.S.
PVC	923	856	904	663	N.S.

M6G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	37.1	24.7	28.2	37.5	N.S
PA	31.6	31.0	34.9	28.8	N.S
SS	26.7	26.4	32.3	33.6	N.S
CS	31.9	28.8	36.0	33.4	N.S
Hep	33.2	33.8	29.0	37.1	N.S
Port	33.4	31.2	C.I.	35.3	N.S
Ren	25.6	24.5	33.4	28.2	N.S
PVC	27.1	32.1	25.6	29.0	N.S

Concentrations of morphine, M3G and M6G in urine (μmol/l)

Time(hr)	Morphine	M3G	M6G	Volume (ml)
0 to 6	7.18	158	3.06	300
6 to 24	5.40	21.9	0.336	1500
24 to 48	5.54	8.45	N.Q.	1300

Table A2.10: Sheep 3 - Morphine sulphate at 10 mg/hr

Concentrations of morphine, M3G and M6G in plasma

Morphine (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	225	240	219	226	254
PA	220	256	435	231	145
SS	213	252	196	N.S.	209
CS	143	279	176	230	179
Hep	49.4	58.9	42.1	50.1	47.0
Port	255	211	173	183	220
Ren	94.6	78.9	60.3	74.7	81.0
PVC	232	241	233	232	149

M3G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	1050	1120	960	1030	1140
PA	1100	1230	648	841	776
SS	893	1420	765	N.S.	1030
CS	852	1180	1050	969	1140
Hep	1150	1300	1060	1020	1200
Port	1310	1370	1310	1040	1110
Ren	927	940	1080	832	1180
PVC	1230	1230	1280	1090	1280

M6G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	43.8	32.5	43.1	39.9	39
PA	27.3	67.4	34.0	42.9	48.1
SS	33.4	45.3	30.1	N.S.	36.2
CS	33.6	80.6	31.6	33.4	42.7
Hep	41.2	33.4	46.4	49.4	35.8
Port	32.3	52.4	47.9	41.8	24.9
Ren	22.1	39.0	32.1	40.7	30.3
PVC	45.7	52.7	49.6	47.5	25.1

Concentrations of morphine, M3G and M6G in urine (μmol/l)

Time(hr)	Morphine	M3G	M6G	Volume (ml)
0 to 6				-
6 to 24		No urine collection		-
24 to 48				-

Table A2.11: Sheep 4 - Morphine sulphate at 2.5 mg/hr

Concentrations of morphine, M3G and M6G in plasma

Morphine (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	68.0	41.7	50.5	51.2	50.8
PA	58.9	47.0	86.2	59.9	75.7
SS	58.5	38.2	56.1	48.0	63.4
CS	59.2	39.6	49.8	50.8	56.4
Hep	22.1	14.7	16.1	18.6	40.0
Port	63.4	44.5	47.0	48.0	53.6
Ren	30.1	24.9	17.2	24.2	25.6
PVC	79.6	39.6	50.1	57.1	59.2

M3G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	358	345	401	371	394
PA	338	351	384	353	423
SS	338	310	394	323	388
CS	360	401	377	349	388
Hep	373	319	366	336	414
Port	353	368	368	340	433
Ren	290	314	349	310	390
PVC	332	290	303	325	407

M6G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
PA	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
SS	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
CS	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
Hep	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
Port	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
Ren	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
PVC	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.

Concentrations of morphine, M3G and M6G in urine (μmol/l)

Time(hr)	Morphine	M3G	M6G	Volume (ml)
0 to 6	1.25	16.0	N.Q.	800
6 to 24	0.866	7.37	N.Q.	2600
24 to 48	N.Q.	2.88	N.Q.	2800

Table A2.12: Sheep 4 - Morphine sulphate at 5 mg/hr

Concentrations of morphine, M3G and M6G in plasma

Morphine (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	78.5	70.1	71.1	57.8	64.8
PA	64.5	79.2	87.6	53.6	81.3
SS	94.3	71.1	72.2	65.5	77.1
CS	102	67.3	78.5	61.3	79.6
Hep	18.9	17.9	23.8	21.7	13.0
Port	63.4	62.4	74.3	55.0	66.9
Ren	70.8	34.7	30.1	43.5	30.5
PVC	104	59.2	74.3	48.4	71.5

M3G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	659	527	698	696	661
PA	678	581	689	657	652
SS	719	581	776	724	719
CS	711	639	752	748	687
Hep	737	611	589	674	830
Port	758	687	663	674	774
Ren	852	540	644	639	585
PVC	836	641	702	780	737

M6G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
PA	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
SS	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
CS	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
Hep	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
Port	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
Ren	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
PVC	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.

Concentrations of morphine, M3G and M6G in urine (μmol/l)

Time(hr)	Morphine	M3G	M6G	Volume (ml)
0 to 6				-
6 to 24		No urine collected		-
24 to 48				-

Table 2.13: Sheep 4 - Morphine sulphate at 10 mg/hr

Concentrations of morphine, M3G and M6G in plasma

Morphine (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	199	251	223	267	212
PA	226	241	225	230	210
SS	219	231	219	195	208
CS	209	235	231	156	188
Hep	85.9	69.0	74.3	66.2	63.1
Port	218	234	211	205	203
Ren	85.5	83.8	92.9	73.9	91.1
PVC	211	238	235	205	228

M3G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	1680	1470	1450	1600	1730
PA	1840	1460	1500	1740	1670
SS	1730	1390	1560	1500	1640
CS	1700	1500	1600	1150	1640
Hep	1900	1560	1620	1930	1920
Port	1710	1400	1580	1740	1670
Ren	1310	1310	1410	1450	1640
PVC	1470	1500	1590	1660	1490

M6G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	48.5	39.9	41.0	78.2	48.5
PA	48.3	37.3	39.9	85.4	48.3
SS	46.2	38.4	51.4	85.4	46.6
CS	42.9	35.5	49.0	56.1	41.2
Hep	48.8	39.7	50.3	83.0	55.5
Port	44.9	47.7	48.3	75.6	53.7
Ren	28.6	38.8	54.6	57.0	39.0
PVC	37.5	36.6	34.2	70.2	42.5

Concentrations of morphine, M3G and M6G in urine (µmol/l)

Time(hr)	Morphine	M3G	M6G	Volume (ml)
0 to 6	7.89	120	0.823	680
6 to 24	3.68	33.2	N.Q.	1600
24 to 48	1.16	9.43	N.Q.	2250

Table A2.14: Sheep 4 - Morphine sulphate at 20 mg/hr

Concentrations of morphine, M3G and M6G in plasma

Morphine (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	396	403	389	424	410
PA	452	424	358	417	343
SS	303	410	414	424	382
CS	301	393	396	449	379
Hep	92.9	128	128	193	132
Port	287	403	466	494	382
Ren	105	134	172	185	165
PVC	354	445	386	435	466

M3G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	2020	2180	2080	2390	2990
PA	2220	2130	1940	2230	2400
SS	1430	2100	2200	2520	2630
CS	1630	1970	2240	2500	2600
Hep	1740	2310	2360	2760	2860
Port	1630	2370	2300	2640	2810
Ren	1630	2030	2410	2570	2690
PVC	1950	2540	2270	2770	3280

M6G (nmol/l)

Time(hr)	5.00	5.25	5.50	5.75	6.00
Art	57.4	56.1	64.1	65.7	85.8
PA	63.3	55.9	64.6	61.5	61.1
SS	61.1	49.8	61.5	65.0	70.9
CS	42.5	48.1	66.1	68.0	67.6
Hep	84.5	54.8	66.3	71.7	93.8
Port	40.3	56.8	65.0	62.4	77.8
Ren	40.3	40.1	69.6	59.4	81.3
PVC	39.2	55.3	58.9	66.1	88.6

Concentrations of morphine, M3G and M6G in urine (µmol/l)

Time(hr)	Morphine	M3G	M6G	Volume (ml)
0 to 6				
6 to 24		Incomplete urine collection		
24 to 48				

Table A2.15: Ratio of the concentrations of morphine, M3G and M6G in blood to those in plasma

Sheep 1 to 4 infused with morphine sulphate, 2.5 to 20 mg/hr

Sheep number	Rate of infusion		Morphine*			M3G*			M6G*		
		H	66.5	133	665	217	2170	5420	102	204	509
1	2.5	0.28	1.39	1.55	1.41	-	0.84	0.73	0.99	-	-
1	5.0	0.28	1.13	1.40	1.68	0.68	0.78	0.82	0.89	-	-
2	2.5	0.33	1.31	1.33	1.40	1.19	0.75	0.70	0.65	-	-
2	10.0	0.23	1.10	1.37	1.34	0.66	0.70	0.68	0.81	-	-
2	20.0	0.24	1.39	1.60	1.36	0.99	0.83	0.74	0.76	-	-
3	2.5	0.25	-	0.99	1.22	0.83	0.85	1.31#	-	0.86	0.81
4	5.0	0.29	-	1.12	1.09	0.78	0.81	0.77	-	0.81	0.74

* nominal concentrations (nmol/l) prepared in drug-free blood collected prior to the infusion

\# the value of 1.31 for M3G measured in the blood of sheep 3 was excluded, as an outlier ($p > 0.05$, Dixon test), from further statistical analysis

Table A2.16: Cardiac output, blood flow to the liver and kidney

Sheep 1 to 4 infused with morphine sulphate, 2.5 to 20 mg/hr

Sheep number	Rate of infusion	Cardiac output*	Liver*	Kidney*
1	2.5	6.48	2.02	1.33
1	5.0	6.49	1.26	0.98
1	10.0	-	-	-
1	20.0	7.30	1.47	0.77
2	2.5	6.06	1.11	0.83
2	5.0	6.34	0.63	0.88
2	10.0	7.27	1.37	0.66
2	20.0	7.69	1.14	0.88
3	2.5	5.23	1.12	0.65
3	5.0	7.01	1.28	1.10
3	10.0	5.57	1.93	1.06
3	20.0	-	-	-
4	2.5	7.83	1.82	1.54
4	5.0	7.22	2.41	1.29
4	10.0	6.81	1.43	0.74
4	20.0	7.11	1.95	1.05

* flows are in l/min

Appendix 3: Data from Studies in Sheep described in Chapter 6.

Tables A3.1 to A3.10 contain the concentrations of morphine, M3G and M6G measured in the plasma and urine from sheep infused with morphine sulphate, and of the concentrations of M3G in plasma and of morphine and M3G in the urine of sheep infused with M3G.

Abbreviations are as for Appendix 2.

Table A3.11 contains the calculated values of Cb/C for morphine, M3G and M6G during infusion with morphine sulphate and with M3G.

Table A3.12 contains values for the fractions of morphine, M3G and M6G unbound in pooled arterial plasma from normal sheep infused with morphine.

Table A3.13 contains the calculated values for cardiac output and blood flows to the liver and kidney.

Table A3.14 contains the concentrations of creatinine in arterial plasma and urine from normal sheep administered separate infusions of morphine and M3G

Table A3.1: Sheep 6 - Morphine sulphate at 10 mg/hr

Concentrations of morphine, M3G and M6G in plasma (nmol/l)

Morphine (nmol/l)

Time (hr)	0.25	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48
Art	1410	297	318	305	284	296	327	325	266	290	295	N.Q.	N.Q.
PA							288	311	310	299	294		
Hep							84.1	87.2	88.5	72.4	72.0		
Port							288	293	332	248	258		
Ren							128	121	142	108	106		
PVC							321	303	293	304	310		

M3G (nmol/l)

Time (hr)	0.25	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48
Art	970	1120	1260	1310	1430	1550	1700	1670	1620	1590	1600	434	N.Q.
PA							1670	1580	1840	1610	1620		
Hep							1730	1620	1760	1560	1670		
Port							1730	1860	1730	1590	1690		
Ren							1610	1500	1490	1490	1400		
PVC							1880	1800	1630	1820	1810		

M6G (nmol/l)

Time (hr)	0.25	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48
Art	38.2	51.7	54.0	52.0	54.5	48.5	56.8	47.1	53.3	54.6	57.1	N.Q.	N.Q.
PA							51.6	50.1	65.4	50.2	57.9		
Hep							52.1	51.5	60.5	70.7	64.2		
Port							51.5	51.3	61.2	52.8	65.3		
Ren							49.9	44.4	40.3	45.4	46.7		
PVC							59.8	52.0	58.3	53.9	75.3		

Concentrations of morphine, M3G and M6G in urine (μmol/l)

Time (hr)	Morphine	M3G	M6G	Urine volume (ml)
0 to 5	5.07	91.2	1.24	645
5 to 6	2.98	45.2	0.752	244
6 to 24	6.17	40.3	0.652	1510
24 to 48	1.25	0.932	N.Q	4220

Table A3.2: Sheep 7 - Morphine sulphate at 10 mg/hr

Concentrations of morphine, M3G and M6G in plasma (nmol/l)

Morphine (nmol/l)

Time (hr)	0.25	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48
Art	915	219	204	178	189	196	176	185	177	170	175	N.Q.	N.Q.
PA							184	201	178	192	173		
Hep							49.5	59.3	50.0	51.8	55.1		
Port							186	173	164	172	174		
Ren							90.2	89.3	98.3	83.8	100		
PVC							180	192	190	185	183		

M3G (nmol/l)

Time (hr)	0.25	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48
Art	600	910	790	760	910	1090	1020	1000	1070	1030	1160	357	N.Q.
PA							1080	1000	1110	930	1280		
Hep							1120	990	1080	1130	1180		
Port							1090	1130	1060	1100	1150		
Ren							930	920	1030	980	1100		
PVC							1040	1060	1150	1040	1170		

M6G (nmol/l)

Time (hr)	0.25	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48
Art	11.5	26.0	24.7	21.3	18.2	25.1	30.1	23.2	35.2	27.2	34.9	N.Q.	N.Q.
PA							31.2	20.3	32.1	25.1	30.6		
Hep							31.1	24.9	31.6	28.8	35.5		
Port							32.6	28.3	30.9	28.2	28.0		
Ren							27.1	23.0	27.8	22.8	33.3		
PVC							34.4	25.2	27.0	24.9	34.6		

Concentrations of morphine, M3G and M6G in urine (μmol/l)

Time (hr)	Morphine	M3G	M6G	Urine volume (ml)
0 to 5	11.9	151	2.08	394
5 to 6	4.20	54.2	0.754	170
6 to 24	56.9	N.Q	N.Q	1180
24 to 48	6.01	N.Q	N.Q	1906

Table A3.3: Sheep 8 - Morphine sulphate at 10 mg/hr

Concentrations of morphine, M3G and M6G in plasma (nmol/l)

Morphine (nmol/l)

Time (hr)	0.08	0.16	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	7	8	9	10	11	12	24	48
Art	819	953	745	249	208	213	198	190	188	177	177	175	165	169	171	51.7	25.4	19.1	9.4	N.Q.	N.Q.	N.Q.	N.Q.
PA											163	193	146	193	170								
Hep											44.0	33.5	47.0	37.6	40.3								
Port											143	185	164	147	155								
Ren											43.3	62.2	48.1	55.6	59.6								
PVC											290	280	249	266	279								

M3G (nmol/l)

Time (hr)	0.08	0.16	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	7	8	9	10	11	12	24	48
Art	203	670	1000	1020	1020	1020	1020	1080	1050	1030	1390	1360	1360	1400	1490	1310	1180	1230	1040	940	850	94	N.Q.
PA											1300	1500	1300	1360	1510								
Hep											1210	1330	1420	1510	1430								
Port											1260	1380	1640	1380	1440								
Ren											1090	1100	1090	1340	1350								
PVC											1180	1310	1350	1300	1320								

M6G (nmol/l) - not quantifiable

Concentrations of morphine, M3G and M6G in urine (μmol/l)

Time (hr)	Morphine	M3G	M6G	Urine volume (ml)
0 to 5	8.52	141	1.83	557
5 to 6	3.06	63.6	0.570	176
6 to 24	0.350	46.3	0.455	1510
24 to 48	N.Q.	3.51	N.Q	1700

Table A3.4: Sheep 10 - Morphine sulphate at 10 mg/hr

Concentrations of morphine, M3G and M6G in plasma (nmol/l)

Morphine (nmol/l)

Time (hr)	0.08	0.16	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48
Art	746	845	691	270	238	227	166	164	157	198	211	171	185	190	169	N.Q	N.Q
PA											161	179	150	199	158		
Hep											72.3	76.1	46.4	52.9	45.2		
Port											161	211	212	169	163		
Ren											83.6	81.2	79.1	70.2	77.8		
PVC											165	186	172	178	173		

M3G (nmol/l)

Time (hr)	0.08	0.16	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48
Art	170	380	620	660	670	700	690	750	800	890	1160	1080	1360	1590	1340	270	N.Q
PA											900	1470	1340	1420	1210		
Hep											960	1400	1690	1410	1380		
Port											1050	1270	1520	1470	1450		
Ren											870	1040	1170	1170	1190		
PVC											980	1200	1260	1490	1300		

M6G (nmol/l)

Time (hr)	0.08	0.16	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48
Art	N.Q.	N.Q.	17.8	16.5	21.1	19.5	24.3	26.2	20.6	17.5	30.3	21.5	26.1	33.3	25	N.Q	N.Q.
PA											26.6	20.1	29.1	37.0	22.5		
Hep											26.2	33.6	35.5	32.3	27.4		
Port											26.3	26.1	27.7	31.0	23.3		
Ren											21.8	21.6	25.8	25.5	17.6		
PVC											26.0	27.8	26.1	35.3	16.2		

Concentrations of morphine, M3G and M6G in urine (μmol/l)

Time (hr)	Morphine	M3G	M6G	Urine volume (ml)
0 to 5	16.2	143	2.95	387
5 to 6	6.24	79.4	1.72	198
6 to 24	0.876	48.0	0.489	1130
24 to 48	N.Q.	11.4	N.Q.	2080

Table A3.5: Sheep 11 - Morphine sulphate at 10 mg/hr

Concentrations of morphine, M3G and M6G in plasma (nmol/l)

Morphine (nmol/l)

Time (hr)	0.08	0.16	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48
Art	696	746	897	307	231	204	136	135	125	137	147	150	135	151	149	N.Q	N.Q
PA											145	148	135	162	143		
Hep											35.7	47.8	70.9	68.3	68.6		
Port											172	214	160	216	228		
Ren											49.4	51.1	50.1	59.5	57.0		
PVC											136	149	123	146	133		

M3G (nmol/l)

Time (hr)	0.08	0.16	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48
Art	180	520	700	850	780	840	880	950	980	1000	1210	1150	1400	1190	1280	190	N.Q
PA											1200	1180	1120	1350	1450		
Hep											1150	1130	1200	1250	1430		
Port											1210	1410	1200	1300	1420		
Ren											1010	1090	1130	1160	1240		
PVC											1370	1210	1180	1210	1300		

M6G (nmol/l)

Time (hr)	0.08	0.16	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48
Art	N.Q.	27.0	33.4	41.2	42.7	45.6	44.3	41.8	42.8	32.7	41.3	36.2	38.1	36.6	34.8	N.Q	N.Q
PA											27.9	38.5	33	45.3	43.7		
Hep											37.4	36.1	37.6	37.2	46.3		
Port											37.9	45.4	38.4	34.7	48.6		
Ren											29.3	37.3	35.6	32.3	40.4		
PVC											32.3	35.6	36.7	35.7	39.6		

Concentrations of morphine, M3G and M6G in urine (μmol/l)

Time (hr)	Morphine	M3G	M6G	Urine volume (ml)
0 to 5	22.9	158	4.09	370
5 to 6	7.16	73.5	1.79	204
6 to 24	24.3	19.5	1.39	2010
24 to 48	3.29	1.37	0.260	2680

Table A3.6: Sheep 6 - M3G at 7.5 mg/hr

Concentrations of M3G in plasma (nmol/l)

M3G (nmol/l)

Time (hr)	0.08	0.16	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48
Art	-	-	4410	-	-	3140	2870	2810	2740	2500	2290	2540	2380	2750	2520	94.8	N.Q.
PA											2570	2400	2450	2440	2440		
Hep											2550	2480	2320	2490	2380		
Port											2190	2370	2530	2250	2510		
Ren											2290	2300	2000	2520	2220		
PVC											2520	2240	2430	2560	2610		

Concentrations of morphine, M3G and M6G in urine (μmol/l)

Time (hr)	Morphine	M3G	M6G	Urine volume (ml)
0 to 5	N.Q.	38.4	N.Q.	1420
5 to 6	N.Q.	26.9	N.Q.	440
6 to 24	0.736	13.4	N.Q.	2180
24 to 48	0.666	0.607	N.Q.	3380

Table A3.7: Sheep 7 - M3G at 4 mg/hr

Concentrations of M3G in plasma (nmol/l)

M3G (nmol/l)

Time (hr)	0.08	0.16	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48
Art	795	1270	1720	1330	1190	1080	883	837	812	832	1340	1200	1400	1200	1350	N.Q.	N.Q.
PA											1310	1240	1400	1250	1350		
Hep											1260	1200	1340	1210	1330		
Port											1310	1220	1400	1220	1190		
Ren											1280	1050	1260	1190	1190		
PVC											1240	1270	1260	1240	1340		

Concentrations of morphine, M3G and M6G in urine (μmol/l)

Time (hr)	Morphine	M3G	M6G	Urine volume (ml)
0 to 5	N.Q.	43.3	N.Q.	656
5 to 6	N.Q.	22.8	N.Q.	196
6 to 24	7.97	8.15	N.Q.	960
24 to 48	0.561	N.Q.	N.Q.	2010

Table A3.8: Sheep 8 - M3G at 4 mg/hr

Concentrations of M3G in plasma (nmol/l)

	M3G (nmol/l)																					
Time (hr)	0.083	0.16	0.25	0.33	0.417	0.5	1	2	3	4	5	5.25	5.5	5.75	6	7	8	9	10	11	12	24	48
Art	765	1500	1670	1270	1180	1150	904	1030	1020	967	1110	1010	940	860	1030	439	292	158	101	N.Q.	N.Q.	N.Q.	N.Q.
PA											1060	990	980	830	1050								
Hep											1000	940	1000	890	980								
Port											1040	910	1030	780	990								
Ren											880	1000	860	830	980								
PVC											930	900	1120	970	1050								

Concentrations of morphine, M3G and M6G in urine (μmol/l)

Time (hr)	Morphine	M3G	M6G	Urine volume (ml)
0 to 5	N.Q.	23.3	N.Q	1160
5 to 6	N.Q.	25.2	N.Q	245
6 to 24	5.22	5.44	N.Q	1390
24 to 48	0.175	0.932	N.Q	1450

Table A3.9: Sheep 10 - M3G at 4 mg/hr

Concentrations of M3G in plasma (nmol/l)

	M3G (nmol/l)																
Time (hr)	0.08	0.16	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48
Art	855	1340	1470	-	1000	1030	893	871	812	824	980	699	832	866	779	N.Q.	N.Q.
PA											829	887	770	870	714		
Hep											839	804	833	881	839		
Port											841	933	858	704	806		
Ren											726	646	707	636	727		
PVC											835	896	846	769	780		

Concentrations of morphine, M3G and M6G in urine (μmol/l)

Time (hr)	Morphine	M3G	M6G	Urine volume (ml)
0 to 5	N.Q.	65.4	N.Q.	1420
5 to 6	N.Q.	32.2	N.Q.	440
6 to 24	N.Q.	10.0	N.Q.	2180
24 to 48	N.Q.	2.10	N.Q.	3380

Table A3.10: Sheep 11 - M3G at 4 mg/hr

Concentrations of M3G in plasma (nmol/l)

	M3G (nmol/l)																
Time (hr)	0.08	0.16	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48
Art	795	1270	1720	1330	1190	1080	883	837	812	832	799	802	836	869	774	N.Q.	N.Q.
PA											747	811	816	793	818		
Hep											781	845	783	787	768		
Port											814	815	815	788	776		
Ren											739	704	770	815	685		
PVC											841	869	789	725	783		

Concentrations of morphine, M3G and M6G in urine (μmol/l)

Time (hr)	Morphine	M3G	M6G	Urine volume (ml)
0 to 5	N.Q.	59.1	N.Q.	625
5 to 6	N.Q.	24.2	N.Q.	226
6 to 24	1.99	4.38	N.Q.	3210
24 to 48	0.773	N.Q.	N.Q.	2450

Table A3.11: Ratio of the concentrations of morphine, M3G and M6G in blood to those in plasma

Sheep infused with morphine sulphate and M3G

	Morphine sulphate					M3G
Sheep number	Morphine*		M3G*	M6G*		M3G*
	35.1 nmol/l	129 nmol/l	558 nmol/l	26.2 nmol/l	98.8 nmol/l	558 nmol/l
6	1.42, 1.52	1.46, 1.50	0.94, 0.95	0.81, 0.87	0.92, 0.93	0.78, 0.89
7	1.09, 1.25	1.30, 1.43	0.91, 1.01	0.94, 1.07	0.89, 0.91	-
8	1.07, 1.11	1.34, 1.91	0.89, 0.97	0.63, 0.81	0.81, 0.90	0.94, 1.19
10	1.15, 1.23	1.13, 1.23	0.63, 0.77	0.66, 0.74	0.69, 0.70	0.89, 0.95
11	0.98, 1.57	1.09, 1.26	0.83, 0.92	0.85, 0.92	0.77, 0.91	0.77, 0.86

* nominal concentrations of morphine, M3G and M6G prepared in drug-free blood

Table A3.12: Fractions of morphine, M3G and M6G unbound in pooled arterial plasma from normal sheep infused with morphine

	Compound [compound infused]		
Sheep number	Morphine [morphine]	M3G [morphine]	M6G [morphine]
6	0.70	0.82	0.92
7	0.78	0.83	1.05
8	0.86	0.83	0.81
10	0.88	0.85	0.83
11	0.76	0.97	0.90

Table A3.13: Cardiac output, blood flow to the liver and kidney

Sheep infused with morphine sulphate and M3G

	Morphine sulphate			M3G		
Sheep number	Cardiac Output*	Liver*	Kidney*	Cardiac output*	Liver*	Kidney*
6	9.04	0.99	1.14	6.72	1.23	1.35
7	7.34	1.92	1.45	8.39	2.16	1.57
8	9.04	3.37	1.13	8.25	2.97	1.55
10	8.17	2.20	1.47	11.0	3.79	1.55
11	10.5	3.16	1.60	10.4	2.50	1.94

* flow in l/min

Table A3.14: Concentrations of creatinine in arterial plasma at 5 and 6 hr and urine collected between 5 and 6 hr from normal sheep administered separate infusions of morphine and M3G

		Concetration of creatinine (mmol/l)		
Sheep number	Compound infused	Plasma at 5 hr	Plasma at 6 hr	Urine
6	Morphine	0.086	0.082	0.84
6	M3G	0.074	0.074	0.89
7	Morphine	0.063	0.068	1.60
7	M3G	0.056	0.057	1.35
8	Morphine	0.066	0.063	1.64
8	M3G	0.067	0.068	1.39
10	Morphine	0.072	0.075	1.86
10	M3G	0.066	0.072	1.52
11	Morphine	0.065	0.063	2.51
11	M3G	0.056	0.059	1.15

Appendix 4: Data from Studies in Sheep described in Chapter 7.

Tables A4.1 to A4.10 contain the concentrations of morphine, M3G and M6G measured in the plasma from sheep infused with morphine sulphate, and of the concentrations of M3G in plasma and of morphine and M3G in the urine of sheep infused with M3G.

Abbreviations are as for Appendix 2.

Table A4.11 contains the concentrations of morphine, M3G and M6G measured in the urine from sheep in renal failure infused with morphine sulphate.

Table A4.12 contains of the concentrations of morphine and M3G in the urine of sheep in renal failure infused with M3G.

Table A4.13 contains the calculated values of Cb/C for morphine, M3G and M6G during infusion with morphine sulphate and with M3G.

Table A4.14 contains values for the fractions of morphine, M3G and M6G unbound in pooled arterial plasma from sheep in renal failure infused with morphine.

Table A4.15 contains the calculated values for cardiac output and blood flows to the liver and kidney of sheep in renal failure administered separate infusions of morphine and M3G.

Table A4.16 contains the concentrations of creatinine in arterial plasma and urine from sheep in renal failure administered separate infusions of morphine and M3G

Table A4.17 contains the amounts of polystyrene and glass beads administered to the sheep for the induction of renal failure

Table A4.18 contains the concentrations of morphine, M3G and M6G in plasma and urine of sheep 5 infused with morphine sulphate when normal, and the cardiac output and blood flow to the liver and kidney

Table A4.1: Sheep 7 with renal failure - Morphine Sulphate at 10 mg/hr

Concentrations of morphine, M3G and M6G in plasma (nmol/l)

Morphine (nmol/l)

Time (hr)	0.25	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144#
Art	616	223	199	155	202	192	173	174	184	198	173	N.Q.	N.Q.	N.S.	N.S.	N.S.	N.S.
PA							182	193	183	195	186						
Hep							39.2	46.0	36.8	40.6	44.8						
Port							169	159	162	172	186						
Ren							68.3	73.5	73.6	73.4	53.8						
PVC							187	171	192	186	155						

M3G (nmol/l)

Time (hr)	0.25	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144#
Art	893	1200	1800	1420	2120	2990	2810	2930	2900	3140	3030	357	N.Q.	N.S.	N.S.	N.S.	N.S.
PA							2910	2910	2390	3060	2770						
Hep							2910	2930	2910	2760	2870						
Port							2860	2800	3060	3290	2970						
Ren							2680	2620	2780	2940	2400						
PVC							2690	2570	2790	3130	2500						

M6G (nmol/l)

Time (hr)	0.25	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144#
Art	33.9	46.6	78.6	53.9	87.9	110	106	118	116	113	110	N.Q.	N.Q.	N.S.	N.S.	N.S.	N.S.
PA							99.0	107	98.0	114	111						
Hep							121	117	103	105	111						
Port							105	105	112	134	122						
Ren							97.0	98.0	102	112	93.0						
PVC							105	105	106	122	103						

Table A4.2: Sheep 8 in renal failure - morphine sulphate at 10 mg/hr

Concentrations of morphine, M3G and M6G in plasma (nmol/l)

Morphine (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	7	8	9	10	11	12	24	48	72	96	120
Art	1070	1120	1350	334	272	241	238	248	257	264	278	271	261	275	263	45.2	37.1	15.8	15.4	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
PA											272	249	291	223	278											
Hep											54.3	53.8	45.6	53.9	52.1											
Port											204	200	228	197	262											
Ren											108	108	102	91	104											
PVC											272	245	225	259	243											

M3G (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	7	8	9	10	11	12	24	48	72	96	120
Art	290	847	1480	1750	1730	1620	2220	2980	3680	4290	4870	4980	5200	5350	5830	5520	5510	4650	5010	5230	4730	3400	1120	538	275	151
PA											5230	4500	5280	4910	6280											
Hep											5080	4980	5380	4530	5140											
Port											4950	4560	5930	4630	5630											
Ren											5190	4830	4740	5120	5290											
PVC											5070	4700	5270	5120	5500											

M6G (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	7	8	9	10	11	12	24	48	72	96	120
Art	N.Q.	N.Q.	17.3	18.9	19.1	28	28.4	38.1	45.7	55.3	71.8	77.1	94.6	83.3	82.2	96.2	100	83.2	86.2	88.2	83.4	62.0	30.1	N.Q.	N.Q.	N.Q.
PA											70.9	65.7	91.5	69.8	82.6											
Hep											74.6	77.6	90.5	71.9	75.8											
Port											83.1	69.7	95.8	76.6	84.4											
Ren											70.8	75.0	62.9	90.4	81.8											
PVC											83.0	72.0	78.2	81.2	62.4											

* concentrations at 144 hr not quantifiable

Table A4.3: Sheep 9 in renal failure - morphine sulphate at 10 mg/hr

Concentrations of morphine, M3G and M6G in plasma (nmol/l)

Morphine (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144
Art	717	1032	1191	401	316	255	259	257	245	252	249	253	267	216	254	N.Q.	N.Q.	N.S.	N.S.	N.S.	N.S.
PA											266	328	290	208	265						
Hep											71.8	69.6	66.3	68.2	76.3						
Port											337	356	281	296	268						
Ren											125	151	99	117	121						
PVC											242	306	258	253	293						

M3G (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144
Art	204	689	1400	1590	1610	1660	2670	2870	4190	4810	5080	5010	5060	5740	5050	4100	3470	N.S.	N.S.	N.S.	N.S.
PA											4860	5570	5820	5410	4740						
Hep											5060	5360	4750	5580	6080						
Port											5220	5580	5350	5570	5470						
Ren											4780	5440	4510	6170	5420						
PVC											5020	5810	5140	5550	5850						

M6G (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144
Art	N.Q.	25.5	46.8	66.0	64.3	65.3	100	111	147	170	184	188	190	205	191	227	123	N.S.	N.S.	N.S.	N.S.
PA											184	204	215	210	188						
Hep											191	208	189	228	239						
Port											193	198	205	201	245						
Ren											170	193	164	216	219						
PVC											190	217	196	209	258						

Table A4.4: Sheep 10 in renal failure - morphine sulphate at 10 mg/hr

Concentrations of morphine, M3G and M6G in plasma (nmol/l)

Morphine (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144
Art	717	1032	1191	401	316	255	259	257	245	252	201	205	224	194	232	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
PA											207	207	218	196	198						
Hep											72.3	62.2	52.0	63.9	54.5						
Port											227	211	214	227	205						
Ren											137	138	138	131	133						
PVC											204	202	221	227	193						

M3G (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144
Art	204	689	1400	1590	1610	1660	2670	2870	4190	4810	3700	4220	4520	4560	4360	3200	2150	1630	1260	614	610
PA											3660	5080	3690	3970	4120						
Hep											4090	3900	4110	4410	4150						
Port											4270	3840	4080	4440	4030						
Ren											4140	4270	4070	3960	4020						
PVC											3910	3780	4470	4330	4130						

M6G (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144
Art	N.Q.	20	23.1	21.3	28.2	35	43.2	53.9	64.7	85.8	89.6	90.8	107	89.3	102	91	61	43.5	18.6	N.Q.	16.4
PA											102	108	90.0	84.2	106						
Hep											104	84.5	104	110	107						
Port											108	82.3	105	96.3	90.9						
Ren											97.7	87.9	103	88.5	91.3						
PVC											94.0	83.1	112	108	103						

Table A4.5: Sheep 11 in renal failure - morphine sulphate at 10 mg/hr

Concentrations of morphine, M3G and M6G in plasma (nmol/l)

Morphine (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144
Art	475	705	701	280	212	218	321	246	235	264	249	253	267	216	254	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
PA											266	328	290	208	265						
Hep											71.8	69.6	66.3	68.2	76.3						
Port											337	356	281	296	268						
Ren											125	151	99	117	121						
PVC											242	306	258	253	293						

M3G (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144
Art	N.Q.	319	522	729	754	807	1600	1970	2560	2960	3480	3920	3800	4010	3480	2880	1880	1620	1500	1200	1050
PA											3590	3690	3760	3930	3560						
Hep											3700	3670	3910	3750	3820						
Port											3590	3930	3450	4090	3540						
Ren											3600	3610	3510	3990	3540						
PVC											3630	3580	3650	3910	3640						

M6G (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144
Art	N.Q.	N.Q.	N.Q.	18.8	16.0	16.2	44.3	57.8	63.6	77.0	92.7	91.0	99.7	101	101	84.1	44.2	34.0	30.7	26.7	20.6
PA											95.7	92.3	97.9	95.1	95.4						
Hep											101	89.1	105	85.3	99.0						
Port											97.1	97.9	86.1	96.7	105						
Ren											91.8	81.5	86.2	87.8	98.0						
PVC											101	85.7	90.3	83.6	104						

Table A4.6: Sheep 12 in renal failure - morphine sulphate at 10 mg/hr

Concentrations of morphine, M3G and M6G in plasma (nmol/l)

Morphine (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144
Art	1010	1280	1320	450	365	333	282	260	269	268	336	300	277	291	294	N.Q.	N.Q.	N.Q.	N.S.	N.S.	N.S.
PA											341	277	297	263	250						
Hep											98.7	78.8	88.2	88.2	102						
Port											N.S.	N.S.	N.S.	N.S.	N.S.						
Ren											226	211	220	215	216						
PVC											332	287	277	293	317						

M3G (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144
Art	135	503	961	1140	1240	1280	1750	2350	2980	3390	5230	4330	4250	4610	5080	6790	4044	3158	N.S.	N.S.	N.S.
PA											3990	4040	4550	3750	3540						
Hep											4790	4580	4270	4300	5230						
Port											N.S.	N.S.	N.S.	N.S.	N.S.						
Ren											4540	4300	4540	4190	4570						
PVC											4690	4180	4100	4640	4900						

M6G (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144
Art	N.Q.	N.Q.	16.0	23.9	25.1	28.6	43.6	59.2	81.4	86.4	146	109	118	122	124	125	55.0	44.0	N.S.	N.S.	N.S.
PA											117	116	107	105	84						
Hep											130	130	90	130	130						
Port											N.S.	N.S.	N.S.	N.S.	N.S.						
Ren											116	122	111	128	101						
PVC											112	120	107	135	123						

Table A4.7: Sheep 13 in renal failure - morphine sulphate at 10 mg/hr

Concentrations of morphine, M3G and M6G in plasma (nmol/l)

Morphine (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144
Art	798	832	1030	1140	422	362	220	212	247	230	190	184	210	218	164	N.Q.	N.Q.	N.Q.	N.S.	N.S.	N.S.
PA											216	173	165	207	261						
Hep											107	66.5	59.1	64.1	96.2						
Port											245	231	186	207	195						
Ren											134	134	128	137	152						
PVC											201	249	221	236	180						

M3G (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144
Art	231	732	1130	1630	1730	2210	2230	3490	3850	4800	4690	4940	5380	5050	4550	3490	3000	1630	N.S.	N.S.	N.S.
PA											5020	4900	4480	5100	4620						
Hep											4800	5500	4590	5190	4780						
Port											5160	5930	5630	5590	5500						
Ren											4980	4840	5090	5000	4710						
PVC											4900	5110	5460	5430	4680						

M6G (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144
Art	N.Q.	N.Q.	23.7	31.3	20.3	55.8	55.0	73.9	82.3	101	91.3	92.3	114	122	111	59.7	33.4	20.3	N.S.	N.S.	N.S.
PA											83.0	80.3	100	114	107						
Hep											87.7	110	102	113	109						
Port											95.2	128	117	123	108						
Ren											81.7	99.1	112	117	111						
PVC											90.2	110	125	112	100						

Table A4.8: Sheep 10 in renal failure - M3G at 4 mg/hr

Concentrations of morphine and M3G in plasma (nmol/l)*

M3G (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144
Art	1600	2070	2950	2540	2660	2610	2580	3130	3550	4060	4060	4620	3620	4210	4400	1280	630	440	N.S.	N.S.	N.S.
PA											4140	4100	4100	4100	4460						
Hep											3960	4430	4110	4310	3870						
Port											3880	4280	4580	4350	4390						
Ren											3750	4130	3770	4020	3960						
PVC											4100	4220	4370	4610	4330						

* morphine at concentrations of 18.0, 17.0 and 19.0 nmol/l in portal vein at 5, 5.5 and 5.75 hr; M6G not quantifiable in plasma

Table A4.9: Sheep 11 in renal failure - M3G at 4 mg/hr

Concentrations of morphine and M3G in plasma (nmol/l)*

M3G (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144
Art	2350	3000	4410	4130	3630	3710	4240	4150	4570	5220	6230	6290	6250	6290	5930	2490	1670	953	N.S.	N.S.	N.S.
PA											5490	6160	5710	5540	5640						
Hep											5900	5950	6620	5220	5460						
Port											6070	5950	5630	5760	6110						
Ren											5480	6670	5810	5810	6410						
PVC											5970	5880	6200	6020	5850						

* morphine, M6G not quantifiable in plasma

Table A4.10: Sheep 13 in renal failure - M3G at 4 mg/hr

Concentrations of morphine and M3G in plasma (nmol/l)*

M3G (nmol/l)

Time (hr)	0.08	0.17	0.25	0.33	0.42	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48	72	96	120	144
Art	941	1580	2160	1790	1780	1620	1750	1950	2450	2750	3680	3460	3480	3460	3790	435	396	268	230	148	N.Q.
PA											3120	2980	3240	3160	3390						
Hep											3480	3120	3180	3580	3310						
Port											4070	3730	3070	3500	4020						
Ren											3740	3010	3800	3420	2850						
PVC											3200	3460	3750	3070	3740						

* morphine, M6G not quantifiable in plasma

Table A4.11: Concentrations of morphine, M3G and M6G in urine of sheep 7 to 13 in renal failure

Morphine Sulphate at 10 mg/hr - concentrations (μmol/l) and volume of urine (ml)

Sheep	Compound	0 to 5 hr		5 to 6 hr		6 to 24 hr		24 to 48 hr		48 to 72 hr		72 to 96 hr		96 to 120 hr		120 to 144 hr	
		Conc.	Vol.	Conc.	Vol.	Conc.	Vol.	Conc.	Vol.	Conc.	Vol.	Conc.	Vol.	Conc.	Vol.	Conc.	Vol.
	Morphine	2.28		0.980		8.13		0.771		0.561		0.421		0.701		N.Q.	
7	M3G	48.0	394	32.1	170	21.6	1180	5.96	1910	N.Q.	3800	N.Q.	3350	N.Q.	1490	N.Q.	1100
	M6G	1.26		1.37		0.520		0.845		N.Q.		N.Q.		N.Q.		N.Q.	
	Morphine	0.526		0.350		N.Q.		0.420		0.771		0.210		N.Q		N.Q.	
8	M3G	24.3	488	20.6	150	17.8	1610	9.01	2980	9.32	1460	5.20	1000	3.01	1700	N.Q.	1900
	M6G	N.Q.		0.150		0.217		N.Q.		N.Q.		0.150		N.Q.		N.Q.	
	Morphine	1.33		0.596		1.61		N.Q.		N.S.		N.S.		N.S.		N.S.	
9	M3G	26.8	270	14.5	155	32.1	695	21.5	1150	N.S.	-	N.S.	-	N.S.	-	N.S.	-
	M6G	1.17		0.650		2.64		1.52		N.S.		N.S.		N.S.		N.S.	
	Morphine	0.280		N.S.		N.Q.		0.771		0.876		N.Q.		0.561		N.Q.	
10	M3G	7.09	650	N.S.	-	8.36	2500	5.96	2900	11.4	2430	4.05	930	5.59	2760	4.55	1320
	M6G	0.260		N.S.		0.260		0.303		0.433		0.260		0.260		N.Q.	
	Morphine	1.79		1.61		0.175		N.Q.		0.245		0.491		0.28		N.Q.	
11	M3G	13.9	299	15.0	128	11.2	3850	8.39	2120	6.52	1520	11.9	980	7.37	1320	7.93	1440
	M6G	0.347		0.433		0.628		0.325		0.238		0.520		N.Q.		N.Q.	
	Morphine	1.02		0.491		0.596		0.386		0.280		N.S.		N.S.		N.S.	
12	M3G	13.0	630	9.10	170	17.4	1420	9.58	1600	6.00	2020	N.S.	-	N.S.	-	N.S.	
	M6G	0.217		0.238		0.303		N.Q.		N.Q.		N.S.		N.S.		N.S.	
	Morphine	1.33		1.05		0.526		1.12		0.771		N.S.		N.S.			
13	M3G	19.4	631	26.0	185	18.9	1960	19.4	1070	13.5	1000	N.S.	-	N.S.	-		
	M6G	0.347		0.715		0.542		0.498		0.282		N.S.		N.S.			

Table A4.12: Concentrations of M3G, morphine and M6G in urine of sheep 10, 11 and 13 in renal failure

M3G at 4 mg/hr - concentrations (μmol/l) and volume of urine (ml)

Sheep	Compound	0 to 5 hr		5 to 6 hr		6 to 24 hr		24 to 48 hr		48 to 72 hr		72 to 96 hr		96 to 120 hr		120 to 144 hr	
		Conc.	Vol.	Conc.	Vol.	Conc.	Vol.	Conc.	Vol.	Conc.	Vol.	Conc.	Vol.	Conc.	Vol.	Conc.	Vol.
	Morphine	N.Q.		N.Q.		13.0		1.72		1.47		N.S.		N.S.		N.S.	
10	M3G	25.5	316	14.3	147	N.Q.	800	33.9	1125	14.9	950	N.S.	-	N.S.	-	N.S.	-
	M6G	N.Q.		N.Q.		N.Q.		N.Q.		N.Q.		N.S.		N.S.		N.S.	
	Morphine	N.Q.		N.Q.		N.Q.		0.25		N.S.		N.S.		N.S.		N.S.	
11	M3G	14.1	510	8.15	186	14.4	1020	5.55	1910	N.S.	-	N.S.	-	N.S.	-	N.S.	-
	M6G	N.Q.		N.Q.		N.Q.		N.Q.		N.S.		N.S.		N.S.		N.S.	
	Morphine	N.Q.		N.Q.		9.08		0.25		N.Q.		N.Q.		N.Q.		N.Q.	
13	M3G	4.9	1010	4.31	342	N.Q.	1520	1.34	2670	1.71	850	0.69	2080	1.21	2280	N.Q.	1870
	M6G	N.Q.		N.Q.		N.Q.		N.Q.		N.Q.		N.Q.		N.Q.		N.Q.	

Table A4.13: Ratio of the concentrations of morphine, M3G and M6G in blood to those in plasma of sheep in renal failure#

Sheep infused with morphine sulphate and M3G

	Morphine sulphate					M3G
Sheep number	Morphine		M3G	M6G		M3G
	70.2 nmol/l	257 nmol/l	1120 nmol/l	52.4 nmol/l	198 nmol/l	1120 nmol/l
7	1.30, 1.52	1.80, 1.35	1.13, 1.04	0.86, 0.67	0.90, 0.98	*
8	-	-	-	-	-	*
9	1.15, 1.32	1.01, 1.05	0.69, 0.67	0.58, 0.67	0.60, 0.67	*
10	1.18, 1.25	1.18, 1.07	0.72, 0.68	0.70, 0.62	0.64, 0.79	-
11	1.17, 1.21	1.20, 1.10	0.75, 0.67	0.69, 0.88	0.85, 0.79	-
12	1.59, 1.66	1.54, 1.53	0.86, 0.90	1.02, 0.86	0.85, 0.85	*
13	1.51, 1.24	1.45, 1.19	0.82, 0.59	0.89, 0.75	0.67, 0.81	0.85, 0.95

* sheep not infused with M3G

\# each experiment conducted in 2 replicates

Table A4.14: Fractions of morphine, M3G and M6G unbound in arterial plasma from sheep in renal failure and infused with morphine and M3G#

	Compound [compound infused]			
Sheep number*	Morphine [morphine]	M3G [morphine]	M6G [morphine]	M3G [M3G]
8	0.76	0.91	0.78	-
9	0.77	1.04	0.85	-
10	0.73	0.98	0.92	0.93
11	0.73	0.88	0.81	0.90
12	0.71	0.94	0.79	-
13	0.78	0.88	0.84	0.90

* there was insufficient plasma from sheep 7 for binding measurements

\# the fractions of morphine, M3G and M6G unbound in arterial plasma of sheep 5 with normal renal function was 0.84, 0.96 and 0.90, respectively

Table A4.15: Cardiac output, blood flow to the liver and kidney#

Sheep infused with morphine sulphate and M3G

	Morphine sulphate			M3G		
Sheep number	Cardiac Output	Liver	Kidney	Cardiac output	Liver	Kidney
7	7.03	1.95	0.56	-	-	-
8	*	1.95	0.37	-	-	-
9	5.14	1.90	0.40	-	-	-
10	9.04	3.31	0.74	*	3.40	0.68
11	7.05	1.94	0.59	4.65	2.13	0.58
12	7.33	0.92	0.38	-	-	-
13	6.97	1.49	0.65	9.08	3.41	1.39

* cardiac output could not be measured, sheep chewed through catheter

values in l/min

Table A4.16: Concentrations of creatinine in arterial plasma at 5 and 6 hr and urine collected between 5 and 6 hr from sheep in renal failure and administered separate infusions of morphine and M3G

		Concetration of creatinine (mmol/l)		
Sheep number	Compound infused	Plasma at 5 hr	Plasma at 6 hr	Urine
7	Morphine	0.164	0.131	1.23
8	Morphine	0.482	0.492	1.76
9	Morphine	0.550	0.558	1.51
10	Morphine	0.467	0.621	N.S.
10	M3G	0.545	0.554	3.41
11	Morphine	0.569	0.577	1.90
11	M3G	0.572	0.540	0.59
12	Morphine	0.550	0.558	0.78
13	Morphine	0.450	0.436	2.46
13	M3G	0.479	0.493	0.38

Table A4.17: Volume of suspension containing polystyrene (PS) or glass (G) beads administered into renal artery of sheep for the induction of renal failure[$]

Day *	Sheep 7	Sheep 8	Sheep 9	Sheep 10	Sheep 11	Sheep 12	Sheep 13
4	-	-	-	3 G, 3 PS	3 G, 3 PS	-	-
5	-	-	5 PS	1 G, 1 PS	1 G, 1 PS	-	-
6	-	-	5 PS	1 G, 1 PS	1 G, 1 PS	-	-
7	2 PS	-	5 PS	1 G, 1 PS	1 G, 1 PS	-	1 G, 1 PS
8	2 PS	-	5 PS	0.5G, 1 PS	0.5G, 1 PS	-	1 G, 1 PS
9	2 PS	-	5 PS	-	2 PS	-	1 PS
10	2 PS	5 PS	5 PS	2 PS	2 PS	1 PS, 1 G	1 PS
11	2 PS	5 PS	5 PS	1 PS, 1 G	1 PS, 1 G	-	0.5 G
12	2 PS	5 PS	5 PS	-	-	1 PS, 1 G	1 G
13	2 PS	5 PS	5 PS	-	-	1 PS, 1 G	2 G
14	4 PS	5 PS	5 PS, 2.5 G	-	-	-	-
15	4 PS	2.5 PS	5 PS	-	-	-	1 G
16	4 PS	5 PS	5 PS	-	-	-	-
17	4 PS	5PS	3 PS, 2 G	-	-	0.5 G	-
18	4 PS	5 PS	2 PS, 2 G	1 PS	1 PS	1 G	-
19	4 PS	5 PS	1 PS, 1 G	-	1 PS, 0.5 G	-	0.5 G
20	5 PS	5 PS, 2.5 G	-	-	-	-	-
21	5 PS	5 PS	1 PS	1 PS	1 PS	-	-
22	-	5 PS	#	2 PS	2 PS	-	-
23	3 PS	1 PS		-	#	#	-
24	2 PS	1 PS		-			-
25	2 PS	1 PS		#			0.5 G
26	2 PS	2 PS					-
27	2 PS	1 PS					-
28	#	-					#
29		1 PS					
30		1 PS					
31		#					

* days after nephrectomy

\# sheep given a lethal injection of pentobarbitone

$ values are the volumes (ml) of suspensions with 120,000 PS beads/ml of water or 100,000 G beads/ml of 5% dextrose

Table A4.18 Sheep 5 - Morphine Sulphate at 10 mg/hr

Concentrations of morphine, M3G and M6G in plasma (nmol/l)

Morphine (nmol/l)

Time (hr)	0.25	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48
Art	616	223	199	155	202	192	210	182	195	190	212	N.Q.	N.Q.
PA							193	183	220	176	220		
Hep							43.2	54.1	61.4	53.0	48.9		
Port							183	164	189	207	181		
Ren							86.2	103	81.9	75.7	63.6		
PVC							212	197	186	221	182		

M3G (nmol/l)

Time (hr)	0.25	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48
Art	893	1200	1800	1420	2120	2990	1130	1090	1950	1100	1220	357	N.Q.
PA							1290	950	1240	1120	1220		
Hep							1230	1070	1370	1430	1210		
Port							1070	1110	1110	1280	1270		
Ren							1070	1070	960	1080	1020		
PVC							1030	1130	1050	1110	1180		

M6G (nmol/l)

Time (hr)	0.25	0.5	1	2	3	4	5	5.25	5.5	5.75	6	24	48
Art	33.9	46.6	78.6	53.9	87.9	110	32.0	31.7	41.1	32.0	32.3	N.Q.	N.Q.
PA							34.1	28.0	36.5	42.9	37.2		
Hep							37.4	40.0	42.9	45.3	34.9		
Port							36.8	34.0	34.5	37.4	35.5		
Ren							36.9	33.3	28.4	28.8	30.5		
PVC							31.0	33.9	34.5	31.5	32.5		

Concentrations of morphine, M3G and M6G in urine (μmol/l)

Time (hr)	Morphine	M3G	M6G	Urine volume (ml)
0 to 5	11.5	151	3.14	411
5 to 6	5.57	82.2	1.80	180
6 to 24	4.49	31.3	0.563	1730
24 to 48	1.54	4.70	N.Q.	1810

Cardiac output, blood flow to the liver and kidney (l/min)

Cardiac output	Liver	Kidney
7.32	1.96	1.70

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