THE EFFECTS OF POSTURE AND SLEEP ON PHARMACOKINETICS

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SUMMARY

The effects of posture and sleep on the pharmacokinetics of five drugs (benzylpenicillin, gentamicin, sulphamethoxazole, trimethoprim and paracetamol) especially chosen for their disposition characteristics, were studied. To assess these effects, the drugs were administered, on separate occasions, to healthy young adults during ambulation, daytime bedrest and, with the exception of benzylpenicillin, sleep. In addition, benzylpenicillin pharmacokinetics were determined following both intravenous and intramuscular administration to the same subjects.

New or improved high-performance liquid chromatography assays were developed for the determination of these drugs in biological fluids. Pharmacokinetic analysis of the drug concentration-time data was performed using traditional compartmental methods as well as a non-compartmental method using statistical moment theory.

Generally, the results showed that posture had the greatest influence on the disposition of drugs with low renal extraction ratios and that sleep appears to have the same effects as bedrest on pharmacokinetics.

With gentamicin, a highly polar compound with a low renal extraction ratio, ambulant subjects exhibited a

larger volume of distribution and clearance than supine subjects. These findings may be due to posture-induced alterations in tissue perfusion and extracellular fluid distribution rather than to changes in renal function. These results may be clinically important; especially the finding that gentamicin C_2 had a longer mean residence time than the other major components since it has been suggested, recently, that gentamicin C_2 is the most nephrotoxic of the components.

Sulphamethoxazole, a weak acid, was found to have a shorter half-life and greater urinary excretion rate during bedrest than during ambulation and a direct relationship was shown between the urinary excretion rate of the drug and both urinary pH and urine flow. Both these factors had higher values during bedrest. Trimethoprim, a weak base, is less sensitive to urine pH changes over the range observed and, consequently, the disposition of this drug was unaffected by posture or sleep.

Unlike gentamicin and sulphamethoxazole, the pharmacokinetics of benzylpenicillin and paracetamol were unaffected by posture. Although the mean plasma benzylpenicillin levels were lower for bedrest than for ambulation, there were no significant differences between ambulation and bedrest for any of the derived pharmacokinetic parameters and thus it is suggested that the posture-dependent changes in the disposition of the penicillins reported by other workers may be

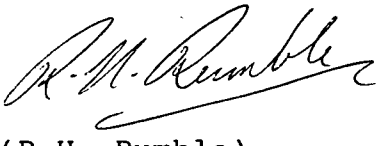
related to the level of exercise undertaken in those studies.

Paracetamol elimination was affected by neither change of posture nor sleep but the rate of absorption appeared to be slowest during sleep. These findings are in accordance with the low hepatic extraction ratio of paracetamol and the slower gastric emptying in supine and sleeping subjects.

Overall, therefore, these studies show that change of posture can cause significant changes in the pharmacokinetics of certain drugs, especially polar compounds with low renal clearance and weak acids whose elimination is sensitive to changes in urine pH and urine flow. In conclusion, it is recommended that the posture of the patient should be taken into account in the design of pharmacokinetic studies and dosage regimes.

This thesis contains no material which has been accepted for the award of any degree or diploma in any College or University.

To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except when due reference is made in the text of the thesis.



(R.H. Rumble)

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CONTENTS

CHAPTER 1

INTRODUCTION

1.1: INTRODUCTION

The impetus for these studies came from two queries directed at staff at the School of Pharmacy, University of Tasmania. Both queries, one from hospital pharmacy and the other from community pharmacy, were concerned with the necessity to maintain a strict dosage regime day and night even if it meant waking the patient.

The determination of a dosage regime to achieve suitable drug levels in the body at specific times of the day may be based on the pharmacokinetics of the drug. The pharmacokinetics of a particular drug depends on both the physico-chemical properties of the drug and the physiological features of the person to whom it is administered. Many factors including age, disease, other drugs, posture, sleep and exercise may affect the physiological variables on which the disposition of the drug depends.

1.2: PHYSIOLOGICAL FACTORS AFFECTING PHARMACOKINETICS

1.2.1: FACTORS AFFECTING ABSORPTION

The great majority of drugs are administered extravascularly and are intended to act systemically. For these drugs, absorption into the systemic circulation is required before the drug can exert its pharmacological effect. Changes in the onset, rate and extent of absorption may contribute to the variability in drug response which may, occasionally, result in toxicity or therapeutic failure.

1.2.1.1: GASTROINTESTINAL ABSORPTION

Most drugs are administered orally and the small intestine is the foremost site of absorption, with very little drug being absorbed from the stomach (Hirtz, 1985). Consequently, the rate of movement of drug into the small intestine determines the time of onset, rate and, occasionally, extent of absorption. Gastric emptying may be affected by many factors. The nature and volume of the gastric contents can have a marked effect. Liquids are emptied faster than solids and it has been suggested that the pylorus has a screening effect for solid particles larger than 2mm (Hirtz, 1985). Several studies have shown that drugs are absorbed more rapidly, although not necessarily more completely, if administered on an empty stomach. Divoll et al (1982) and Heading et al (1973) observed that paracetamol was absorbed more slowly when

administered with food than when it was administered to fasting subjects.

The rate of gastric emptying is slowed by drugs such as propantheline and narcotic analgesics thus delaying absorption of other drugs (Clements et al, 1978; Nimmo, 1976) while metoclopramide enhances gastric emptying resulting in accelerated absorption of drugs (Nimmo, 1976).

Disease can also either enhance or slow the gastric emptying rate. Chronic calcular cholecystitis increases the rate of gastric emptying (Nimmo, 1976) while various other conditions including diabetes mellitus, myxoedema, malnutrition (Nimmo, 1976), migraine (Volans, 1974) and cardiac failure (Benet, Greither & Meister, 1976) retard the passage of gastric contents into the duodenum.

Gastric emptying is slower in bedrested subjects than ambulant or upright subjects. Even the postion in which a subject lies can influence gastric emptying rate. Yu (1975) observed that, in neonates, the stomach emptied more rapidly in the prone and right lateral positions than in the supine and left lateral positions. More recently, Burn-Murdoch, Fisher & Hunt (1980) found that the rate of gastric emptying of a saline solution was faster in subjects who were lying on their right side than in subjects sitting which, in turn, was faster than in subjects lying on their left side. However, when the administered solution contained glucose (100g/l), posture had no effect on the rate of gastric emptying, possibly because feedback mechanisms from duodenal

receptors controlled the emptying of the energy-dense meal (Burn-Murdoch et al, 1980). Slower onset of absorption in supine subjects than in ambulant subjects has been found for paracetamol (Nimmo & Prescott, 1978), amoxycillin (Roberts & Denton, 1980), theophylline (Warren, Cuss & Barnes, 1985) and zopiclone (Channer, Dent & Roberts, 1984). Roberts & Denton (1980) also found that when amoxycillin was administered immediately prior to sleep, the lag time for the onset of absorption was longer than for bedrested subjects, suggesting even slower gastric emptying during sleep.

The bioavailability of acid-labile drugs and drugs metabolised while in the gastrointestinal tract, such as penicillin, erythromycin and levodopa, may be reduced when gastric emptying is slow. On the other hand, for poorly-soluble drugs such as griseofulvin , nitrofurantoin, and digoxin, and drugs whose absorption is saturable, for example riboflavin, a slow rate of gastric emptying actually increases the extent of absorption by providing either a greater opportunity for the drug to dissolve or longer contact time between the drug and the absorption sites (Gibaldi, 1984). The bioavailability of most drugs is usually unaffected by the rate of gastric emptying.

Once a drug has entered the intestine, it must pass through the intestinal wall into the blood of the portal vein and be transported to the liver before entering the systemic circulation. Even though gastrointestinal absorption of a

drug may be complete, the extent of absorption of intact drug into the systemic circulation may be less than complete if the drug is metabolised by enzymes in either the gut wall or the liver. The presystemic clearance depends on several physiological factors, including enzyme capacity of the gut wall and of the liver, splanchnic blood flow, plasma protein binding and gastrointestinal motility (Pond & Tozer, 1984). Many clinically important drugs undergo considerable presystemic clearance after an oral dose. Some examples of these drugs are propranolol, metoprolol, amitriptyline, hydralazine, morphine, lignocaine, organic nitrates and nifedipine.

The enzyme-inducing effects of drugs and smoking have been reported to result in reduced bioavailability of alprenolol and lignocaine (Pond & Tozer, 1984). Reduced enzyme capacity of the liver may account for the increased bioavailability of chlormethiazole, labetolol, metoprolol, dextropropoxyphene and propranolol in patients with cirrhosis of the liver (Blaschke & Rubin, 1979).

Blood flow assures continuous absorption by removing the drug that passes through the gastrointestinal membrane. With highly lipid-soluble drugs, penetration may be so rapid that the absorption becomes perfusion-limited (Rowland & Tozer, 1980a). Food causes a transient increase in the splanchnic blood flow. Svensson et al (1983) observed that the splanchnic blood flow was elevated for 280 minutes with a mean increase of 69% at 40 minutes following a high-protein meal. The bioavailability of propranolol (McLean et

al, 1981; Jackman et al, 1981), metoprolol and hydralazine (Melander, 1978) is higher when the drug is administered with food, although Walden et al (1981) did not find a significant increase in hydralazine bioavailability in subjects dosed one hour after food. McLean et al (1978) showed by computer simulation that these results may be due to the transient nature of the increase in splanchnic blood flow. Not all drugs which undergo extensive presystemic clearance have an increased bioavailability when administered with food. This has been found for prazosin, amitriptyline and dextropropoxyphene (Melander & McLean, 1983). Liver blood flow is reduced in cardiac failure (Benet et al, 1976) and may be altered in an unpredictable manner in liver disease (Pond & Tozer, 1984).

Changes in posture and exercise alter hepatic blood flow but, so far, there are no reports on posture, sleep or exercise modifying the bioavailability of a drug through altered splanchnic blood flow. The effects of posture and exercise on hepatic blood flow are discussed further in section 1.2.3.

1.2.1.2: INTRAMUSCULAR ABSORPTION

The second most common site of extravascular administration of drugs intended to have a systemic action is intramuscular.

The usual sites of injection are the arm (deltoid), lateral

thigh (vastus lateralis) or the buttocks (gluteus maximus). The effect of injection site on the rate of absorption of drugs has been studied by several workers. Injections into the thigh result in higher plasma drug concentrations than injections into the buttocks for diazepam (Assaf, Dundee & Gamble, 1974), cephacetrile, gentamicin and cephaloridine (Reeves et al, 1974). Injection into the deltoid gave higher lignocaine peak plasma concentrations than thigh injection which, in turn, gave higher concentrations than injection into the buttocks (Cohen et al, 1972; Schwartz et al, 1974). These findings correlate well with the rank order of the blood flow to each muscle group determined by Evans et al (1975) using the ^{133}Xenon washout technique. In an earlier work, Bederka, Takemori & Miller (1971) injected a variety of substances (molecular weights 20 - 585) into muscle of rats and concluded that muscle blood flow rather than diffusion is the rate-limiting step for the absorption of drugs administered intramuscularly. Variation in muscle blood flow was regarded as being the major reason for the considerable variation observed in the disposition of morphine injected into the gluteus maximus (Rigg et al, 1978).

On moving from the supine to an upright position, there is a shift of blood flow away from the liver and kidneys (Culbertson et al, 1951; Chapman et at, 1948). No details appear to be available on the effect of posture alone on skeletal muscle flow. Exercise certainly causes an increased blood flow to the working muscle. Ahlborg & Felig (1982) reported a seven-fold increase in leg muscle blood

flow during bicycle exercise. Riches (1954) observed higher plasma levels of streptomycin in active, ambulant patients than when the patients remained at rest. Also, Schmidt & Roholt (1966) found higher and earlier peak benzylpenicillin concentrations in ambulant patients than in the same patients when bedrested, indicating a faster rate of absorption of the drug during exercise.

1.2.2: FACTORS AFFECTING DISTRIBUTION

The pharmacokinetic parameter which characterizes the distribution of a drug is the apparent volume of distribution (V) which is used to relate the plasma concentration (C) to the amount of drug in the body (Ab) (V=Ab/C). The apparent volume of distribution rarely corresponds to a real volume. Drug distribution may be to any combination of tissues and fluids in the body and there are many factors that can affect the determined apparent volume of distribution.

Drugs are distributed throughout the body by means of the blood circulation. Almost all drugs easily cross capillary walls and enter the extracellular fluid except in specialized regions such as the brain where the capillaries differ in their permeability characteristics and, so, present a barrier to drugs not having lipophilic properties. Movement of drugs from the extracellular fluid into cells depends on the physico-chemical properties of the drugs and the extracellular pH. Blood flow, then, is the rate-

limiting step in the distribution of most drugs into the tissues. Polar drugs diffusing across tightly knit lipoid membranes are the exception. In cardiac failure, autoregulation maintains a relatively normal blood flow to the brain and myocardium and relatively less to the kidney, muscle and splanchnic tissues (Benowitz & Meister, 1976). Changes in the distribution of procainamide, quinidine, lignocaine and digoxin have been reported in patients with cardiac failure (Wilkinson, 1976; Benowitz & Meister, 1976). Swartz & Sidell (1973) found that, under conditions of heat and exercise stress, the volumes of distribution for pralidoxime and p-aminohippurate increased, particularly the volume of the tissue compartment. It was suggested that, due to the altered blood flow during exercise and stress, the drugs were distributed to tissues not receiving much drug at rest.

For water-soluble drugs, changes in the extracellular fluid volume can alter their apparent ditribution. The extracellular fluid volume is expanded in cardiac failure (Benowitz, 1984) and following a high salt intake (Gauer, Henry & Behn, 1970). Neonates and young children have a higher proportion of their body mass as water than adults while in the elderly this proportion is smaller than in young adults (Klotz, 1976). On adopting an upright position there is a shift of plasma fluid from the vascular system to the interstitial space. Nearly 500ml may leave the vasculature after sixty minutes standing (Hagan, Diaz & Horvath, 1978). Exercise causes a further shift of fluid. Greenleaf et al (1979) observed a decrease in plasma volume

of greater than 500ml in subjects undertaking supine exercise. Prescott (1975) reported higher plasma levels of paracetamol in bedrested patients than in ambulant subjects. As there were no differences in the rates of absorption or elimination between the two groups, these results were attributed to a larger volume of distribution in ambulant subjects due to either increased peripheral perfusion or a shift in plasma fluids associated with the change of posture and exercise.

The movement of fluid from the vascular system with change of posture or exercise also results in haemoconcentration with the most pertinent change being an approximately 21% increase in plasma protein concentration (Hagan et al, 1978). The plasma concentration of drugs highly bound to plasma proteins would increase during haemoconcentration resulting in a smaller apparent volume of ditribution. Dettli & Spring (1966) observed an apparently very low elimination rate of sulphasymazine in patients who got out of bed before a twelve-hour sample was taken. They attributed these findings to the effect of haemo-concentration. Half of the extracellular protein is found in the interstitial fluid with a large proportion of it being in the skin and muscle due to the large volumes of extracellular fluid in these tissues (Gauer et al, 1970). Prolonged bedrest or immobilisation produces a shift of protein to the extravascular areas. This also occurs following extensive burns as a result of increased capillary permeability (Jusko, 1976).

Many drugs have a high affinity for tissue proteins so any displacement of drug from these binding sites will affect plasma levels and the apparent volume of distribution of the drug. Pedersen et al (1983) observed a rise of 63% in plasma digoxin levels when subjects were immobilised for two hours following normal physical activity. On resumption of normal activities and with strenuous exercise, the plasma digoxin levels gradually declined to the pre-immobilisation levels. Changes in tissue binding of the drug were considered to be the cause of the observed plasma level changes.

1.2.3: FACTORS AFFECTING HEPATIC ELIMINATION

Most drugs are metabolised to some extent with the liver being the major site of drug metabolism, although this may also occur in several other tissues.

The hepatic clearance of a drug depends on three physiological variables -- the blood flow to the liver; the intrinsic ability of the liver to irreversibly metabolise the drug; and the binding of the drug to plasma proteins and cellular components in the blood (Nies, Shand & Wilkinson, 1976). The clearance of drugs of high hepatic extraction ratio is dependent on liver blood flow because the flow is small relative to the intrinsic ability of the liver to remove the drug. For drugs of low extraction ratio, the intrinsic ability of the liver to remove the drug is small relative to the blood flow and the clearance of these drugs is independent of liver blood flow (Rowland & Tozer, 1980b).

Factors which alter hepatic blood flow will affect the disposition of drugs with flow-dependent clearance such as lignocaine, verapamil, many beta-adrenergic blocking agents, narcotic analgesics and tricyclic antidepressants (George, 1979). The hepatic blood flow decreases in the elderly resulting in a reduction in the clearance of propranolol, lignocaine and chlormethiazole (George, 1979). The influence of hepatic disease on hepatic blood flow is complex. It appears, however, that hepatic blood flow is reduced in patients with chronic liver disease and, as a consequence, the clearance of many highly extracted drugs is also reduced in these patients (Williams, 1984). Pessayre et al (1978) determined that the reduced clearance of d-propranolol in cirrhotic patients was due to a combination of impaired ability of the liver to metabolise the drug and reduced liver blood flow. The clearance of intravenously administered lignocaine is lower in patients with cardiac failure as this disease causes a drop in liver blood flow in proportion to the cardiac index (Benowitz & Meister, 1976).

Culbertson et al (1951) examined the effect of posture on hepatic blood flow and observed a decrease in estimated liver blood flow of approximately 38% when subjects adopted an upright postue. In a study on the effect of posture on the elimination of a high hepatic extraction ratio drug, midazolam, the clearance of the drug in supine subjects was about twice that observed in ambulant subjects (Klotz & Ziegler, 1982). The liver blood flow also falls during exercise. Lundbergh & Strandell (1974) observed a 39% fall

in liver blood flow in normal subjects and a 59% fall in patients with infectious hepatitis during supine exercise. Swartz, Sidell & Cucinell (1974) examined liver blood flow during exercise, heat and fluid-restriction stresses using indocyanine green clearance as the indicator of liver blood flow. Indocyanine green clearance decreased with increasing stress. In the same set of experiments, the disposition of antipyrine, a low extraction ratio drug, was not greatly affected by these changes in liver blood flow.

It is generally considered that only drug not bound to plasma proteins is available for elimination by the liver. Since dissociation from protein is very rapid, changes in protein binding usually have no effect on the elimination of drugs with high extraction ratio but the clearance of drugs with low extraction ratio and high affinity for plasma proteins will be sensitive to changes in the extent of protein binding. Factors affecting protein binding have already been discussed (section 1.2.2). The clearance of warfarin, disopyramide and phenprocoumon have been shown to vary with the fraction unbound (Tozer, 1984).

Drugs with low hepatic extraction ratio are also sensitive to changes in the intrinsic clearance ability of the liver. The intrinsic clearance can be enhanced by factors that induce the activity of drug-metabolising enzymes and reduced by factors that inhibit those enzymes. Paracetamol has been used as a model drug for type II metabolism by several workers. The clearance of paracetamol is higher in subjects exposed to enzyme-inducing agents such as cigarette smoking

and alcohol (Mucklow et al, 1980), oral contraceptive steroids (Mitchell et al, 1983; Mucklow et al, 1980; Miners, Attwood & Birkett, 1983), diet containing charcoal-grilled beef (Anderson et al, 1983), phenytoin, carbamazepine and sulphinpyrazone (Miners, Attwood & Birkett, 1984b). Enzymatic activity may be reduced in liver disease. Reduced clearance of paracetamol was observed in patients with severe liver disease (Forrest et al, 1979) and Gilbert's syndrome (Douglas, Savage & Rawlins, 1978). Antipyrine, another low extraction ratio drug, also has reduced clearance in severe liver disease (Farrell et al, 1978).

Posture does not appear to cause any changes in liver enzyme activity, although Elfstrom & Lindgren (1978) reported increased clearance of phenazone (antipyrine) when subjects were supine. The increase was small and was attributed to the small effect increased blood flow would have on a low extraction ratio drug rather than change of enzyme activity.

1.2.4: FACTORS AFFECTING RENAL ELIMINATION

The kidney is a very important organ of drug elimination for both parent drug and its metabolites. All drugs in the systemic circulation are filtered at the glomerulus but the renal clearance of a drug depends on the balance between its glomerular filtration, active tubular secretion and passive reabsorption.

At the glomerulus, only non protein bound drug is filtered,

so, depending on whether the drug is reabsorbed or not, any of the conditions that modify drug protein binding (section 1.2.2) will alter the rate of drug clearance. This is of clinical importance for drugs normally highly bound to plasma proteins such as phenytoin, valproic acid and warfarin (Brater & Chennavasin, 1984). The other determinants of a kidney's capacity to filter drugs are the number of filtering nephrons and the integrity of the glomeruli of those nephrons. The effects of disease that may alter glomerular function on pharmacokinetics have been reviewed recently by Brater & Chennavasin (1984) and Gambertoglio (1984).

Although changes in renal blood flow may affect the active secretion and passive reabsorption of drugs, these will only be of importance for drugs of high renal extraction ratio. The glomerular filtration rate does not alter significantly with changes in renal plasma flow (Knox et al, 1975). A number of clinically important drugs undergo active secretion. These have been listed by Brater & Chennavasin (1984) and include the penicillins, nitrofurantoin, thiazides and many non-steroidal antiinflammatory agents amongst the acids and cimetidine, amiloride, triamterene, pethidine and amantadine amongst the bases.

Renal blood flow is affected by posture, exercise and stress. Brun, Knudsen & Raaschou (1945) observed a fall in kidney blood flow of 20 - 45% when subjects moved from a supine to an upright position. In a comparison of renal blood flow during bedrest and during various grades of

exercise while upright, Chapman et al (1948) observed a decline in renal blood flow of 15 - 35% depending on the level of exercise. Radigan & Robinson (1949) also observed a substantial drop in kidney blood flow with exercise and an even greater fall during exercise under heat stress. In that study, glomerular filtration rate was unaffected by exercise in ambient temperatures but decreased under heat conditions. Breiby et al (1983) found no change in kidney blood flow when subjects moved from a supine position to a sitting position. The penicillins are high renal extraction ratio drugs and Levy (1967) attributed the higher benzyl-penicillin levels in ambulant compared to bedrested subjects, observed by Schmidt & Roholt (1966), to a decreased renal clearance associated with a reduced renal blood flow. Roberts & Denton (1980) found a similarly reduced renal clearance of amoxycillin in ambulant subjects compared to supine and sleeping subjects. That changes in renal plasma flow may be responsible for these results is possibly confirmed by the study of Breiby et al (1983) where sitting caused no change in either renal blood flow or the clearance of ampicillin. Exercise has been found to reduce the renal clearance of atenolol (Mason et al, 1980), pralidoxime and para-aminohippurate (Swartz & Sidell, 1973).

The reabsorption of weak acids and weak bases will depend on their physico-chemical properties, the pH of the distal tubular urine and the urine flow. Diet, drugs and the condition of the patient can influence urinary pH. The elimination of several sulphonamides (Dettli, 1973; Vree et

al, 1978b), amphetamine (Beckett & Rowland, 1964) and tetracycline (Jaffe et al, 1973) have been found to vary with urine pH or urine flow. Sleep produces an acidosis which is reflected in the urine (Elliott, Sharp & Lewis, 1959) while in bedrested patients urinary pH is higher than in ambulant or sleeping subjects (Roberts & Denton, 1980)

1.2.5: CIRCADIAN RHYTHMS

Most body functions exhibit circadian rhythms and many of these (e.g. cardiac output, gastrointestinal motility, urinary and gastric pH, liver and renal blood flow) may influence the absorption and disposition of drugs. Studies showing these circadian effects on the pharmacokinetics of drugs have been extensively reviewed (Ritschel, 1984; Reinberg & Smolensky, 1982; Levy, 1982). As some of the pharmacokinetic changes have been associated with diurnal rhythms, the effects of posture have not always been separated from the effects of a true rhythm. An example is the disposition of theophylline. A diurnal rhythm in theophylline plasma levels following an oral dose of sustained-release theophylline was described by Smolensky et al (1982). Taylor et al (1983) reported a diurnal rhythm in theophylline absorption. A recent study, however, has shown that the diurnal variation in theophylline levels can be explained, at least in part, by differences in posture (Warren et al, 1985). These findings highlight the need to take posture into account when describing the pharmacokinetics of a drug.

CHAPTER 2

AIMS

Posture has been shown to affect the pharmacokinetics of many drugs and some reports of diurnal rhythms in drug disposition may, in fact, be due to changes in posture. The aims, therefore, of this study were to examine , in a systematic manner, the effects of posture on the pharmacokinetics of a number of selected drugs and for those drugs, separate the effect of sleep from the effects of posture.

The drugs were chosen on the basis of their disposition characteristics and their clinical importance. The following drugs were studied: gentamicin, benzylpenicillin, sulphamethoxazole, trimethoprim, and paracetamol.

CHAPTER 3

GENTAMICIN

3.1: INTRODUCTION

3.1.1: DRUG PROFILE

Gentamicin is an effective and widely used bactericidal antibiotic, particularly useful in the treatment of serious gram-negative bacterial infections. The clinically available form of gentamicin is a complex consisting of three major components (gentamicins C_1,C_{1a} and C_2) and several minor components (Wilson, Richard & Hughes, 1973a; Anhalt, Sancilio & McCorkle, 1978). In commercial batches of the drug, there are wide variations in the relative proportions of these components (Anhalt et al, 1978; Wilson et al, 1973a) and the United States Pharmacopeia (1984) is the only official monograph which sets limits on the relative amount of each component allowed in the complex. Little attention appears to have been paid to the *in vivo* antibacterial potency of the different components. *In vitro* studies of the susceptibilities of various microorganisms to the individual components (C_1, C_{1a}, C_2) have indicated a range of minimum inhibitory concentration ratios (Waitz & Weinstein, 1969).

A limitation on the use of gentamicin is its narrow therapeutic index, with high peak concentrations being associated with ototoxicity and high trough concentrations appearing to increase the risk of nephrotoxicity (Evans et al, 1980). There may be differences in toxicity between the major components. Mosegaard, Welling & Madsen (1975) suggested that the use of gentamicin complex may result in a greater incidence of renal function impairment than the use of gentamicin C_1 alone. More recently, Kohlhepp et al (1984) compared the nephrotoxicity in rats of gentamicin complex with that of its major components. They found the nephrotoxicity of gentamicin complex to be due largely to the C_2 component with gentamicin C_{1a} being much less nephrotoxic and gentamicin C_1 producing minimal nephrotoxicity. So far, dosing regimes do not take into account either differences in component content of the product or differences in the pharmacology of the components.

In attempts to overcome the potential toxicity problems, several dosing nomograms have been developed which take into account many patient factors including renal function, sex, and lean body weight (Schumacher, 1975; Barza & Lauermann, 1978; Korsager, 1980). In spite of these nomograms, there is still large intersubject variability in serum concentrations of gentamicin following a calculated dose (Kaye, Levison & Labovitz, 1974; Fischer, Hedrick & Riff, 1984). None of the nomograms take into account the posture

of the patient.

3.1.2: ASSAYS

Several assay methods are available for monitoring gentamicin in biological fluids in the clinical setting. Traditionally, microbiological assay has been the most common assay technique used but this method is slow, often inaccurate and subject to interference by other antibiotics (Reeves & Bywater, 1975). Recently a number of new assay methods have been published that are faster, more specific and more accurate. These methods include radioimmunoassay, fluorescent immunoassay, enzyme immunoassay and the adenylation methods (Ratcliff et al, 1981; Matzke et al, 1982). The immunoassay methods utilize the principle of competitive binding and are supplied in kit form containing all the necessary reagents and antibiotic standards. A batch of five samples can be assayed, using these or the adenylation assays, in less than two hours, with the enzyme immunoassay taking as little as twenty minutes (Ratcliff et al, 1981). The methods have been compared by several authors (Matzke et al, 1980; Ratcliff et al, 1981; Hospes, Boskma & Brouwers, 1982; Matzke et al, 1982; Rotschafer et al, 1983). Matzke et al (1980) found that although the results of the assay techniques correlated well with the expected values, a given value from one assay may not be reproducible when the sample is assayed by a different technique and that intra-assay variations of ±24-31% may be experienced. Rotschafer et al (1983) found assay-dependent differences in calculated pharmacokinetic parameters which

resulted in markedly different dosage recommendations. These assays do not include a chromatography step prior to analysis and hence the individual gentamicin components (C_1, C_{1a} and C_2) are quantified as a combined amount.

Wilson et al (1973a) quantified the individual components of gentamicin using combined thin-layer chromatography and microbiology techniques. High-performance liquid chromatography (HPLC) equipment is becoming more common in laboratories and a number of HPLC assays for gentamicin in biological fluids have been developed (Peng et al, 1977; Maitra et al, 1977; Anhalt, 1977; Anhalt & Brown, 1978; Back,Nilsson-Ehle,Nilsson-Ehle, 1979; Larsen, Marinelli & Heilesen, 1980; Walker & Coates, 1981; Barends, Zwaan & Hulshoff, 1981; Marple & Oates, 1982; Kabra, Bhatnagar & Nelson, 1983; Essers, 1984). However, few HPLC assays separate the individual gentamicin components and of those that do, some of the methods are limited by the requirement for post-column derivatisation (Anhalt, 1977; Anhalt & Brown, 1978), the use of unsuitable mobile phases (Maitra et al, 1977; Back et al, 1979) or the use of sophisticated automation equipment (Essers, 1984).

3.1.3: PHARMACOKINETICS

Gentamicin is a highly polar compound and is not absorbed from the gastrointestinal tract, hence, administration is either intramuscular or intravenous. The disposition of gentamicin has been described by one-compartment, two-

compartment and three-compartment models. Although many authors consider the one-compartment model sufficiently accurate to use in the design of dosage nomograms (Hull & Sarubbi, 1976; Korsager, 1980; Bauer et al, 1983; Berg et al, 1983), other workers claim that a minimum of two compartments are necessary to adequately describe gentamicin disposition (Evans et al, 1980; Schentag et al, 1977).

Following administration, gentamicin rapidly distributes into extracellular fluid but peripheral distribution into certain tissues (especially the kidney) is much slower and accumulation in those tissues may continue for several days on multiple dosing (Schentag et al, 1977). The initial distribution of gentamicin is related to the lean body mass of the subject and gentamicin has been found to have an apparent volume of distribution of approximately 0.24 l/kg (ideal body weight) (Gyselynck, Forrey & Cutler, 1971; Hull & Sarubbi, 1976) although for obese patients, some authors add a correction factor to account for small but significant distribution into the adipose tissue (Korsager, 1980; Bauer et al, 1983). The reported protein binding of gentamicin has ranged from 0 to 30% but it appears that binding to plasma proteins is negligible (Pechere & Dugal, 1979).

Gentamicin is not metabolised but is eliminated solely by the kidney (Schentag et al, 1977). The major route of elimination is by glomerular filtration although there is evidence that some tubular reabsorption occurs (Schentag et al, 1977). Thus, it is considered to have a low renal

extraction ratio.

The pharmacokinetics of the individual gentamicin components have never been fully described. Nation et al (1978) compared gentamicin C_1 with gentamicin (C_{1a},C_2) levels in patients. However, the components C_{1a} and C_2 were not resolved in that study and the evaluation was restricted to the analysis of gentamicin in a limited number of blood samples collected at random from patients. Based on this limited evaluation, Nation et al (1978) concluded that the disposition of all three components of the gentamicin complex is the same or very similar. Mosegaard et al (1975) had previously suggested that substantial differences may exist between the pharmacokinetics of gentamicin complex and gentamicin C_1. Differences in both the volume of distribution and serum clearance of gentamicin and gentamicin C_1 were reported.

In the present studies, the effects of posture and sleep on the disposition of gentamicin complex and the individual components were examined. The effects of sex and menstrual cycle on gentamicin pharmacokinetics were also studied.

3.2: ANALYSIS

3.2.1: MATERIALS AND METHODS

Gentamicin sulphate containing 563 mg gentamicin base per milligram of powder with a component ratio of C_1 25.4%, C_{1a} 31.7%, C_2 42.9%, was supplied by Essex Laboratories, Sydney, Australia. All solvents were specially purified to HPLC grade. Water was de-ionised and glass distilled daily. All other chemicals were reagent grade. O-phthalaldehyde reagent was prepared according to the method of Maitra et al (1977).

3.2.1.1: Chromatographic equipment

Separation was performed using a Waters M6000A solvent delivery system fitted with a U6K injector. A C_{18} µBondapak column (particle size 10µm; 300 mm x 3.9 mm I.D.;Waters Associates, Milford, MA, USA) was used in combination with a guard column (µBondapak C_{18}/Porasil B; Waters Associates; 23 mm x 3.9 mm I.D.). Detection of fluorescent products in the eluent was performed using a Schoeffel model FS970LC fluorometer (Schoeffel Instrument Corp, Westwood, NJ, USA) with fluorescence excitation at 260 nm and emission detection at 418 nm. Detector signal was recorded on a dual-channel Omniscribe recorder (Houston Instruments, Austin, TX, USA). The mobile phase was 1% triethylamine (TEA) solution (adjusted to pH6.2 ± 0.1 with phosphoric acid) - methanol (79:21) mixture. The flow rate was 2 ml/min. Injections were made with a 25 µl Hamilton Syringe.

3.2.1.2: Sample preparation

Preparation of derivatised gentamicin from serum samples was performed by the method of Maitra et al (1977) with some modification. In this method a disposable Pasteur pipette was plugged with silanized glass wool and about 150 mg of dry silicic acid was added to form a silicic acid column of 1.0 cm height. The column was treated with 1.0 ml of water. Serum (0.5 ml) was diluted to 2.0 ml with water, vortexed, and applied to the silicic acid column. The serum sample tube was rinsed with 1.0 ml water which was then applied to the column and eluted with the help of pressure from a rubber bulb. The eluate was discarded. Immediately o-phthalaldehyde reagent (0.5ml) was applied to the column and allowed to stand for 30 seconds. The reagent was eluted with the aid of pressure from the rubber bulb and the eluate was discarded. The derivatised gentamicin was then eluted from the column by adding 1.5ml of methanol. The eluent was vortexed, centrifuged and stored in the dark until injected. 20µl of the sample was injected onto the column.

3.2.1.3: Preparation of standard curves

Plasma standards were prepared by spiking drug-free plasma with known amounts of a freshly-prepared aqueous solution of gentamicin to produce concentrations of 1-20 mg/l total gentamicin (representing 0.14 - 2.86 mg/l C_1, 0.18 - 3.57 mg/l C_{1a}, 0.24 - 4.83 mg/l C_2).

The standards were then assayed in the described manner.

Standard curves were prepared by plotting the peak heights of the components versus concentration.

3.2.2: RESULTS

Chromatograms of blank serum and serum from a subject following intravenous administration of gentamicin are shown in Figure 3.1. The three major gentamicin components were well separated from each other and eluted in the order of C_1, C_{1a}, and C_2 at 4.8, 6.8 and 8.6 minutes, respectively.

Peak heights and serum concentrations were linearly related for each of the three components over the concentration range used for the standard curves ($r>0.99$) (Figure 3.2).

The intra- and inter-day reproducibility of the assay are shown in Table 3.1.

3.2.3: DISCUSSION

At the time of this study, the only HPLC methods capable of separating the major gentamicin components were those of Anhalt (1977), Maitra et al (1977) and Back et al (1979). The method of Anhalt (1977) used post-column derivatisation which, although reliable, has some disadvantages including the need for additional pumping equipment and a mixing chamber (not available in this laboratory); a high consumption of reagent; and an increase in baseline noise due to the pumping of derivatising agent through the detector (Essers, 1984). Pre-column derivatisation is

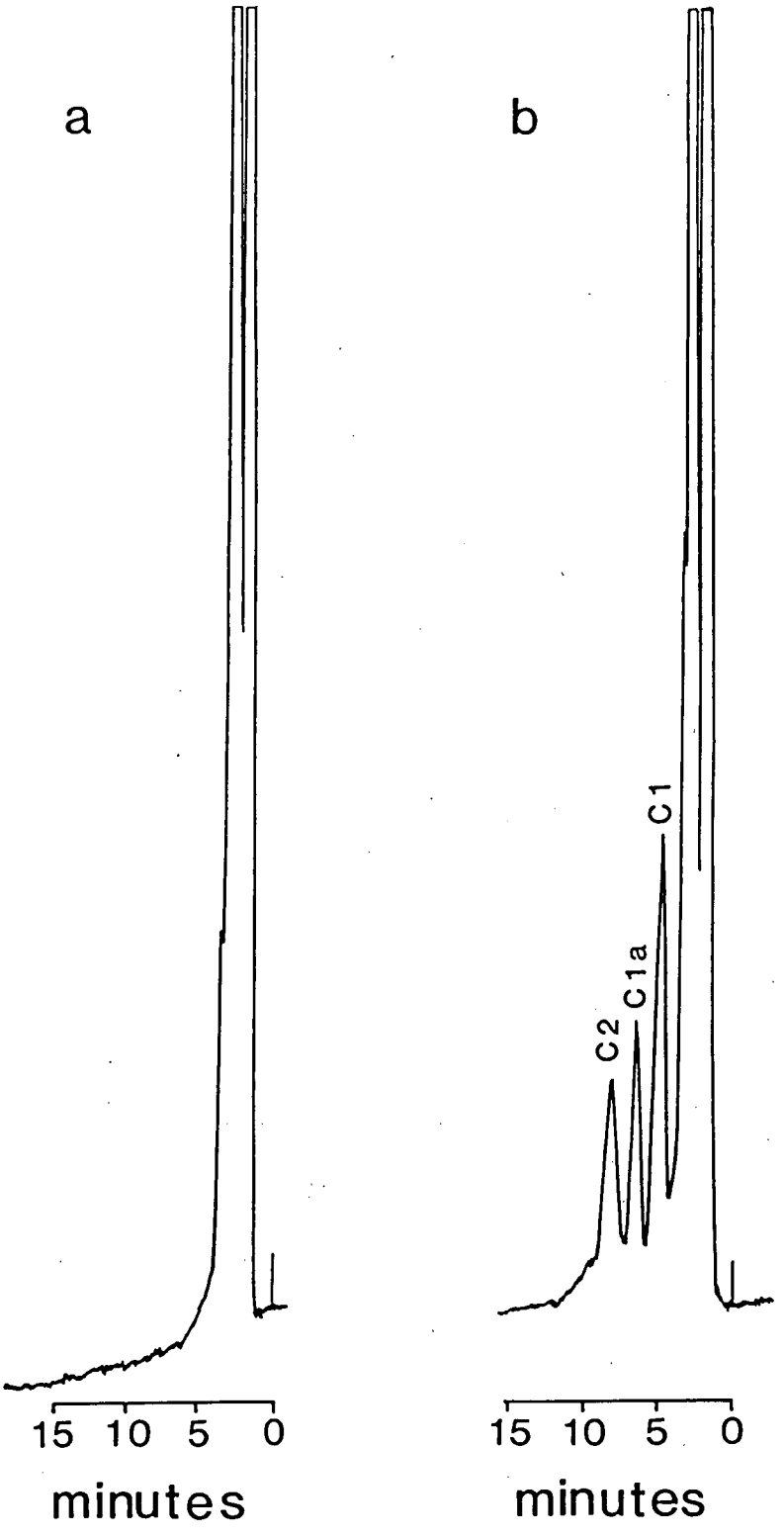

Figure 3.1 Chromatograms of (a) blank serum and (b) serum from the same subject 80 minutes after intravenous administration of 1mg/kg lean body weight dose of gentamicin. The gentamicin concentration is estimated to be 0.38 mg/l (C_1), 0.57 mg/l (C_{1a}) and 0.45 mg/l (C_2)

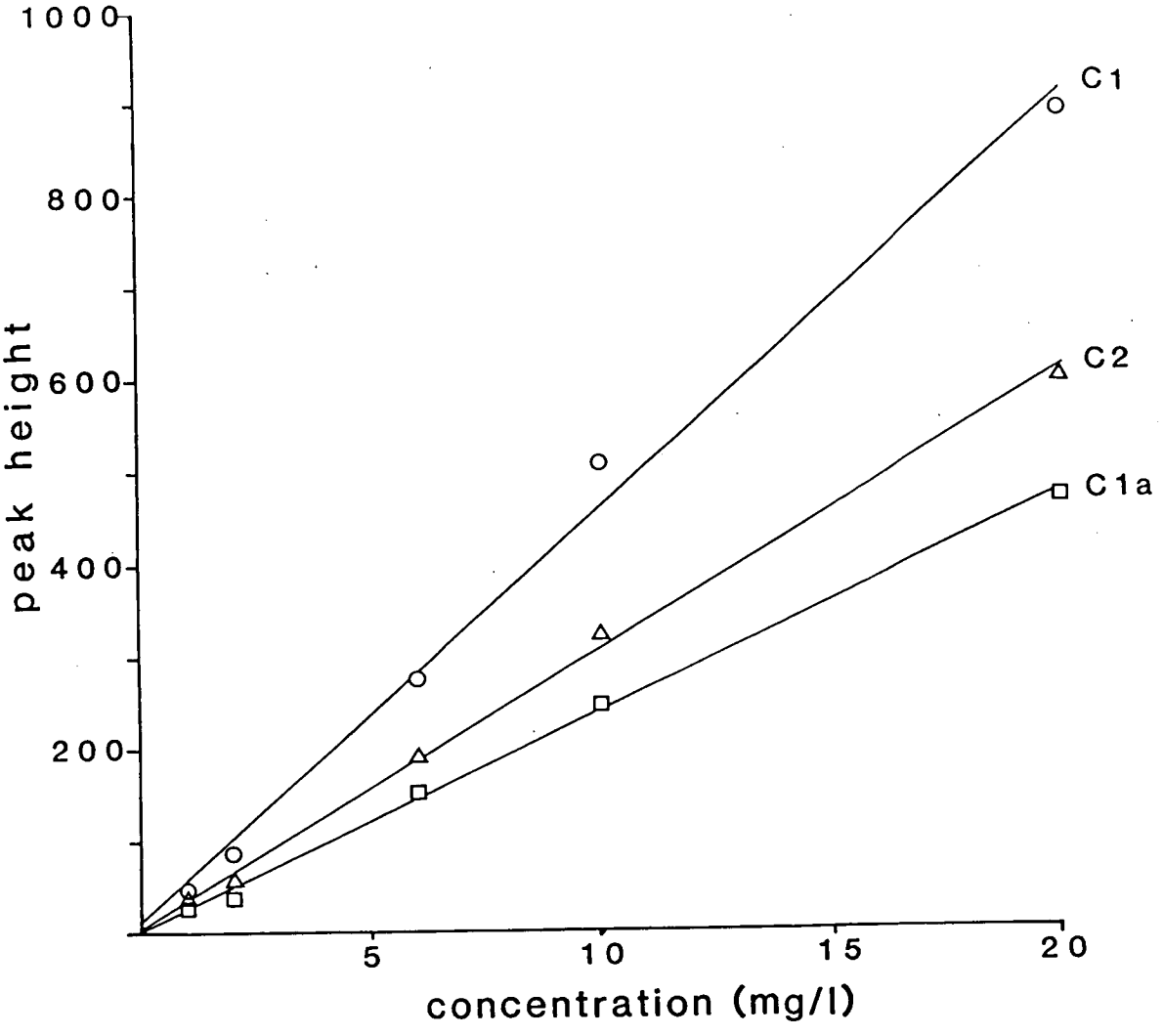

Figure 3.2 Standard curves for gentamicins C_1, C_{1a} and C_2. The concentration axis represents the concentration of total standard powder per millilitre of serum. The actual concentrations are given in the table below.

	Concentration (mg/l)		
Total sample	C_1	C_{1a}	C_2
5	0.715	0.893	1.208
10	1.430	1.785	2.415
15	2.145	2.677	3.623
20	2.860	3.570	4.830

Table 3.1 Intra- and inter-day variation of gentamicin in serum

Concentration (mg/l)		CV (%) of component		
		C_1	C_{1a}	C_2
2	Intra-day (n=5)	6.76	11.66	7.02
	Inter-day (n=6)	8.87	19.76	14.82
10	Intra-day (n=5)	6.05	5.13	6.36
	Inter-day (n=10)	6.49	7.22	10.35

simpler, requiring no additional machinery and is likely to eliminate more interfering material (Maitra et al, 1979). The methods of Maitra et al (1977) and Back et al (1979) use pre-column derivatisation and are identical except in the manner of sample extraction. Back et al (1979) modified the method of sample extraction to allow the assay of several aminoglycosides by the same technique. In the method of Maitra et al (1977) the derivatising agent, o-phthal-aldehyde, is included in the eluent subsequently injected onto the column. However, during the development of the present assay, the inclusion of this reagent led to a decrease in sample peak height throughout the day. As the o-phthalaldehyde reagent is alkaline (pH 10.4), its repeated injection may have destroyed the silica skeleton of the stationary phase of the pre-column and column. It was found that this problem was overcome by discarding the derivatising eluate, then eluting the derivatised gentamicin with methanol. To assess that this method still provided satisfactory recovery, comparison was made of samples containing 0.1 ml of drug solution, 0.5 ml o-phthalaldehyde reagent and 0.9 ml methanol (total volume 1.5 ml), vortexed, centrifuged then injected onto the column, with samples prepared as described (1.5 ml methanol eluent). This comparison showed that the peak heights were higher when the reagent was not included. In the automated method of Essers (1984), the problem was overcome by pre-saturating the derivatising solution with silica.

Maitra et al (1977) used excitation and emission wavelengths

of 340 nm and 418 nm, respectively. In this study it was found that these wavelengths resulted in an unacceptably low peak height : noise ratio. Excitation at 260 nm and emission at 418 nm provided negligible background noise and good sensitivity, giving optimal peak height : noise ratios.

In the development of this assay, the mobile phase of Maitra et al (1977) of EDTA-methanol was used initially and this provided good resolution with retention times (C_1, 5.6 min; C_{1a}, 7.2 min; C_2, 9.6 min) similar to those of the published method (C_1 4.7 min, C_{1a} 7.2 min, C_2 9.5 min). However, continued use of this mobile phase over a few days led to loss of peak resolution. This was restored by either increasing the water content of the mobile phase with associated longer retention times or increasing the EDTA concentration. The pH of the mobile phase (pH 8.75) was higher than the top of the optimum pH range (pH 2 - 7) for bonded-phase silica-based column packings (Giese, 1983). At this pH the silica matrix is likely to dissolve and release of bonded groups from the surface may occur. These factors may account for the loss of resolution experienced in this study. A change from EDTA to 1% triethylamine (TEA) adjusted to pH 6.2 ± 0.1 with phosphoric acid gave resolution similar to that initially experienced using the EDTA-methanol mobile phase. This mobile phase provided stable chromatograms over several weeks with the same column and injection of hundreds of samples. Comparison of chromatograms using EDTA-methanol and using TEA-methanol show similar resolution and sequence of elution of the

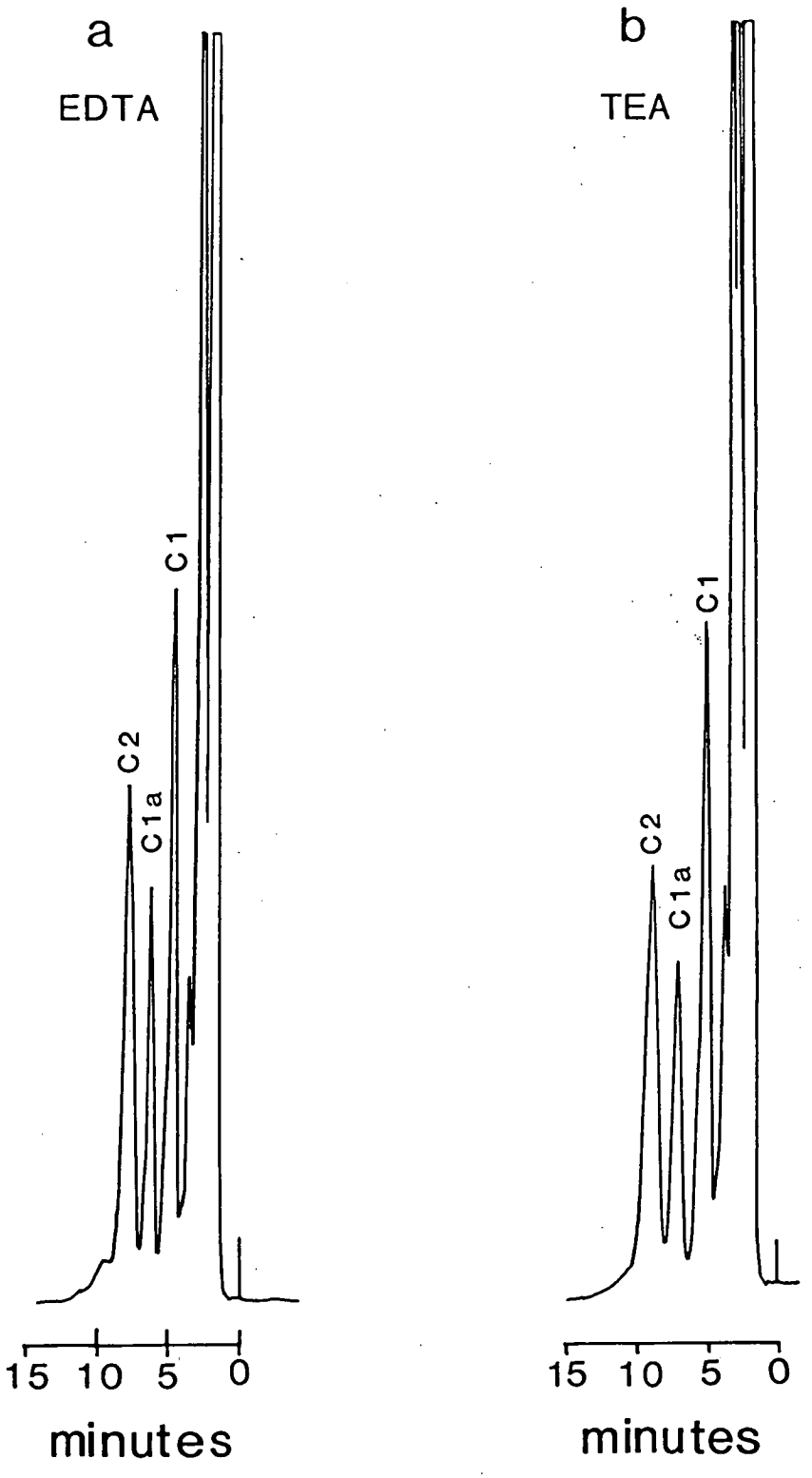

Figure 3.3 Chromatograms of serum spiked with C_1 (1.43mg/l), C_{1a} (1.785mg/l) and C_2 (2.415mg/l) and assayed (a) using the mobile phase of Maitra et al (1977) containing EDTA and (b) the mobile phase of the present method containing triethylamine (TEA)

components (Figure 3.4).

This method provides a simple, rapid assay for the determination of the three major components of gentamicin (C_1, C_{1a} and C_2) with sufficient sensitivity for use in clinical and pharmacokinetic studies.

3.3: PHARMACOKINETIC STUDY

3.3.1: CLINICAL PROTOCOL

3.3.1.1: Subjects

Twelve healthy, young adult volunteers (six male, six female) aged 21 to 29 years participated in the study. Prior to the investigation, a detailed medical history was taken. No subjects gave a history of liver or renal disease. Physical examination, haematological tests and liver and renal function tests for all subjects were normal. Informed consent was obtained from each subject; approval was obtained, before commencing the study, from the Human Ethics Committee of the University of Tasmania.

3.3.1.2: Procedure

Each subject was assessed for lean body mass by measurement of skin fold thickness at four sites (Durnin & Womersley, 1974). Gentamicin (1 mg/kg lean body mass) (Essex Laboratories, Sydney, Australia) was administered by slow intravenous bolus (over 3 minutes) to each subject on three separate occasions - during bedrest in the day, immediately

prior to sleep at night (usually at midnight) and during daytime ambulation. One subject (ABI) did not participate in the sleep study. To assess the effects of menstrual cycle on gentamicin disposition, four of the female subjects were administered the dose on a second occasion of daytime bedrest. Changes in posture were not allowed throughout each section of the study. At least two weeks elapsed between each section of the study.

3.3.1.3: Sample collection

An indwelling catheter was inserted into the antecubetal vein of the arm not used for dosing, approximately one hour before dosing. Patency of the catheter was maintained by flushing with a small volume of heparinised saline (10 IU/ml) after each blood sample was withdrawn. Subjects then adopted the posture required for the study. 5 ml blood samples were withdrawn at 0, 3, 15, 30, 45, 60, 80, 100, 120, 180, 240, 300, 360 and 480 minutes. To avoid possible interaction between gentamicin and heparin (Myers et al, 1978), the first 1.0 ml of blood was drawn separately and discarded then the samples were collected into non-heparinised tubes and allowed to clot at room temperature before being centrifuged. The serum was drawn off using sterile Pasteur pipettes and stored at -20°C until assayed.

3.3.1.4: Pharmacokinetics and statistics

The serum total gentamicin concentration (C) versus time (t) data were fitted to a biexponential equation of the

following form

$$C = Ae^{-\alpha t} + Be^{-\beta t} \qquad (3.1)$$

corresponding to a 2-compartment model by non-linear regression analysis. The calculated values of A, B, α, β were used to estimate the half-life of the α phase ($t_{\frac{1}{2}\alpha}$) and of the β phase ($t_{\frac{1}{2}\beta}$), the area under the curve (AUC), the body clearance (CL_B), the apparent volume of distribution (V) and its components, the apparent volumes of the central compartment (V_C) and tissue compartment (V_T) (Gibaldi & Perrier, 1982a).

In addition, the disposition of total gentamicin and the individual gentamicin components (C_1, C_{1a}, C_2) was characterised by non-compartmental methods using statistical moment theory. The terminal elimination rate constant (β) was determined from the slope of the terminal linear segment of a semilogarithmic plot of serum concentration versus time. The zero moment of the drug concentration-time curve, the area under the curve (AUC), was calculated according to trapezoidal rule from concentration-time data following drug administration (Gibaldi and Perrier, 1982b). The first moment, the mean residence time (MRT), was calculated according to Equation 3.2 (Gibaldi and Perrier, 1982b).

$$MRT = \frac{AUMC}{AUC} = \frac{{}_0\int^{\infty} tCdt}{{}_0\int^{\infty} Cdt} \qquad (3.2)$$

The apparent total serum clearance (CL_B) was calculated by dividing the dose of the component (D_{iv}) by the area under the curve (AUC). The volume of distribution at steady-state (V_{SS}) for each component and total gentamicin was calculated from Equation 3.3 (Gibaldi and Perrier, 1982b)

$$V_{ss} = CL_B.MRT = \frac{D_{iv}.AUMC}{AUC^2} \qquad (3.3)$$

The elimination half-life ($t_{\frac{1}{2}\beta}$) was also calculated for each of the gentamicin components in the usual manner ($t_{\frac{1}{2}\beta} = 0.693/\beta$).

Urine flow was the mean value observed during the first eight hours after dosing.

Statistical comparisons of the pharmacokinetic parameters for total gentamicin and the gentamicin components were made by one-factor analysis of variance with repeated measures (Ferguson, 1976). Statistical comparison of the effect of sex and stage of menstrual cycle on the disposition of gentamicin was made by one-way analysis of variance or t-test, as appropriate (Ferguson, 1976).

3.3.2: RESULTS

The mean serum total gentamicin levels versus time profiles during ambulation, bedrest and sleep are presented in Figure 3.5. The mean concentration-time curve for ambulant

subjects was lower at all times than the curves for sleep and bedrest. The individual experimental and pharmacokinetic data for gentamicin in subjects during ambulation and sleep are shown in Tables 3.2 and 3.3, respectively. The data for male and female subjects during bedrest are shown in Tables 3.4 and 3.5, respectively. The mean experimental and pharmacokinetic data for total gentamicin derived from individual concentration-time curves are shown in Table 3.6.

Pharmacokinetic analysis of total gentamicin according to a two-compartment model showed significant differences between ambulation and supine (bedrest and sleep) values for all the parameters determined except total body clearance. In each case the values of the parameters obtained during bedrest did not differ significantly from those obtained during sleep. Non-compartmental analysis based on statistical moment theory showed statistically significant differences between the values determined for ambulant subjects and those for supine subjects (bedrest and sleep) for the area under the curve (AUC), volume at steady state (V_{ss}) and total body clearance (CL_B).

Figure 3.6 shows the concentration-time curves for each of the major gentamicin components during ambulation, sleep and bedrest. Again, at all times the gentamicin component concentrations during ambulation were lower than during bedrest and sleep. The mean pharmacokinetic data determined

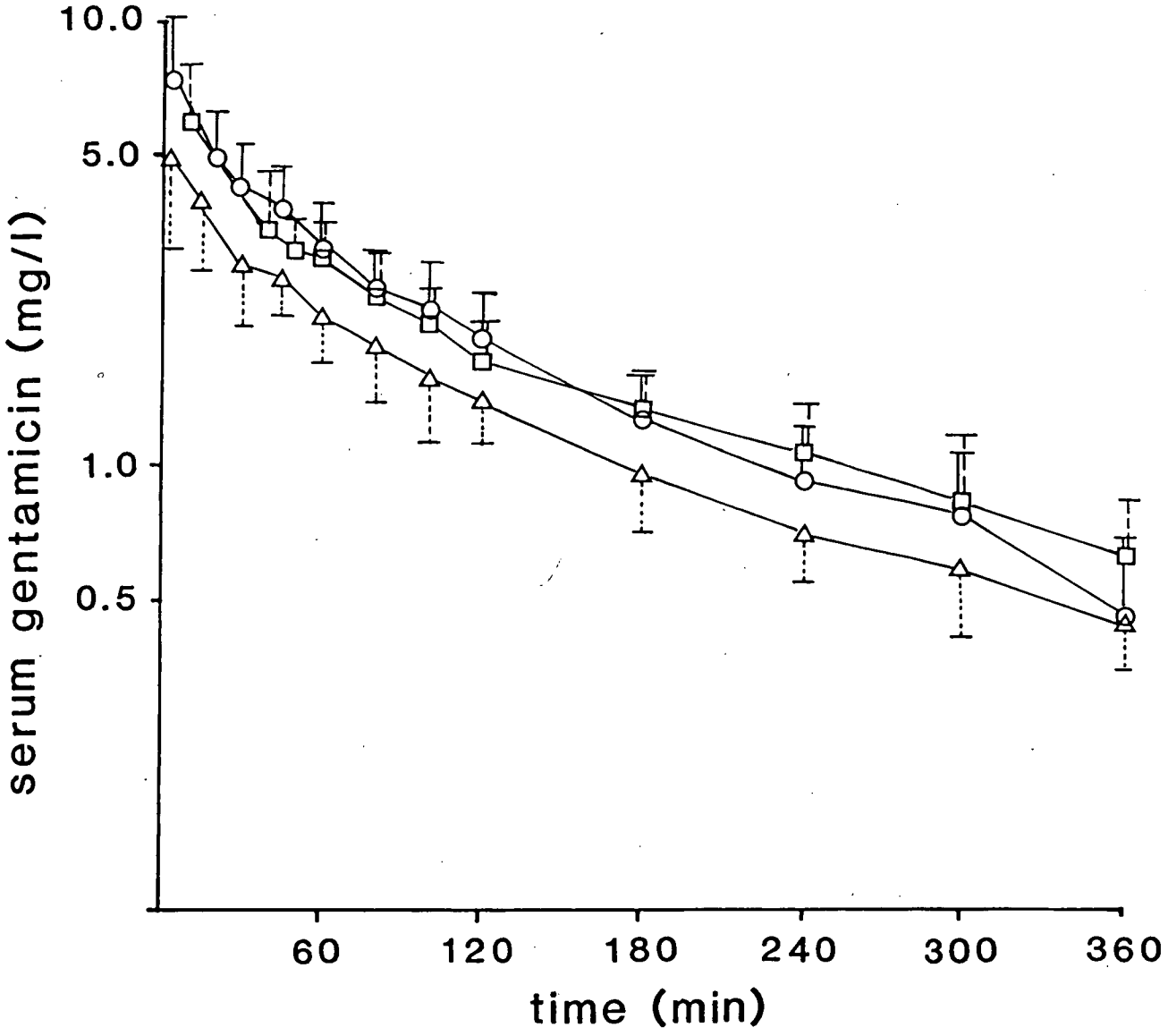

Figure 3.4 Mean (±SD) serum total gentamicin concentrations found in healthy adult volunteers following administration of a 1mg/kg (lean body mass) dose of gentamicin during △ ambulation, ○ bedrest and □ sleep

Table 3.2 Experimental and pharmacokinetic data describing the disposition of gentamicin in ambulatory subjects

Parameter[a]	Subjects											
	PT	CH	MP	MR	BP	RL	CF	FW	JR	AB	MH	ABI
Age (y)	22	21	21	22	21	21	21	23	22	21	21	29
Sex	M	M	M	M	M	M	F	F	F	F	F	F
TBW (kg)	71.8	67.5	69.0	61.3	72.1	63.3	58.1	62.1	60.5	60.9	65.6	61.6
LBM (kg)	63.3	57.6	61.3	55.2	64.7	57.7	41.5	43.8	44.0	39.5	46.2	46.2
AUC (min.mg/l)	402	740	507	1140	802	830	543	658	455	562	877	814
$t_{½\alpha}$ (min)	17.33	115.69	29.75	62.75	20.19	61.94	18.47	5.80	10.96	13.79	49.98	56.04
$t_{½\beta}$ (min)	118.7	564.9	159.7	693.0	185.5	427.1	137.2	178.3	119.8	140.8	724.2	884.7
V (l/kg TBW)	0.376	0.940	0.404	0.790	0.299	0.677	0.260	0.276	0.276	0.235	0.839	1.176
V (l/kg LBM)	0.426	1.101	0.455	0.877	0.334	0.742	0.365	0.391	0.380	0.362	1.191	1.569
CL_B[b] (ml/min)	157.51	77.81	121.00	48.40	80.64	69.51	76.42	66.54	96.77	70.34	52.66	56.77
V_{ss} (l/kg LBM)	0.336	0.579	0.356	0.590	0.242	0.507	0.312	0.359	0.330	0.319	0.862	0.471
MRT (min)	141.5	515.3	188.1	690.8	198.0	438.1	172.5	262.1	155.9	183.7	748.6	205.3
CL_B[c] (ml/min)	150.22	64.75	116.08	47.16	79.03	66.80	75.04	59.93	93.22	68.52	53.18	106.01
Urine flow (ml/min)	0.54	0.54	0.15	0.62	0.52	0.66	0.54	0.62	1.25	1.39	0.57	3.60

[a] TBW - total body weight; LBM - lean body mass; AUC - area under the serum-concentration-time curve; $t_{½\alpha}$ - distribution half-life; $t_{½\beta}$ - elimination half-life; V - volume of distribution; CL_B - total body clearance; V_{ss} - volume of distribution at steady-state; MRT - mean residence time

[b] CL_B - total body clearance calculated using the AUC estimated in the compartmental pharmacokinetic analysis.

[c] CL_B - total body clearance calculated using the AUC estimated by the trapezoidal rule.

Table 3.3 Experimental and pharmacokinetic data describing the disposition of gentamicin in sleeping subjects

Parameter[a]	Subjects											
	PT	CH	MP	MR	BP	RL	CF	FW	JR	AB	MH	ABI
Age (y)	22	21	21	22	21	21	21	23	22	21	21	29
Sex	M	M	M	M	M	M	F	F	F	F	F	F
TBW (kg)	71.8	67.5	69.0	61.3	72.1	63.3	58.1	62.1	60.5	60.9	65.6	61.6
LBM (kg)	63.3	57.6	61.3	55.2	64.7	57.7	41.5	43.8	44.0	39.5	46.2	46.2
AUC (min.mg/l)	1014	885	837	638	944	1161	549	626	628	727	664	-
$t_{½\alpha}$ (min)	13.89	17.37	21.46	15.24	18.39	12.46	67.12	17.95	9.99	29.95	2.71	-
$t_{½\beta}$ (min)	160.9	159.3	143.7	117.4	171.2	153.4	118.4	168.0	112.9	155.4	154.4	-
V (l/kg TBW)	0.201	0.222	0.220	0.239	0.235	0.174	0.222	0.273	0.189	0.200	0.236	-
V (l/kg LBM)	0.236	0.260	0.248	0.266	0.262	0.191	0.311	0.387	0.259	0.308	0.335	-
CL_B[b] (ml/min)	62.40	65.07	73.24	86.58	68.50	49.68	75.59	70.02	70.06	54.31	69.55	-
V_{ss} (l/kg LBM)	0.187	0.249	0.212	0.240	0.223	0.204	0.303	0.344	0.216	0.238	0.269	-
MRT (min)	192.7	240.2	180.1	153.3	214.6	223.0	171.1	220.2	136.4	175.0	192.5	-
CL_B[c] (ml/min)	61.46	59.80	72.05	86.48	67.14	52.75	73.51	68.37	69.72	53.61	64.47	-
Urine flow (ml/min)	1.34	1.20	0.60	1.43	0.77	1.96	1.01	0.65	1.20	1.16	1.01	-

[a] Parameters as defined in Table 3.2

[b] CL_B - total body clearance calculated using the AUC estimated in the compartmental pharmacokinetic analysis

[c] CL_B - total body clearance calculated using the AUC estimated by the trapezoidal rule

Table 3.4 Experimental and pharmacokinetic data describing the disposition of gentamicin in male subjects during bedrest

Parameter[a]	Subject PT	CH	MP	MR	BP	RL
Age (y)	22	21	21	22	21	21
TBW (kg)	71.8	67.5	69.0	61.3	72.1	63.3
LBM (kg)	63.3	57.6	61.3	55.2	64.7	57.7
AUC (min.mg/l)	742	829	748	837	1294	868
$t_{½\alpha}$ (min)	5.70	9.17	20.47	7.11	38.00	38.94
$t_{½\beta}$ (min)	142.4	139.3	102.2	87.2	263.1	280.4
V (l/kg TBW)	0.244	0.207	0.175	0.135	0.263	0.425
V (l/kg LBM)	0.277	0.243	0.197	0.150	0.293	0.466
CL_B[b] (ml/min)	85.27	69.50	81.93	65.92	49.98	66.44
V_{ss} (l/kg LBM)	0.238	0.218	0.156	0.140	0.163	0.290
MRT (min)	184.8	187.6	124.1	124.4	166.7	257.8
CL_B[c] (ml/min)	81.37	66.77	77.00	62.13	63.25	64.82
Urine flow (ml/min)	1.74	1.41	1.91	1.96	0.93	1.15

[a] Parameters as defined in Table 3.2

[b] CL_B - total body clearance calculated using the AUC estimated in the compartmental pharmacokinetic analysis

[c] CL_B - total body clearance calculated using the AUC estimated by the trapezoidal rule

Table 3.5 Experimental and pharmacokinetic data describing the disposition of gentamicin in female subjects during bedrest

Parameter[a]	Subject									
	CF	ABI	FW		JR		AB		MH	
Age (y)	21	29	23		22		21		21	
TBW (kg)	58.1	61.6	62.1		60.5		60.9		65.6	
LBM (kg)	41.5	46.2	43.8		44.0		39.5		46.2	
Day of MC[b]	N/A	N/A	6	20	11	25	5	19	13	27
AUC (min.mg/l)	432	583	991	517	603	761	801	811	824	357
$t_{½\alpha}$ (min)	11.37	7.75	15.67	23.90	4.42	24.80	6.86	12.78	27.57	16.98
$t_{½\beta}$ (min)	66.8	139.5	153.0	132.9	80.4	159.4	129.2	151.8	214.7	73.6
V(l/kg TBW)	0.160	0.259	0.157	0.262	0.140	0.220	0.151	0.175	0.265	0.209
V(l/kg LBM)	0.224	0.345	0.223	0.371	0.192	0.302	0.233	0.270	0.376	0.297
CL_B[c] (ml/min)	96.00	79.20	44.20	84.79	72.98	57.78	49.29	48.70	56.10	129.39
V_{ss} (l/kg LBM)	0.215	0.348	0.206	0.343	0.166	0.250	0.193	0.234	0.292	0.373
MRT (min)	97.5	203.2	206.9	175.2	107.7	194.4	166.1	195.9	243.7	159.1
CL_B[d] (ml/min)	91.32	79.21	43.64	85.82	67.87	56.55	45.79	47.10	55.40	108.19
Urine flow (ml/min)	1.12	1.15	3.58	2.96	3.21	2.23	1.30	2.92	2.51	2.76

[a] Parameters as defined in Table 3.2

[b] Day of MC - day of menstrual cycle

[c] CL_B - total body clearance calculated using the AUC estimated in the compartmental pharmacokinetic analysis

[d] CL_B - total body clearance calculated using the AUC estimated by the trapezoidal rule

Table 3.6 Comparison of mean estimates (±SD) of pharmacokinetic parameters for gentamicin from data in Tables 3.2, 3.3, 3.4 and 3.5

Parameter[a]	Ambulation (n = 12)	Sleep (n = 11)	Bedrest (n = 12)	Significant difference
TBW (kg)	64.48 ± 4.64	64.75 ± 4.78	64.48 ± 4.64	
LBM (kg)	51.75 ± 9.11	52.25 ± 9.38	51.75 ± 9.11	
AUC (min.mg/l)	649 ± 213	788 ± 194	796 ± 216	N/S
$t_{½\alpha}$ (min)	38.56 ± 31.96	20.59 ± 16.87	16.09 ± 12.44	$p<0.05$[d]
$t_{½\beta}$ (min)	361.2 ± 283.3	146.8 ± 21.0	149.9 ± 69.2	$p<0.01$[d]
V (l/kg TBW)	0.546 ± 0.323	0.219 ± 0.027	0.215 ± 0.083	$p<0.01$[d]
V (l/kg LBM)	0.683 ± 0.413	0.279 ± 0.053	0.268 ± 0.090	$p<0.01$[d]
CL_B[b] (ml/min)	81.20 ± 31.10	67.73 ± 10.17	68.07 ± 16.01	N/S
V_{ss} (l/kg LBM)	0.439 ± 0.174	0.244 ± 0.046	0.219 ± 0.064	$p<0.05$[d]
MRT (min)	325.0 ± 217.6	190.8 ± 31.7	172.5 ± 51.7	N/S
CL_B[c] (ml/min)	74.16 ± 34.19	66.31 ± 9.61	66.55 ± 14.20	$p<0.05$[d]
Urine flow (ml/min)	0.92 ± 0.91	1.12 ± 0.39	1.83 ± 0.86	$p<0.05$[e]

[a] Parameters as defined in Table 3.2

[b] CL_B - total body clearance calculated using the AUC estimated in the compartmental analysis

[c] CL_B - total body clearance calculated using the AUC estimated by the trapezoidal rule

[d] Ambulation significantly different from bedrest and sleep

[e] Ambulation significantly different from bedrest but not sleep

for each of the components during each of the three states studied are shown in Table 3.7.

For gentamicins C_1 and C_{1a} there were significant differences in the area under the curve, apparent volume of distribution at steady-state and total body clearance. For gentamicin C_2 the significant differences existed in the apparent volume of distribution and the total body clearance. In each case the values of the parameters for ambulation differed significantly from the values for sleep and bedrest. The values of the parameters determined for sleep were not significantly different from those for bedrest except for the values of the apparent volume of distribution at steady state for gentamicin C_{1a} where the value for sleep was significantly greater than the value for bedrest.

Statistical comparison of the values of the pharmacokinetic parameters obtained for the different components within each state studied showed no significant differences between the components for the apparent volume of distribution at steady state and the total body clearance. During ambulation and bedrest the mean residence time for gentamicin C_2 was significantly longer than the mean residence times for gentamicin C_1 and C_{1a}. During sleep the mean residence time for C_2 was significantly longer than the mean residence time for C_1 but was not significantly different from that for C_{1a} (Table 3.7).

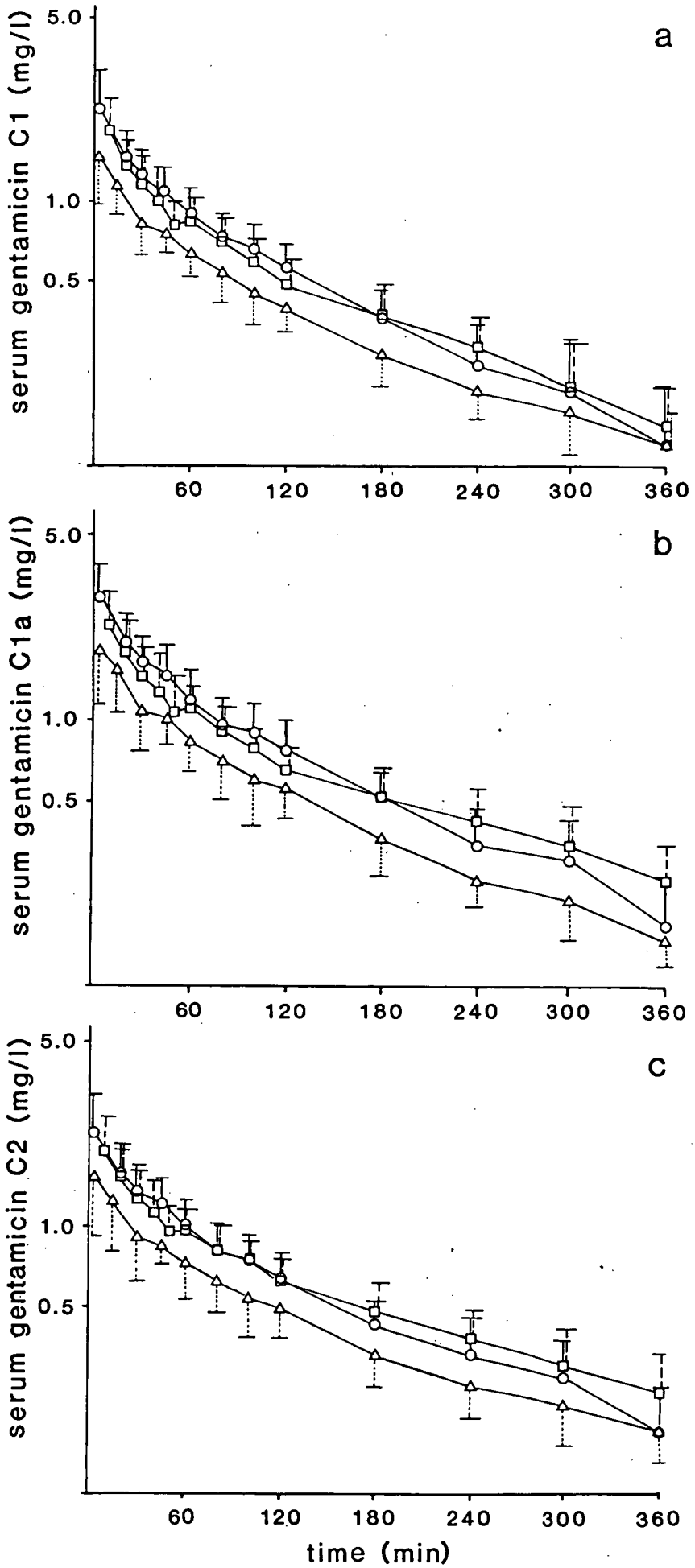

Figure 3.5 Mean (±SD) serum concentration versus time profiles observed during △ ambulation, ○ bedrest and □ sleep for (a) gentamicin C_1, (b) gentamicin C_{1a} and (c) gentamicin C_2.

Table 3.7 Mean (±SD) pharmacokinetic data for gentamicin C_1, C_{1a} and C_2 determined during ambulation, sleep and bedrest

Parameter[a]		Ambulation (n = 12)	Sleep (n = 11)	Bedrest (n = 12)	Significant difference
AUC	C_1	168.2 ± 37.4	218.9 ± 54.5	224.7 ± 53.8	p<0.05
(min.mg/l)	C_{1a}	219.9 ± 48.4	315.3 ± 76.9	303.1 ± 63.2	p<0.01
	C_2	216.3 ± 54.9	271.8 ± 68.5	273.0 ± 60.7	N/S
V_{ss}	C_1	16.47 ± 2.52	11.90 ± 3.19	10.54 ± 3.19	p<0.01
(l)	C_{1a}	16.08 ± 3.71	13.06 ± 2.07	10.50 ± 3.32	p<0.01
	C_2	17.58 ± 3.19	12.45 ± 2.07	11.24 ± 3.31	p<0.01
Significant difference		N/S	N/S	N/S	
CL_B	C_1	96.1 ± 32.6	72.6 ± 9.5	71.2 ± 16.1	p<0.05
(ml/min)	C_{1a}	92.9 ± 21.4	64.8 ± 9.6	67.6 ± 15.9	p<0.01
	C_2	80.9 ± 26.9	63.3 ± 12.8	63.0 ± 14.7	p<0.05
Significant difference		N/S	N/S	N/S	
MRT	C_1	179.7 ± 34.2	167.4 ± 51.5	154.3 ± 54.0	N/S
(min)	C_{1a}	175.2 ± 25.2	202.9 ± 26.9	157.7 ± 38.2	N/S
	C_2	227.9 ± 49.6	202.7 ± 31.4	185.2 ± 53.2	N/S
Significant difference		p<0.01	p<0.05	p<0.05	

[a] Parameters as defined in Table 3.2

In the comparison of males and females, it was found that the total body weight of males (67.5 ± 4.43 kg) was significantly greater than that of females (61.5 ± 2.45 kg) ($p<0.05$). Ideal body weights were also significantly different (males, 60.0 ± 3.71 kg; females, 43.5 ± 2.64 kg) ($p<0.01$). Figure 3.7 shows the mean (±SD) gentamicin component levels versus time profiles for males and for females at 2 different stages of their menstrual cycle. The pharmacokinetic parameters determined from those profiles are presented in Table 3.8. The clearance (CL_B) of gentamicin C_1 for females in the second half of their menstrual cycle was significantly different from that of females during the first half of their menstrual cycle ($p<0.05$) (Table 3.8). When the clearance of gentamicin C_1 was corrected for lean body mass the clearance (CL_B) for males was significantly different from the clearance for females, independent of stage of menstrual cycle (Table 3.8). There were no significant differences between the clearance of total gentamicin, gentamicin C_{1a}, gentamicin C_2 for males and females.

The volume of distribution at steady state (V_{ss}), adjusted for lean body mass, for gentamicin C_{1a} for females in the second half of their menstrual cycle was significantly different from the V_{ss} for males and from the V_{ss} for females in the first half of their menstrual cycle. There was no significant difference between males and females during the first half of their menstrual cycle for V_{ss} for

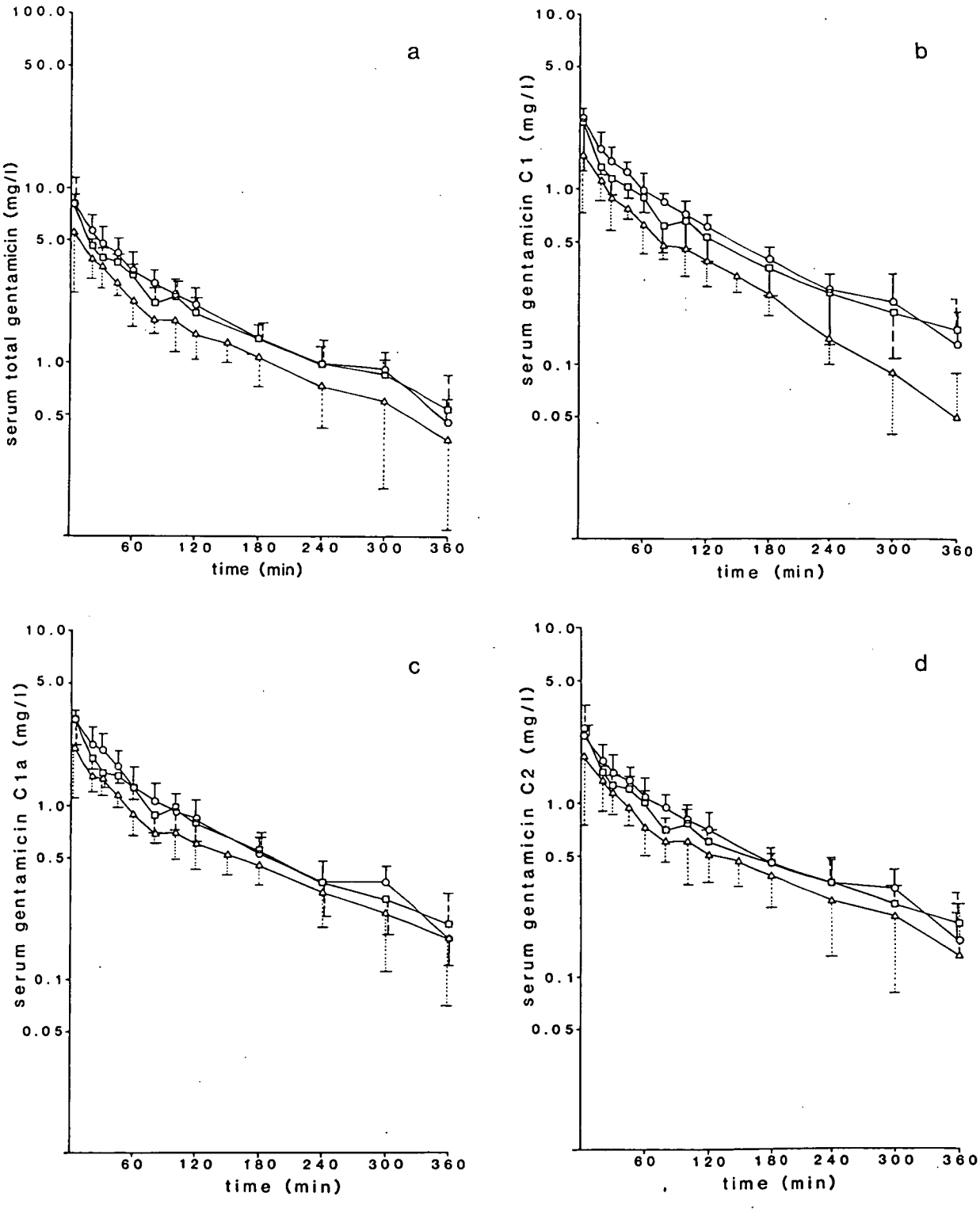

Figure 3.6 Mean (±SD) gentamicin concentrations versus time found in ○ males, □ females during the follicular phase of their menstrual cycle, and △ females during the luteal phase of their menstrual cycle for (a) total gentamicin, (b) gentamicin C_1, (c) gentamicin C_{1a} and (d) gentamicin C_2

Table 3.8 Mean (±SD) pharmacokinetic parameters for total gentamicin and the gentamicin components estimated for male subjects and for female subjects at two phases of their menstrual cycle

Parameter[a]	Sex[c]	Gentamicin			
		Total	C_1	C_{1a}	C_2
AUC	M	873 ± 87.3	250 ± 22.3	325 ± 46.3	260 ± 45.9
	F_1	837 ± 146.1	232 ± 53.7	318 ± 54.7	286 ± 45.7
	F_2	639 ± 200.6	141 ± 40.9[f]	256 ± 83.6	223 ± 101.4
CLB[b] (ml/min)	M	69.22 ± 7.99	71.22 ± 6.08	72.27 ± 12.66	65.82 ± 11.62
	F_1	53.18 ± 11.05	58.39 ± 15.55	52.95 ± 10.85	50.47 ± 10.19
	F_2	74.42 ± 27.91	99.23 ± 36.00[d]	70.98 ± 28.90	76.00 ± 38.32
CL_B[b]	M	1.155 ± 0.110	1.190 ± 0.108	1.204 ± 0.183	1.095 ± 0.169
(ml/min/kg LBM)	F_1	1.224 ± 0.230	1.341 ± 0.319	1.217 ± 0.206	1.163 ± 0.219
	F_2	1.695 ± 0.551	2.262 ± 0.703[e]	1.615 ± 0.574	1.720 ± 0.786
V_{ss} (l/kg TBW)	M	0.178 ± 0.052	0.159 ± 0.040	0.165 ± 0.039	0.173 ± 0.052
	F_1	0.149 ± 0.039	0.156 ± 0.052	0.133 ± 0.022	0.159 ± 0.028
	F_2	0.209 ± 0.052	0.182 ± 0.046	0.189 ± 0.027	0.191 ± 0.034
V_{ss} (l/kg LBM)	M	0.201 ± 0.058	0.180 ± 0.047	0.186 ± 0.046	0.195 ± 0.058
	F_1	0.214 ± 0.054	0.224 ± 0.072	0.191 ± 0.035	0.228 ± 0.039
	F_2	0.300 ± 0.068	0.261 ± 0.063	0.271 ± 0.036[f]	0.274 ± 0.041
$t_{½\beta}$ (min)	M	169.1 ± 82.5	120.0 ± 52.7	130.1 ± 32.2	136.5 ± 46.0
	F_1	144.4 ± 55.8	137.1 ± 54.5	122.5 ± 36.8	163.4 ± 48.9
	F_2	129.4 ± 38.2	81.0 ± 17.1	137.7 ± 42.7	137.2 ± 56.2

[a] Parameters as defined in Table 3.2

[b] Determined by statistical moment theory

[c] M - males (n=6); F_1 - females (n=4) during the follicular phase of their menstrual cycle; F_2 - females (n=4) during the luteal phase of their menstrual cycle

[d] F_2 significantly different from F_1 but not M ($p<0.05$)

[e] M significantly different from F_1 and F_2 ($p<0.05$)

[f] F_2 significantly different from M and F_1 ($p<0.05$)

gentamicin C_{1a}. The differences in V_{ss} between males and females for the other gentamicin components and total gentamicin did not reach significance. Nor did any differences in V_{ss} between females during the different stages of their menstrual cycles (Table 3.8). Differences in sex and in stage of menstrual cycle had no significant effect on the other parameters (MRT, $t_{\frac{1}{2}\beta}$) determined for the gentamicin components (Table 3.8).

3.3.3: DISCUSSION

The values for the compartmental pharmacokinetic parameters for total gentamicin in this study (Table 3.6) are similar to those observed by several other workers following administration of gentamicin to subjects with normal renal function (Cutler et al, 1972; Kaye et al, 1974; Sawchuck et al, 1977; Leroy et al, 1978; Bauer & Blouin, 1983; Bauer et al, 1983). As found by Kaye et al (1974) and Michelson et al (1976), there is a wide inter-subject variation in the disposition kinetics for gentamicin, especially for ambulant subjects (Table 3.6). The non-compartmental pharmacokinetic parameters, mean residence time (MRT) and volume at steady state (V_{ss}), have not been described by other workers.

The pharmacokinetics of gentamicin have been described previously by one-compartment, two-compartment and three-compartment models. The inadequacy of the one-compartment model in providing useful parameters for dosing has been discussed by several workers (Wilson et al, 1973b; Evans et

al, 1980; Schentag et al, 1977). In examining the disposition of gentamicin over a normal dosing interval, a two-compartment model appears to adequately describe the pharmacokinetics of the drug. However, parameters obtained from a two-compartment model do not account for the slow, continuing rise in peak and trough levels observed on repeated dosing. Gentamicin appears to slowly distribute into tissues, particularly the kidney, saturation of the tissues taking several days (Schentag et al, 1977). The present study describes the disposition of gentamicin over a normal dosing interval (8 hr) during which ambulation and sleep could be maintained.

The higher volume of distribution observed in ambulant subjects (Table 3.6) may reflect the greater perfusion of peripheral areas during ambulation since skeletal muscle blood flow may increase nearly four-fold with exercise (Vatner & Pagiani, 1976) with a decrease in blood flow to the kidney and liver (Chapman et al, 1948; Culbertson et al, 1947; Brengelmann, 1983). Schentag et al (1977) described skeletal muscle as a large volume, low concentration organ for gentamicin distribution. A similar change in volume of distribution with exercise was observed for another highly polar, renally excreted drug, pralidoxime (Swartz & Sidell, 1973).

The total clearance determined by compartmental analysis did not show any significant difference between the different states examined although the mean clearance during

ambulation was greater than the clearance in either supine state. In the non-compartmental pharmacokinetic analysis, the total body clearance of gentamicin in ambulant subjects was significantly greater than in supine subjects (Table 3.6). These differences in level of significance are probably due to model-dependent differences in the determination of the AUC. In the compartmental analysis the AUC is determined from the regression parameters (AUC = $A/\alpha + B/\beta$) whereas the AUC in the non-compartmental analysis was calculated by the trapezoidal rule. For the trapezoidal rule, the area from the time of the last data point to infinite time was calculated using the β determined from the slope of the terminal linear portion of the semilogarithmic plot of concentration-time data. With the longer distribution half-life ($t_{\frac{1}{2}\alpha}$) during ambulation (Table 3.6), it is possible that the β calculation for that phase includes a greater distribution factor than during either sleep or bedrest in spite of only data beyond 180 minutes post-dose being used in the calculations. Substitution of the β from the two-compartment analysis in the calculation of AUC by the trapezoidal rule suggests that this may be the case. In that substitution the AUC for sleep was 802.7 ± 184.9 (compared to 800.1 ± 177.0) and for bedrest was 802.0 ± 166.8 (compared to 800.6 ± 154.2) whereas for ambulation the difference was greater, 694.1 ± 232.2 (compared to 617.4 ± 155.1), (Table 3.6).

The clearance of gentamicin during ambulation tended to be

greater than during either bedrest or sleep (Tables 3.6 and 3.7). Factors that could account for changes in gentamicin clearance with changes in posture or sleep are alterations in glomerular filtration rate, urinary pH, urine flow or changes in the distribution rate giving an apparent change in clearance. As glomerular filtration rate appears to be independent of posture or exercise (Radigan & Robinson, 1949; Swartz & Sidell, 1973) and gentamicin clearance is not affected by urinary pH (Mariel et al, 1972) and in the present study there was no correlation between urine flow rate and clearance ($r = 0.25$, $p = 0.15$), the changes in clearance may be reflecting altered distribution.

Gentamicin is a complex consisting of three major components, gentamicins C_1, C_{1a} and C_2 and several minor components (Wilson et al, 1973a; Anhalt et al, 1978) and most official monographs do not set any limits on the relative proportions of the components allowed in the complex. The United States Pharmacopeia (1984) allows the proportion of each component within a commercial batch of the drug to vary within quite wide limits (C_1, 25-50%; C_{1a}, 15-40%; C_2, 20-50%). Variations between commercial batches have been confirmed by several workers (Anhalt et al, 1978; Kantor & Selzer, 1968; Wilson et al, 1973a). Thus, each time gentamicin is administered, the disposition of the total complex depends on the individual pharmacokinetic properties of three chemical entities and their relative proportion in the complex. Michelson et al (1976) suggested that these variations in percentage of the major components

between commercial batches of gentamicin and possible differences in the pharmacokinetic properties may account for the wide variability of serum gentamicin levels reported in the literature. If the antibacterial potency of the components also varied, then this could have important clinical consequences. In fact, in vitro studies of the susceptibilities of various microorganisms to the individual components C_1, C_{1a}, C_2 have indiciated a range of minimum inhibitory concentration ratios (Waitz & Weinstein, 1969).

Very few studies have examined the pharmacokinetics of the gentamicin components and none have studied the effect of posture or sleep on their disposition. Mosegaard et al (1975) compared the efficacy and pharmacokinetics of gentamicin complex and gentamicin C_1. In that study the efficacy of gentamicin complex and gentamicin C_1 appeared to be similar, however, there appeared to be some differences in the disposition of the two gentamicin dosage forms. Serum concentrations of gentamicin complex were generally higher than those for gentamicin C_1. The volume of distribution of gentamicin C_1 (25 ± 1.5% of body weight) was 47% greater than that of gentamicin complex (17 ± 1% of body weight) and the total body clearance of gentamicin C_1 (57 ± 8 ml/min) was also considerably greater than that of gentamicin complex (35 ± 4 ml/min). However, the half-lives of the two agents were comparable.

Nation et al (1978) compared the disposition of gentamicin

C_1 and the other two components (C_{1a}, C_2) combined. The components C_{1a} and C_2 could not be resolved in that study with the evaluation being restricted to reporting the ratios of the peak height for C_1 to the peak height for C_{1a}, C_2 for a limited number of blood samples from patients. Based on these results and a study in a single rabbit, Nation et al (1978) concluded that as there was little difference in the ratios in the patient samples and little change over 5 hours in the rabbit samples that the disposition of gentamicin C_1 was similar to gentamicins C_{1a} and C_2.

Examination of the results for the components in this study (Table 3.7) showed that, for each component, the volume at steady state (V_{ss}) and total body clearance (CL_B) were significantly greater during ambulation than during either supine state. Although there was a trend towards gentamicin C_2 to have a greater V_{ss} and smaller CL_B than the other components, this trend did not reach statistical significance in any of the states examined. These trends were, however, reflected in the mean residence time (MRT) where the MRT for gentamicin C_2 was significantly longer than for gentamicin C_1 in all states and than gentamicin C_{1a} during ambulation and bedrest. There may be some correlation between gentamicin C_2 having the longest mean residence time and the toxicity of this component observed by Kohlhepp et al (1984). Preferential binding to the kidney or greater accumulation of this component in the body on multiple dosing may result in its greater toxicity.

Very few systematic human studies on the influence of sex and the effect of menstrual cycle on drug kinetics have been reported (Giudicelli & Tillement, 1977). Giudicelli & Tillement (1977) suggested that the half-life of gentamicin was shorter in females than in males. In a study of 1,640 patients, Zaske et al (1982) found that females eliminated gentamicin more rapidly than males and that there were sex-related differences in half-life, clearance and volume of distribution. The half-life of another aminoglycoside, kanamycin, was also reported to be shorter in females than in males (Nielson et al, 1973). In the present study, although the mean half-life of total gentamicin in females is shorter than the mean half-life in males (Table 3.8), there was no significant difference in half-life or any other pharmacokinetic parameter determined for total gentamicin between males and females. Figure 3.7 shows that the mean serum gentamicin levels for females investigated in this study were lower than those for males at all times. However, more detailed examination of the data reveals that only the AUC for gentamicin C_1 for females differed significantly from that for males (Table 3.8). In this study a dose was used in accordance with the reported better correlation between the volume of distribution and subjects' lean body mass than with total body weight (Hull & Sarubbi, 1976). Other workers have suggested that a hybrid of lean body mass and total body weight should be used to calculate aminoglycoside doses to account for the extracellular fluid contained in adipose tissue (Bauer et al, 1980; Blouin et al, 1979; Korsager, 1980; Schwartz et al, 1978). The males

in the present study had little adipose tissue (TBW:LBM, range 1.10 - 1.17) whereas the females had a considerably higher fraction of adipose tissue (TBW:LBM, range 1.38 - 1.54). The females had a slightly higher volume of distribution (0.28 ± 0.07 l/kg LBM) than males (0.27 ± 0.11 l/kg LBM) based on ideal body weight suggesting some distribution into adipose tissue, although this difference did not reach significance. In the work of Zaske et al (1982), the dose was administered on a basis of total body weight and pharmacokinetic analysis used a one-compartment model. If the females in that study had a higher proportion of their body weight as adipose tissue than the males, a smaller volume of distribution per kilogram of total body weight in females might be expected (Schwartz et al, 1978). Recently, Bauer et al (1978) studied aminoglycoside kinetics in morbidly obese subjects and observed that obese patients had considerably higher volumes of distribution than normal weight subjects but that it was difficult to apply a fixed correction factor and some subjects were best dosed using ideal body weight while others should be dosed using total body weight.

There have been very few studies into the influence of menstrual cycle on the disposition of drugs. Most of the studies have investigated drugs eliminated by metabolism such as paracetamol (Wojcicki et al, 1979), antipyrine (Riester et al, 1980) and nitrazepam (Jochemsen et al, 1982). Although for nitrazepam and paracetamol there were minor changes in some of the pharmacokinetics of the drugs

associated with different phases of the menstrual cycle, these changes were not statistically significant (Jochemsen et al, 1982; Wojcicki et al, 1979). Tegeris & Panteleakis (1973) reported that, following oral administration of the drug, menstruating women had lower plasma levels of ampicillin (a drug eliminated predominantly by the kidney) at certain times after dosing than when non-menstruating. Complete details of the study were not given. Towards the end of a menstrual cycle, there appears to be localised oedema and changes in fluid distribution rather than changes in total body water, although many women report weight gain and generalised oedema in the pre-menstruum (Reid & Yen, 1981; Friedman, 1984). These changes in fluid distribution throughout the menstrual cycle may lead to alterations in the apparent volume of distribution of polar, water-soluble drugs such as gentamicin. Although the number of subjects in the present study was small, this was the trend observed. However this change was only significant for gentamicin component C_{1a}. The clearance of the drug also tended to be higher during the luteal phase thus causing no apparent change in the elimination half-life (Table 3.8).

The only component for which the clearance during the second half of the menstrual cycle was significantly higher than during the first half of the cycle, was gentamicin C_1. The mean clearance of this component during the luteal phase was nearly double the mean clearance during the follicular phase. The mean clearances of the other components altered by 30 to 50%. A doubling of the clearance would result in a

halving of the steady state concentration of that component on chronic dosing. Such an effect could have clinical consequences especially in batches of gentamicin containing 50% gentamicin C_1.

Although the differences in disposition between the major gentamicin components appear to be minor, the finding of gentamicin C_2 having a longer mean residence time than the other components may be clinically important in the light of the findings of Kohlhepp et al (1984) which showed that gentamicin C_2 is the most nephrotoxic of the components. The effects of posture could result in ambulant subjects requiring a larger loading dose (50 - 100% greater) than supine subjects and possibly higher maintenance doses to offset the higher clearance during ambulation. These results highlight the need to routinely monitor gentamicin levels rather than rely on nomograms or fixed dosage schedules.

CHAPTER 4

BENZYLPENICILLIN

4.1: INTRODUCTION

4.1.1: DRUG PROFILE

Benzylpenicillin (Penicillin G) is the only natural penicillin in clinical use. In spite of the introduction of newer β-lactam penicillins with either a wider spectrum of activity or increased activity against penicillinase-producing bacteria, benzylpenicillin is still frequently considered to be the drug of choice for the treatment of infections due to susceptible organisms (Wise, 1982; Ball, 1982). The penicillins bind to target sites on the cytoplasmic membranes of susceptible bacteria and interfere with peptidoglycan synthesis. This action results in a weakened cell wall which bursts from internal pressure. Thus, the penicillins have a bactericidal action (McAllister, 1982).

Benzylpenicillin is virtually free of toxicity at all usual doses. At very high doses (greater than 20 million units per day) some central nervous system side effects have been noted but these are more likely to occur in the presence of renal insufficiency or pre-existing central nervous system

lesions (Mandell & Sande, 1980). The most common adverse reactions to benzylpenicillin are those involving hypersensitivity. Although severe hypersensitivity reactions such as anaphylaxis may occur, the usual reactions are mild to moderate skin manifestations (Ibister, 1971).

4.1.2: ASSAYS

Several high-performance liquid chromatographic (HPLC) methods for the determination of benzylpenicillin in dosage forms or as pure drug have been reported (White, Carroll & Zarembo, 1977; Nachtmann & Gstrein, 1980; Ghebre-Sellassie, Hem & Knevel, 1982). In studies examining various aspects of the pharmacokinetics of benzylpenicillin, the usual method for assay of the drug has been microbiological (Schmidt & Roholt, 1966; McCarthy & Finland, 1960; Cole, Kenig & Hewitt, 1973; Kates et al, 1980). Microbiological assays are slow and also suffer from a lack of selectivity and low precision (Westerlund, Carlqvist & Theodorsen, 1979). HPLC has the advantage of being rapid, highly selective and usually more precise than microbiological assays. HPLC assays have been developed for a number of penicillins in biological fluids (Westerlund et al, 1979; Vree et al, 1978c; Thijssen, 1980; Uno et al, 1981; Teare et al, 1982) but only that of Westerlund et al (1979) assays benzylpenicillin. The method of Westerlund et al (1979) involves post-column derivatisation, a process which requires special equipment and techniques which are often not available. The method described below (section 4.2) describes a simple, rapid and selective HPLC assay for the

determination of benzylpenicillin in plasma and urine.

4.1.3: PHARMACOKINETICS

Benzylpenicillin is acid-labile and, thus, much of an orally administered dose is destroyed by the gastric secretions. Absorption following an oral dose is variable and unpredictable (Barza & Weinstein, 1976). Consequently, benzylpenicillin is now administered predominantly by parenteral means.

Once in the sytemic circulation, benzylpenicillin is widely distributed throughout the body with an apparent volume of distribution equivalent to about 50% of body water (Mandell & Sande, 1980). It is about 60% bound to plasma proteins (Barza & Weinstein, 1976).

Benzylpenicillin is rapidly eliminated from the body, mainly by the kidney where it is actively secreted into the renal tubule as well as filtered at the glomerulus. The active secretion can be blocked by administration of probenecid. Some metabolism occurs and Cole et al (1973) found approximately 19% of an intramuscularly administered dose was converted to penicilloic acid.

The disposition of benzylpenicillin apppears to be affected by posture or exercise. Schmidt & Roholt (1966) observed that the serum levels of benzylpenicillin, following intramuscular administration, were higher during ambulation

than during bedrest. Levy (1967) suggested that these differences could be due to posture-dependent alterations in certain physiological parameters such as renal blood flow. Kates et al (1980) studied the effects of prolonged recumbency on benzylpenicillin pharmacokinetics and found that seven days recumbency had no effect on the disposition of the drug. The effects of posture on the disposition of other penicillin have been studied. Roberts & Denton (1980) found that following an oral dose, serum amoxycillin levels were significantly higher during ambulation than during either bedrest or sleep. Pharmacokinetic analysis showed increased total serum clearance and renal clearance in supine subjects. Those results were attributed to a diminished renal blood flow when subjects are ambulant. Breiby et al (1983) administered ampicillin intravenously to subjects while sitting and while supine and found no changes in clearance but some differences in distribution with change in posture. It is of interest that the two studies in which the greatest changes in drug disposition were observed, were those in which the penicillins were administered extravascularly (Schmidt & Roholt, 1966; Roberts & Denton, 1980).

This study was undertaken to examine the effects of posture (bedrest and ambulation) on the pharmacokinetics of benzylpenicillin following both intravenous and intramuscular administration. Comparison of the resultant pharmacokinetics allows the effects of posture on absorption to be separated from the effects of posture on disposition. As Roberts & Denton (1980) reported no significant

differences between the pharmacokinetics of amoxycillin during bedrest and those during sleep, the disposition of benzylpenicillin during sleep was not evaluated in the study.

4.2: ANALYSIS

4.2.1: MATERIALS & METHODS

4.2.1.1: Chemicals

Benzylpenicillin was supplied by Commonwealth Serum Laboratories (Australia). Methanol and acetonitrile were specially purified for HPLC and supplied by Waters Associates (Milford, MA, USA). All other chemicals were analytical grade. The water used was de-ionized then glass distilled. Benzylpenicilloic acid, the major metabolite of benzylpenicillin, was prepared according to the method of Cole et al (1973).

4.2.1.2: HPLC instrumentation and conditions

Reversed-phase HPLC was performed using a Waters M-6000A solvent delivery system fitted with a U6K injector. A C_{18} µBondapak column (particle size 10 µm; 300 mm x 3.9 mm I.D; Waters Associates, Milford, MA, USA) was used in combination with a guard column (µBondapak C_{18}/Porasil B; Waters Associates, 23 mm x 3.9 mm I.D.) in all studies. UV absorbance at 214 nm was monitored with a Waters Model 441 UV absorption detector fitted with a Zinc lamp. The absorbance was recorded on a dual-channel Omniscribe

recorder (Houston Instruments, Austin, TX, USA). Injections were made with a 25 µl Hamilton syringe. The mobile phase was 0.015M phosphate buffer (pH 7.0 ± 0.1)-methanol (72:30) mixture. The flow rate was 1 ml/min.

4.2.1.3: Sample Preparation

Plasma and urine samples were processed by transferring a 200µ aliquot of the sample into a glass tube (disposable borosilicate glass culture tubes; 6 x 50 mm; Kimble, IL, USA) and adding an equal volume of acetonitrile. The sample was vortexed for 1 minute then centrifuged at 1500g for 5 min. An aliquot (20µl for plasma, 10µl for urine) of clear supernatant was injected onto the column.

4.2.1.4: Preparation of Standard Curves

Plasma standards were prepared at the start of each day by spiking drug-free plasma with known amounts of freshly prepared aqueous solution of benzylpenicillin to produce concentrations of 0.5-50 mg/l. The standards were then assayed in the described manner. Urine standards were prepared in a similar manner in drug-free urine to produce concentrations of 20-4000 mg/l. Standard curves were prepared by plotting the peak height of benzylpenicillin versus concentration.

4.2.2: RESULTS

Typical chromatograms of blank plasma and plasma from a subject following intravenous administration of benzylpenicillin are shown in Figure 4.1.

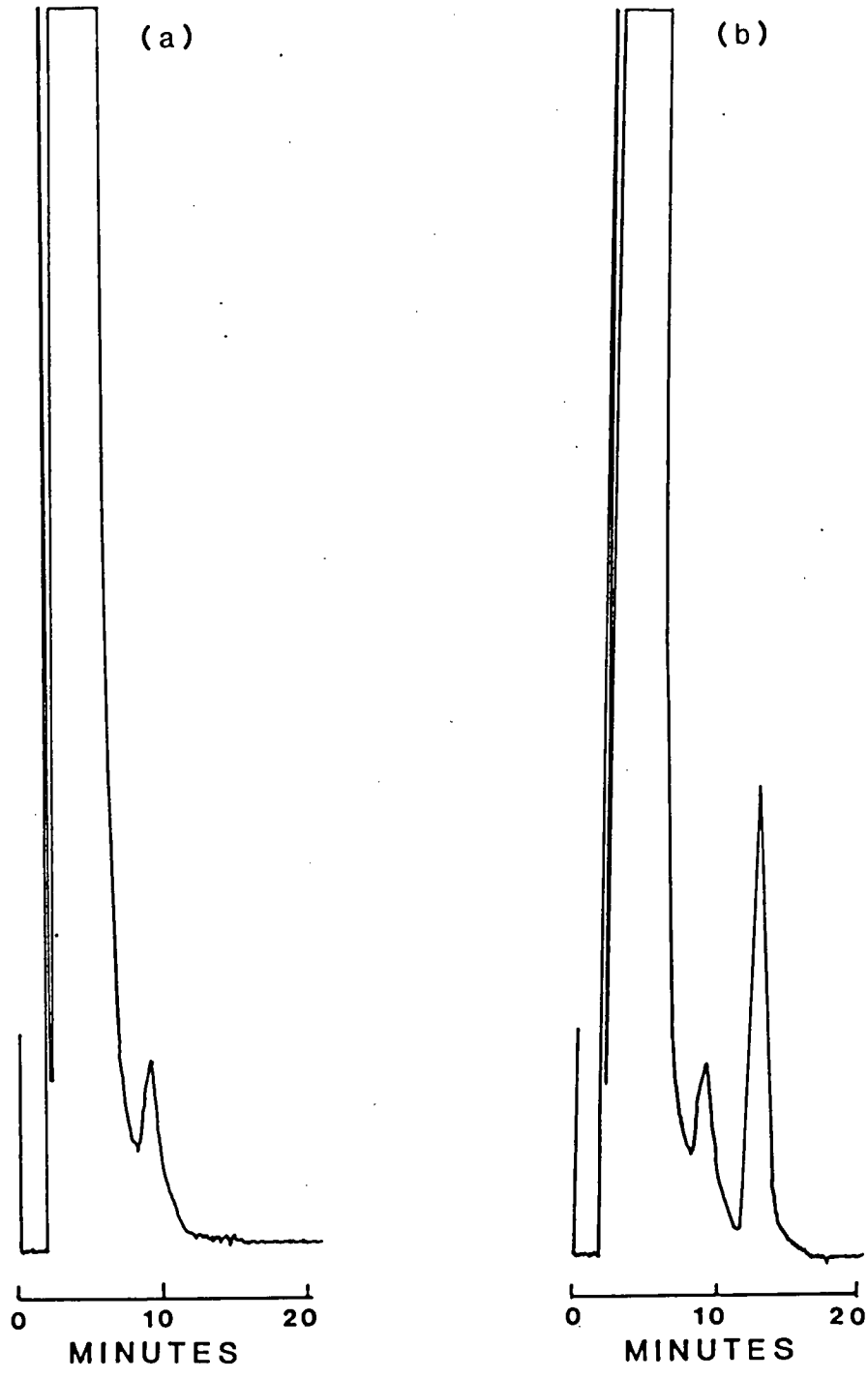

Figure 4.1 Chromatograms of (a) blank plasma and (b) plasma from the same subject 30 minutes after intravenous administration of 600 mg benzylpenicillin. The plasma concentration of benzylpenicillin is estimated to be 7.3 mg/l

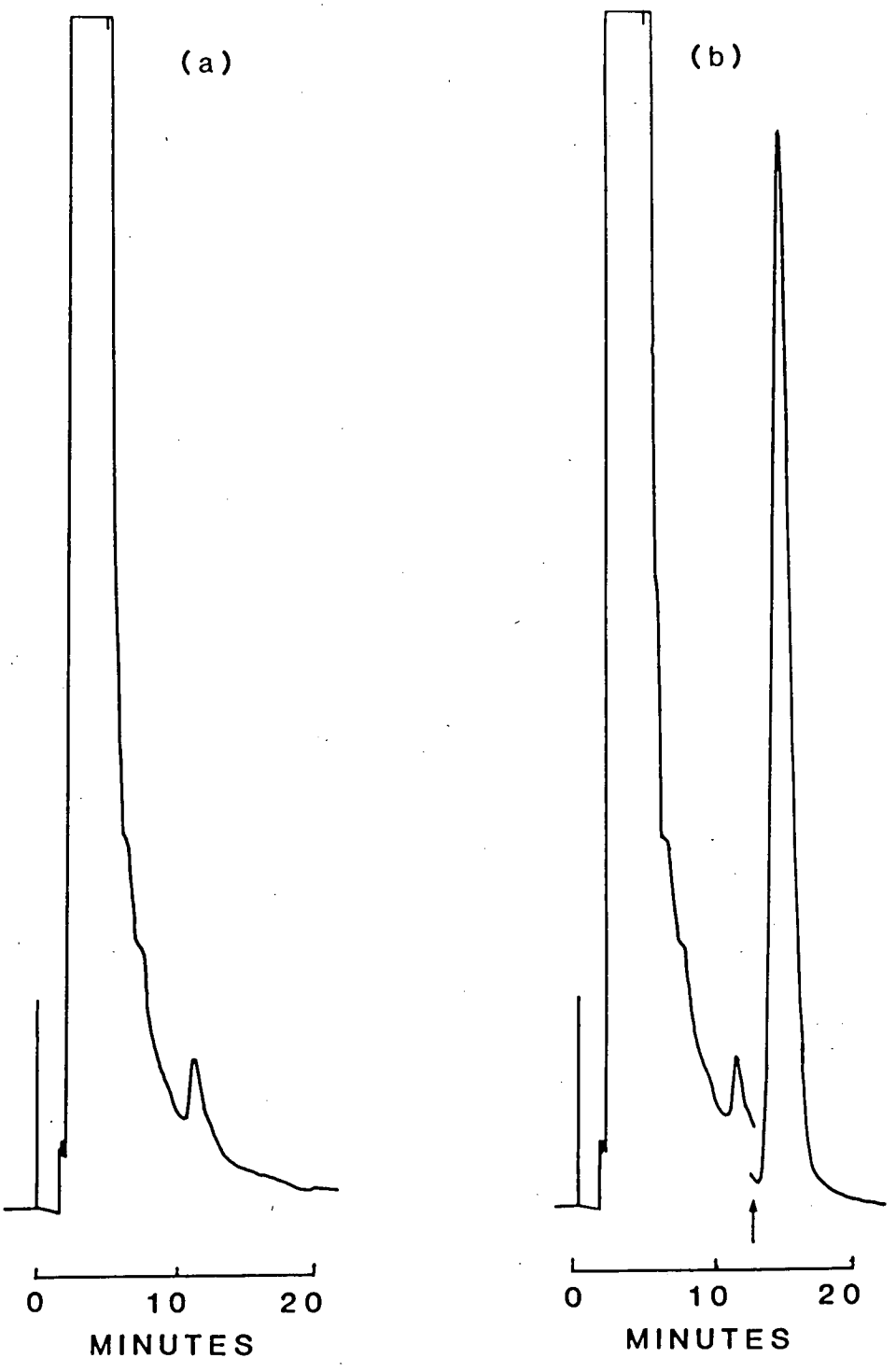

Figure 4.2 Chromatograms of (a) blank urine and (b) urine from the same subject collected from 0 - 8 hours after intravenous administration of 600 mg benzylpenicillin. The urine concentration of benzylpenicillin is estimated to be 1310 mg/l. The arrow indicates change in absorbance scale.

The retention time for benzylpenicillin is 12.4 minutes. Figure 4.2 shows chromatograms of blank urine and urine following administration of benzylpenicillin, from the same subject.

Over the concentration ranges studied (0-50 mg/l for plasma and 0-4000 mg/l for urine), linearity of response was found to be good ($r>0.99$) and consistently reproducible for standard curves based on peak height following injection of a known volume of supernatant (Figures 4.3 & 4.4).

Under the conditions of this assay, the detection limit for benzylpenicillin in plasma was 0.2 mg/l. 0.5 mg/l could be determined quite readily and reproducibly. In urine the detection limit was about 10 mg/l.

The intra- and inter-day reproducibility of the assay for plasma are shown in Table 4.1. The intra-day coefficients of variation for benzylpenicillin in urine at concentrations of 2000 mg/l, 200 mg/l and 20 mg/l were 2.74%, 1.69% and 8.5%, respectively ($n = 6$).

4.2.3: DISCUSSION

Several HPLC assays for benzylpenicillin in non-biological fluids have used UV detection at 254 nm (Ghebre-Sellassie et al, 1982; White et al, 1977; Blaha et al, 1976). It was found using the present system that a greater than twenty-fold increase in sensitivity could be achieved by monitoring the effluent at 214 nm rather than at 254 nm without increased interference or baseline noise. UV detection in

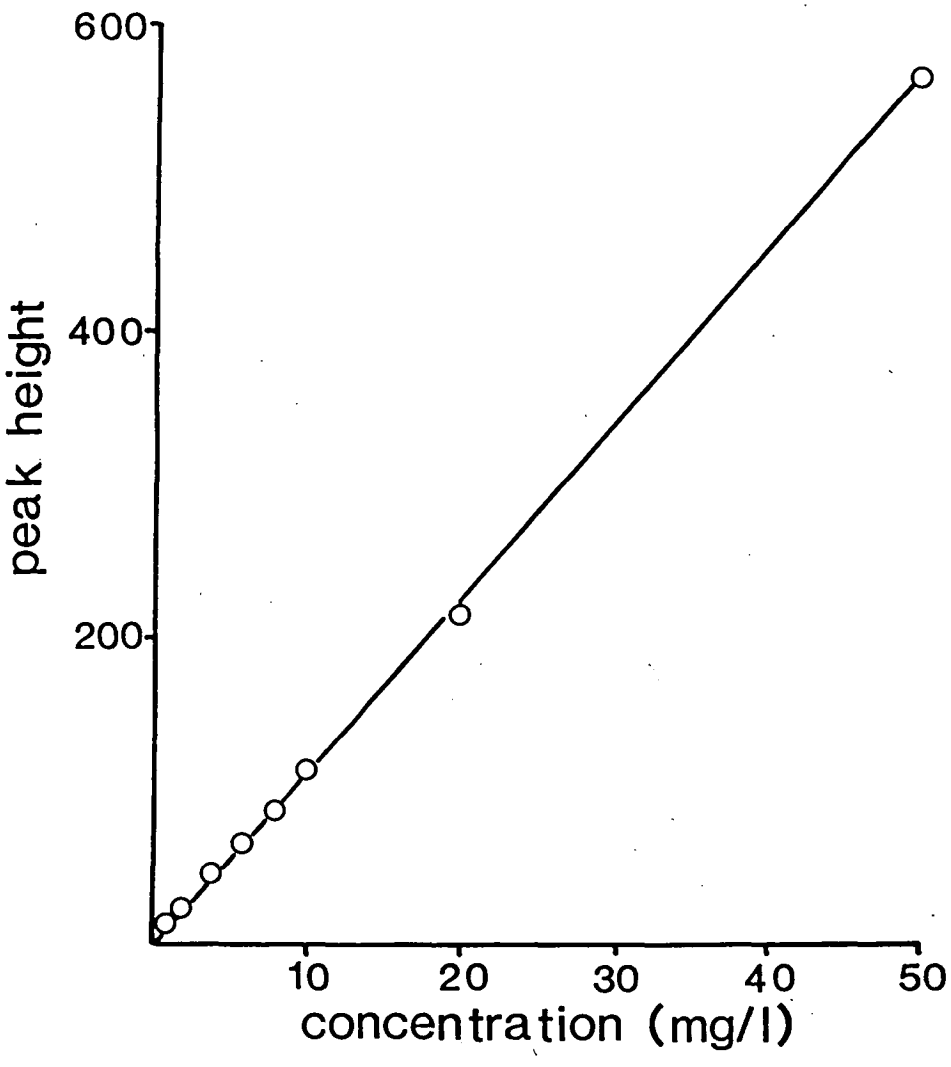

Figure 4.3 Standard curve for benzylpenicillin in plasma

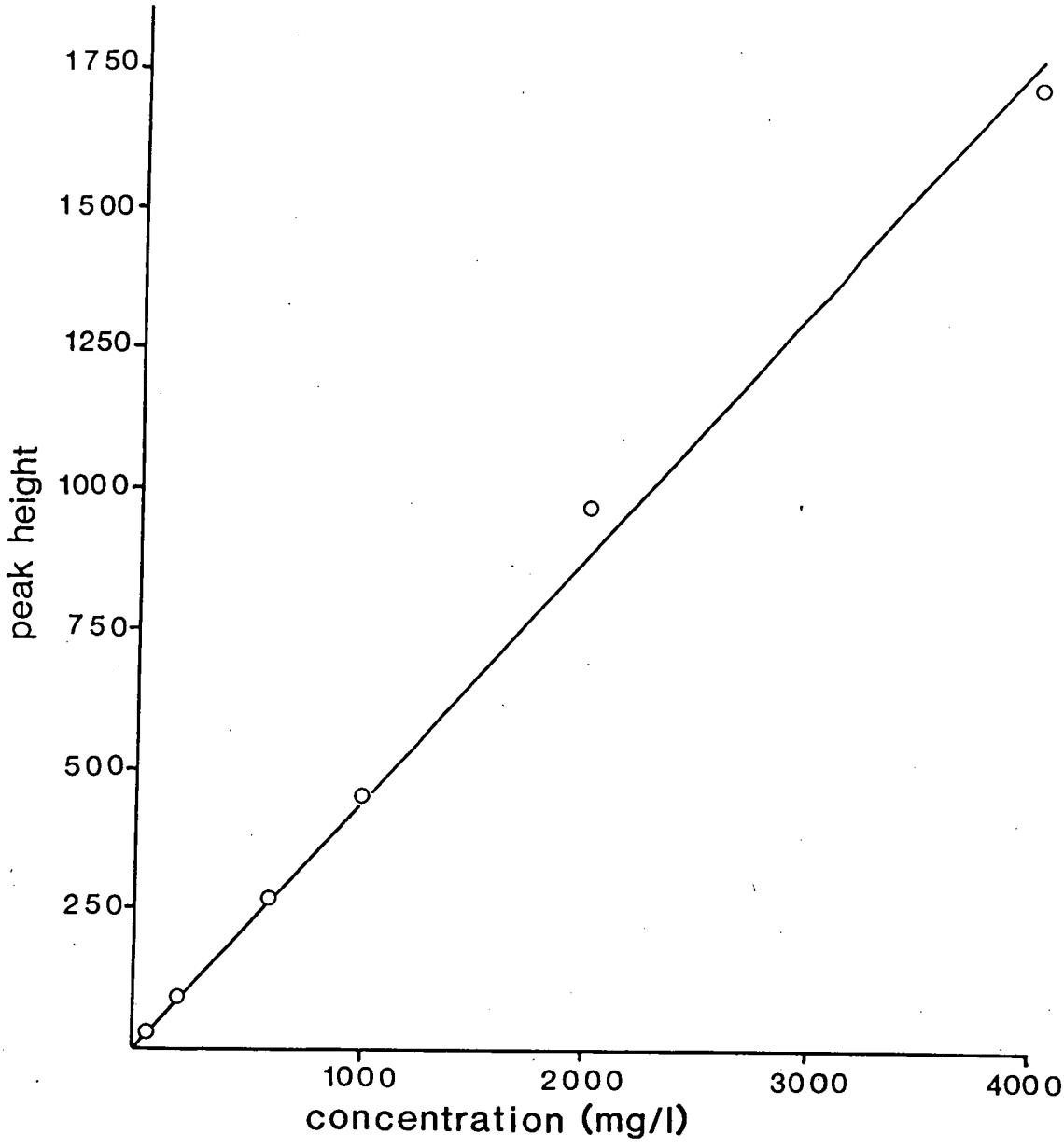

Figure 4.4 Standard curve for benzylpenicillin in urine

Table 4.1 Intra- and inter-day variation of benzylpenicillin in plasma.

Concentration mg/l	Intra-day CV(%) (n=6)	Inter-day CV(%) (n=5, over 2 weeks)
20	2.53	3.59
10	2.26	4.89
4	1.69	5.81
1	7.30	9.16

the range of 210-230 nm has been used by other workers (Vree et al, 1978c; Thijssen, 1980; Teare et al, 1982; Uno et al, 1981) to quantitate various other penicillins in biological fluids.

A number of agents may be used to precipitate plasma protein. In HPLC assays for other penicillins, perchloric acid (Vree et al, 1978c), trichloroacetic acid (Westerlund et al, 1979) and acetonitrile (Teare et al, 1982) have been used. In the present study, acetonitrile was found to completely precipitate plasma protein and provided the cleanest chromatogram. Acetonitrile was also added to urine samples to reduce the number of endogenous peaks. The usual method for the determination of benzylpenicillin in biological fluids is by microbiological means. Such assays have a low precision with an experimental error of ± 15% and their selectivity is reduced if active metabolites or other compounds with antibacterial activity are present (Westerlund et al, 1979). Except at plasma benzylpenicillin concentrations of 1 mg/l or less, the present assay had an intra-day coefficient of variation of less than 5% and the inter-day coefficient of variation was less than 10% for all concentrations (Table 4.1). The inter-day coefficient of variation could be reduced to that of the intra-day by preparing standard curves on each day of assay. The selectivity of this method was studied by measuring the retention times of other common penicillins. Those times are shown in Table 4.2. Modification of the polarity of the mobile phase could make this system suitable for the assay of these compounds.

Table 4.2 Retention times of various penicillins

Penicillin	Retention time (minutes)
Ampicillin	6.0
Methicillin	9.2
Benzylpenicillin	12.4
Amoxycillin	14.4
Oxacillin	26.0
Cloxacillin	37.2
Flucloxacillin	40.0

This method was used in the following study to follow the disposition of benzylpenicillin administered intravenously and intramuscularly. The plasma benzylpenicillin-time profiles for volunteers following intravenous administration showed levels similar to those obtained by Kates et al (1980) following intravenous administration of the same dose but using a microbiological assay.

In summary, this HPLC assay provides a selective, reliable and reproducible method for the rapid determination of benzylpenicillin in plasma and urine. The method does not require time-consuming or complex extraction or derivatisation techniques.

4.3: PHARMACOKINETIC STUDY

4.3.1: CLINICAL PROTOCOL

4.3.1.1: Subjects

Eight healthy, young adult volunteers (four male, four female) aged 21-23 years, participated in the study. Prior to the investigation, a detailed medical history was taken with particular attention to possible penicillin allergy. Subjects with a history of drug allergy, eczema and hayfever were excluded from the study. No subjects gave a history of liver or renal disease. Physical examination, haematological tests and tests for liver and renal function

showed no abnormality. No other drugs were taken during the two weeks preceding the study or during the study. Informed consent was obtained from each subject and approval from the Human Ethics Committee of the University of Tasmania was obtained before commencing the study.

4.3.1.2: Procedure

Each subject was given 600mg benzylpenicillin on four separate occasions - by intravenous administration during bedrest and during ambulation and by intramuscular administration during bedrest and during ambulation. To avoid any possible variations in physiological parameters due to food intake the studies were commenced after an overnight fast and no food was permitted for two hours after dosing. At least one week elapsed between each study. The investigation was conducted in a cross-over design and each subject served as its own control. The dose was administered after the subjects had been either lying or standing for one hour. Changes in posture were not allowed throughout each phase of the study, with ambulant subjects being allowed to sit only for short periods. As caffeine appeared to interfere with the assay for benzylpenicillin, subjects refrained from caffeine-containing beverages overnight before each study and during each study.

4.3.1.3: Sample collection

An indwelling catheter was inserted into an antecubetal vein, approximately one hour before dosing. During the intravenous phases of the study the cannulae were inserted into the opposite arm to that used for dosing. Patency of

the cather was maintained by flushing with a small volume of heparinised saline (10 IU/ml) after each blood sample was collected. 5ml blood samples were drawn at 0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 240 minutes after dosing. To avoid contamination of the blood sample with heparinised saline, the first 1.0ml of blood was drawn separately and discarded. All samples were placed into heparinised tubes and immediately centrifuged. The plasma was stored at -20°C until assayed.

All urine excreted by the subjects 0-8 hours and 8-24 hours was collected, measured and an aliquot stored at -20°C until assayed. Samples of serum and urine were assayed for benzylpenicillin as soon as possible after collection.

4.3.1.4: Pharmacokinetic and statistical analysis

Intravenous data

The plasma benzylpenicillin concentration (C) versus time (t) data, obtained following intravenous administration of the drug, were fitted to a biexponential equation of the following form:

$$C = Ae^{-\alpha t} + Be^{-\beta t} \qquad (4.1)$$

corresponding to a 2-compartment open model. All data were fitted using a non-linear least-squares regression computer program (FUNFIT). The calculated values of A, B, α and β were used to estimate the half-life of the β-phase ($t_{\frac{1}{2}\beta}$), body clearance (CL_B), apparent volume of distribution (V),

volumes of the central compartment (V_c) and tissue compartment (V_t), distribution rate constants (k_{12} and k_{21}) and the elimination rate constant (K_{10}).

The area under the concentration-time curve (AUC) used in the compartmental analysis was calculated using the regression parameters from Equation 4.1.

$$AUC = A/\alpha + B/\beta \qquad (4.2)$$

whereas the AUC and AUMC used in statistical moment analysis were calculated by the trapezoidal rule with appropriate correction for the partial area from the final data point to infinity based on the slope of the terminal exponential phase (Gibaldi & Perrier, 1982b). The mean residence time (MRT) is given by (Gibaldi & Perrier, 1982b)

$$MRT = \frac{AUMC}{AUC} = \frac{{}_0\int^{\infty} tCdt}{{}_0\int^{\infty} Cdt} \qquad (4.3)$$

The renal clearance of benzylpenicillin (CL_R) was estimated from the ratio of amount of unchanged drug excreted in the urine to infinite time (X_u^{∞}) to the area under the plasma benzylpenicillin concentration versus time curve (AUC) (Equation 4.4). For this calculation it was assumed that urine collected over 24 hours was equivalent to infinite time.

$$CL_R = \frac{X_u^{\infty}}{AUC} \qquad (4.4)$$

The dependence of renal clearance on flow was examined using the "well-stirred" model for clearance (Gibaldi & Perrier 1982a) and the corresponding equation given by Roberts & Denton (1980).

$$CL_R = \frac{f_s . CL_{int} . Q_R}{f_s . CL_{int} + Q_R} \qquad (4.5)$$

where f_s is the fraction of unbound drug, CL_{int} is the renal intrinsic clearance and Q_R is the effective renal blood flow. As benzylpenicillin excretion is primarily by tubular secretion and is largely independent of protein binding (Craig and Welling, 1977) the term f_s is approximated to unity. Equation 4.5 was used to examine the dependence of renal benzylpenicillin clearance on renal blood flow changes induced by posture.

Intramuscular data

The overall elimination rate constant (β) was determined from the slope of the terminal linear segment of a semi-logarithmic plot of plasma concentration versus time. The elimination half-life ($t_{\frac{1}{2}}$) was then calculated in the usual manner ($t_{\frac{1}{2}} = 0.693/\beta$).

The area under the plasma benzylpenicillin concentration versus time curve (AUC) was determined using the trapezoidal rule with appropriate correction for the partial area from the final data point to infinity based on the slope of the terminal exponential phase (Gibaldi & Perrier, 1982b). Since benzylpenicillin was administered intramuscularly in

the present study, its apparent volume of distribution could not be estimated directly. The ratio of the apparent volume of distribution for benzylpenicillin (V) to its extent of absorption into the systemic circulation (F) was calculated according to Equation 4.6.

$$V/F = \frac{D}{\beta . AUC} \qquad (4.6)$$

where D is the administered dose and AUC is the area under the plasma benzylpenicillin concentration versus time curve.

For each subject, the area under the plasma benzylpenicillin concentration versus time curve following intramuscular administration (AUC_{im}) was compared with the area under the plasma benzylpenicillin concentration versus time curve following intravenous administration of the same dose (AUC_{iv}). From this comparison, the bioavailability of benzylpenicillin from the intramuscular site could be determined.

$$F = \frac{AUC_{im}}{AUC_{iv}} \qquad (4.7)$$

By substitution of F into Equation 4.6, the apparent volume of distribution for each subject was determined. The bioavailability of benzylpenicillin was also used to calculate the total body clearance (CL_B) of benzylpenicillin

according to Equation 4.8.

$$CL_B = \frac{F.D}{AUC} \qquad (4.8)$$

The renal clearance of benzylpenicillin, following intramuscular administration, was calculated according to Equation 4.4 and the dependence of the renal clearance on renal plasma flow was examined using Equation 4.5.

Using data from the intravenous dosing phases of this study, the fraction remaining to be absorbed at various times was determined according to the Loo-Riegelman method (Loo & Riegelman, 1968). The rate of absorption was calculated from linear regression of these data. Extrapolation of the linear regression of the data allowed the lag time for absorption to be determined. The lag time was taken to be the time at which the fraction remaining to be absorbed equalled 1.00. The peak time (corrected for lag time) was also used to reflect the rate of absorption of benzylpenicillin.

The non-compartmental pharmacokinetic parameter, mean residence time (MRT), based on statistical moment theory, was calculated from the AUC and AUMC and is given by Equation 4.3. The mean absorption time (MAT) was determined from Equation 4.9 (Riegelman & Collier, 1980) for the seven subjects using the mean residence times determined from both intramuscular and intravenous data.

$$MAT = MRT_{im} - MRT_{iv} \quad (4.9)$$

The mean urinary flow rate for each subject was calculated from the urine volume collected during the first eight hours after drug administration.

Statistical comparison of the effect of bedrest and ambulation on various pharmacokinetic parameters was made by Wilcoxon's test for paired observation.

4.3.2: RESULTS

The mean plasma benzylpenicillin levels versus time profiles for bedrest and ambulation following intravenous administration of benzylpenicillin are shown in Figure 4.5. Due to problems with venous spasm preventing blood sample collection from one subject, these profiles represent the data of only seven subjects. Although at all times the mean plasma benzylpenicillin concentrations were lower during bedrest than during ambulation, these concentrations were not significantly different ($p>0.05$).

Following intramuscular administration of the drug, the plasma benzylpenicillin concentrations during bedrest were, again, lower at all times, except at 10 minutes after dosing, than during ambulation (Figure 4.6). The concentrations were only significantly different at 45

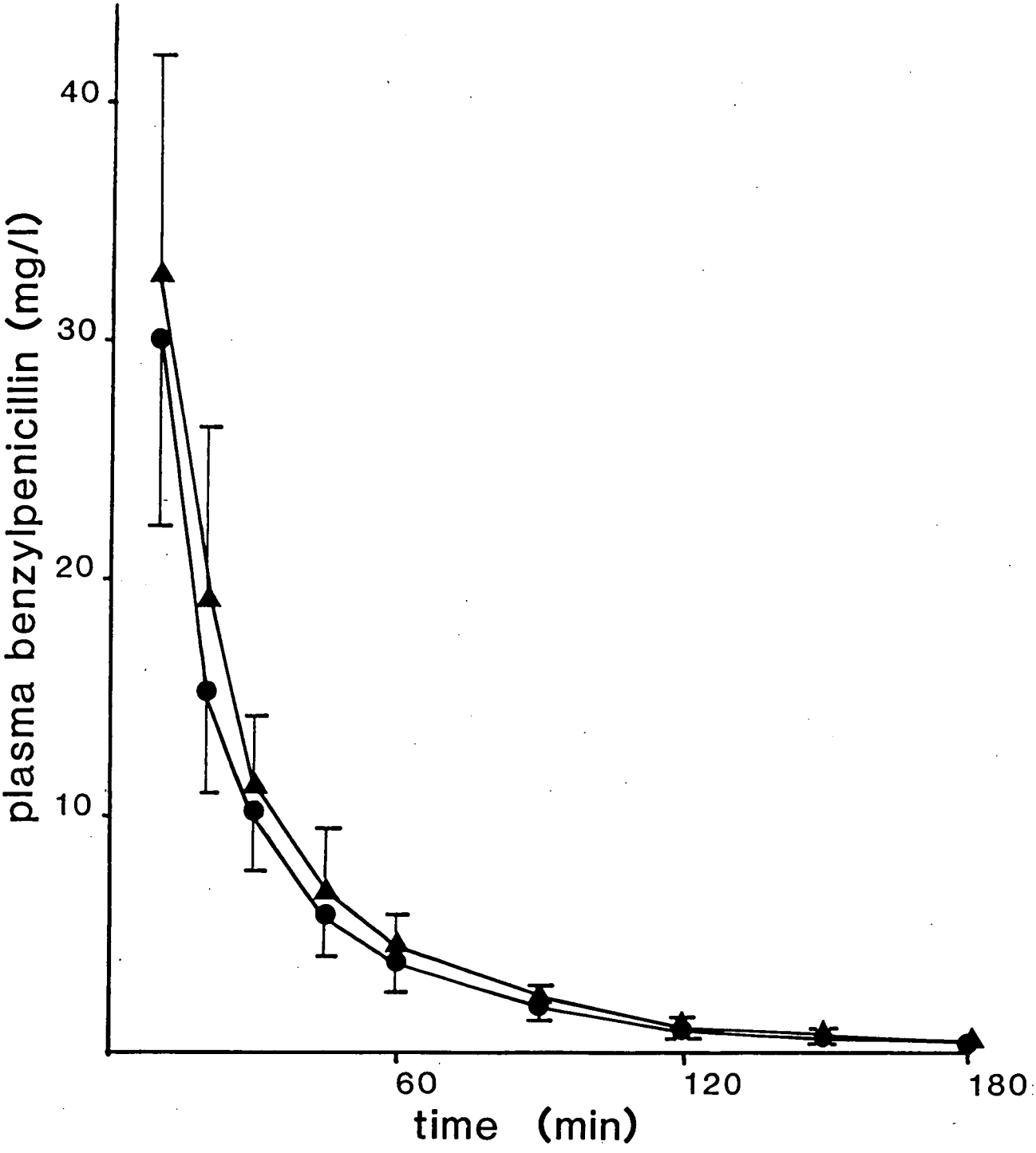

Figure 4.5 Mean (±SD) plasma benzylpenicillin concentrations found in seven healthy subjects during Δ ambulation and O bedrest following intravenous administration of 600 mg benzylpenicillin

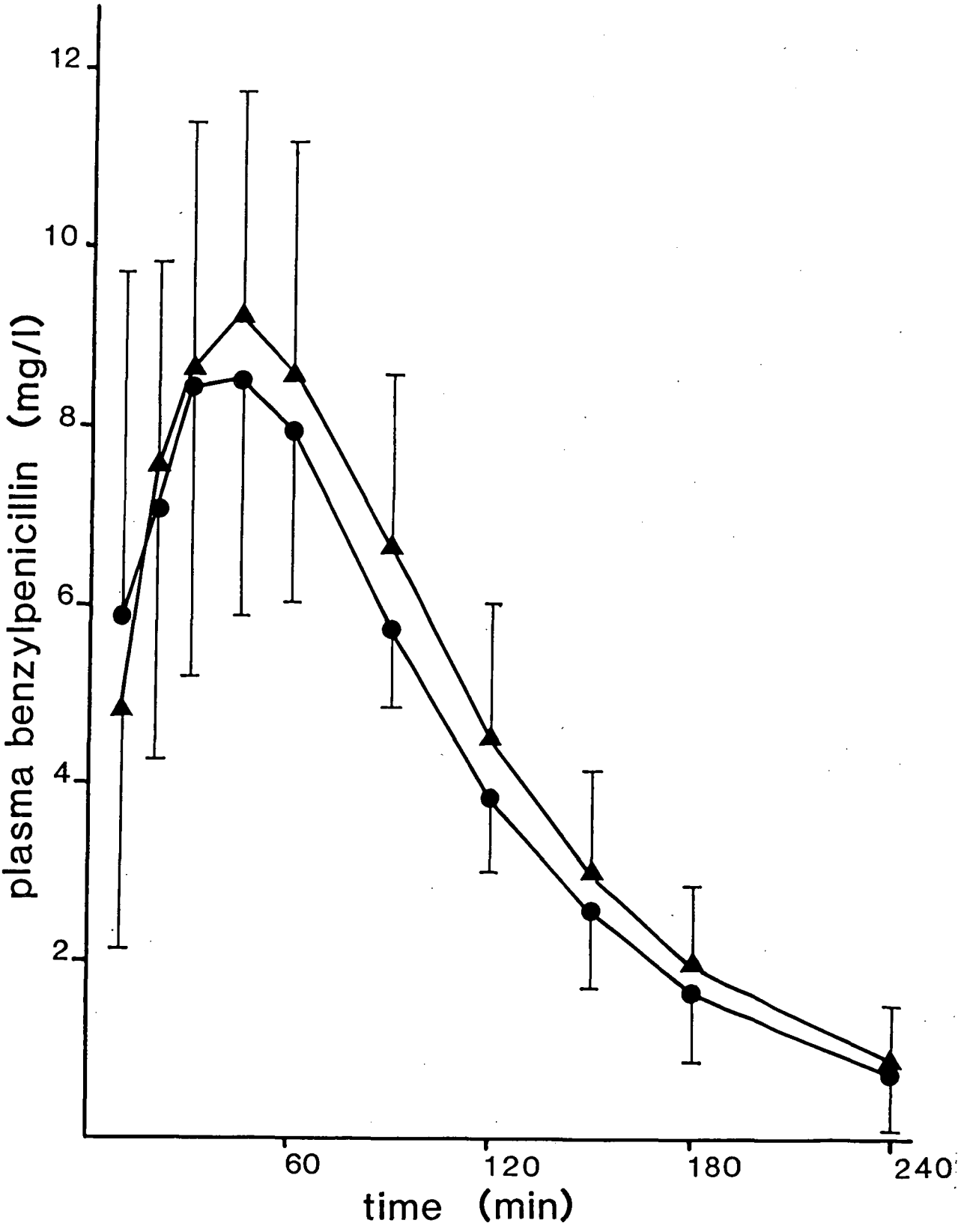

Figure 4.6 Mean (±SD) plasma benzylpenicillin concentrations found in eight healthy volunteers during Δ ambulation and O bedrest following intramuscular administration of 600 mg benzylpenicillin

minutes post-dosing ($p<0.05$).

The mean experimental and pharmacokinetic data for each of the parameters determined during bedrest and ambulation, following both intravenous and intramuscular dosing, are shown in Tables 4.3 and 4.4, respectively. Individual experimental and pharmacokinetic data are presented in Tables 4.5 (i.v., ambulation), 4.6 (i.v., bedrest), 4.7 (i.m., ambulation) and 4.8 (i.m., bedrest).

There was no significant difference between the values obtained during bedrest and ambulation for any of the pharmacokinetic parameters determined when the dose was administered intravenously.

In the pharmacokinetic analysis of the data obtained following intramuscular administration of benzylpenicillin, several of the pharmacokinetic parameters required the use of data from the intravenous-dosing phase of the study. The results for those parameters represent only the seven subjects for whom complete intravenous data was available. Comparison of bedrest and ambulation values of the pharmacokinetic parameters determined after intramuscular dosing showed that the area under the plasma benzylpenicillin concentration versus time curve (AUC_{im}) for ambulation was significantly larger than the AUC_{im} for bedrest ($p<0.05$). Otherwise there were no significant differences between any of the pharmacokinetic parameters determined from the intramuscular data.

Table 4.3 Comparison of mean estimates (±SD) of pharmacokinetic data for benzylpenicillin in seven subjects following intravenous administration of the dose (from Tables 4.5 & 4.6)

Parameter[a]	Ambulation	Bedrest
k_{12} ($10^{-2}min^{-1}$)	1.34 ± 0.73	1.74 ± 0.89
k_{21} ($10^{-2}min^{-1}$)	2.39 ± 1.19	2.43 ± 0.99
k_{10} ($10^{-2}min^{-1}$)	4.58 ± 1.23	4.98 ± 1.03
AUC (min.mg/l)	1278 ± 273	1153 ± 262
V (l/kg)	0.58 ± 0.41	0.73 ± 0.76
V_c (l/kg)	0.17 ± 0.07	0.17 ± 0.06
$t_{½\beta}$ (min)	53.6 ± 32.3	54.2 ± 36.1
CL_B (ml/min)	487.4 ± 100.5	543.6 ± 122.6
CL_R (ml/min)	309.4 ± 93.4	324.1 ± 145.3
CL_{NR} (ml/min)	178.0 ± 51.3	218.2 ± 66.5
f	0.63 ± 0.12	0.58 ± 0.17
MRT (min)	36.23 ± 13.45	35.27 ± 10.21

[a] k_{12} and k_{21} - apparent first-order intercompartmental distribution rate constants; k_{10} - apparent first order elimination rate constant; $t_{½\beta}$ - elimination half-life; AUC - area under the plasma concentration-time curve; V - apparent volume of distribution; V_c - volume of the central compartment; CL_B - total body clearance; f - fraction of the dose excreted unchanged in the urine; CL_R - renal clearance; CL_{NR} - nonrenal clearance; MRT - mean residence time

Table 4.4 comparison of mean estimates (±SD) of pharmacokinetic data for benzylpenicillin in eight subjects following intramuscular administration of the dose (from Tables 4.7 & 4.8)

Parameter[a]	Ambulation	Bedrest
$t_{½\beta}$ (min)	52.5 ± 11.7	50.9 ± 19.3
AUC[c] (min.mg/l)	1175 ± 256	1032 ± 204
V/F (l/kg)	0.63 ± 0.22	0.64 ± 0.19
F[b]	0.93 ± 0.11	0.95 ± 0.10
V[b] (l/kg)	0.55 ± 0.18	0.62 ± 0.25
CL_B[b] (ml/min)	487.6 ± 100.9	544.0 ± 122.7
f	0.46 ± 0.12	0.55 ± 0.09
CL_R (ml/min)	264.3 ± 99.7	314.6 ± 87.4
CL_{NR}[b] (ml/min)	233.6 ± 81.3	236.9 ± 108.4
MRT (min)	100.9 ± 21.4	96.7 ± 29.8
MAT[b] (min)	65.7 ± 33.3	63.8 ± 32.3

[a] $t_{½\beta}$ - elimination half-life; AUC - area under the plasma concentration-time curve; V/F - apparent volume of distribution (V) divided by the fraction of dose absorbed (F); F - fraction of the dose absorbed; V - apparent volume of distribution; CL_B - total body clearance; f - fraction of the dose excreted unchanged in the urine; CL_R - renal clearance; CL_{NR} - nonrenal clearance; MRT - mean residence time; MAT - mean absorption time

[b] n = 7

[c] $p<0.05$

Table 4.5 Experimental and pharmacokinetic data describing the disposition of benzylpenicillin in ambulant subjects following intravenous administration of the dose.

Parameter[a]	Subject						
	AU	HW	AC	BM	CJ	SB	CL
Sex	F	F	F	M	M	M	M
Age	21	23	21	21	22	21	22
Weight (kg)	54	60	67	70	72	68	75
k_{12} ($10^{-2}min^{-1}$)	0.70	2.37	1.18	1.35	0.54	2.28	0.94
k_{21} ($10^{-2}min^{-1}$)	1.27	3.92	2.73	3.72	0.71	1.97	2.43
k_{10} ($10^{-2}min^{-1}$)	3.78	5.62	5.02	4.38	2.76	6.47	4.05
AUC (min.mg/l)	1721	986	1190	1333	974	1520	1224
V (l/kg)	0.64	0.44	0.38	0.28	1.48	0.43	0.38
V_c (l/kg)	0.17	0.18	0.15	0.15	0.31	0.09	0.16
$t_{½\beta}$ (min)	68.4	30.2	35.1	30.5	120.2	50.8	40.0
CL_B (ml/min)	348.7	608.5	504.0	450.3	615.9	394.8	490.0
CL_R (ml/min)	157.2	423.0	373.2	313.0	386.3	275.7	237.7
CL_{NR} (ml/min)	191.5	185.5	130.8	137.3	229.6	119.1	252.3
f	0.45	0.70	0.74	0.70	0.63	0.70	0.48
MRT (min)	39.77	27.20	26.73	29.80	65.03	31.32	33.78

[a] Parameters as defined in Table 4.3

Table 4.6 Experimental and pharmacokinetic data describing the disposition of benzylpenicillin in bedrested subjects following intravenous administration of the dose.

Parameter[a]	Subject						
	AU	HW	AC	BM	CJ	SB	CL
Sex	F	F	F	M	M	M	M
Age	21	23	21	21	22	21	22
Weight (kg)	54	60	67	70	72	68	75
k_{12} ($10^{-2}min^{-1}$)	0.88	0.94	2.12	2.10	3.15	2.17	0.80
k_{21} ($10^{-2}min^{-1}$)	2.32	0.64	3.56	2.87	2.79	3.14	1.69
k_{10} ($10^{-2}min^{-1}$)	4.04	4.53	6.20	5.50	5.36	5.85	3.37
AUC (min.mg/l)	1573	796	1414	1139	1001	1127	1021
V (l/kg)	0.42	2.43	0.27	0.41	0.54	0.39	0.64
V_c (l/kg)	0.17	0.28	0.10	0.14	0.16	0.13	0.23
$t_{½\beta}$ (min)	41.1	133.8	30.0	38.0	45.4	34.5	56.3
CL_B (ml/min)	381.4	753.7	424.2	526.5	599.4	532.3	587.6
CL_R (ml/min)	229.7	527.5	129.5	285.4	427.2	220.3	449.3
CL_{NR} (ml/min)	151.7	226.2	294.7	241.1	172.2	303.0	138.3
f	0.60	0.70	0.31	0.54	0.71	0.41	0.76
MRT (min)	33.29	52.84	24.10	29.22	38.41	27.16	42.90

[a] Parameters as defined in Table 4.3

Table 4.7 Experimental and pharmacokinetic data describing the disposition of benzylpenicillin in ambulant subjects following intramuscular administration of the dose.

Parameter[a]	Subject							
	AU	HW	AC	EM	BM	CJ	SB	CL
Sex	F	F	F	F	M	M	M	M
Age	21	23	21	21	21	22	21	22
Weight (kg)	54	60	67	73	70	72	68	75
$t_{½\beta}$ (min)	49.2	65.4	43.2	54.0	52.8	31.8	68.4	55.2
AUC (min.mg/l)	1600	920	1325	970	1434	921	1175	1053
V/F (l/kg)	0.49	1.02	0.44	0.83	0.46	0.43	0.75	0.61
F	0.93	0.93	1.06	-	1.06	0.93	0.77	0.85
V (l/kg)	0.46	0.95	0.47	-	0.49	0.40	0.58	0.52
CL_B (ml/min)	347.5	606.9	504.8	-	450.5	619.2	396.9	487.6
f	0.28	0.63	0.38	0.43	0.44	0.47	0.62	0.46
CL_R (ml/min)	106.2	409.0	178.7	336.8	188.7	314.3	317.6	263.5
CL_{NR} (ml/min)	241.3	197.9	326.1	-	261.8	304.9	79.3	224.1
MRT (min)	101.3	114.3	88.2	94.01	97.3	64.9	139.0	108.1
MAT (min)	61.5	87.1	61.5	-	67.5	0.08	107.7	74.3

[a] Parameters as defined in Table 4.4

Table 4.8 Experimental and pharmacokinetic data describing the disposition of benzylpenicillin in bedrested subjects following intramuscular administration of the dose.

Parameter[a]	Subject							
	AU	HW	AC	EM	BM	CJ	SB	CL
Sex	F	F	F	F	M	M	M	M
Age	21	23	21	21	21	22	21	22
Weight (kg)	54	60	67	73	70	72	68	75
$t_{½\beta}$ (min)	33.6	46.8	51.6	36.6	42.6	35.4	76.2	84.6
AUC (min.mg/l)	1299	838	1254	720	1060	877	1097	1110
V/F (l/kg)	0.41	0.80	0.53	0.61	0.50	0.49	0.88	0.88
F	0.83	1.06	0.89	-	0.93	0.87	0.97	1.09
V (l/kg)	0.34	0.85	0.47	-	0.47	0.43	0.85	0.96
CL_B (ml/min)	380.7	755.0	425.6	-	529.4	596.8	531.4	588.8
f	0.51	0.49	0.58	0.44	0.63	0.72	0.46	0.53
CL_R (ml/min)	235.6	350.9	275.9	367.3	252.7	496.6	254.0	283.6
CL_{NR} (ml/min)	145.1	404.1	149.7	-	276.7	100.2	277.4	305.2
MRT (min)	68.3	93.7	100.9	78.8	73.9	74.8	135.8	147.0
MAT (min)	35.0	40.9	76.8	-	44.7	36.4	108.6	104.1

[a] Parameters as defined in Table 4.4

Examination of the absorption profile for benzylpenicillin following intramuscular administration, using the Loo-Riegelman method, failed to show any differences between the rate of absorption during bedrest and during ambulation (Figure 4.7). Table 4.9 gives the mean for peak concentration (C_p), the time to peak concentration (t_p),the lag time for absorption (t_L), the time to reach peak plasma benzylpenicillin concentration corrected for lag time (t_{corr}) and the rate constant for absorption (k_a). In the determination of several of these parameters, data from the intravenous dosing phases of the study were utilized, so, for those parameters the values in Table 4.9 are the means for only seven subjects. There were no significant differences between ambulation and bedrest for any of these parameters.

It was found that the urinary flow rate during bedrest (mean ± SD: 1.56 ± 0.32 ml/min) was significantly greater than during ambulation (mean ± SD: 0.98 ± 0.40 ml/min) ($p<0.05$) in the intravenous phases of the study. However, the renal clearance of benzylpenicillin was not related to urine flow rate ($r = 0.172$, $p>0.5$) (Figure 4.8). In the intramuscular dosing phases of this study, there was no significant difference between the urinary flow rate during ambulation (mean ± SD: 0.84 ± 0.11 ml/min) and the urinary flow rate during bedrest (mean± SD: 0.88 ± 0.37 ml/min). Again, the renal clearance of benzylpenicillin was not related to urine flow rate ($r = 0.004$, $p>0.5$).

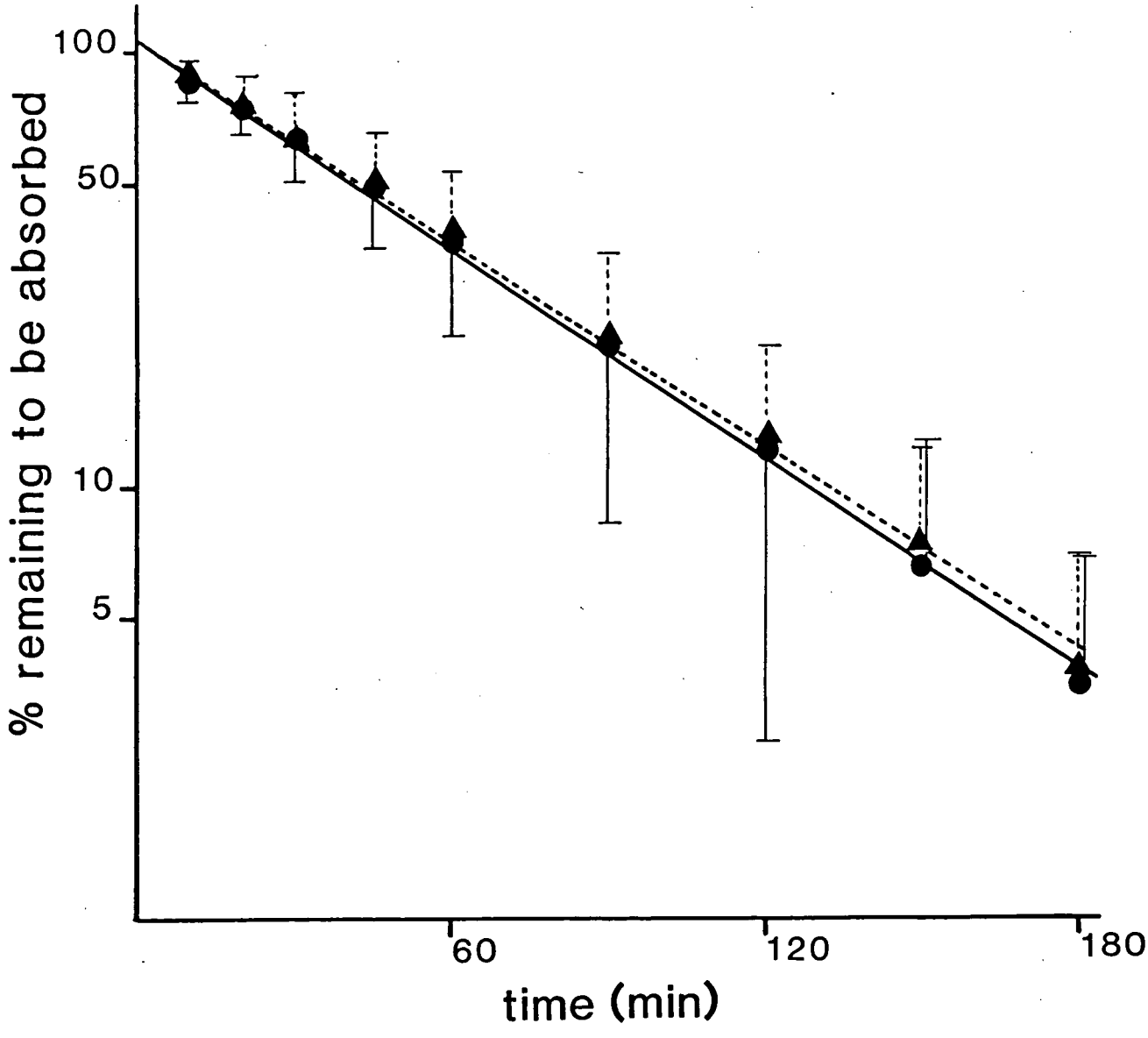

Figure 4.7 Fraction of the dose remaining to be absorbed versus time following intramuscular administration of 600 mg benzylpenicillin into the buttock during Δ ambulation and O bedrest

Table 4.9 Mean (±SD) pharmacokinetic data describing the absorption of benzylpenicillin following intramuscular administration of the drug

Parameter[a]	Ambulation	Bedrest
Cp (mg/l)	9.59 ± 2.55	8.76 ± 2.92
tp (min)[b]	42.9 ± 9.6	35.7 ± 15.1
t_L (min)[b]	6.40 ± 4.51	7.70 ± 3.6
t_{corr} (min)[b]	36.5 ± 7.7	28.0 ± 13.4
k_a (min^{-1})[b]	0.021 ± 0.010	0.0228 ± 0.009

[a] Cp - peak plasma concentration of benzylpenicillin; tp - time to Cp following intramuscular administration of benzylpenicillin; t_L - lag time; t_{corr} - time to reach peak plasma benzylpenicillin concentration corrected for lag time; k_a - rate constant for absorption

[b] n = 7

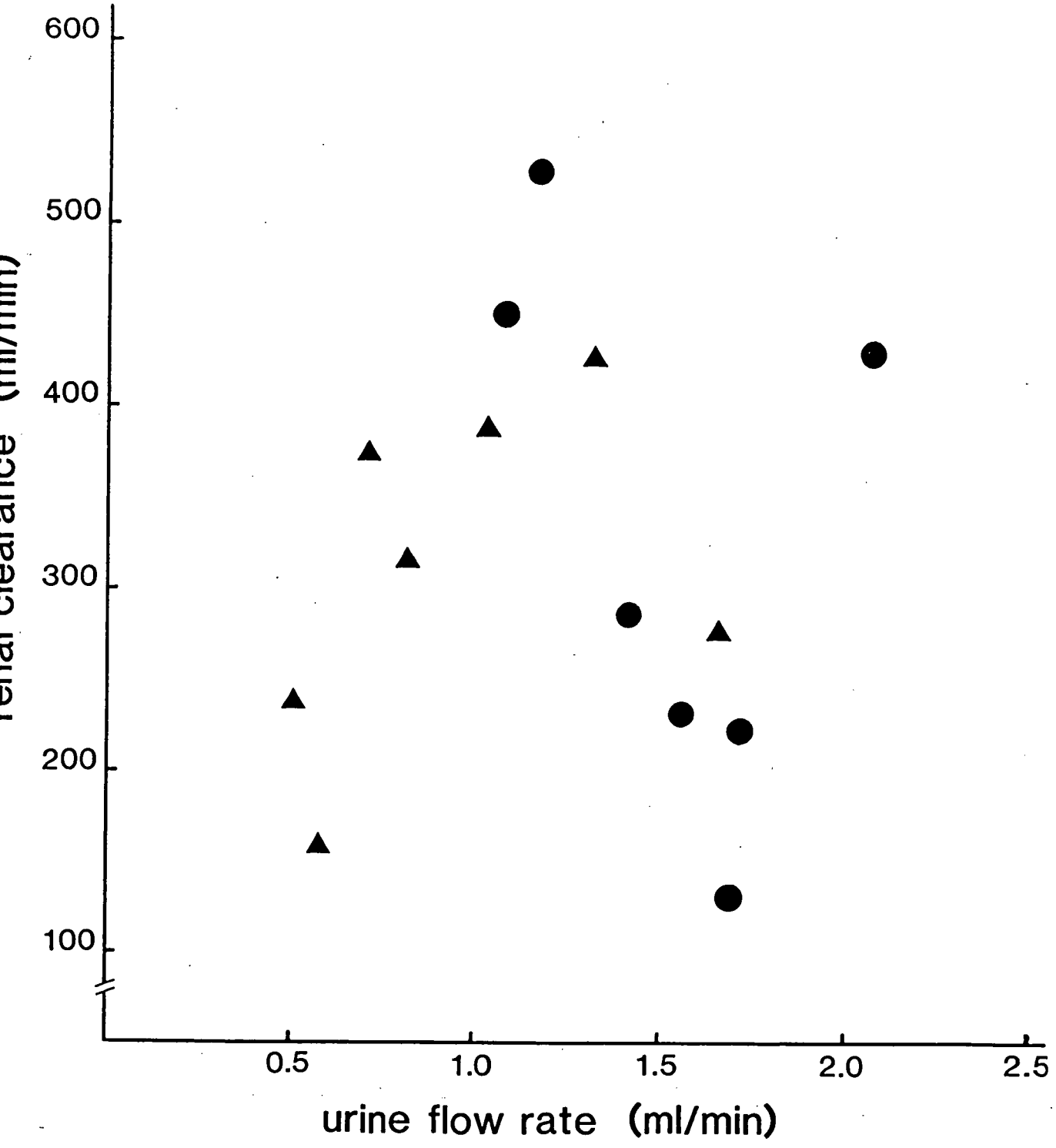

Figure 4.8 Renal clearance of benzylpenicillin (CL_R) versus urine flow rate during △ ambulation and ○ bedrest

In the present study there was no apparent difference in any of the pharmacokinetic parameters between male and female subjects.

4.3.3: DISCUSSION

Limited information is available on the disposition of benzylpenicillin following parenteral administration. In this work, a specific HPLC analytical procedure to quantify the parent drug only was used. The metabolites, penicilloic acid and 6-aminopenicillanic acid, are found in the urine after oral administration of pencillin G (Cole et al, 1973). The pharmacokinetic parameters obtained from this study are comparable with those determined in other studies. The mean residence time and mean absorption time data presented here have not been reported previously. The mean values for the terminal half-life found in the present study following intravenous administration of the drug (53.6 min during ambulation and 54.2 min during bedrest) and following intramuscular administration of the drug (52.5min and 50.9 min during ambulation and bedrest,respectively) are similar to those found by Kates et al (1980) (49.2 min and 51.2 min) and also those determined by Levy (1967) in ambulant and supine subjects following intramuscular administration of benzylpenicillin (50.4 min and 55.8 min respectively).

The mean values for the volume of distribution during bedrest [47.0 l (i.v.) and 42.0 l (i.m.)] and during ambulation [38.6 l (i.v.) and 36.4 l (i.m.)] were very similar to those determined by Levy (1967) (47 l during

bedrest and 37 l during ambulation). Levy, also, was unable to show a statistically significant difference between the volume of distribution during bedrest and ambulation. Table 4.10 provides a comparison of the pharmacokinetic values derived by Levy (1967) and those of the present study. The other pharmacokinetic parameters (k_{12}, k_{21}) derived from intravenous data describing drug distribution were also unaffected by posture. Rowland & Tozer (1980c) suggest that the distribution of certain drugs may be perfusion or diffusion rate controlled. Benzylpenicillin falls into the latter category and is therefore unlikely to be affected by regional changes in blood flow occurring with posture.

In previous studies of the effects of posture on the disposition of the penicillins, Levy (1967) in a pharmacokinetic analysis of the report by Schmidt & Roholt (1966) who administered benzylpenicillin intramuscularly, determined that the rate constant for non-renal elimination, k_m, was significantly increased and the fraction of the dose excreted unchanged was significantly lower in ambulation than in bedrest. These findings might reflect changes in metabolism or changes at the absorption site. More recently, Roberts & Denton (1980) studied the effects of posture on orally administered amoxycillin. They observed an increased total body clearance of amoxycillin during bedrest but this assumed that there was no change in the bioavailability of the drug with change in posture. In the intramuscular dosing phases of the present study, the area

Table 4.10 Comparison of pharmacokinetic data from Levy (1967) with data determined in this study

Parameter[a]	Ambulation			Bedrest		
	IV	IM	LEVY	IV	IM	LEVY
$t_{\frac{1}{2}\beta}$ (min)	53.6 ± 32.3	52.5 ± 11.7	50.4	54.2 ± 36.1	50.9 ± 19.3	55.8
f	0.63 ± 0.12	0.46 ± 0.12	0.67	0.58 ± 0.17	0.55 ± 0.09	0.82
V (l)	38.63 ± 30.39	36.4 ± 10.6	37	46.97 ± 44.58	42.0 ± 18.9	47
CL_R (ml/min)	309.4 ± 93.4	264.3 ± 99.7	340	324.1 ± 145.3	314.6 ± 87.4	480
CL_{NR} (ml/min)	178.0 ± 51.3	233.6 ± 81.3	166.5	218.2 ± 66.5	236.9 ± 108.4	101.8

[a] Parameters as defined in Table 4.3

under the plasma benzylpenicillin concentration versus time curve during bedrest was significantly less than during ambulation. This AUC reflects both availability and clearance (Equation 4.8). Neither availability nor total body clearance (estimated using intravenous data) were found to be significantly altered by changes in posture. The lack of change highlights the intra-individual variability in benzylpenicillin kinetics. Significant changes are observed when only a single parameter such as AUC is compared on two occasions. The modification of this data by incorporating other data from the intravenous phases of the study (and its associated error) results in a loss of the statistical difference between bedrest and ambulation. Thus, the data available does not allow the source of the difference in AUC to be determined. The pharmacokinetic parameters (bioavailability, rate of absorption, mean absorption time) describing the absorption of intramuscular benzylpenicillin, were derived using both intramuscular and intravenous data and, therefore, have a greater error than would be associated with the direct determination of a single parameter. However, if posture-dependent changes in absorption kinetics do occur but not be shown by this study, these changes must be quite small and are not likely to be clinically significant.

The mean renal clearances of benzylpenicillin found in this study (Tables 4.3 & 4.5) are intermediate between glomerular filtration and renal plasma flow and are consistent with the suggestion that benzylpenicillin is removed from the body by both active tubular secretion and glomerular filtration

(Barza & Weinstein, 1976).

Levy (1967) determined that the renal clearance of benzylpenicillin was significantly increased in bedrest, but posture had no effect on total body clearance and Roberts & Denton (1980) observed both an increased renal clearance and increased total body clearance of amoxycillin during bedrest.

Although, following intramuscular administration of benzylpenicillin, six of the eight subjects had a higher renal clearance of benzylpenicillin during bedrest than during ambulation, this trend was not sufficient for the difference to be significant. No trend was observed when the drug was administered intravenously. Levy (1967) and Roberts & Denton (1980) suggested that the changes in renal clearance observed in their studies may be due to changes in renal blood flow with changes in posture. Several haemodynamic changes occur with changes in posture such as haemoconcentration, decreased plasma volume (Hagan et al, 1978) and reduced renal blood flow (Selkurt, 1963) in the upright subject. According to Equation 4.5, the renal clearance of benzylpenicillin is dependent on renal blood flow. If it is assumed that the renal intrinsic clearance and red blood cell - plasma partition coefficient for benzylpenicillin are unaffected by posture, and using the mean data for renal clearance (Table 4.3) and a bedrest renal plasma flow of 650 ml/min (Roberts & Denton, 1980), a decrease in renal plasma flow on adopting an upright posture

of approximately 28% is estimated from Equation 4.5. In the intravenous phases of this study this decrease was only about 10%. Roberts & Denton (1980) calculated a decrease in renal plasma flow of approximately 40% for their subjects and for the data of Levy (1967). The level of exercise as well as posture can influence renal plasma flow. In the supine position, the renal plasma flow is 15-20% higher than in the erect position with no exercise (Selkurt, 1963). During light to moderate exercise, renal plasma flow decreased by 15-27% compared to supine renal plasma flow (Chapman et al, 1948). As the level of exercise increases, the renal plasma flow decreases even further (Radigan & Robinson, 1949). The ambulant subjects in the present study only undertook light exercise, being confined mostly to the room in which the study was conducted or walking in a corridor. The larger decline in renal plasma flow in ambulant subjects estimated by Roberts & Denton (1980) may reflect a greater level of exercise undertaken in the ambulatory phase of their study than in the present study. From the results of the present study, it appears that posture per se or a change of posture accompanied by light exercise is insufficient to alter the renal clearance of benzylpenicillin. During the course of this study, Breiby et al (1983) reported a study on the effect of posture (bedrest and sitting at rest) on the pharmacokinetics of ampicillin following intravenous administration of the drug. They observed changes in distribution but no significant changes in clearance with change in posture. In that study, measurement of the glomerular filtration rate and renal blood flow failed to show any significant changes with

change in posture from lying down to sitting at rest.

The present study demonstrates that, although significant differences may be found with posture using measurements from extra-vascular studies alone, these differences disappear when intravenous data are used to gain a greater insight into the reasons for these differences. The results do suggest, however, that the absorption kinetics of benzylpenicillin after intramuscular administration is independent of posture. The major source of the posturally determined differences in plasma AUC appear to be differences in the clearance. Although this study showed a trend towards reduced renal clearance in upright subjects undertaking light exercise when compared to bedrested subjects, the change failed to reach significance. The changes in the pharmacokinetics of the penicillins with changes in posture observed by other workers are probably related to the level of exercise undertaken by the subjects in those studies.

CHAPTER 5

SULPHAMETHOXAZOLE and TRIMETHOPRIM

5.1: INTRODUCTION

5.1.1: DRUG PROFILES

Both trimethoprim and sulphamethoxazole are broad-spectrum antibiotics with activity against gram-negative and gram-positive microorganisms. However, their action is bacteriostatic rather than bactericidal. Sulphamethoxazole interferes in the bacterial folic acid metabolism by competing with para-aminobenzoic acid and thus blocking the biogenesis of dihydrofolate. Trimethoprim inhibits dihydrofolate reductase by competing with dihydrofolate (Brumfitt, Hamilton-miller & Kosmidis, 1973). This sequential blockade of tetrahydrofolate production is thought to be the cause of the synergistic action observed when these antibiotics are combined (Brumfitt et al, 1973). Trimethoprim, in vitro, is 10 to 40 times more active, weight for weight, than sulphamethoxazole and it has been found that similar ratios (1:10 to 1:40) of trimethoprim to sulphamethoxazole provide optimal synergism (Brumfitt et al, 1973). To achieve these ratios in plasma, trimethoprim and sulphamethoxazole are given in a fixed 1:5 ratio (co-trimoxazole). Recently, it has been suggested that

trimethoprim has some bactericidal effects and its combination with sulphamethoxazole antagonizes these effects rendering the combination bacteriostatic (Friesen, Hekster & Vree, 1981). Also, there is some uncertainty as to whether the combination is synergistic or merely additive (Friesen et al, 1981).

Co-trimoxazole is particularly effective in the treatment of urinary tract infections, pneumocystis and otitis media and provides a valuable second line agent for the treatment of many other infections.

This combination of trimethoprim and sulphamethoxazole is generally well tolerated. The majority of reported reactions are gastrointestinal or dermatological and these are usually minor. Toxicity is seldom life-threatening and is usually completely reversible. Friesen et al (1981) suggest that most of the reported adverse reactions to the sulphamethoxazole-trimethoprim combination are due to sulphamethoxazole.

5.1.2: ASSAYS

A number of analytical methods have been used for the determination of trimethoprim in biological fluids including microbiological (Bushby & Hitchings, 1968), spectrofluorometric (Schwartz, Koechlin & Weinfeld, 1969; Lichtenwalner et al, 1979) and high-performance liquid chromatography (Weinfeld & Macasieb, 1979; Watson et al,

1980). The most widely used assay for the determination of sulphonamides in biological fluids has been a colorimetric assay based on the Bratton-Marshall reaction (Bratton & Marshall, 1939) but, recently, high-performance liquid chromatographic methods have been developed for sulphonamide determination (Johnson, Jeter & Clairborne, 1975; Sharma, Perkins & Bevill, 1976) and for determination of both trimethoprim and sulphamethoxazole (Vree et al, 1978a; Bury & Mashford, 1979; Gochin, Kanfer & Haigh, 1981). Only the methods of Vree et al (1978a) and Gochin et al (1981) also quantitate the major metabolite of sulphamethoxazole, N_4-acetylsulphamethoxazole. The method of Gochin et al (1981) used solvent extraction in the preparation of samples while that of Vree et al (1978a) uses protein precipitation. For the present study, protein precipitation was chosen for its speed and simplicity, however the extensive dilution used by Vree et al (1978a) was undesirable. Using standard protein precipitation techniques, neither the mobile phase of Vree et al (1978a) nor that of Gochin et al (1981) gave sufficient resolution of the compounds from endogenous peaks. Consequently a new assay was developed.

5.1.3: PHARMACOKINETICS

Both trimethoprim and sulphamethoxazole are well absorbed after oral administration although there is considerable individual variation in absorption rates (Patel & Welling, 1980).

These two drugs have quite different physico-chemical

properties. Sulphamethoxazole (a weak acid, pKa 5.6) is less lipid-soluble than trimethoprim (a weak base, pKa 7.3) so does not distribute as extensively into tissues. Trimethoprim preferentially enters certain tissues, particularly the kidney, liver, spleen and prostate (Brumfitt et al, 1973). Consequently, trimethoprim has a much greater volume of distribution (100 to 200 l) than sulphamethoxazole (12 to 18 l) (Patel & Welling, 1980). The different distribution characteristics of the two drugs results in a sulphamethoxazole : trimethoprim ratio in the plasma ranging from 16:1 to 31:1 when a standard ratio of 5:1 is administered (Patel & Welling, 1980). In the tissues this beneficial ratio is lost due to the poor penetration of sulphamethoxazole. Both drugs bind to plasma proteins, trimethoprim being approximately 45% bound and sulphamethoxazole 66% bound.

Both drugs are metabolised but to quite different extents. Only about 20% sulphamethoxazole is excreted unchanged in the urine while approximately 60% of the dose is excreted as N_4-acetylsulphamethoxazole and 15% as glucuronides (Rieder, 1973). Trimethoprim is only about 20% metabolised with the remainder of the dose being excreted unchanged in the urine. A number of metabolites have been described for trimethoprim (Rieder, 1973). As the excretion of both compounds is relatively slow, the elimination half-life of sulphamethoxazole being 7-9 hours and that of trimethoprim being 6-17 hours in patients with normal renal function, the combination is usually administered on a twice daily basis.

The renal clearances of both sulphamethoxazole and trimethoprim are dependent on urinary pH with the renal clearance of sulphamethoxazole also being dependent on urine flow (Vree et al, 1978b; Craig & Kunin, 1973).

Since differences in the pharmacokinetics of drugs do occur in bedrest, sleep and ambulation, it is of some importance to study the influence of these factors on drugs such as trimethoprim and sulphamethoxazole which have markedly different physico-chemical properties yet are administered in a fixed combination.

5.2: ANALYSIS

5.2.1: MATERIALS AND METHODS

5.2.1.1: Chemicals

Sulphamethoxazole, N_4-acetylsulphamethoxazole and trimethoprim were supplied by Roche Products, Dee Why, NSW. Methanol and acetonitrile were specially purified for HPLC and supplied by Waters Associates (Milford, MA, USA). All other chemicals were analytical grade. The water used was de-ionised and glass distilled.

5.2.1.2: HPLC instrumentation and conditions

Plasma assay: Reversed-phase HPLC was performed using a Waters M-6000A solvent delivery system fitted with a U6K injector. A μBondapak phenyl column (particle size 10 μm; 300 mm x 3.9 mm I.D.; Waters Associates, Milford, MA, USA)

was used in combination with a guard column (μBondapak C_{18}/PorasilB; Waters Associates, 23 mm x 3.9 mm I.D.). UV absorbance at 230 nm was monitored with a Waters Model 441UV absorption detector fitted with a cadmium lamp. The absorbance was recorded on a dual-channel Omniscribe recorder (Houston Instruments, Austin, TX, USA). Injections were made with a 100 μl Hamilton syringe. The mobile phase was 0.067M phosphate buffer (pH 5.9) and acetonitrile (100:14) mixture. The flow rate was 1.8 ml/min.

Urine assay: This assay was performed using, a Constametric 1 HPLC pump (Laboratory Data Control), fitted with a Rheodyne injector, an ODS-HC SIL-X-1 column (particle size 10 μm; 250 mm x 26 mm ID; Perkin Elmer, Norwalk, CONN, USA) and a guard column (Spherisorb C_{18}; Phase Sep, Clwyd, UK; 23 mm x 3.9 mm ID). UV absorbance at 230 nm was monitored with a Perkin Elmer LC55 Spectrophotometer (Perkin Elmer, Norwalk, CONN, USA). The absorbance was recorded on a Curken Chart Recorder (Curken Scientific Inc, Danbury, CONN, USA). Injections were made using a 25 μl Hamilton syringe. The mobile phase was 0.067M potassium dihydrogen phosphate solution (pH 4.2) and acetonitrile mixture (100:6.6). The flow rate was 1.5 ml/min.

5.2.1.3: Sample preparation

Plasma assay: A 400 μl aliquot of plasma was transferred into a glass tube (disposable borosilicate glass culture tubes; Kimble, IL, USA). To this was added 200 μl methanol and 40μl perchloric acid (33%) solution. The sample was vortex mixed for 1 minute then centrifuged at 1500 g for 5

minutes. An aliquot (100 µl) of clear supernatant was injected onto the column.

Urine assay: Equal volumes (200 µl) of urine sample and acetonitrile were added to a glass tube, vortex mixed for 1 minute then centrifuged at 1500 g for 10 minutes. An aliquot (10µl) of the clear supernatant was injected onto the column.

5.2.1.4: Preparation of standard curves

Plasma standards were prepared by spiking drug-free plasma with known amounts of aqueous solutions of sulphamethoxazole, N_4-acetylsulphamethoxazole, and trimethoprim. The standards were prepared such that those containing high concentrations of sulphamethoxazole had low concentrations of N_4-acetylsulphamethoxazole and vice versa, as might be observed in clinical samples. The concentration range of the standards were sulphamethoxazole, 1.0 - 50.0 mg/l; N_4-acetyl-sulphamethoxazole, 0.5 - 20.0 mg/l; trimethoprim, 0.2 - 5.0 mg/l. The standards were then assayed in the described manner. Urine standards were prepared in a similar manner in drug-free urine to produce concentrations of 6.25 - 100 mg/l, 12.5 - 200 mg/l and 6.25 - 100 mg/l for sulphamethoxazole, N_4-acetylsulphamethoxazole and trimethoprim, respectively. Standard curves were prepared by plotting the peak height of drug versus concentration.

5.2.2: RESULTS

5.2.2.1: Plasma assay

Chromatograms of blank plasma and plasma spiked with sulphamethoxazole, N_4-acetylsulphamethoxazole and trimethoprim are shown in Figure 5.1. The three compounds were well separated from each other and free from interference by endogenous peaks. The retention times were 7.8 min, 9.7 min, 14.7 min for N_4-sulphamethoxazole, sulphamethoxazole and trimethroprim, respectively. Peak heights and plasma concentrations were linearly related for each of the three compounds over the concentration range studied ($r>0.99$) (Figure 5.2). Sensitivity was 0.1 mg/l for N_4-acetylsulphamethoxazole, 0.2 mg/l for sulphamethoxazole and 0.075 mg/l for trimethoprim.

Recovery was assessed by assaying aqueous and plasma standard solutions in the described manner and comparing the resultant peak heights. The recovery of sulphamethoxazole and trimethoprim were found to be 103 ± 4.9% (S.D.) and 96.6 ± 4.6% (S.D.), respectively over the range of concentrations used for the standard curves. The recovery of N_4-acetylsulphamethoxazole was lower [85.3 ± 3.6% (S.D.)]. However, the extent of recovery was consistent as indicated by the reproducibility of the assay. The intra- and inter-day reproducibility of the assay are shown in Table 5.1.

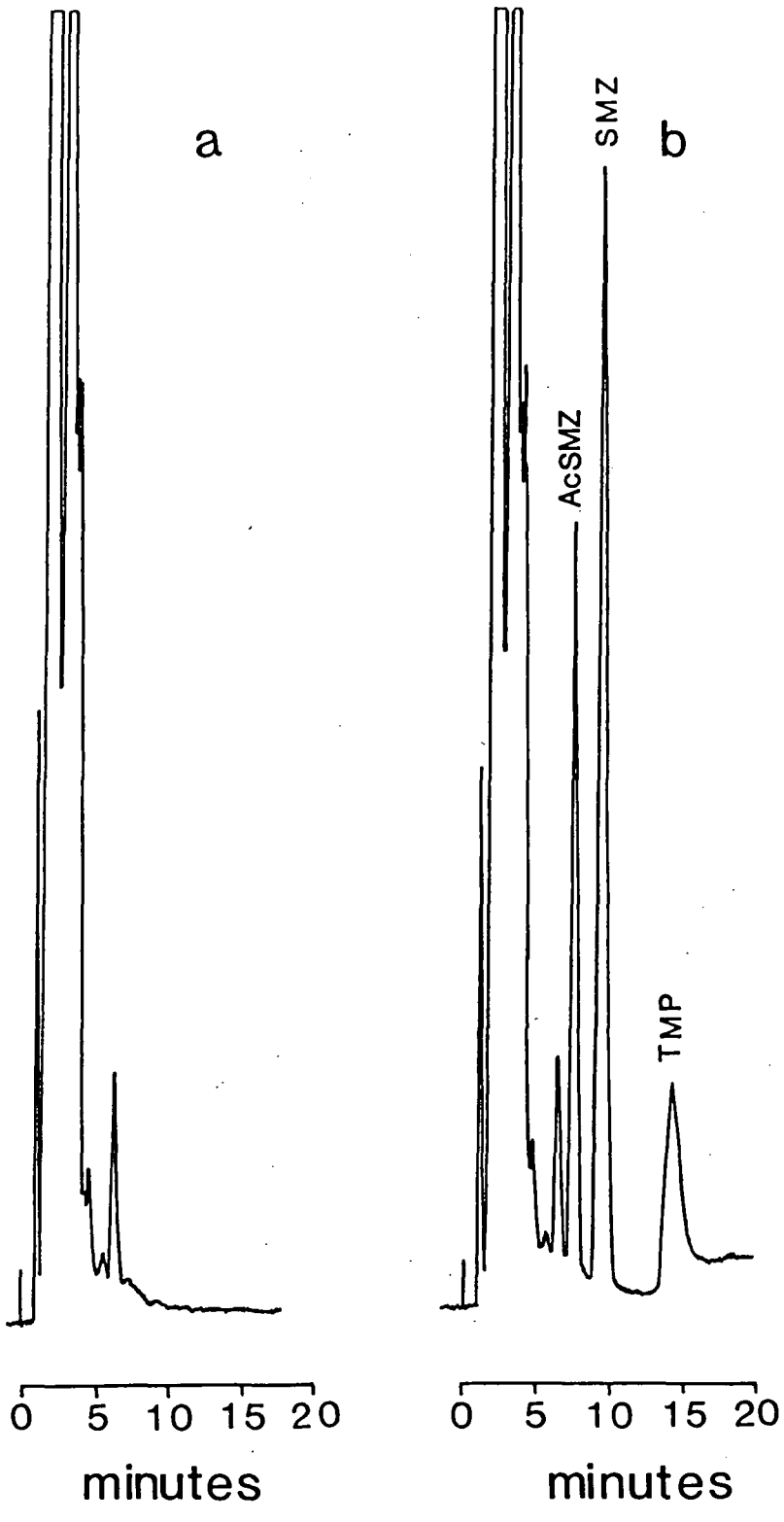

Figure 5.1 Chromatograms of (a) blank plasma and (b) plasma from the same subject spiked with N_4-acetylsulphamethoxazole (AcSMZ) 5mg/l, sulphamethoxazole (SMZ) 10mg/l, and trimethoprim (TMP) 0.5mg/l

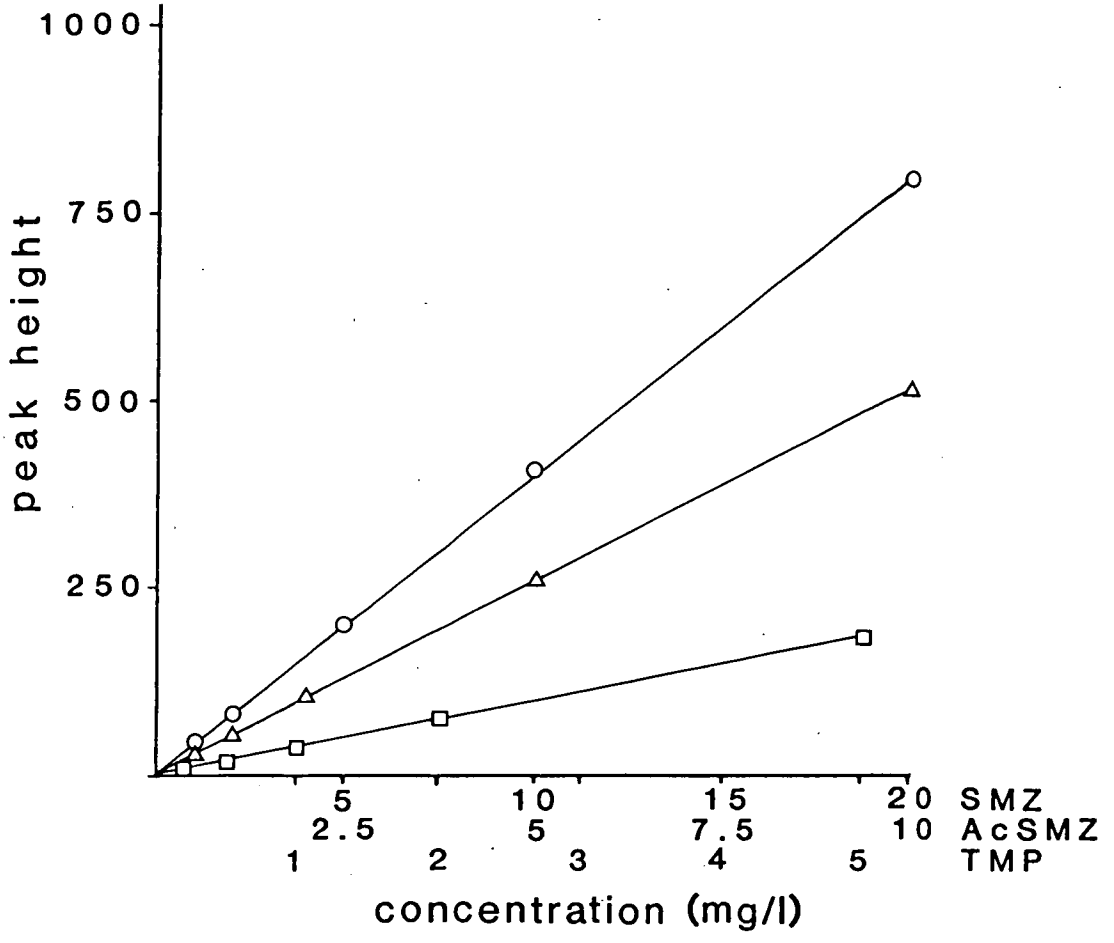

Figure 5.2 Standard curves for ○ sulphamethoxazole (SMZ), △ N_4-acetylsulphamethoxazole (AcSMZ) and □ trimethoprim (TMP) in plasma

Table 5.1 Intra- and inter-day variation in the assay of trimethoprim (TMP), sulphamethoxazole (SMZ) and N_4-acetyl-sulphamethoxazole (AcSMZ) in plasma.

Compound	Concentration (mg/l)	CV(%) Intra-day (n = 6)	Inter-day (n = 10)
TMP	5.0	1.20	
	2.0	4.61	11.97
	1.0	4.40	12.55
	0.5	3.01	12.19
	0.2	9.80	8.06
SMZ	50.0	1.57	
	20.0	1.74	7.33
	10.0	1.86	9.03
	5.0	2.24	7.53
	2.0	1.77	8.82
AcSMZ	20.0	2.71	
	10.0	2.14	6.90
	5.0	1.46	6.25
	2.0	1.48	6.96
	1.0	2.32	6.19

5.2.2.2: Urine assay

Chromatograms of blank urine and urine from a subject following oral administration of co-trimoxazole are shown in Figure 5.3. Using this system the compounds eluted in a sequence different from the plasma assay. The retention times were trimethoprim, 5.0 min; sulphamethoxazole, 9.3 min; and N_4-acetylsulphamethoxazole, 18.5 min. There was no interference from endogenous peaks. Standard curves obtained by plotting the peak height of each compound versus their respective concentration were linear ($r>0.99$) over all the concentration ranges studied (Figure 5.4). The assay limits for trimethoprim, sulphamethoxazole and N_4-acetylsulphamethoxazole were 4 mg/l, 5 mg/l and 10 mg/l, respectively. The intra- and inter-day reproducibility of the assay are shown in Table 5.2.

5.2.3: DISCUSSION

The described methods provide simple, rapid means for assaying trimethoprim, sulphamethoxazole and its major metabolite, N_4-acetylsulphamethoxazole in biological fluids. These methods are well suited to the clinical monitoring of the drugs.

The sample preparation methods for both plasma and urine are simple. Given that the sensitivity of the assay is sufficient, HPLC allows direct injection of plasma and urine onto the column. However, doing so allows large amounts of contaminants into the system. The most common methods of sample preparation are solvent extraction or protein

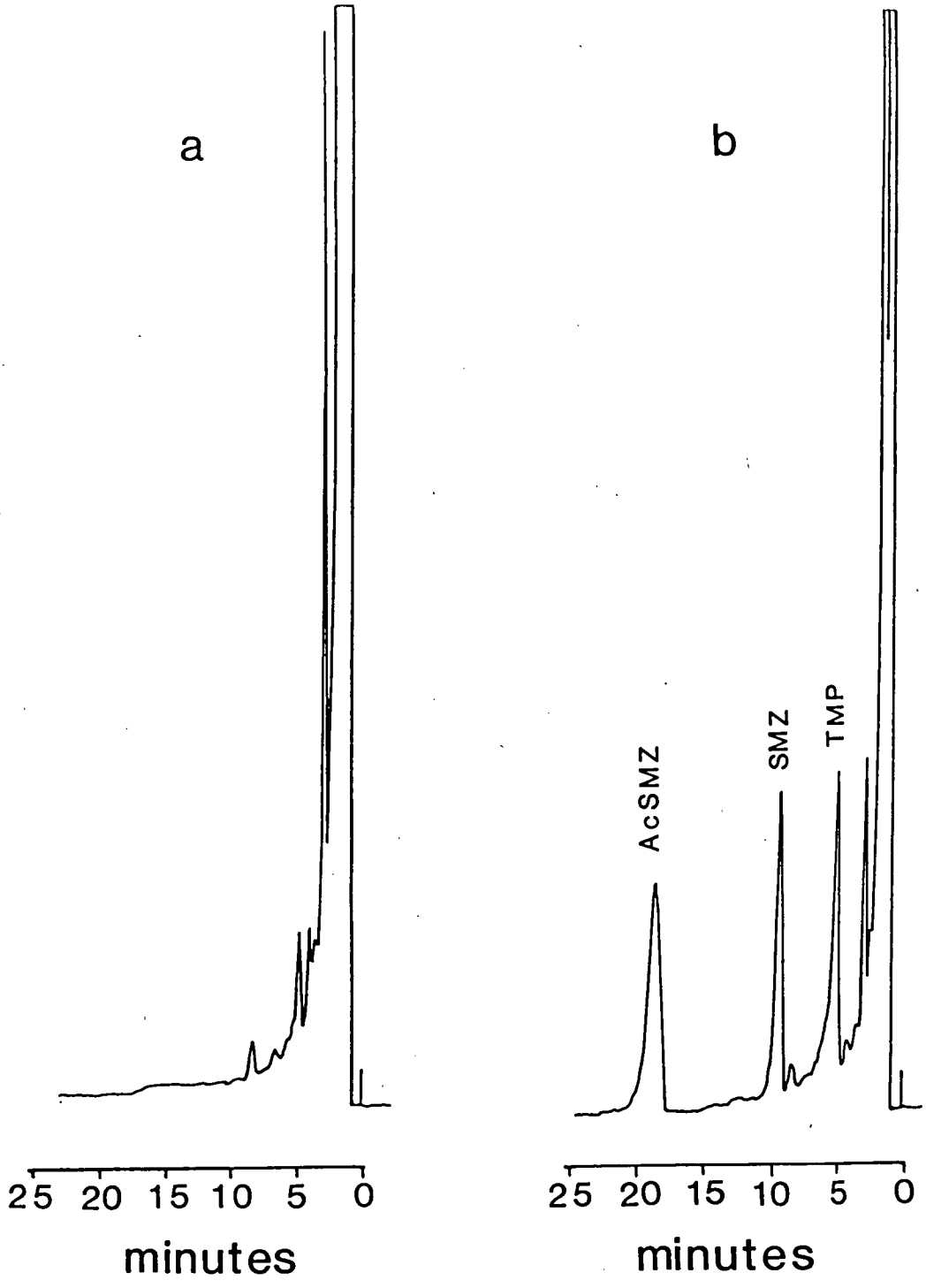

Figure 5.3 Chromatograms of (a) blank urine and (b) urine from the same subject collected from 0 - 12 hours after oral administration of 800 mg sulphamethoxazole and 160 mg trimethoprim. The estimated urine concentrations are trimethoprim (TMP) 32.88 mg/l, sulphamethoxazole (SMZ) 64.91 mg/l, N_4-acetylsulphamethoxazole (AcSMZ) 131.3 mg/l.

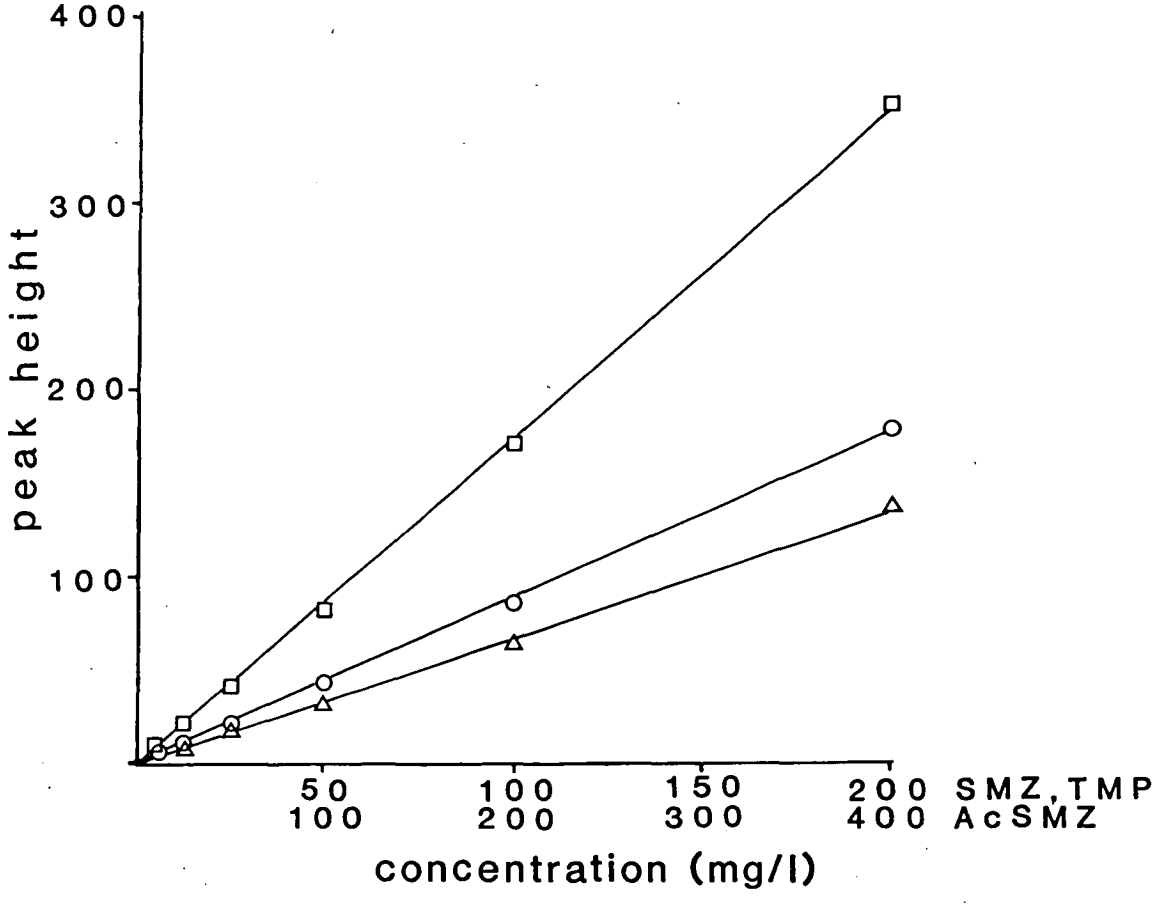

Figure 5.4 Standard curves for ○ sulphamethoxazole (SMZ), □ trimethoprim (TMP) and △ N_4-acetylsulphamethoxazole (AcSMZ) in urine

Table 5.2 Intra- and inter-day variation in the assay of trimethoprim (TMP), sulphamethoxazole (SMZ) and N_4-acetyl-sulphamethoxazole (AcSMZ) in urine.

Compound	Concentration (mg/l)	CV(%) Intra-day (n = 6)	Inter-day (n = 11)
TMP	100	2.39	5.41
	50		4.51
	25	2.88	4.91
	12.5		7.86
	6.25	13.74 (n = 9)	
SMZ	100	5.11	6.15
	50		7.09
	25	2.54	8.27
	12.5		7.45
	6.25	12.69 (n = 9)	
AcSMZ	200	2.36	6.69
	100		7.04
	50	5.67	11.30
	25		6.33
	12.5	13.07 (n = 9)	

precipitation (Li Wan Po & Irwin, 1980). Solvent extraction has been used by some workers in sample preparation for the assay of trimethoprim alone or with sulphamethoxazole and N_4-acetylsulphamethoxazole (Gochin et al, 1981; Ascalone, 1981; Watson et al, 1980; Weinfeld & Macasieb, 1979). Solvent extraction frequently achieves higher sensitivity but is relatively slow. Protein precipitation is rapid and has been used in co-trimoxazole assays by Vree et al (1978a) and Bury & Mashford (1979). Commonly used protein precipitants are trichloroacetic acid, tungstic acid, perchloric acid and acetonitrite (Li Wan Po & Irwin, 1980; Giese, 1983). Vree et al (1978a) diluted the serum samples ten fold with water before adding sufficient perchloric acid solution to dilute it a further five times. Using this dilution they found the recovery of sulphamethoxazole to be 88 ± 4% and trimethoprim 83 ± 2%. The recovery was less if the sample was not diluted. Bury & Mashford (1979) used trichloroacetic acid (4 M) to precipitate proteins, requiring only 50 µl to be added to 1 ml plasma sample. However, their method required separate chromatographic conditions to assay trimethoprim and sulphamethoxazole. The recovery of sulphamethoxazole was only 81%. In the present study, trichloroacetic acid, acetonitrile and perchloric acid were tested as potential protein precipitants. Trichloroacetic acid was found to produce an interfering peak and acetonitrile caused poor drug peak shape especially at low drug concentrations. Perchloric acid was chosen but methanol was also added to remove any protein bound drug. This combination of methanol and perchloric acid has been

used successfully previously (Rumble, Roberts & Wanwimolruk, 1981).

In this study the detection wavelength was 230 nm. This wavelength was also used by Gochin et al (1981) and Watson et al (1980) because at this wavelength the absorbance of trimethoprim is about five times that of sulphamethoxazole on a weight basis (Gochin et al, 1981). This is important as the plasma concentration ratio of sulphamethoxazole to trimethoprim is approximately 20:1. Vree et al (1978a) used a wavelength of 225 nm while Bury & Mashford (1979) used 254 nm. These wavelengths are not optimal for trimethoprim. This was confirmed by a UV scan of solutions of trimethoprim and sulphamethoxazole in mobile phase.

The system used for plasma assay was tested for suitability for assaying urine samples. There was some interference from endogenous peaks which may have been overcome by pH manipulation. A change of laboratories necessitated using different equipment for the assay of urine samples, so, greater manipulation of pH and acetonitrile content of the mobile phase was necessary to assay the compounds in urine using the ODS-HC SIL-X-1 column. Experience with the two columns shows that the method is suitable for phenyl or C_{18} columns with some variation in pH or proportion of acetonitrile.

This method was found to provide consistent results over several weeks of routine use with low interday variability. This variability was reduced further by assaying several

standards each day.

5.3: PHARMACOKINETIC STUDY

5.3.1: CLINICAL PROTOCOL

5.3.1.1: Subjects

Six healthy, young adult volunteers (three male, three female) aged 19-29 years, participated in the study. Prior to the investigation, a detailed medical history was taken. No participants gave a history of drug allergy, liver or renal disease or any concurrent illness. Physical examination, haematological tests and liver and kidney function tests were normal for all subjects. Each subject gave informed consent and approval was obtained from the Human Ethics Committee of the University of Tasmania before commencing the study.

5.3.1.2: Procedure

Co-trimoxazole (800 mg sulphamethoxazole and 160 mg trimethoprim; Bactrim DS, Roche, Dee Why, Australia) was administered as a single oral dose with 250 ml water on three separate occasions - during daytime bedrest, immediately prior to sleep (about midnight) and during daytime ambulation. No food or drink was permitted for two hours before and two hours after dosing. At least two weeks separated each study.

5.3.1.3: Sample Collection

An indwelling catheter was inserted into an antecubetal vein approximately one hour before dosing. Subjects then adopted the posture required for the study. This posture was strictly maintained for the duration of the study. 5 ml blood samples were collected into heparinised tubes at 0, 10, 20, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600, 720 minutes after dosing (blood collection ceased after 600 minutes during the sleep study). The samples were centrifuged and the plasma drawn off then stored at -20°C until assayed. Urine was collected 0-12, 12-24, 24-48 and 48-72 hours (0-10, 10-24, 24-48, 48-72 hours for sleep study). The volume and pH of each sample was measured and an aliquot retained at -20°C until assayed. Samples were assayed according to the method described above.

5.3.1.4: Pharmacokinetics and statistical analysis

For each drug, where there were four or more data points following the measured peak drug concentration, the overall elimination rate constant (β) was determined from the slope of the terminal linear segment of a semilogarithmic plot of plasma concentration versus time. The elimination half-life ($t_{\frac{1}{2}}$) was calculated in the usual manner ($t_{\frac{1}{2}} = 0.693/\beta$). The area under the plasma concentration-time curve from time zero to ten hours (AUC_{10}) was calculated by the trapezoidal rule. For data where β had been calculated, the area under the plasma concentration-time curve from time zero to infinity (AUC_{∞}) was calculated by the trapezoidal rule and adding the usual "area to infinity" correction (last estimate of plasma drug concentration divided by β). The

apparent total body clearance (CL_B) for these data was calculated by dividing the dose by the AUC_∞. Since the drugs were administered orally in the present study, the volumes of distribution for the drugs could not be estimated directly. Where β had been calculated, the present data did enable the ratio of the apparent volume of distribution (V) to the extent of absorption into the systemic circulation (F) for that drug to be calculated according to Equation 5.1.

$$V/F = \frac{D}{\beta.AUC} \qquad (5.1)$$

where D is the oral dose.

The rate of absorption was assessed from the observed time to peak drug concentration and the observed peak drug concentration. The amount of drug excreted unchanged in the urine during the study period (ten or twelve hours) and the volume of urine excreted during that time were used to determine the urinary excretion rate for each drug for each phase of the study. Urine flow rate was calculated as the mean value observed during the first urine collection period following drug administration.

Statistical comparison of the effect of bedrest, sleep and ambulation on various pharmacokinetic parameters was made by analysis of variance or t-test as appropriate.

5.3.2: RESULTS

The mean plasma sulphamethoxazole concentration versus time profiles during ambulation, bedrest and sleep are presented in Figure 5.5 and the profiles for its major metabolite N_4-acetylsulphamethoxazole and for trimethoprim are shown in Figures 5.6 and 5.7, respectively. The individual experimental and pharmacokinetic data for these drugs during ambulation, bedrest and sleep are presented in Tables 5.3, 5.4 and 5.5, respectively.

The mean data for each of the states of ambulation, bedrest and sleep are presented in Table 5.6. As the plasma collection periods of this study were only conducted over 10-12 hours as this was the maximum time interval that subjects could remain asleep or ambulant, calculation of the terminal elimination rate constant and, thus, terminal half-life ($t_{\frac{1}{2}}$), total body clearance (CL_B) and volume of distribution (V/F) for sulphamethoxazole was not possible for many subjects, especially during the sleep phase of the study. Sufficient post-peak concentration data were available for determination of these parameters for trimethoprim for all subjects in each of the three phases of the study. There were no significant differences between the values obtained during ambulation, bedrest and sleep for any of the pharmacokinetic parameters for sulphamethoxazole and trimethoprim (Table 5.6) except for the half-life and total body clearance for sulphamethoxazole. The half-life of sulphamethoxazole during bedrest was significantly shorter than during ambulation and the clearance of the drug

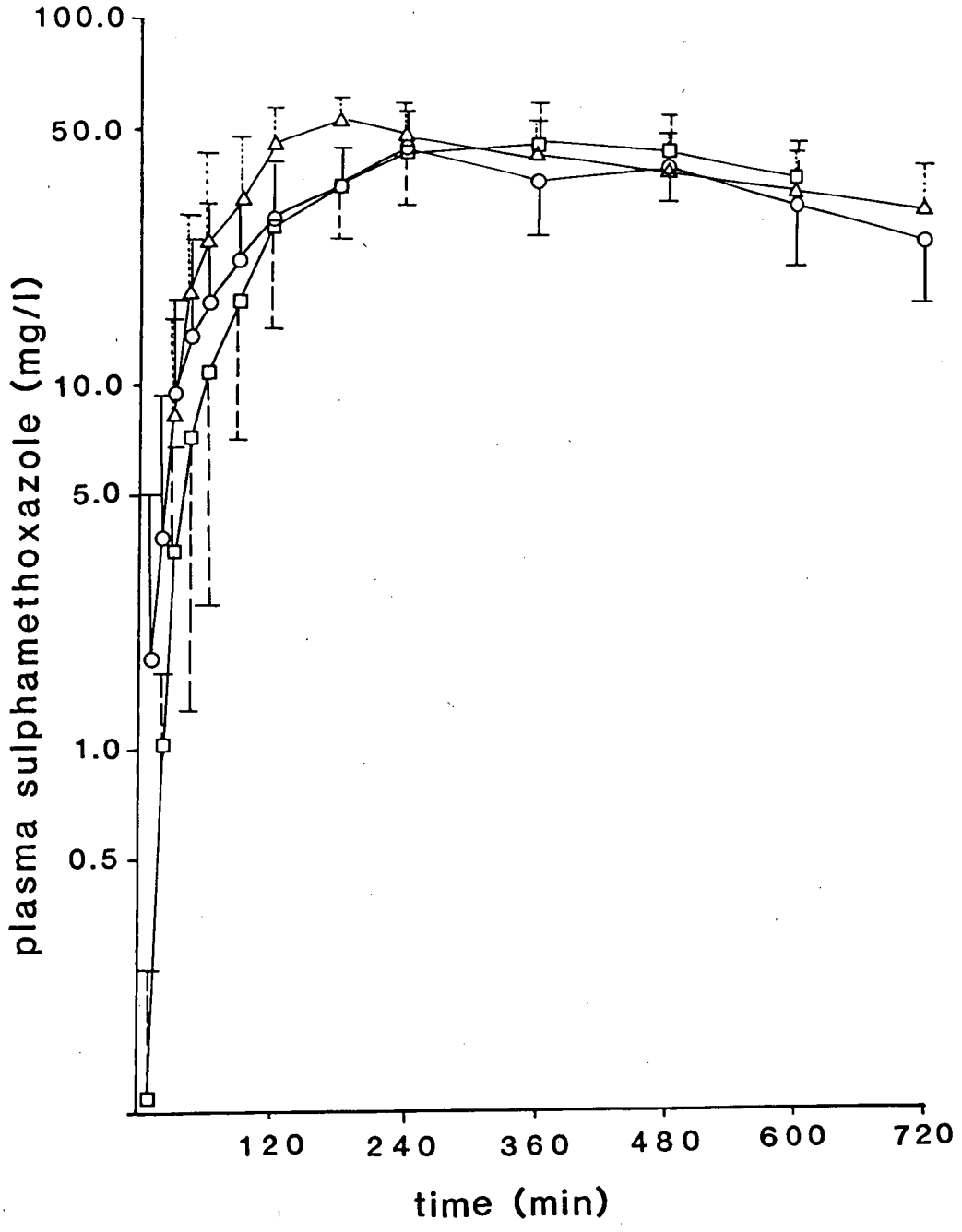

Figure 5.5 Mean (±SD) plasma sulphamethoxazole concentration - time profiles observed during Δ ambulation, O bedrest and □ sleep in six healthy volunteers following oral administration of 800mg sulphamethoxazole with 160mg trimethoprim

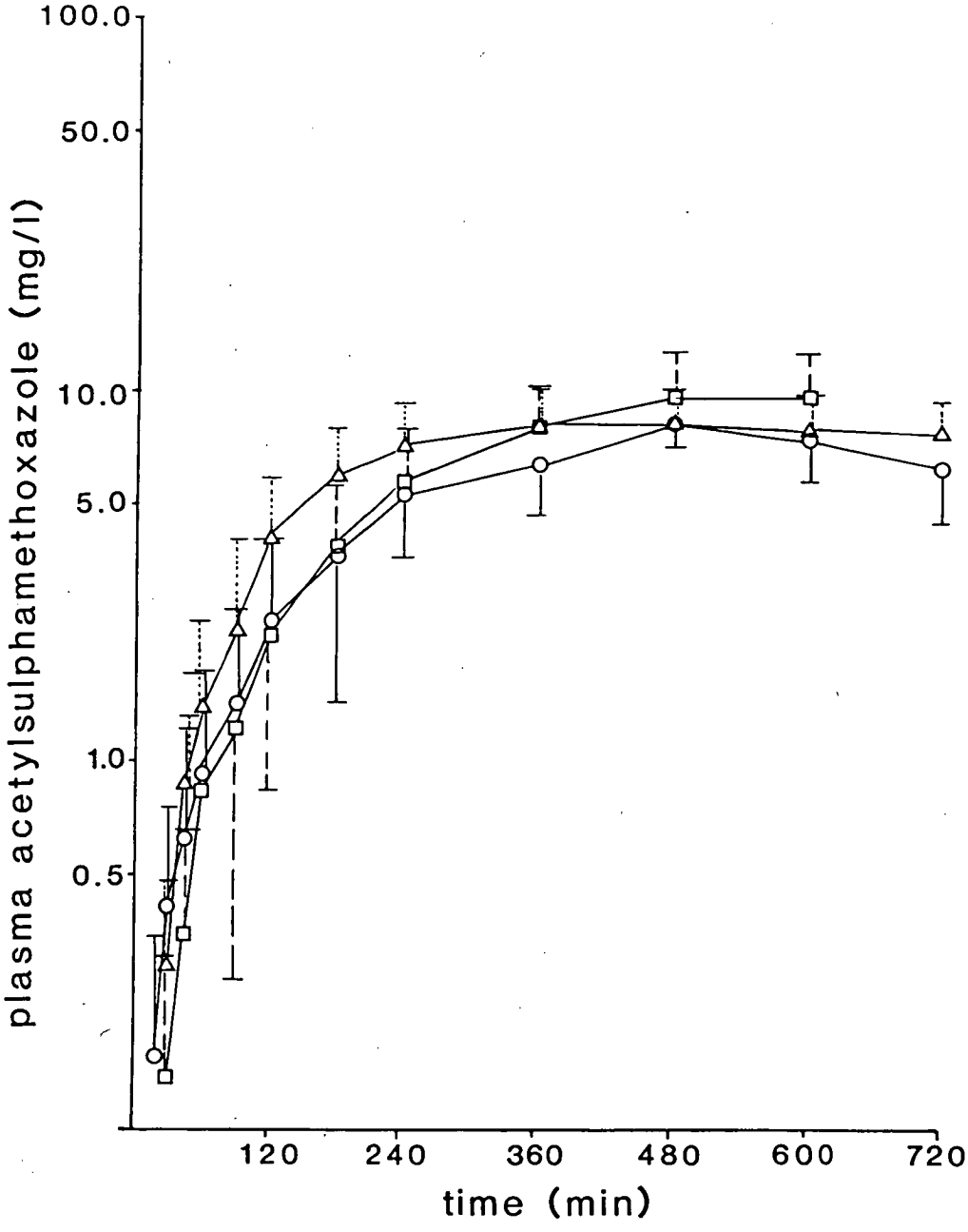

Figure 5.6 Mean (±SD) plasma concentration versus time profiles of the major metabolite of sulphamethoxazole (N_4-acetylsulphamethoxazole) during △ ambulation, ○ bedrest and □ sleep following administration of 800mg sulphamethoxazole and 160mg trimethoprim to six healthy subjects

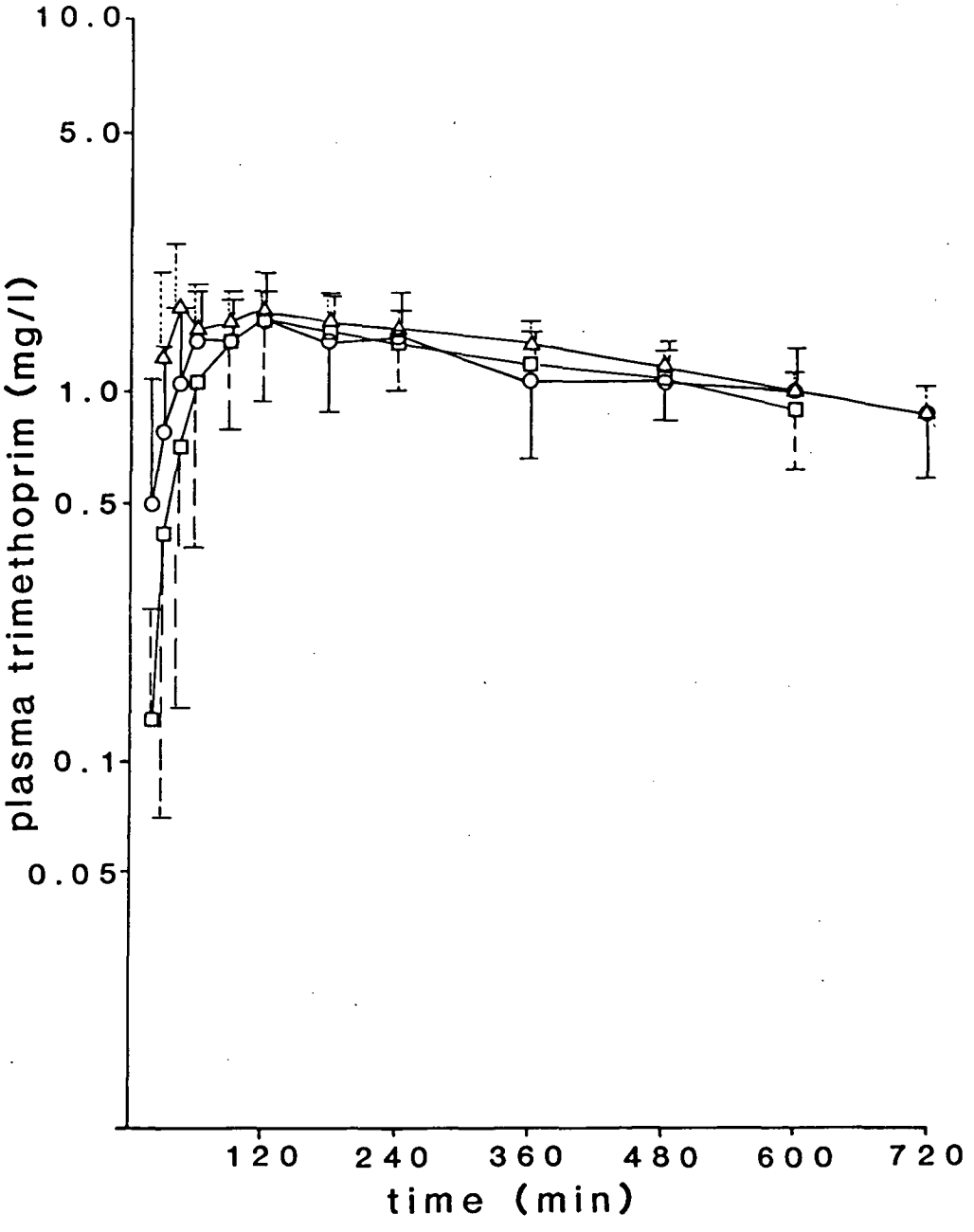

Figure 5.7 Mean (±SD) plasma trimethoprim concentrations found in six healthy volunteers following administration of 160mg trimethoprim with 800mg sulphamethoxazole during △ ambulation, ○ bedrest and □ sleep

Table 5.3 Experimental and pharmacokinetic data describing the disposition of sulphamethoxazole, N_4-acetylsulphamethoxazole and trimethoprim in ambulant subjects.

Parameter[a]	Subject					
	CW	HM	JS	AA	CD	JG
Age (y)	19	19	21	19	29	21
Sex	F	F	F	M	M	M
Sulphamethoxazole						
AUC_{10} (min.mg/l)	23492	23566	29960	17686	24186	20284
$t_{\frac{1}{2}}$ (h)	8.95	9.31	18.63	9.17	10.79	13.28
CL_B (ml/min)	16.63	13.88	6.96	22.68	15.36	15.42
V/F (l)	12.89	11.19	11.23	18.00	14.36	17.72
C_p (mg/l)	60.02	52.51	65.36	49.30	56.36	56.57
t_p (min)	190	340	192	160	92	135
N_4-acetylsulphamethoxazole						
AUC_{10} (min.mg/l)	4548	2989	3776	3984	4844	2123
Trimethoprim						
AUC_{10} (min.mg/l)	812.85	837.38	881.03	716.43	670.28	805.25
$t_{\frac{1}{2}}$ (h)	11.00	7.49	11.55	6.75	9.69	15.82
CLB (ml/min)	89.12	101.86	77.23	132.95	116.81	74.41
V/F (l)	84.88	66.14	77.23	77.75	100.70	101.93
C_p (mg/l)	1.73	1.88	1.93	1.58	1.46	2.92
t_p (min)	50	135	45	100	62	35

[a] AUC_{10} - area under plasma concentration-time curve from time 0 to 10 hours; $t_{\frac{1}{2}}$ - half-life; CL_B - total body clearance; V/F - apparent volume of distribution (V divided by the fraction of dose absorbed (F); C_p - observed peak plasma drug concentration; t_p - observed time to C_p following drug administration.

Table 5.4 Pharmacokinetic data describing the disposition of sulphamethoxazole, N_4-acetylsulphamethoxazole and trimethoprim in bedrested subjects.

Parameter[a]	Subject					
	CW	HM	JS	AA	CD	JC
Sulphamethoxazole						
AUC_{10} (min.mg/l)	19739	17909	24558	21147	16758	18775
$t_{\frac{1}{2}}$ (h)	7.80	N/A	13.43	5.95	8.31	9.17
CL_B (ml/min)	18.32	N/A	11.27	25.99	24.14	21.04
V/F (l)	12.38	N/A	13.10	13.40	17.37	16.70
C_p (mg/l)	65.77	40.64	57.02	47.66	43.97	42.39
t_p (min)	240	480	360	120	180	240
N_4-acetylsulphamethoxazole						
AUC_{10} (min.mg/l)	3311	2419	3020	3916	3391	2434
Trimethoprim						
AUC_{10} (min.mg/l)	668.30	773.93	948.95	791.73	343.65	663.65
$t_{\frac{1}{2}}$ (h)	9.87	9.96	12.29	11.44	4.60	12.98
CL_B (ml/min)	105.61	88.68	69.61	88.62	301.27	97.05
V/F (l)	90.26	76.45	74.05	87.74	120.03	109.04
C_p (mg/l)	1.91	1.81	2.29	1.86	0.74	1.73
t_p (min)	90	240	120	60	90	63

[a] Parameters as defined in Table 5.3

Table 5.5 Pharmacokinetic data describing the disposition of sulphamethoxazole, N_4-acetylsulphamethoxazole and trimethoprim in sleeping subjects.

Parameter[a]	Subject					
	CW	HM	JS	AA	CD	JC
Sulphamethoxazole						
AUC_{10} (min.mg/l)	24232	24643	27370	16667	18974	15050
$t_{½}$ (h)	N/A	11.00	N/A	N/A	9.71	N/A
CL_B (ml/min)	N/A	14.18	N/A	N/A	17.78	N/A
V/F (l)	N/A	13.50	N/A	N/A	14.94	N/A
C_p (mg/l)	53.70	51.34	69.34	34.20	42.50	36.26
t_p (min)	360	180	360	360	240	360
N_4-acetylsulphamethoxazole						
AUC_{10} (min.mg/l)	4677	4848	3153	3886	3462	1584
Trimethoprim						
AUC_{10} (min.mg/l)	804.93	864.68	896.75	510.20	551.65	498.68
$t_{½}$ (h)	14.44	10.69	8.68	18.93	12.69	21.39
CL_B (ml/min)	71.35	91.73	90.84	87.83	161.42	75.84
V/F (l)	89.19	84.94	68.30	143.98	177.38	140.44
C_p (mg/l)	1.84	2.03	2.50	1.08	1.48	1.28
t_p (min)	120	65	120	90	120	180

[a] Parameters as defined in Table 5.3

Table 5.6 Comparison of mean estimates (± SD) of pharmacokinetic parameters for sulphamethoxazole, N_4-acetylsulphamethoxazole and trimethoprim from data in Tables 5.3, 5.4 & 5.5.

Parameter[a]	Ambulation	Bedrest	Sleep
Sulphamethoxazole			
AUC_{10} (min.mg/l)	23196 ± 4142	19814 ± 2769	21156 ± 4948
$t_{½}$ (h)	11.69 ± 3.77	8.93 ± 2.76[b]	N/A
CL_B (ml/min)	15.16 ± 5.05	20.15 ± 5.77[b]	N/A
V/F (l)	14.23 ± 3.05	14.59 ± 2.27	N/A
C_p (mg/l)	56.69 ± 5.63	49.58 ± 9.84	47.89 ± 13.10
t_p (min)	184.8 ± 84.7	270.0 ± 130.0	310.0 ± 79.7
N_4-acetylsulphamethoxazole			
AUC_{10} (min.mg/l)	3711 ± 1011	3082 ± 584	3602 ± 1190
Trimethoprim			
AUC_{10} (min.mg/l)	787.20 ± 78.72	698.37 ± 202.56	687.82 ± 186.82
$t_{½}$ (h)	10.43 ± 3.26	10.19 ± 3.00	14.47 ± 4.87
CL_B (ml/min)	98.73 ± 23.04	125.14 ± 87.11	96.50 ± 32.88
V/F (l)	84.77 ± 14.15	92.93 ± 18.20	117.37 ± 42.65
C_p (mg/l)	1.92 ± 0.52	1.72 ± 0.52	1.70 ± 0.52
t_p (min)	71.2 ± 38.6	110.5 ± 67.1	115.8 ± 38.5

[a] Parameters as defined in Table 5.3

[b] $p<0.05$ (paired t-test)

during bedrest was greater than during ambulation.

Insufficient sulphamethoxazole data were available from the sleep phase of the study for comparison.

The urinary data is presented in Table 5.7. One subject (CD) failed to collect all urine in the sleep phase of the study. The urinary excretion rates of both sulphamethoxazole and N_4-acetylsulphamethoxazole during bedrest were significantly greater than during sleep but not ambulation. The urinary excretion rate of trimethoprim showed no significant differences between ambulation, bedrest and sleep. The urine pH during bedrest was significantly higher than either ambulation and sleep while the urine flow rate and the urinary sulphamethoxazole:trimethoprim ratio during bedrest were significantly higher than during ambulation and sleep.

5.3.3: DISCUSSION

The disposition of both sulphamethoxazole and trimethoprim following a single dose is best described by a two-compartment open model (Naber, Vergin & Weigand, 1981). Since the present study was conducted over a relatively short period for which subjects could maintain sleep or ambulation and the drugs were administered orally, good estimates of the individual kinetic constants describing the biexponential pharmacokinetics could not be obtained. In the case of trimethoprim, sufficient data were available in each of the three states to enable calculation of the model-independent pharmacokinetic parameters of terminal half-

Table 5.7 Mean (± SD) urinary drug excretion rate, urine flow and urine pH during ambulation, bedrest and sleep

Parameter[a]	Ambulation (n = 6)	Bedrest (n = 6)	Sleep (n = 5)
R_{ur} (SMZ) (mg/h)	3.69 ± 1.64	7.72 ± 4.12[b]	1.71 ± 0.93
R_{ur} (AcSMZ) (mg/h)	13.13 ± 4.34	16.39 ± 4.86[b]	7.92 ± 3.37
R_{ur} (TMP) (mg/h)	3.52 ± 0.91	3.63 ± 1.47	3.37 ± 0.59
Urine pH	6.44 ± 0.35	7.25 ± 0.26[c]	6.38 ± 0.50
Urine flow (ml/min)	1.05 ± 0.38	1.49 ± 0.43[b]	0.89 ± 0.28
SMZ:TMP	1.08 ± 0.49	2.30 ± 1.25[b]	0.50 ± 0.28

[a] R_{ur} - renal excretion rate; SMZ - sulphamethoxazole; AcSmz - N_4-acetylsylphamethoxazole; TMP - trimethoprim; SMZ:TMP - ratio of amount of sulphamethoxazole excreted in the urine during the first urine collection period to the amount of trimethoprim excreted over the same time.

[b] Bedrest significantly different from sleep but not from ambulation. ($p<0.05$)

[c] Bedrest significantly different from sleep, ambulation. ($p<0.05$)

life, clearance and apparent volume of distribution. For sulphamethoxazole, these parameters could not be calculated for all subjects during bedrest and sleep. Non-compartmental pharmacokinetic analysis of the data using statistical moment theory was not possible due to insufficient data describing the elimination phase for each compound. Yamaoka, Nakagawa and Uno (1978) calculated that if elimination phase data is acquired until the plasma concentration of a drug is 5% of its maximum, the error in mean residence time (MRT) is about 10%. In this study the final measured concentrations of sulphamethoxazole and trimethoprim are about 50% of their maxima, so, the error in calculating the mean residence time would be unacceptable.

5.3.3.1: Sulphamethoxazole pharmacokinetics

The mean plasma sulphamethoxazole concentrations obtained at various times following oral administration of 800 mg of sulphamethoxazole, in combination with trimethoprim, to healthy subjects (Figure 5.5) are similar to those reported previously (Naber et al, 1981; Welling et al, 1973; Vergin et al, 1981). The values for the pharmacokinetic parameters determined for sulphamethoxazole (Table 5.6) are also similar to those reported by other workers (Spreux-Veroquaux et al, 1983; Naber et al, 1981; Kremers, Duvivier & Heusghem, 1974). Although no significant differences were found for the rates of sulphamethoxazole absorption between the states (Table 5.6), the actual mean values for time to reach peak plasma concentration (t_p) suggest that absorption occurs more rapidly during ambulation than during bedrest and that absorption is slowest during sleep. This is

probably related to slower gastric emptying in supine subjects than in ambulant subjects. Similar slower rate of absorption in supine subjects has been observed for other drugs by other workers (Roberts & Denton, 1980; Nimmo & Prescott, 1978). The faster mean rate of absorption during ambulation results in a higher mean peak concentration (Cp) of sulphamethoxazole. However, the peak concentrations for each of the three states were not significantly different.

Due to the slower absorption rate and shorter plasma monitoring period during the sleep phase of the study, it was not possible to determine the terminal half-life, total clearance or volume of distribution for enough subjects to make comparisons between the values for these parameters obtained during sleep and those obtained during bedrest and ambulation (Table 5.5). Comparison of the disposition of sulphamethoxazole during bedrest and ambulation showed that the terminal half-life was significantly shorter and the total body clearance was significantly higher during bedrest than during ambulation. The apparent volume of distribution divided by the extent of absorption (V/F) was not significantly affected by the change in posture. This faster elimination of the drug during bedrest was associated with a significantly higher urine flow rate and a significantly higher urinary pH (Table 5.7). Sulphamethoxazole is a weak acid with a pKa of 5.6 (Wormser, 1978). With these physico-chemical properties, the renal excretion may be assumed to be dependent on urinary pH and uring flow (Cafruny, 1977). Welling et al (1973)

administered urinary acidifier or alkaliniser at the same time as drug administration to subjects and observed that alkaline loading increased the urinary recovery of sulphamethoxazole and that the half-life was shorter but not significantly so. More recently, Vree et al (1978b) found that when the urine was maintained alkaline (pH 7 to 8) the half-life of sulphamethoxazole was shorter (9 h) than when the urine was maintained at a pH of 5.5 to 6.0 (11 h). Vree et al (1978b) also found that at a particular urinary pH (alkaline or acid), the renal excretion rate of sulphamethoxazole was influenced by urine flow. In the present study, the relationship between the urinary excretion rate of sulphamethoxazole on urinary pH and urine flow was examined (Figures 5.8 and 5.9). There was a linear relationship between the urinary excretion rate and urine flow ($r = 0.790$, $p<0.001$) and between the urinary excretion rate and urine pH ($r = 0.790$, $p<0.001$). The urinary excretion rate of N_4-acetylsulphamethoxazole appeared not to be influenced by urine flow rate ($r = 0.389$, $p = 0.123$) and only marginally by urine pH ($r = 0.686$, $p<0.01$). Vree et al (1978b) and Vree et al (1979) found the renal excretion rate of N_4-acetylsulphamethoxazole was not influenced by either urine flow rate or urine pH. The pKa of N_4-acetyl-sulphamethoxazole is 5.00 and its lipid solubility is about one-fifth that of sulphamethoxazole (Vree et al, 1979). The renal elimination of weakly acidic drugs with pKa between 3.0 and 7.5 is influenced by urinary pH if the undissociated form is lipid soluble (Cafruny, 1977). Although the lipid solubility of N_4-acetylsulphamethoxazole is lower than that

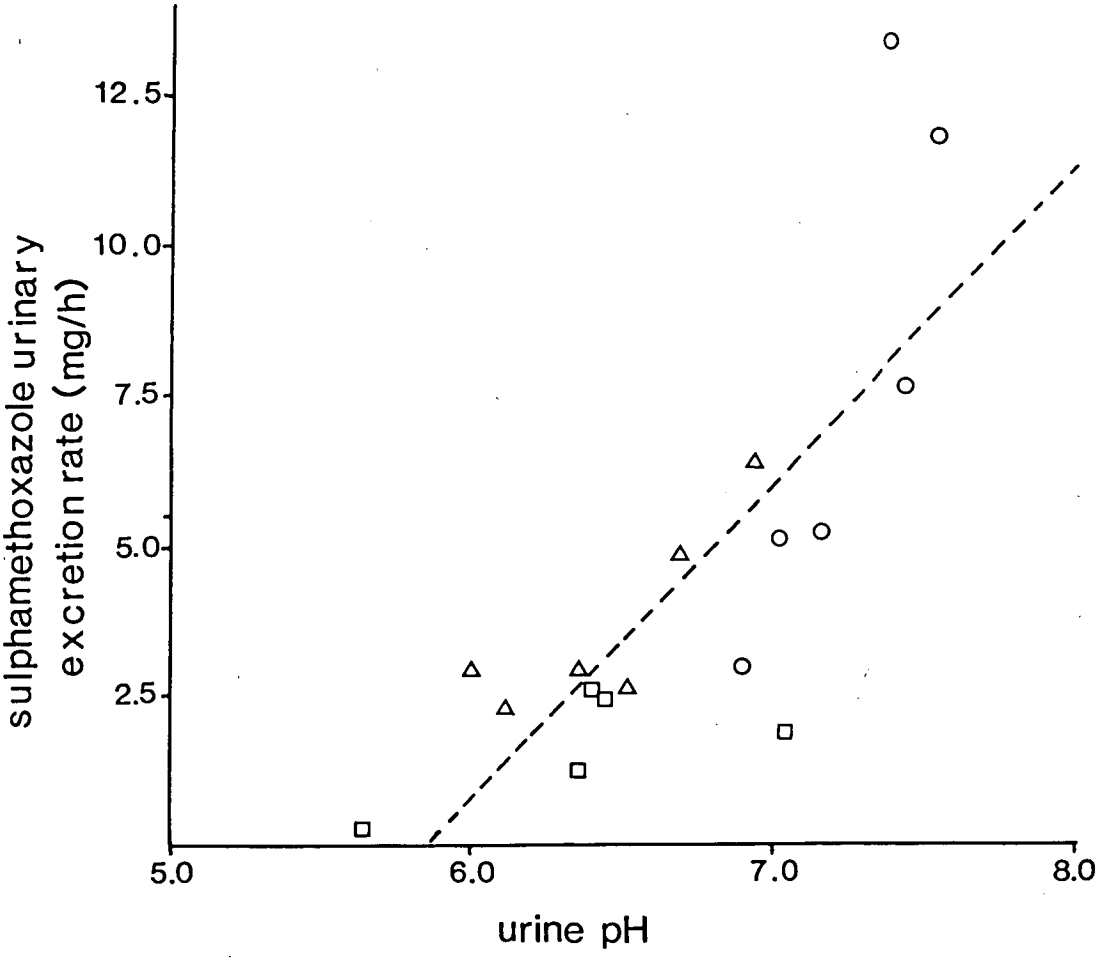

Figure 5.8 Urinary excretion rate (R_{ur}) of sulphamethoxazole versus urine pH during △ ambulation, □ sleep and ○ bedrest

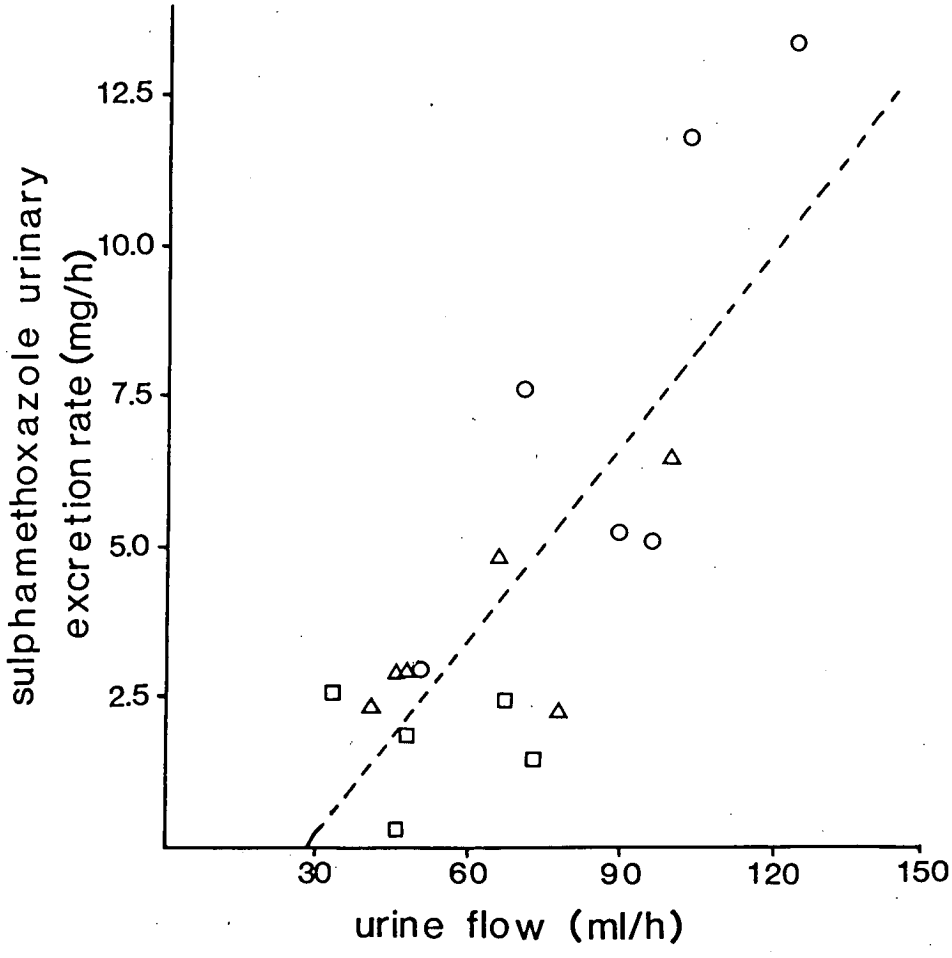

Figure 5.9 Urinary excretion rate (R_{ur}) of sulphamethoxazole versus urine flow rate during △ ambulation, ○ bedrest and □ sleep

of the parent drug, Vree et al (1978b) and Vree et al (1981) found that its renal clearance (40-60 ml/min) in subjects with normal renal function was considerably lower than the glomerular filtration rate suggesting some reabsorption occurred in the tubule. Thus, it is not unexpected that pH should have some influence on its elimination rate.

5.3.3.2: Trimethoprim pharmacokinetics

From the plasma trimethoprim concentration-time profiles (Figure 5.7) and the calculated values for the pharmacokinetic parameters (Table 5.6), the disposition of trimethoprim appears to be unaffected by posture or sleep.

The peak concentration of trimethoprim was always achieved earlier than the peak concentration of sulphamethoxazole. This phenomenon has been observed by other workers (Rogers et al, 1980; Naber et al, 1981; Kaplan et al, 1973). The peak concentrations and values for the other pharmacokinetic parameters appear to be in close agreement with published values for subjects with normal renal function (Patel & Welling, 1980; Wormser, 1978).

In contrast to sulphamethoxazole, the urinary excretion rate of trimethoprim appears to be unaffected by changes in urine flow ($r = 0.198$, $p = 0.447$) or by changes in urine pH ($r = 0.272$, $p = 0.291$). Sharpstone (1969) and Bergan & Brodwall (1972) also found no correlation between urine flow and renal excretion of trimethoprim but several workers have observed a significant relationship between urine pH and the renal elimination of trimethoprim (Craig & Kunin, 1973;

Bergan & Brodwall, 1972; Welling et al, 1973). However, in those studies many urine samples had quite low pH (pH<6.0) whereas, in the present study, only one subject had a urinary pH of less than 6.0 during the study period. Bergan & Brodwall (1972) suggest that at urinary pH greater than 6.2 net reabsorption occurs and it is only below this point that net tubular secretion takes place. It may be that the pH of the urine samples in the present study were too high for a relationship between the renal excretion rate of trimethoprim and urine pH to be evident.

The dependence of sulphamethoxazole elimination on urine pH and urine flow results in a significantly greater urinary sulphamethoxazole:trimethoprim ratio during bedrest than during sleep. Craig & Kunin (1973) reported ratios greater than 5:1 in alkaline urine. The highest ratio in the present study was 4.14:1. Variations in the sulphamethoxazole:trimethoprim ratio in urine are unlikely to have any clinical consequences over a normal dosing interval as the concentrations of both drugs are usually several times the minimum inhibitory concentration required for most susceptible organisms found in the urine (Patel & Welling, 1980).

CHAPTER 6

PARACETAMOL

6.1: INTRODUCTION

6.1.1: DRUG PROFILE

Paracetamol (acetaminophen) is an effective and widely used analgesic and antipyretic agent. It has little anti-inflammatory action at normal therapeutic doses used in humans although an anti-inflammatory effect has been demonstrated at high doses in animal models (Flower, Moncada & Vane, 1980). The specific mechanism of action of paracetamol is unknown. It has been suggested, however, that its analgesic and antipyretic effects and lack of anti-inflammatory effect may be associated with an ability to inhibit prostaglandin synthetase in the brain but little ability to inhibit peripheral tissue prostaglandin synthetase (Jackson, MacDonald & Cornett, 1984).

At recommended doses, serious adverse reactions to paracetamol are uncommon. Following massive overdose of paracetamol, liver damage ranging from minor to severe may occur. The liver damage occurs when liver glutathione levels drop to less than 20-30% of normal during the metabolism of paracetamol to the cysteine and mercapturic

acid conjugates. When this occurs, a reactive metabolite binds to hepatic proteins leading to hepatic necrosis (Davis, Labadarios & Williams, 1976). Treatment with N-acetylcysteine within 10 to 24 hours of overdosage can prevent hepatic damage occurring. Some recent reports indicate that cimetidine, but not ranitidine, inhibits the metabolism of paracetamol to the reactive metabolite and prevents liver glutathione depletion (Abernathy et al, 1983; Mitchell, Schenker & Speeg, 1984). Cimetidine may be a suitable agent in the treatment of paracetamol overdosage.

6.1.2: ASSAYS

There are several assay techniques available for the determination of paracetamol in biological fluids. The hepatotoxicity of paracetamol when ingested in large amounts has lead to many of the assays being developed for the determination of plasma concentrations following overdose rather than following therapeutic doses. Non-chromatographic methods include UV spectrophotometry and colorimetry. These methods are more susceptible to interference and poor or variable extraction than chromatographic methods. Also, determination of individual metabolites is not possible. Chromatographic techniques have included both gas-liquid chromatography and high-performance liquid chromatography. Non-chromatographic, gas-liquid chromatographic and early high-performance liquid chromatographic methods have been reviewed by Wiener (1978). A survey of recent reports on paracetamol pharmacokinetics (Divoll et al, 1982; Abernathy et al, 1983; Ameer et al,

1983; Anderson et al, 1983; Mitchell et al, 1983; Miners et al, 1984b) showed that high-performance liquid chromatography was now the assay technique of choice. Ideally, for full pharmacokinetic studies, the assay methods used should be capable of measuring paracetamol metabolites as well as the parent drug in both plasma or serum and urine. Several HPLC methods have been described for the determination of paracetamol and its metabolites in urine (Howie et al, 1977; Knox & Jurand, 1977; Knox & Jurand, 1978; Wilson et al, 1982; Miners et al, 1984a) but only those of Mrochek et al (1974) and Adriaenssens & Prescott (1978) are capable of measuring the paracetamol metabolites in plasma.

The methods of Mrochek et al (1974) involves anion-exchange chromatography and takes 24 hours per sample. The method described below provides a rapid determination of paracetamol and its major metabolites, the sulphate and glucuronide conjugates, in serum.

6.1.3: PHARMACOKINETICS

Paracetamol is a moderately water-soluble and lipid-soluble weak acid with a pKa of 9.5, thus it is largely un-ionised in biological fluids. Paracetamol is rapidly absorbed from the small intestine, its onset and rate of absorption being dependent on the rate of gastric emptying (Clements et al, 1978). A variable proportion of the dose is lost through first-pass metabolism and the bioavailability appears to be

dose-dependent, increasing from 63% after 500mg dose to almost 90% after 1g and 2g doses (Rawlins, Henderson & Hijab, 1977) and, also, to be formulation-dependent with a 650mg dose having 87% bioavailability when administered as a solution but only 79% when given as tablets (Ameer et al, 1983).

Paracetamol distributes throughout most tissues and fluids and has a volume of distribution of approximately 0.9 l/kg (Perucca & Richens, 1979). There is no apparent binding of paracetamol to plasma proteins at drug concentrations found after therapeutic doses. At higher plasma concentrations similar to those observed after toxic doses, paracetamol is 15-21% bound to plasma proteins (Gazzard et al, 1973).

Paracetamol is extensively metabolised with only 2 to 5% of a dose being excreted unchanged in the urine. Following a therapeutic dose the major metabolites are the sulphate (33%) and glucuronide (54%) conjugates with the cysteine (4%) and mercapturate (5%) being relatively minor metabolites (Forrest et al, 1979). In neonates and young children, glucuronidation is a less important metabolic pathway for the elimination of paracetamol; paracetamol sulphate being the major metabolite (Miller, Roberts & Fischer, 1976).

Shively & Vesell (1975) suggested that there is a temporal variation in the elimination of paracetamol. However, a recent study by Malan, Moncrieff & Bosch (1985) found that the time of dosing had no effect on the disposition of

paracetamol. It has been reported that the absorption of paracetamol may be slower during sleep and bedrest than during ambulation (Mattok & McGilveray, 1973; Nimmo & Prescott, 1978) but the effect of posture was not separated from that of sleep. Prescott (1975) reported that the plasma concentrations of paracetamol in convalescent patients in bed were higher than in ambulant healthy volunteers after an oral dose of 1.5g. The lower plasma paracetamol concentrations in the ambulant healthy volunteers were attributed to an increase in the volume of distribution produced by exercise performed by these subjects (Prescott, 1975).

In the present study, the effects of posture and sleep on the disposition of paracetamol and its metabolites were examined. Paracetamol is a model for drugs with low hepatic extraction ratios which undergo type II metabolism.

6.2: ANALYSIS

6.2.1: MATERIALS & METHODS

6.2.1.1: Chemicals

Paracetamol and its metabolites, paracetamol sulphate, glucuronide and cysteine were supplied by Sterling Laboratories, Newcastle on Tyne, Great Britain. Methanol and acetonitrile were specially purified for HPLC and supplied by Waters Associates (Milford, MA, USA). Water was de-ionised then glass distilled. All other materials used in the determination of paracetamol and its metabolites in

biological fluids were of analytical quality.

6.2.1.2: HPLC instrumentation and conditions

Reversed-phase high-performance liquid chromatography was performed using a Waters model M6000A solvent delivery system fitted with a U6K injector, precolumn (μBondapak C_{18}/Porasil B; 23 mm I.D.; Waters Associates, Milford, MA, USA) and a μBondapak C_{18} column (particle size 10 μm; 300 mm x 3.9 mm I.D; Waters Associates). UV absorbance at 254 nm was monitored with a Waters Model 450 variable wavelength absorbance detector. The absorbance was recorded on a dual-channel Omniscribe recorder (Houston Instruments, Austin, TX, USA). Injections were made with a 25 μl Hamilton syringe.

6.2.1.3: Serum paracetamol and metabolites

For the determination of serum paracetamol, paracetamol glucuronide and paracetamol sulphate, the mobile phase was 0.1M potassium dihydrogen phosphate - methanol - formic acid (90%) - ethylacetate mixture (90:15:0.15:0.1) at a flow rate of 1 ml/min.

Samples were prepared by adding 20 μl perchloric acid (30%) containing 2 mg/ml phenol (internal standard) to 200 μl serum. The sample was vortex mixed for one minute then centrifuged at 1500g for 5 minutes. 10μl of the supernatant was injected onto the column.

6.2.1.4: Urinary paracetamol and metabolites

Urinary paracetamol and its glucuronide, sulphate and cysteine conjugates were determined by the method of Miners et al (1984a). In this method the mobile phase was 2.5% acetonitrile and 97.5% 20 mmol/l orthophosphoric acid (adjusted to pH4.7 with potassium hydroxide) at a flow rate of 2 ml/min.

Urine samples were pretreated by adding 20 µl perchloric acid (30%) to 200 µl urine, vortex mixing for 1 minute, then centrifuging. 5 µl of the supernatant was injected onto the column.

6.2.1.5: Preparation of standard curves

Plasma standards were prepared by spiking drug-free plasma with known amounts of freshly-prepared aqueous solutions of paracetamol and its metabolites to produce concentrations of 1-20 mg/l for each compound. The standards were then assayed in the described manner. Urine standards were prepared in a similar manner to produce concentrations of 5-500 mg/l of each compound. Standard curves for plasma were prepared by plotting the peak height ratio (drug:internal standard) versus concentration and standard curves for urine were prepared by plotting the peak height of the compound versus concentration.

6.2.2: RESULTS & DISCUSSION

Chromatograms of blank serum and serum from a subject following oral administration of 500 mg paracetamol are shown in Figure 6.1. The retention times for paracetamol glucuronide, paracetamol sulphate, paracetamol and phenol were 4.8, 7.2, 8.8 and 17 minutes, respectively. The peak for the glucuronide metabolite eluted on the shoulder of the plasma peak which made accurate determination of low concentrations of this metabolite difficult. To make the glucuronide peak more visible the chart speed was 5 mm per minute for the first 5.5 minutes following each injection then reduced to 2.5 mm per minute (Figure 6.1). Figure 6.2 shows chromatograms of blank urine and urine following oral administration of 500 mg paracetamol. The retention times for paracetamol glucuronide, paracetamol sulphate, paracetamol cysteine and paracetamol were 3.0, 6.8, 8.2 and 9.2 minutes, respectively. These retention times are in the same sequence but slightly longer than those observed by Miners et al (1984a).

Several HPLC assays for the determination of paracetamol either alone or with its metabolites use UV detection at 254 nm (Knox & Jurand, 1977; Adriaenssens & Prescott, 1978; Demotes-Mainard, 1984) while all other assay reports reviewed use UV detection between 240 nm and 250 nm (Howie et al 1977; Horvitz & Jatlow, 1977; O'Connell & Zurzola, 1982; Wilson et al, 1982). Using 254 nm in this assay provided satisfactory sensitivity without interfering endogenous peaks.

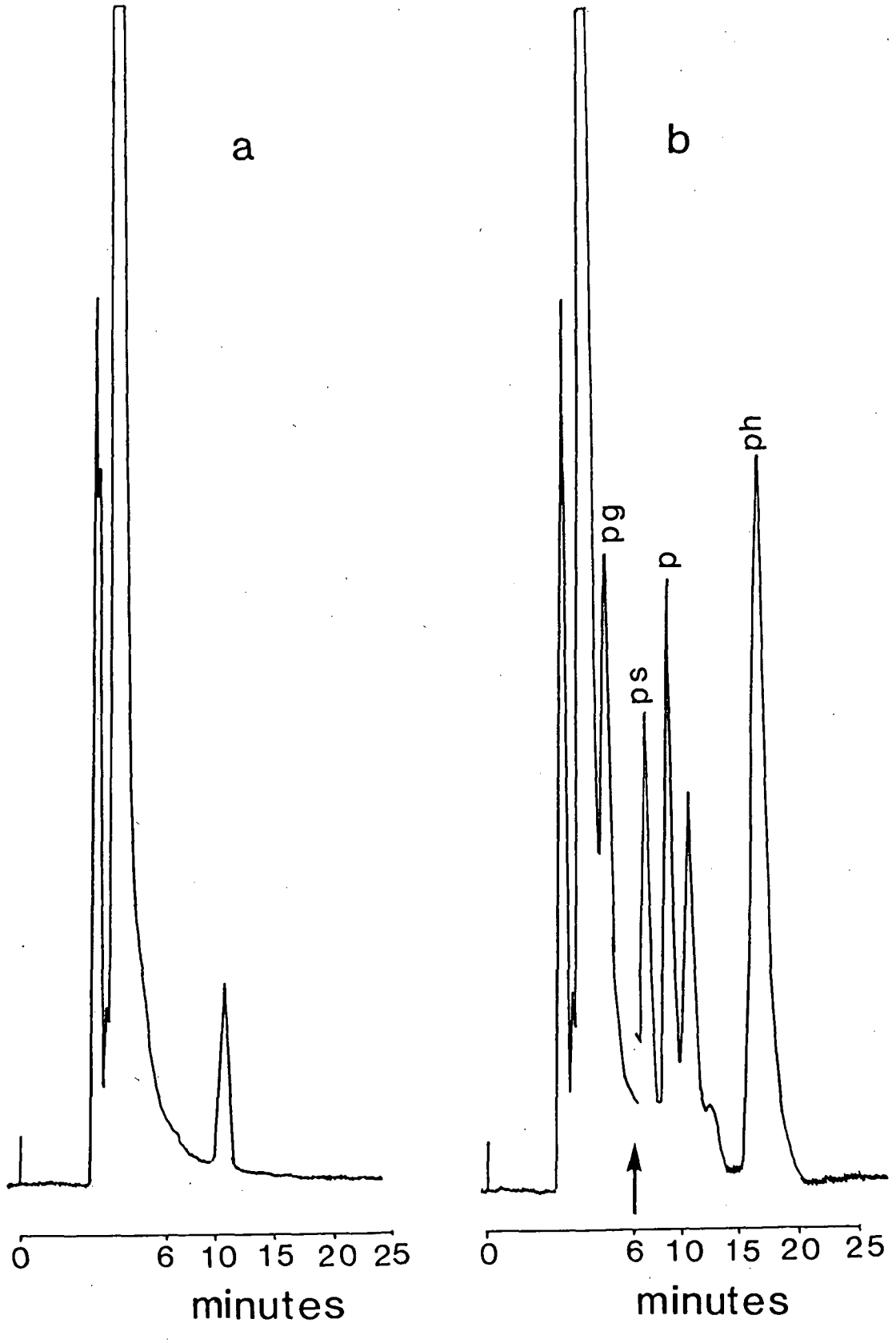

Figure 6.1 Chromatograms of (a) blank serum and (b) serum from the same subject 120 minutes after oral administration of 500mg paracetamol. Estimated concentrations are: paracetamol glucuronide (pg) 3.06 mg/l; paracetamol sulphate (ps) 3.59mg/l; paracetamol (p) 4.00 mg/l. The internal standard is phenol (ph). The arrow indicates both change in chart speed and change in absorbance scale

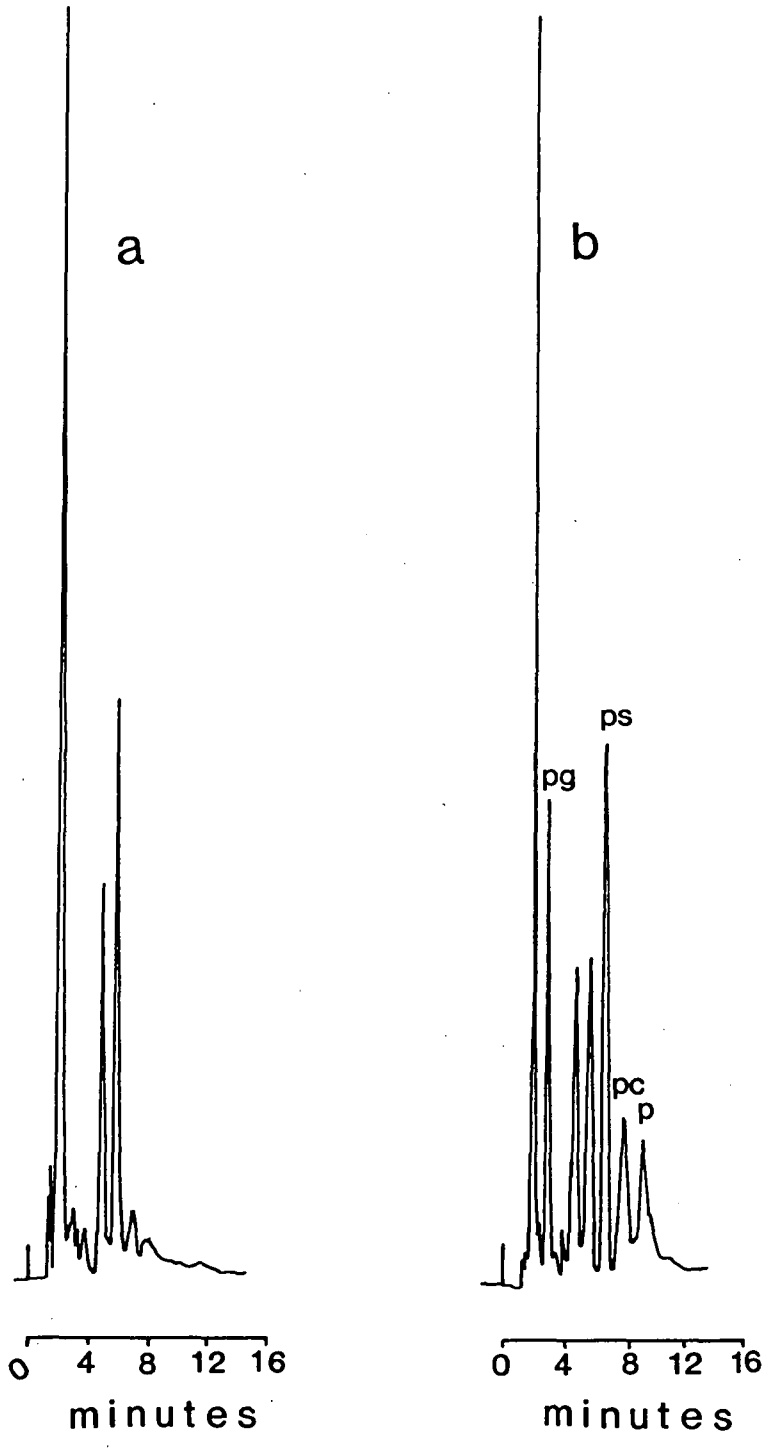

Figure 6.2 Chromatograms of (a) blank urine and (b) urine from the same subject collected 0 - 24 hours after oral administration of 500mg paracetamol. Estimated concentrations are: paracetamol glucuronide (pg) 484.8 mg/l; paracetamol sulphate (ps) 216.2 mg/l; paracetamol cysteine (pc) 25.7mg/l; paracetamol (p) 8.7mg/l

To reduce contamination of the column with serum proteins and lipids, serum samples maybe pretreated, usually by extraction of the drug using an immiscible organic solvent or by precipitation of the plasma proteins by a suitable agent. Commonly used agents to precipitate serum proteins include trichloroacetic acid, tungstic acid, perchloric acid and acetonitrile (Li Wan Po & Irwin, 1980; Giese, 1983). In the assay of paracetamol, trichloroacetic acid (Howie et al, 1977) and perchloric acid (Adriaenssens & Prescott, 1978) have been used. Most other HPLC assays for paracetamol in plasma or serum have used solvent extraction. Protein precipitation has the advantages of being rapid and simple and, providing that release of drug from plasma proteins is complete, is not subject to variable extraction of the drug. Paracetamol is not bound to plasma proteins at therapeutic plasma concentrations (Forrest et al, 1982), so protein precipitation is an excellent manner of sample preparation for the assay of this drug.

The mobile phase used in this assay was derived from information in the report by Knox & Jurand (1977) which studied the influence of changes in mobile phase components on the retention patterns of urinary paracetamol and its metabolites. The final choice of mobile phase provided optimum resolution of the compounds from endogenous peaks and each other.

Over the concentration ranges studied (0-20 mg/l for serum and 0-500 mg/l for urine), linearity of response was found

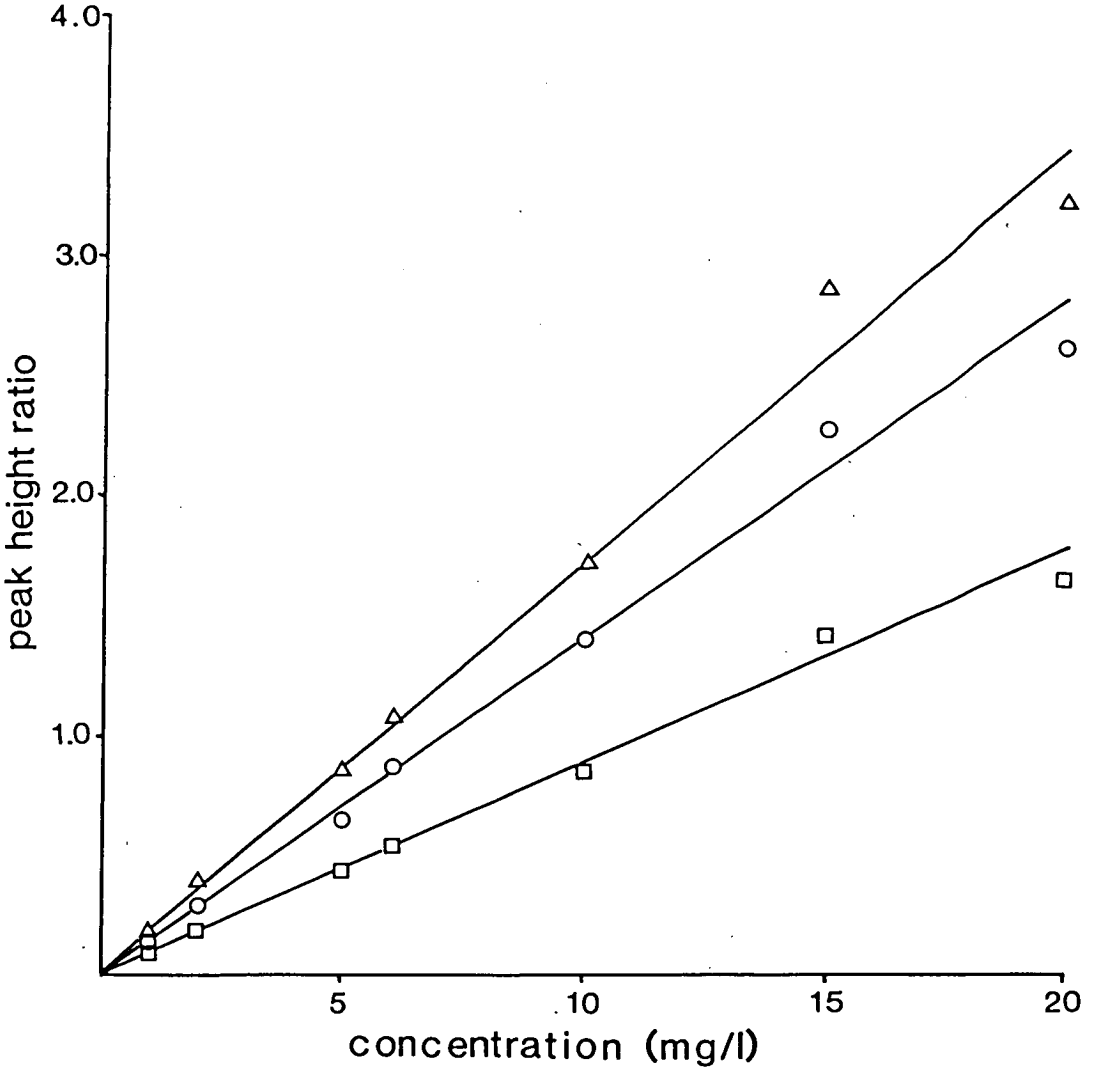

Figure 6.3 Serum standard curves for △ paracetamol, ○ paracetamol glucuronide and □ paracetamol sulphate

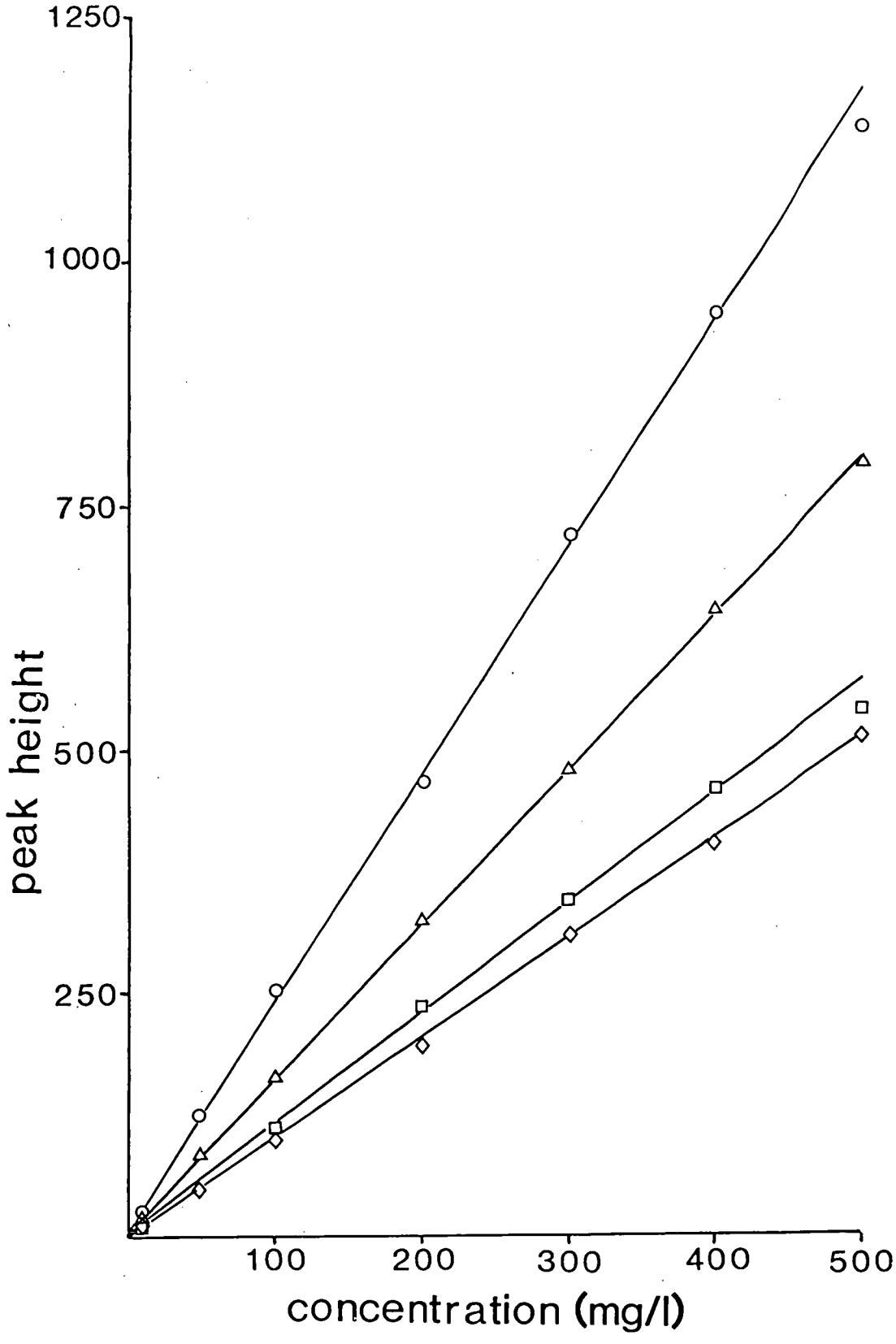

Figure 6.4 Urine standard curves for △ paracetamol, ○ paracetamol glucuronide, □ paracetamol sulphate, ◇ paracetamol cysteine

to be good ($r>0.99$) (Figures 6.3 & 6.4) and consistently reproducible for standard curves based on the peak height ratio of drug to internal standard for plasma and on peak height alone for urine. Under the conditions of this assay, the assay limits for paracetamol and its glucuronide and sulphate metabolites in serum were approximately 0.5, 1.0 and 1.0 mg/l, respectively. In urine, the assay limits were approximately 5 mg/l for the cysteine and sulphate metabolites and 2 mg/l for paracetamol and the glucuronide metabolite.

6.3: PHARMACOKINETIC STUDY

6.3.1: CLINICAL PROTOCOL

6.3.1.1 Subjects

Eight healthy male adult volunteers aged 21 to 26 years participated in the study. Prior to the investigation, a detailed medical history was taken. No subjects gave a history of liver or renal disease and physical examination, haematological tests and liver and renal function tests were normal for all subjects. Before the study was commenced, informed consent was obtained from each subject and approval was obtained from the Human Ethics Committee of the University of Tasmania.

6.3.1.2 Procedure

Each subject ingested 500mg paracetamol (Sterling Laboratories, Sydney, Australia) with 250ml of water on

three separate occasions - during ambulation in the day, during bedrest in the day and immediately prior to sleep at night (usually at 12 midnight). No food was permitted for two hours before and two hours after dosing. At least one week elapsed between each study.

6.3.1.3: Sample collection

Blood was sampled via an indwelling catheter, inserted into an antecubetal vein approximately one hour before dosing. The catheter permitted blood sampling with minimum disturbance to the subject. Patency of the catheter was maintained by flushing it with a small volume of heparanised saline (10 IU/ml) after each blood sample was taken. Blood was collected at 0, 15, 30, 45, 60, 90, 120, 180, 240 and 360 minutes after dosing. During blood collection, the first 1.0ml was drawn separately and discarded then the remainder of the sample was collected into non-heparinised tubes and allowed to clot at room temperature before being centrifuged. Serum was drawn off with a Pasteur pipette and stored at -20° until assayed. All urine excreted by the subjects over 24 hours after dosing was collected and pooled. An aliquot of the pooled urine was retained and stored at -20° until assayed.

6.3.1.4: Pharmacokinetic and statistical analysis

The overall elimination rate constant (β) for paracetamol was determined from the slope of the terminal linear segment of a semilogarithmic plot of serum concentration versus time. This was then used to calculate the elimination half-life ($t_{\frac{1}{2}} = 0.693/\beta$). The apparent total body clearance

(CL_B) of paracetamol was calculated by dividing the dose by the area under the serum paracetamol versus time curve from zero to infinity (AUC). The AUC was determined by the trapezoidal rule (Gibaldi & Perrier 1982b). The time to peak serum paracetamol concentration and the peak serum paracetamol concentration were used to assess the rate of absorption of the drug. As paracetamol was administered orally, its apparent volume of distribution could not be estimated directly. The ratio of the apparent volume of distribution for paracetamol (V) to its availability to the systemic circulation (F) was calculated according to Equation 6.1.

$$V/F = \frac{D}{\beta . AUC} \qquad (6.1)$$

where D is the administered oral dose.

The non-compartmental pharmacokinetic parameter, mean residence time (MRT), based on statistical moment theory, was calculated from the AUC and AUMC and is given by Equation 6.2 (Gibaldi & Perrier, 1982b):

$$MRT = \frac{AUMC}{AUC} = \frac{{}_0\int^{\infty} tCdt}{{}_0\int^{\infty} Cdt} \qquad (6.2)$$

The areas under the serum paracetamol glucuronide and paracetamol sulphate versus time curves were also calculated by the trapezoidal rule. As it was not possible to accurately determine the slope of the terminal portion of

the semilogarithmic plots of serum metabolite concentration versus time, this slope was assumed to be the same as that of the parent drug (Cummings, King & Martin, 1967) for the calculation of the "area to infinity" portion of the area under the curve (last estimate of serum metabolite concentration divided by β).

The renal clearance (CL_R) of paracetomol, paracetamol glucuronide and paracetamol sulphate were estimated from the ratio of amount of unchanged drug or metabolite excreted in the urine to infinite time (X_u^∞) to the AUC for parent drug or metabolite (Equation 6.3). For this calculation, it was assumed that urine collected up to 24 hours was equivalent to infinite time.

$$CL_R = \frac{X_u^\infty}{AUC} \qquad (6.3)$$

As the metabolites, paracetamol glucuronide and paracetamol sulphate are eliminated renally and apparently not further metabolised, it was assumed that the renal clearance of the metabolite adequately described the total clearance of that metabolite. The clearance of formation (CL_m) was then calculated from the following equation (Rowland & Tozer, 1980d)

$$CL_m = \frac{AUC_m . CL_{(m)}}{AUC_d} \qquad (6.4)$$

Where CL_m is the clearance associated with the metabolism of

parent drug to the particular metabolite, AUC_m and AUC_d are the areas under the serum concentration versus time curves for metabolite and parent drug, respectively, and $CL_{(m)}$ is the total clearance of that metabolite.

Statistical comparisons of the effect of bedrest, sleep and ambulation on various pharmacokinetic parameters was made by one-factor analysis of variance with repeated measures (Ferguson, 1976). The source of significant differences was identified using a studentized range test.

6.3.2: RESULTS

The mean serum paracetamol levels versus time profiles are shown in Figure 6.5. There were no significant differences between the profiles.

The individual experimental and pharmacokinetic data for paracetamol in subjects during ambulation, bedrest and sleep are shown in Tables 6.1, 6.2 and 6.3, respectively. The mean data for each of the parameters determined during each state are shown in Table 6.4.

There were no significant differences between the values obtained during ambulation, bedrest and sleep for any of the pharmacokinetic parameters determined except the non-compartmental parameter, mean residence time (MRT). For this parameter the values obtained during ambulation and sleep were significantly different ($p<0.05$) but differences between ambulation and bedrest and between bedrest and sleep

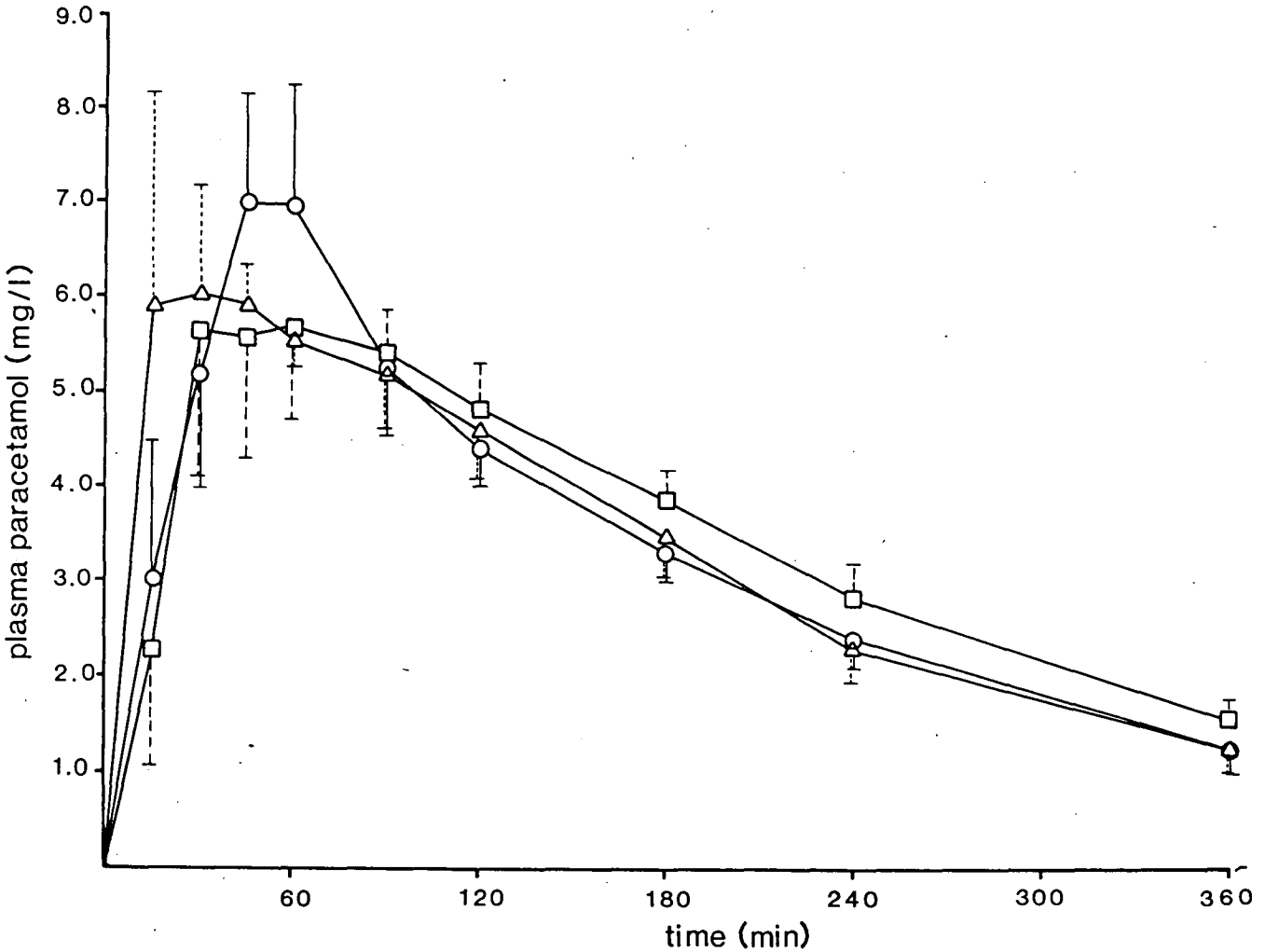

Figure 6.5 Mean (±SD) serum paracetamol concentrations found in healthy adult volunteers following oral administration of 500mg paracetamol during △ ambulation, ○ bedrest and □ sleep

Table 6.1 Experimental and pharmacokinetic data describing the disposition kinetics of paracetamol in ambulatory male subjects

Parameter[a]	Subject							
	1	2	3	4	5	6	7	8
Age (y)	23	21	22	26	21	23	23	23
Weight (kg)	72.3	71.8	61.0	70.5	72.7	76.4	72.7	68.1
AUC (min.mg/l)	1837	1287	2052	1359	2102	1472	987	1102
$t_{½}$(min)	130.0	148.3	144.4	115.5	177.8	114.9	103.1	98.8
CL_B (ml/min)	272.1	388.4	243.7	368.0	237.9	339.7	506.8	453.6
V/F (l/kg)	0.71	1.16	0.83	0.87	0.84	0.74	1.04	1.29
CL_R (ml/min)	4.46	N/A	1.56	3.39	7.90	3.67	3.04	4.54
MRT (min)	192.5	231.5	246.6	145.0	292.4	160.7	155.8	139.3
f	0.022	N/A	0.015	0.011	0.032	0.018	0.013	0.028
Urine flow (ml/min)	1.61	1.29	0.45	0.54	0.72	0.42	0.52	1.94
Urine pH	6.0	5.5	5.1	5.7	5.7	5.5	6.4	5.7

[a] AUC - area under the serum paracetamol concentration versus time curve from zero to infinite time; $t_{½}$ - half-life; CL_B - total body clearance; V/F - apparent volume of distribution (V) divided by the fraction of dose absorbed (F); CL_R - renal clearance; MRT - mean residence time; f - fraction of dose excreted unchanged in urine

Table 6.2 Experimental and pharmacokinetic data describing the disposition kinetics of paracetamol in male subjects during bedrest

Parameter[a]	Subject							
	1	2	3	4	5	6	7	8
Age (y)	23	21	22	26	21	23	23	23
Weight (kg)	72.3	71.8	61.0	70.5	72.7	76.4	72.7	68.1
AUC (min.mg/l)	1836	1058	2534	1363	1679	1222	957	1053
$t_{\frac{1}{2}}$ (min)	140.3	72.2	173.9	120.6	156.6	114.3	99.5	105.4
CL_B (ml/min)	272.4	472.5	197.3	366.9	297.7	409.3	512.8	474.7
V/F (l/kg)	0.76	0.69	0.81	0.91	0.92	0.88	0.01	1.44
CL_R (ml/min)	6.86	3.02	5.25	3.16	2.92	N/A	4.00	7.41
MRT(min)	214.6	193.9	270.5	173.4	239.0	190.7	153.9	177.4
f	0.038	0.026	0.048	0.030	0.028	N/A	0.020	0.031
Urine flow (ml/min)	0.99	3.00	2.34	1.84	1.00	3.74	1.20	2.28
Urine pH	6.6	6.2	6.5	5.8	7.1	6.6	6.7	7.1

[a] Parameter definitions as in Table 6.1

Table 6.3 Experimental and pharmacokinetic data describing the disposition kinetics of paracetamol in male subjects during sleep

Parameter[a]	Subject							
	1	2	3	4	5	6	7	8
Age (y)	23	21	22	26	21	23	23	23
Weight (kg)	72.3	71.8	61.0	70.5	72.7	76.4	72.7	68.1
AUC (min.mg/l)	1766	1319	1899	1199	3014	1380	1131	1458
$t_{½}$ (min)	126.5	187.9	152.3	116.2	181.5	115.6	117.1	164.4
CL_B (ml/min)	283.1	378.9	263.3	417.0	165.9	362.2	442.2	343.0
V/F (l/kg)	0.71	1.43	0.95	0.99	0.60	0.79	1.03	1.63
CL_R (ml/min)	2.55	2.58	1.84	4.50	1.96	3.98	4.78	1.85
MRT (min)	213.8	295.2	229.3	261.7	267.4	188.5	238.0	223.2
f	0.022	0.032	0.017	0.018	0.013	0.018	0.023	0.010
Urine flow (ml/min)	0.65	1.50	0.43	0.88	0.63	0.86	1.07	0.58
Urine pH	5.6	5.9	6.3	5.9	5.8	5.8	6.9	5.3

[a] Parameter definitions as in Table 6.1

Table 6.4 Comparison of mean estimates (± SD) of pharmacokinetic parameters for paracetamol in eight male subjects from data in Tables 6.1, 6.2 and 6.3

Parameter[a]	Ambulation	Bedrest	Sleep
AUC (min.mg/l)	1525 ± 425	1465 ± 531	1646 ± 613
$t_{\frac{1}{2}}$(min)	129.1 ± 26.6	122.8 ± 32.8	142.2 ± 30.3
CL_B (ml/min)	351.3 ± 97.9	375.5 ± 112.0	331.9 ± 90.5
V/F (l/kg)	0.94 ± 0.21	0.92 ± 0.23	1.02 ± 0.35
CL_R (ml/min)	4.08 ± 1.96	4.66 ± 1.88	3.01 ± 1.23
MRT (min)	195.5 ± 55.8	201.7 ± 38.1	239.6 ± 33.8[b]
f	0.020 ± 0.008	0.032 ± 0.009	0.019 ± 0.007
Urine flow(ml/min)	0.94 ± 0.21	2.14 ± 0.32[c]	0.83 ± 0.12
Urine pH	5.7 ± 0.13	6.6 ± 0.15[c]	5.9 ± 0.17

[a] Parameter definitions as in Table 6.1

[b] Sleep significantly different from ambulation but not bedrest ($p<0.05$)

[c] Bedrest significantly different from ambulation, sleep ($p<0.01$)

were not significantly different. It was found that the urinary flow rate and urinary pH of bedrested subjects were significantly greater than during either sleep or ambulation.

In Table 6.5 the mean time to peak (t_p) for paracetamol and the mean peak serum paracetamol concentration (C_p) for ambulation, bedrest and sleep are shown. The mean times to peak for sleeping subjects were greater than for bedrest subjects which were greater than for ambulant subjects and the mean peak concentration for ambulant subjects were higher than for bedrest subjects which were higher than for sleeping subjects. However, owing to the large individual variation in these absorption parameters, these parameters were not found to be significantly different in any of the states.

The mean serum paracetamol glucuronide levels versus time profiles and mean serum paracetamol sulphate levels versus time profiles are shown in Figures 6.6 and 6.7, respectively. Pharmacokinetic parameters determined for the metabolites are shown in Table 6.5. There were no significant differences between the renal clearances or the clearance of formation determined for either metabolite in each state of ambulation, bedrest or sleep. As can be seen from Figures 6.6 and 6.7 and Table 6.5, the mean time to peak concentration follows the same sequence as for the parent drug. Only the time to peak concentration for paracetamol glucuronide shows any significant difference

Table 6.5 Comparison of mean estimates (±SD) of pharmacokinetic parameters for paracetamol absorption and for paracetamol metabolites in eight male subjects.

Parameter[a]	Ambulation	Bedrest	Sleep
C_p paracetamol (mg/l)	9.47 ± 4.18	8.65 ± 2.64	7.29 ± 2.67
t_p paracetamol (min)	35.6 ± 27.7	54.5 ± 29.7	72.0 ± 53.5
t_p glucuronide (min)	105.0 ± 22.6	128.6 ± 48.0	165.0 ± 62.1[c]
t_p sulphate (min)	96.9 ± 33.5	84.4 ± 42.4	97.5 ± 54.5
t_{corr} glucuronide (min)	70.6 ± 24.6	65.4 ± 55.6	101.9 ± 54.7
t_{corr} sulphate (min)	39.4 ± 33.0	31.3 ± 18.8	28.1 ± 32.5
$CL_{formation}$ glucuronide (ml/min)	89.6 ± 30.3[b]	73.9 ± 45.4[b]	64.3 ± 19.0
$CL_{formation}$ sulphate (ml/min)	105.0 ± 39.9[b]	73.6 ± 29.9[b]	88.0 ± 40.2
CL_R glucuronide (ml/min)	174.0 ± 115.8	138.7 ± 99.9	65.0 ± 21.5
CL_R sulphate (ml/min)	125.0 ± 65.2	85.5 ± 28.0	105.3 ± 30.6

[a] C_p - peak serum paracetamol concentration; t_p - time to peak concentration for paracetamol or metabolites;
$CL_{formation}$ - clearance of formation; CL_R - renal clearance;
$t_{corr} = t_p$(metabolite) - t_p (paracetamol)

[b] n = 7

[c] sleep significantly different from ambulation but not bedrest ($p<0.05$)

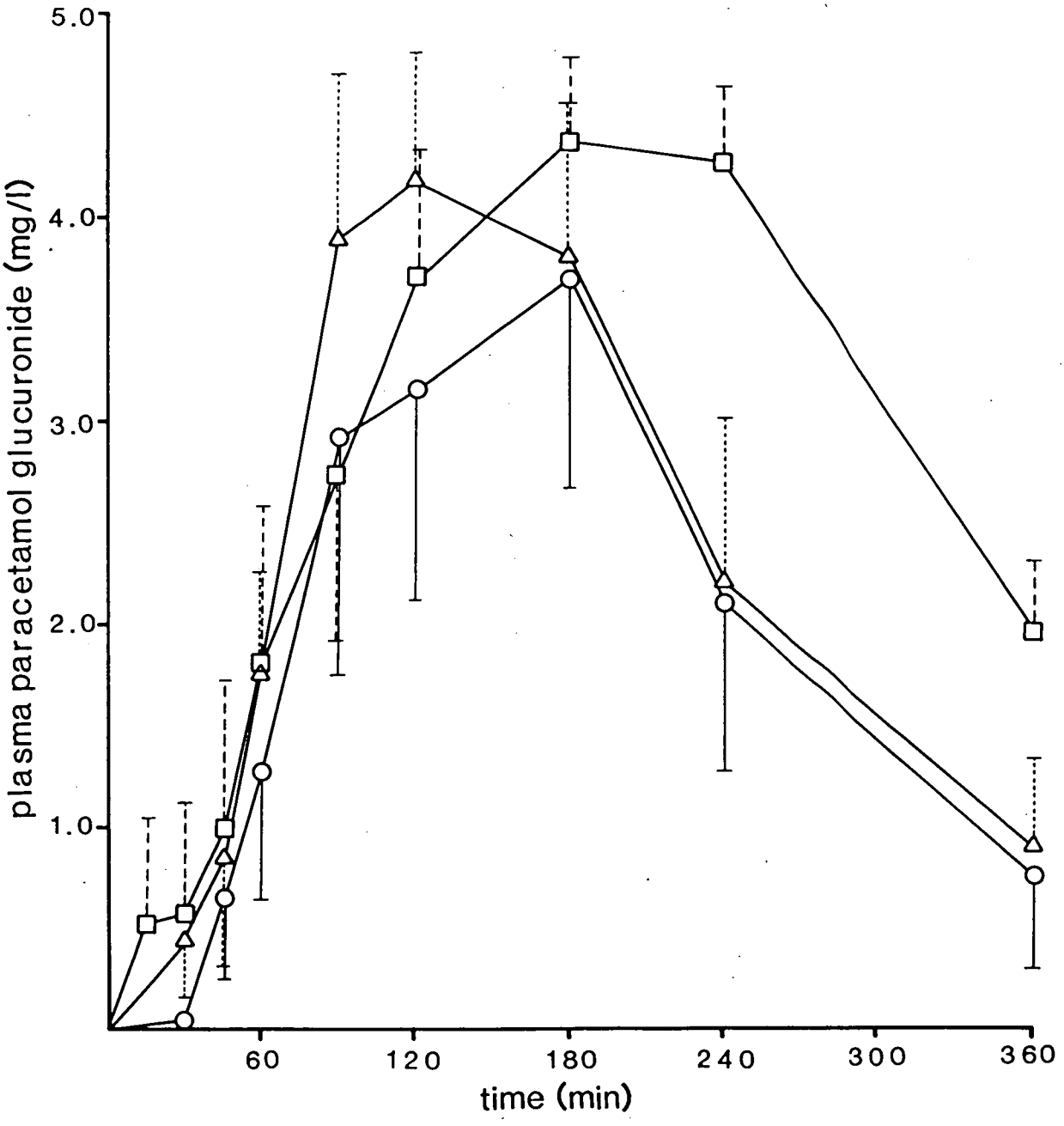

Figure 6.6 Mean (±SD) serum paracetamol glucuronide concentrations found in healthy adult volunteers following oral administration of 500mg paracetamol during △ ambulation, ○ bedrest and □ sleep

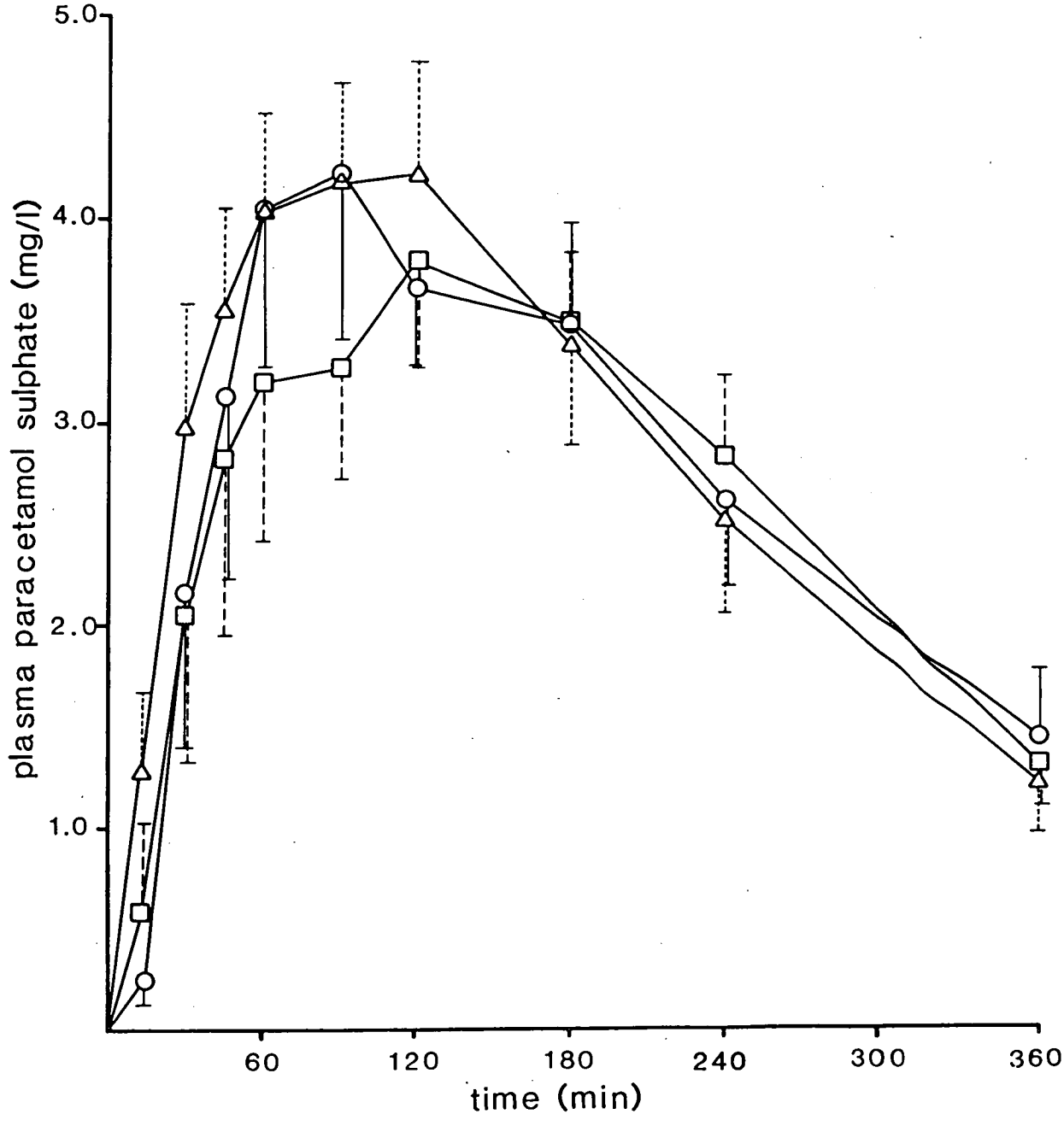

Figure 6.7 Mean (±SD) serum paracetamol sulphate concentrations found in healthy adult volunteers following oral administration of 500mg paracetamol during △ambulation, ○ bedrest and □ sleep

with change of posture, the time to peak during sleep being significantly greater than the time to peak during ambulation. When the time to peak data for the metabolites are corrected for the time to peak for paracetamol, these differences disappear (Table 6.5).

6.3.3: DISCUSSION

The half-lives for paracetamol found in the three phases of this study (range 72.2 - 187.9 min.) are similar to those observed by many other workers (Forrest et al, 1979; Cummings et al, 1967; Shively & Vesell, 1975; Forfar et al, 1980) following both oral and intravenous administration of the drug. The mean apparent body clearance and volume of distribution of paracetamol for ambulation, bedrest and sleep (Table 6.4) are also similar to those observed by other workers (Divoll et al, 1982; Shively & Vesell, 1975; Perucca & Richens, 1979). The non-compartmental pharmacokinetic parameter, mean residence time (MRT), has not been described previously for paracetamol.

The present study showed no significant differences in the plasma concentrations of paracetamol during ambulation and bedrest. Significantly higher concentrations of paracetamol in bedridden convalescent patients than in ambulant volunteers has been reported by Prescott (1975), differences in the apparent volume of distribution being suggested as the explanation. As this study and an earlier one (Mattok & McGilveray, 1973) have failed to show any effect of posture on paracetamol disposition, the differences reported by

Prescott (1975) probably reflect either different subject characteristics or other effects.

Shively & Vesell (1975) have reported that the disposition of paracetamol showed temporal variation. However, the changes are slight. After dosing at 6 am, the mean elimination half-life was 15% longer than the dose at 2 pm; the difference being attributed to a change in the apparent volume of distribution. The longer half lives also appeared to correspond to high plasma cortisol levels (Shively & Vesell, 1975). No significant differences in paracetamol disposition for the night time (sleep) and day time (bedrest) studies were observed in the present work, yet the times of dosing correspond to the times for minimal and maximal plasma cortisol levels (Moore Ede, 1973). Evidence of differences in the absorption rate between the states was found in this study. The mean residence time (MRT) was significantly longer during sleep than during ambulation ($p<0.05$) (Table 6.4). Other studies have also reported the absorption of paracetamol to be slower during sleep and bedrest than during ambulation (Mattock & McGilveray, 1973; Nimmo & Prescott, 1978). As V/F, elimination half life and body clearance of paracetamol do not differ significantly between the states of bedrest, ambulation and sleep (Table 6.4), the differences in MRT probably reflect differences in mean absorption time (MAT) rather than mean disposition time (MRT_{IV})-(MRT = MAT + MRT_{IV}) (Riegelman & Collier, 1980). The differences in the times to peak concentration for paracetamol and its metabolites in the three states are also

consistent with absorption rate differences between sleep, bedrest and ambulation. Mean peak times of 72, 55 and 36 min were found for paracetamol for the states of sleep, bedrest and ambulation, respectively (Table 6.5). The time to peak metabolite concentration (t_p) for paracetamol glucuronide was significantly longer during sleep than during ambulation (Table 6.5). However, when this was corrected for the time to peak serum paracetamol concentration, this difference disappeared. The time to peak paracetamol sulphate concentration showed no significant difference between any of the states. As the amount of total drug recovered from the urine during the study did not differ significantly between the states, changes in posture and sleep appear to have had no significant effect on the extent of paracetamol absorption. Other pharmacokinetic parameters for the major metabolites of paracetamol, paracetamol glucuronide and paracetamol sulphate, were not significantly altered by postural changes or sleep. Prescott (1980) reported the mean renal clearance of paracetamol sulphate and glucuronide to be 166 ml/min and 130 ml/min, respectively, following a dose of 20 mg/kg. In the present study the mean ambulant renal clearances of these metabolites were similar (Table 6.5) although there was a large intersubject variation. The clearance of formation does not appear to have been reported elsewhere.

The results of this study suggest that there are no significant changes in the disposition of paracetamol and its metabolites with changes in posture and sleep. The absorption of paracetamol tends to be slower in supine

subjects than in ambulant subjects. However, these changes are not likely to be of any clinical significance.

An important implication of this study is that drugs falling into the category of low binding, low hepatic extraction ratio are unlikely to be affected by changes in posture or sleep. The lack of change in the pharmacokinetics of paracetamol and its conjugates is consistent with the known physiology of the body. For instance, both the basal metabolic rate and glomerular filtration rate appear relatively constant throughout the day and night (Wagner, 1975). Changes in urine pH, urine flow and haemodynamics with change of posture or during sleep have been reported (Roberts & Denton, 1980) and it is the pharmacokinetics of drugs most dependent on these variables which are likely to show any changes with posture and/or sleep.

CHAPTER 7

CONCLUSIONS

7.1: POSTURE

The findings of these studies show that the pharmacokinetics of drugs can be modified by changes in posture.

Posture appears to have only minor effects on oral absorption. The mean times to peak concentration for paracetamol, sulphamethoxazole, and trimethoprim were longer during bedrest than ambulation but not significantly so. Posture did not have any significant effects on the intramuscular absorption of benzylpenicillin.

The distribution of the highly polar drug, gentamicin, was altered by changes in posture. On adoption of an upright posture, haemoconcentration occurs and there is a shift in blood flow away from the liver and kidney to the periphery. These changes in tissue perfusion and extravascular fluid may account for the larger volume of distribution for gentamicin observed in ambulant subjects than observed in supine subjects. The distribution of the other drugs studied appeared to be unaffected by changes in posture.

The only drug studied with extensive hepatic elimination was

paracetamol which has a low hepatic extraction ratio. The elimination of paracetamol in ambulant subjects did not differ significantly from that in supine subjects.

The effects of posture on the renal elimination of drugs depended on the disposition characteristics of the drugs. The higher urine pH and urine flow in bedrested subjects resulted in a shorter half-life and greater urinary excretion rate for sulphamethoxazole in supine subjects than in ambulant subjects. These results are in accordance with the physico-chemical properties of sulphamethoxazole, a weak acid of pKa 5.7. The urinary excretion rate of trimethoprim, a weak base, is also dependent on urine pH but is less sensitive to changes than sulphamethoxazole over the range of urine pH observed in these studies. Consequently, changes in posture did not affect the disposition of trimethoprim.

Gentamicin is eliminated solely by glomerular filtration and has a low renal extraction ratio. As the glomerular filtration rate is not affected by posture or exercise (Breiby et al, 1983; Swartz & Sidell, 1973), the minor posture-dependent changes in clearance observed in these studies are probably due to altered tissue uptake associated with modified distribution rather than variation in renal clearance.

Moving from a supine to an upright position causes a 15-20% decrease in renal blood flow. Benzylpenicillin is an

example of drugs with high renal extraction ratios whose elimination may be influenced by changes in renal perfusion. In these studies, the renal clearance of benzylpenicillin was unaffected by change of posture. These findings suggest that the posture-dependent changes in renal clearance of the penicillins reported by Roberts & Denton (1980) and Schmidt & Roholt (1966) were due to a higher level of exercise in the ambulant phase of those studies which would further reduce renal blood flow.

7.2: SLEEP

In the studies where the effects of sleep were examined, sleep had, generally, the same effects on drug disposition as bedrest.

7.3: FUTURE WORK

As hepatic blood flow is altered by changes in posture, the effects of posture on the elimination of high hepatic extraction ratio drugs requires examination. Klotz & Ziegler (1982) reported that the total body clearance of midazolam was much higher in supine subjects than in ambulant subjects. It would be interesting to confirm their results using another drug with similar disposition characteristics. One of the difficulties in selecting a suitable drug is that many drugs with high hepatic clearance (such as propranolol, lignocaine, verapamil and hydralazine) are vasoactive and may affect their own disposition.

Exercise causes many haemodynamic changes and may be an important factor in the disposition of drugs. The findings of Swartz & Sidell (1973) and Swartz et al (1974) indicate that exercise may alter the disposition of drugs with either high renal clearance or high hepatic clearance. Tissue perfusion is modified by exercise. This may alter the distribution of certain drugs. This has already been shown for digoxin (Pedersen et al, 1983). Future studies could examine the effects of graded exercise on the disposition of drugs.

Dettli & Spring (1966) observed an apparently much slower elimination of the highly protein-bound drug, sulphasymazine, when patients got out of bed before their blood sample time. This finding was attributed to the haemoconcentration that occurs on moving from a supine to an upright posture. For highly protein-bound drugs these changes could produce misleading results in blood-level monitoring and the pharmacokinetics of these drugs may appear altered by changes in posture. A systematic examination of the effects of posture on the blood levels and disposition of clinically important highly-bound drugs such as phenytoin and warfarin should be undertaken.

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