THE DISPOSITION OF PARACETAMOL N-ACETYL-D-L-METHIONATE IN HEALTHY SUBJECTS AND RENALLY IMPAIRED PATIENTS

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AUTHENTICATION

I hereby declare that this thesis was composed by myself and that the work described, other than that acknowledged to have been performed by others, is my own.

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ABSTRACT

Methionine is a gluthathione precursor and has been shown to be effective in preventing liver damage after paracetamol overdosage. Sterling Winthrop have linked paracetamol and N-acetyl-D-L-methionine as the N-acetyl-D-L-methionine ester of paracetamol in an attempt to make a safer form of paracetamol. As part of this compound's development the disposition of paracetamol N-acetyl-D-L-methionate was studied firstly in healthy subjects and then in patients with chronic renal failure. Each study was conducted as a double blind crossover clinical trial where each volunteer received 1 g of an effervescent formulation of paracetamol as (1) paracetamol (1 g) and (2) paracetamol N-acetyl-D-L-methionate (2.146 g).

Existing HPLC methods for measurement of paracetamol and its metabolites in plasma and urine were validated and used. In addition, a method was developed for measurement of paracetamol N-acetyl-D-L-methionate in plasma using solid phase extraction and analysis by HPLC with u.v. detection. Using these methods the stability of paracetamol N-acetyl-D-L-methionate was determined and the rate of hydrolysis in aqueous samples was shown to be accelerated as pH and temperature increased. The rate of hydrolysis in plasma and whole blood at 37°C was much greater, particularly in whole blood.

Following administration of 1 g of paracetamol to healthy subjects, paracetamol was rapidly absorbed, distributed and extensively metabolised. The major metabolites were the glucuronide and sulphate conjugates of paracetamol with the capacity for sulphation probably

reduced because of saturation of the pathway. A minor fraction of the dose was metabolised to a reactive intermediate which was then conjugated with glutathione and metabolised to form the cysteine and mercapturic acid conjugates of paracetamol. Elimination of paracetamol was consistent with glomerular filtration and passive reabsorption of paracetamol and active secretion of the sulphate and glucuronide conjugates. The total 24 h urinary recovery of paracetamol was essentially complete. This group provided control data consistent with other reports in the literature.

Following administration of 2.146 g of paracetamol N-acetyl-D-L-methionate to healthy subjects the parent compound was rapidly hydrolysed by ubiquitous esterases and the peak plasma paracetamol concentration was reduced and delayed. There was a corresponding delay in the appearance of paracetamol metabolites. The relative bioavailability of paracetamol however, was not altered and paracetamol absorption was essentially complete following administration of paracetamol in this form. The extent of paracetamol metabolism was the same as that obtained after administration of paracetamol alone and the amount recovered in urine as the glutathione derived conjugates was not increased even although methionine is a glutathione precursor. Sulphate conjugation was however significantly increased following administration of paracetamol N-acetyl-D-L-methionate. This may be explained by methionine providing a source of inorganic sulphate thus preventing its depletion or alternatively that the paracetamol sulphate pathway was less readily saturated as paracetamol was released more slowly. The increase in sulphate conjugation was at the expense of glucuronide conjugation. Elimination of

paracetamol and its metabolites was not altered following administration of paracetamol N-acetyl-D-L-methionate.

Following administration of 1 g of paracetamol to 7 non-dialysis and 5 haemodialysis patients with chronic renal failure absorption of paracetamol was normal, however, there was gross cumulation of metabolites and the late elimination phase of the parent compound was also impaired compared to healthy subjects. Elimination of metabolites in the haemodialysis patients was negligible. Cysteine and mercapturic acid conjugates of paracetamol were present at low concentrations in plasma and were eliminated at similar rates in the non-dialysis patients. In the haemodialysis patients elimination of mercapturic acid was negligible, whereas cysteine concentrations decreased over the period 8-24 h. The cysteine conjugate was, therefore, removed by a route other than urinary excretion. It may be that at the high metabolite concentrations attained in renal failure patients, the cysteine conjugate and possibly the other metabolites of paracetamol may undergo limited enterohepatic circulation with subsequent reabsorption of the parent compound, thus explaining the abnormal late elimination phase of paracetamol. Metabolism of paracetamol, however, was not altered in patients with chronic renal failure with the fractional urinary recovery of paracetamol and its metabolites in non-dialysis patients similar to that obtained in healthy subjects. The capacity for active tubular transport was reduced to a greater extent than passive reabsorption and the total 24 h urinary recovery of paracetamol was reduced in the non-dialysis patients. On long term paracetamol therapy plasma paracetamol concentrations would probably be higher than in the present study and

there would be gross cumulation of paracetamol metabolites. Multiple dose studies, therefore, require to be performed.

Following on, the disposition of paracetamol N-acetyl-D-L-methionate in patients with chronic renal failure was compared with the disposition of paracetamol administered alone to the same renal failure patients. The appearance of paracetamol was delayed and the peak plasma paracetamol concentrations reduced following administration of paracetamol N-acetyl-D-L-methionate. All other pharmacokinetic variables for paracetamol N-acetyl-D-L-methionate were, however, not significantly different from those obtained after administration of paracetamol alone to the same patients. Therefore, the relative bioavailability, metabolism and elimination of paracetamol administered in this form were not altered in patients with chronic renal failure. In particular the portion of the dose metabolised to form the cysteine and mercapturic acid conjugates remained unchanged. Unlike in healthy subjects, sulphate conjugation was not increased following administration of paracetamol N-acetyl-D-L-methionate in non-dialysis patients. As inorganic sulphate is retained in these patients it is probable that depletion of inorganic sulphate following administration of paracetamol alone was prevented, thus avoiding saturation of the sulphate pathway.

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CHAPTER I

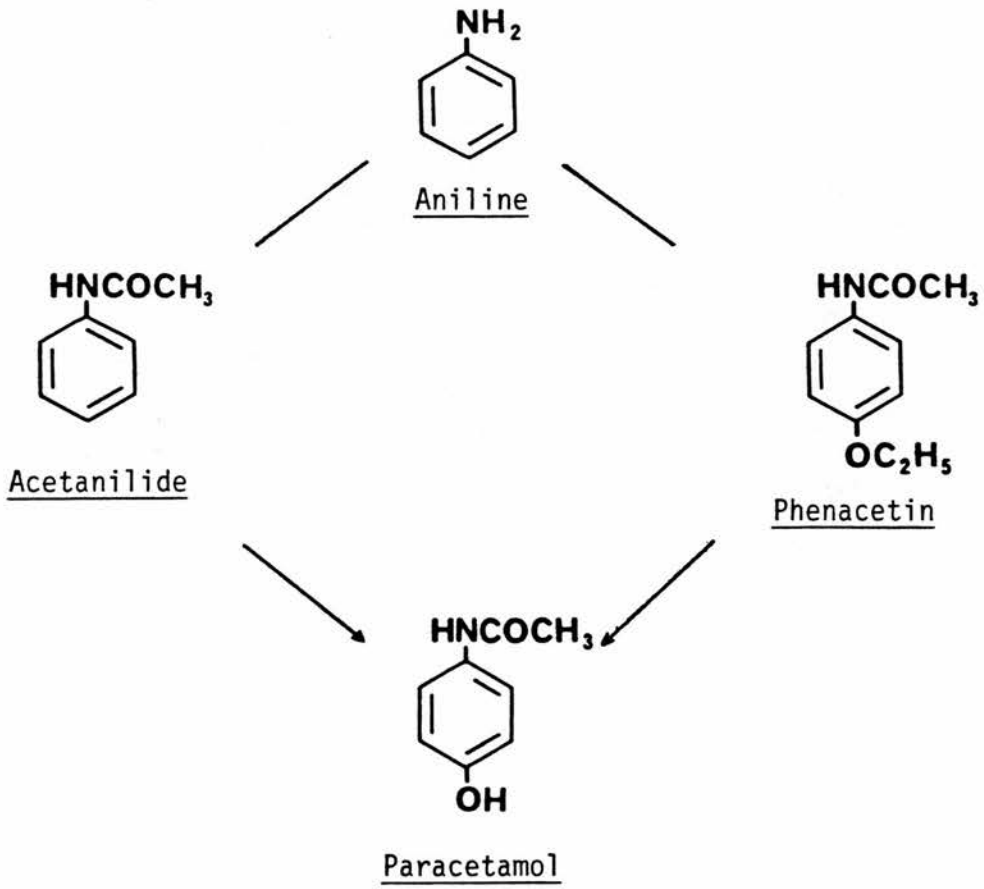
Introduction and Objectives

SECTION 1

PARACETAMOL

a) History

In the late 19th century, the antipyretic properties of aniline derivatives were investigated following the chance discovery that acetanilide lowered body temperature in patients with fever. Acetanilide was introduced into clinical practice in 1886 and the following year phenacetin became available.



It is probable that paracetamol was first used clinically in 1893 by von Mering (Smith, 1958). At this time, however, paracetamol was not considered a suitable substitute for acetanilide or phenacetin. The observation that acetanilide and phenacetin also had analgesic properties resulted in their use continuing well into the 20th century.

It was investigation into the metabolic fate of acetanilide and phenacetin by Brodie and Axelrod in the late 1940's that brought about the re-emergence of paracetamol. These investigations showed that acetanilide (Brodie and Axelrod, 1948) and phenacetin (Brodie and Axelrod, 1949) were both predominantly metabolised to paracetamol and excreted in conjugated form in man. At the same time, paracetamol was shown to have similar analgesic potency to acetanilide (Flinn and Brodie, 1948). It was therefore concluded that the antipyretic and analgesic properties of acetanilide and phenacetin were mainly due to their conversion to paracetamol. A further advantage of paracetamol was that unlike acetanilide and phenacetin, it did not cause methaemoglobinemia.

Paracetamol was marketed in Great Britain in 1956. Acceptance was initially slow and in 1963 it was included in the British Pharmacopoeia. Clinical studies showed paracetamol to be well tolerated with antipyretic and analgesic activities similar to aspirin (Beaver, 1965).

Since 1963, there has been a marked increase in the use of paracetamol with a corresponding decline in the use of aspirin (Spooner

and Harvey, 1976). In April 1985, paracetamol was included in the limited list of analgesics for mild to moderate pain drawn up by the Department of Health.

Paracetamol is a very safe and effective antipyretic and analgesic agent when taken in therapeutic doses. When taken in overdose, however, paracetamol can cause severe and occasionally fatal liver damage. The first cases of liver damage caused by paracetamol overdosage were reported more than 20 years ago (Davidson and Eastham, 1966; Thomson and Prescott, 1966). With the increasing availability of paracetamol, further reports were soon to follow.

The mechanism by which paracetamol causes hepatotoxicity was discovered by Mitchell and his colleagues (Jollow et al, 1973; Mitchell et al, 1973 a; b; Potter et al, 1973). A minor proportion of a dose of paracetamol is converted to a reactive intermediate by cytochrome P-450 mixed function oxidase. The reactive intermediate is then conjugated with glutathione and excreted in the urine as cysteine and mercapturic acid conjugates. In overdose, however, glutathione becomes depleted and the reactive metabolite is free to covalently bind to the vital hepatic macromolecules causing cell death.

The results of Mitchell's work provided a rational approach to the treatment of paracetamol toxicity. Prescott and Critchley (1983) have reviewed the treatment of paracetamol poisoning and described the successful use of glutathione precursors and other sulphydryl compounds.

Chemical data for paracetamol are shown in Appendix 1.1.

b) Pharmacology

Paracetamol is a well tolerated mild analgesic and antipyretic in both adults and children with only weak anti-inflammatory properties (Beaver, 1965). It is particularly suited for the relief of moderate pain intensity such as headache, musculoskeletal pain and dysmenorhea (British National Formulary, 1989).

Algesia, pyrexia and inflammation are thought to be mediated by prostaglandins, and it is the ability of antipyretic analgesics to inhibit prostaglandin biosynthesis that gives them their effect. Different pharmacological potencies and properties within the antipyretic analgesic drug group may be explained by the prostaglandin producing enzyme systems from different tissues, possessing different sensitivities towards these drugs (Ferreira and Vane, 1974; Flower, 1974).

Although this explanation may provide part of the answer, the mechanisms that cause pain, fever and inflammation are not fully understood, nor are the pharmacological actions of any of the antipyretic analgesics. This area of research is therefore inconclusive with a great number of conflicting publications appearing in the literature. This is particularly the case for aniline derivatives (Brune, 1983). The pharmacological properties and efficacy of paracetamol are, therefore, more easily assessed through clinical trials.

Studies assessing the analgesic efficacy of paracetamol have been reviewed (Beaver, 1965; Cooper, 1981; Mehlisch, 1983) and the overall conclusion is that paracetamol is significantly better than placebo and equianalgesic and equipotent to aspirin. Examples of investigations substantiating these findings include studies in cancer pain (Wallenstein and Houde, 1954) and oral surgery pain models (Cooper, 1981).

Studies of the efficacy of paracetamol and aspirin in lowering body temperature have also been reviewed (Lovejoy, 1978). Compared to placebo, paracetamol and aspirin demonstrate significant and similar antipyretic effects. Temperature declined from 0.5 h following paracetamol administration with a duration of action of approximately 3 h. Maximum reductions in temperature were in the order of 2.7% (Colgan and Mintz, 1957) and 2.9% (Steele et al, 1972).

Paracetamol has little or no anti-inflammatory activity in the treatment of rheumatoid arthritis (Batterman and Grossman, 1955; Hajnal et al, 1959). Paracetamol does, however, exhert anti-inflammatory effects against post traumatic inflammation. Following surgical removal of bilateral wisdom teeth, paracetamol reduced post operative swelling on the third day by 29% compared to placebo in addition to reducing local hyperpyrexia and pain (Skjelbred and Lokken, 1979). It may be, therefore, that paracetamol has a clinical use for some types of inflammation not associated with arthritis (Cooper, 1981).

c) Absorption, Distribution, Metabolism and Excretion

Absorption: Paracetamol is usually taken orally and it is absorbed from the upper gastro-intestinal tract by passive diffusion. Transport across the mucosal membrane is dependent on paracetamol being in a lipid soluble, unionised state at physiological pH. It is a weak organic acid (pKa 9.5), which is predominantly unionised in biological fluids and therefore available for gastro-intestinal absorption. Other conditions such as mucosal blood supply and surface area may also affect absorption (Prescott, 1974).

The primary sites of absorption have been investigated using animal models (Weikel and Lish, 1959; Josting et al, 1976; Bagnal et al, 1979; Pang et al, 1986). Methodological differences and variable physiological conditions have, however, led to variation in results. It is probable that the gastro-intestinal tract is capable of absorbing paracetamol over most of its length, but the upper small intestine is the optimum site and as much as 76% can be absorbed in 30 min (Josting et al, 1976). These findings are supported by studies in man where the rate of gastric emptying was shown to influence the rate of absorption (Heading et al, 1973).

Paracetamol was administered orally as a solution to 8 healthy subjects at a dose level of 20 $mg.kg^{-1}$ following an overnight fast (Prescott, 1980). Under these optimal conditions, absorption was rapid with the mean peak plasma concentration at 30 min. Paracetamol absorption may be greatly influenced by formulation and there is also

considerable individual variation (McGilveray et al, 1971; Prescott, 1974; Ritcher and Smith, 1974; Ameer et al, 1983).

The effect of age, weight, sex, posture, drugs and disease on paracetamol absorption has been reviewed (Forrest et al, 1983). The most influential factor is the rate of gastric emptying. Food, drugs and disease states which reduce gastric emptying consequently reduce the rate of paracetamol absorption and vice versa, however, the total amount absorbed is unaltered. Binding agents such as activated charcoal and cholystyramine reduce the total amount of paracetamol absorbed.

Distribution: Following absorption, paracetamol is rapidly distributed throughout most tissues and body fluids.

Two hours after the administration of 2.7 g of phenacetin to a dog, paracetamol was more or less equally distributed between tissue water and plasma water (Brodie and Axelrod, 1949). These results were subsequently confirmed in a similar study where 5 dogs each received 300 $mg.kg^{-1}$ of paracetamol orally (Gwilt et al, 1963). In neither study was there an accumulation of paracetamol in any of the tissues investigated. Low paracetamol concentrations were observed in fat and cerebrospinal fluid and the mean whole blood to plasma ratio at 45 min was 1.06. There is no appreciable binding of paracetamol to erythrocytes nor to plasma proteins at concentrations less than 60 $\mu g.ml^{-1}$. The extent of plasma protein binding at 280 $\mu g.ml^{-1}$ was 15-21% (Gazzard et al, 1973). The glucuronide and sulphate

conjugates of paracetamol also do not bind to plasma proteins (Lowenthal et al, 1976).

Metabolism: Most drugs are primarily dependent on metabolism for their elimination and are converted in the liver to polar metabolites which are more readily excreted by the kidney.

A potential site for drug metabolism is the gastro-intestinal tract. Intestinal metabolism of paracetamol has been observed in rats (Josting et al, 1976; Pang et al, 1986) and man (Rogers et al, 1987). Glucuronide and sulphate conjugates have been detected, but only in insignificant quantities. In addition, following oral and intravenous administration of paracetamol in man, the percentage urinary recovery of paracetamol and its metabolites was similar (Clements et al, 1984). These findings suggest the gastro-intestinal tract is an insignificant site for paracetamol metabolism.

From the small intestine, paracetamol passes to the systemic circulation via the portal vein and the liver. The oral bioavailability of paracetamol in man is 70-80% (Rawlins et al, 1977; Perucca and Richens, 1979; Clements et al, 1984). Quantitative urinary recoveries and minimal intestinal metabolism indicate that bioavailability is limited by first pass metabolism by the liver. The first pass metabolism of paracetamol is said to be dose dependent (Rawlins et al, 1977) with an oral bioavailability of 63% after 0.5 g and 89% after 1.0 g. However, these findings have not been confirmed in more recent studies (Clements et al, 1984).

The major route of paracetamol metabolism is by conjugation at the hydroxyl group. Glucuronyl and sulphotransferases in the presence of their uridine diphosphoglucuronic acid (UDPGA) and 3'-phosphoadenosine-5'-phosphosulphate (PAPS) cofactors form the glucuronide and sulphate conjugates of paracetamol respectively. Unlike the glucuronide pathway sulphate conjugation exhibits dose dependent saturation within the therapeutic dose range (Prescott, 1984). In addition, the capacity for sulphation is probably dependent on the availability of inorganic sulphate (Levy, 1986) a precursor of PAPS.

A minor fraction of paracetamol is metabolised to form a reactive intermediate metabolite which is probably N-acetyl-p-benzoquinone imine (Miner and Kissinger, 1979; Dahlin et al, 1984). The exact mechanism by which the intermediate is formed is unclear (Potter and Hinson, 1986). It is normally recovered by conjugation with reduced glutathione.

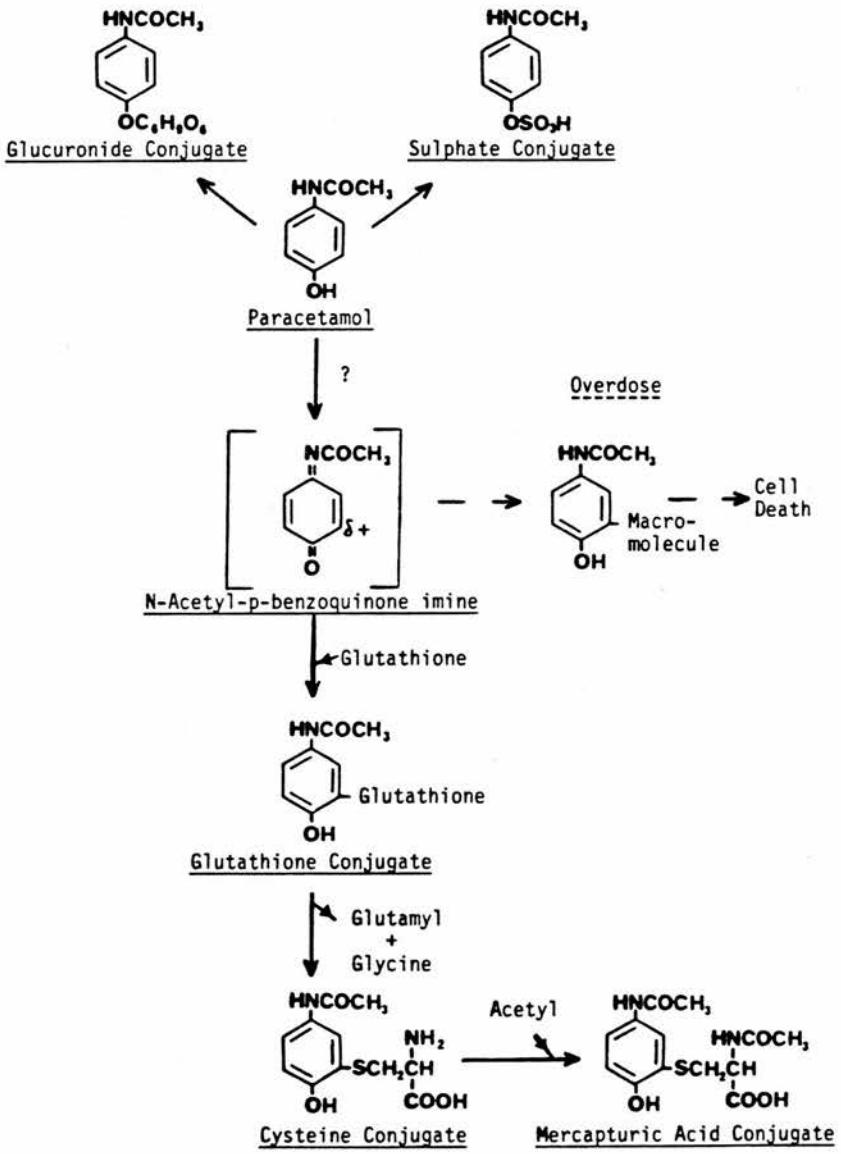
The ability to inactivate the intermediate by conjugation with hepatic glutathione is dose dependent. Following overdosage, glutathione becomes depleted leaving the reactive intermediate free to bind to vital hepatic macromolecules causing cell death. With therapeutic doses, however, glutathione deactivates the reactive intermediate forming a paracetamol gluthathione conjugate (Mitchell et al, 1973b, 1974; Potter et al, 1974). This is converted to the cysteine conjugate of paracetamol by loss of glutamyl and glycine moieties catalysed by glutamyl transferase and dipeptidase respectively. The cysteine conjugate may then be acetylated by N-acetyl transferase to form the mercapturic acid conjugate of paracetamol.

Other minor metabolites accounting for approximately 1% of a therapeutic dose have been identified in man. These include conjugates of 3-hydroxy and 3-methoxyparacetamol (Andrews et al, 1976) and 3-thiomethylparacetamol (Klutch et al, 1978). Following overdosage, 3-methoxyparacetamol has also been identified (Knox and Jurand, 1977, 1978).

After a therapeutic dose of paracetamol the 24 h urinary recovery is about 85-95% in healthy subjects. The glucuronide, sulphate, cysteine and mercapturic acid conjugates and parent compound account for approximately 55, 30, 4, 4 and 4% respectively (Prescott, 1980). The proposed metabolic pathway for paracetamol in man is shown in Figure 1.1.

As indicated earlier, paracetamol metabolism is dose dependent. The effect of dose on paracetamol metabolism has been demonstrated by investigating the urinary metabolite profiles of overdose patients on supportive therapy who did not develop severe liver damage. In this group there was less sulphate and more glucuronide conjugation with no significant change in the proportions of cysteine and mercapturic acid conjugates produced compared to healthy subjects receiving therapeutic doses. In a similar group of patients where severe liver damage did develop, there was an increase in cysteine and mercapturate conjugation, reflecting an increased proportion of paracetamol being converted to the toxic intermediate. This increase was at the expense of glucuronide conjugation (Prescott, 1980, 1983; Forrest et al, 1982).

FIGURE 1.1 Paracetamol Metabolism in Man (Adapted from Mitchell et al, 1974)



Paracetamol metabolism is also age dependent as young children less able to conjugate phenolic drugs with glucuronic acid, compensate by predominantly converting them to the sulphate conjugate (Levy et al, 1975; Notarianni et al, 1987).

In patients with severe liver disease receiving therapeutic doses of paracetamol the plasma paracetamol to glucuronide and sulphate ratio is greater than in healthy subjects. The 24 h urinary metabolite profiles, however, were not significantly different from those obtained from healthy subjects (Forrest et al, 1979). In patients with Gilbert's Syndrome the ability to conjugate paracetamol with glucuronic acid was impaired (Douglas et al, 1978). These findings, however, have not been confirmed recently (Ullrich et al, 1987).

The influence of alcohol on paracetamol metabolism has been reviewed (Seeff et al, 1986). Chronic and acute use of alcohol appears to increase and decrease respectively the proportion of paracetamol metabolised to the reactive intermediate.

Drugs which induce microsomal enzymes alter paracetamol metabolism. Compared to healthy subjects, patients receiving anticonvulsants or rifampicin exhibited enhanced glucuronide conjugation with no significant change in sulphate conjugation. Urinary excretion of cysteine and mercapturic acid conjugates were similar in each group (Prescott et al, 1981).

Inter-individual variation in paracetamol metabolism was greater than intra-individual variation (Caldwell et al, 1980; Clements et al,

1984). The contribution to inter-individual variation by sulphate and glucuronide conjugation was much less than that of the cysteine and mercapturic acid pathway (Critchley et al, 1986).

The influence of genetic factors on paracetamol metabolism has been investigated in healthy twins. The data suggests that unidentified environmental conditions account for inter-individual variations in paracetamol metabolism, rather than genetic factors (Nash et al, 1984). The investigators, however, only concentrated on the sulphate and glucuronide conjugation of paracetamol and omitted to consider its conversion to the cysteine and mercapturic acid conjugates.

Ethnic studies of paracetamol metabolism have shown that the fractional recovery of the cysteine and mercapturic acid conjugates is less in Africans than in Caucasians. This may be due to genetic or environmental factors such as dietary differences, particularly protein intake (Critchley et al, 1986).

Excretion: Paracetamol and its conjugates are excreted rapidly in man. Unchanged drug appears to be filtered at the glomerulus followed by passive reabsorption producing a low renal clearance which is dependent on the urine flow rate. The renal clearance of the conjugates of paracetamol is greater than the glomerular filtration rate and independent of the urine flow rate indicating active renal tubular secretion with negligible reabsorption. The excretion of paracetamol and its conjugates is independent of urinary pH (Prescott and Wright, 1973). These findings have also been observed in the dog (Duggin and Mudge, 1975).

The renal clearance of paracetamol sulphate has been shown to decrease as plasma concentration increases and is, therefore, dose dependent (Clements et al, 1984; Morris and Levy, 1984).

Investigation of the biliary excretion of paracetamol in man is difficult. Following 1 g of oral paracetamol only the glucuronide and cysteine conjugates representing 0.1 and 0.6% of the dose respectively were recovered in bile after 12 h. This, however, represents 19.4% of the total excretion of the cysteine conjugate. Taking into account the limitations of the experiment the biliary recovery reported is maybe an underestimate (Jayasinghe et al, 1986). Earlier studies of the biliary excretion of paracetamol in man proved inconclusive (Siegers et al, 1984).

d) Toxicity and Overdosage

As no preclinical toxicity studies were performed with paracetamol, it was not until paracetamol had gained in popularity that its hepatotoxic properties following acute overdosage were discovered. Reports of paracetamol hepatotoxicity in cats (Eder, 1964) and rats (Boyd and Bereczky, 1966) were soon followed by reports of severe and fatal liver damage following paracetamol overdosage in man (Davidson and Eastham, 1966; Thomson and Prescott, 1966). Since then the incidence of paracetamol overdosage has gradually increased. In Edinburgh the number of annual admissions to the Regional Poisoning Treatment Centre increased from 15 in 1967 to a peak of 309 in 1979. In 1983, 13% of overdose admissions involved paracetamol (Prescott, 1983).

The mechanism of paracetamol hepatotoxocity was discovered by Mitchell and colleagues in 1973. Initial work in rats and mice demonstrated that pretreatment with compounds which stimulated the cytochrome P-450 mixed function oxidase system potentiated hepatic necrosis, whereas pretreatment with inhibitors of this enzyme system reduced liver necrosis. It was, therefore, proposed that necrosis was mediated by a toxic metabolite and not by paracetamol itself (Mitchell et al, 1973a). Further experiments using [^{3}H]-paracetamol demonstrated that necrosis was caused by covalent binding of a reactive intermediate metabolite to vital hepatic macromolecules and that the degree of covalent binding was proportional to the severity of necrosis (Jollow et al, 1973). In vitro studies confirmed the covalent binding of a metabolite generated by cytochrome P-450 mixed function oxidase to microsomal proteins (Potter et al, 1973). Finally, the fundamental role of glutathione in protecting against paracetamol induced hepatic necrosis was demonstrated. Mice pretreated with diethylmaleate (which depletes hepatic glutathione) potentiated hepatic necrosis, whereas treatment with cysteine, a glutathione precursor, prevented hepatic damage. Administration of paracetamol caused dose-dependent depletion of hepatic glutathione. Furthermore, hepatic necrosis was potentiated by treatments which depleted glutathione while treatments that maintained glutathione concentrations protected against liver damage. Covalent binding of the reactive metabolite did not occur until glutathione was depleted by about 70% (Mitchell et al, 1973b). Marked species differences in susceptibility to hepatotoxicity of paracetamol were correlated directly with the extent of hepatic glutathione depletion (Davis et al, 1974). The protective role of glutathione in man was

confirmed where an increase in the urinary excretion of glutathione derived metabolites was observed following increasing doses of paracetamol. It was also estimated that approximately 10 to 15 g of paracetamol would be required to deplete glutathione levels (Mitchell et al, 1974) to a critical level. There is marked individual variation in susceptibility to paracetamol toxicity in man (Prescott and Critchley, 1983).

Following paracetamol overdosage, consciousness is not impaired and there are no specific symptoms apart from nausea and vomitting. However, from 24-36 h there may be abdominal pain and hepatic tenderness which persists for 2 to 3 days (Prescott, 1983). It is not until 12 to 36 h after ingestion that biochemical evidence of acute hepatic injury becomes apparent. This is characterised by gross elevation of plasma aspartate and alanine aminotransferase (AST and ALT), mild hyperbilirubenemia and a prolonged prothrombin time (Proudfoot and Wright, 1970; Prescott et al, 1971; Clark et al, 1973a). In patients who recover, liver function returns to normal within 7 to 20 days. Severely poisoned patients may develop hepatic failure on the third to sixth day with deepening jaundice, deterioration of consciousness which is often followed by death (Canalese et al, 1981). Liver biopsies and post mortem studies reveal extensive centrilobular hepatic necrosis with little or no inflammatory reaction (Davidson and Eastham, 1966; Clark et al, 1973b; James et al, 1975; Portmann et al, 1975). Other complications of severe paracetamol overdosage include haematological abnormalities, renal failure, pancreatitis and cardiotoxicity. These have been reviewed in detail (Prescott, 1983).

Initial clinical assessment of the severity of paracetamol poisoning is difficult as there are no early symptoms and no reliance can be placed on the number of tablets claimed to be taken by the patient (Prescott, 1978). The most reliable early indication of liver damage is probably prolongation of the plasma paracetamol elimination half life. Hepatic damage is to be expected if the half life exceeds 4 h (Prescott et al, 1971). However, it is not practicable to measure the half life therefore a 'treatment line' joining a semilogarithmic plot of 200 $mg.litre^{-1}$ at 4 h to 30 $mg.litre^{-1}$ at 15 h is used as an index of the severity of poisoning. Severe liver damage as defined by plasma AST or ALT activity exceeding 1000 $U.litre^{-1}$ occurs in about 60% of patients with plasma paracetamol concentrations above this line. Patients with plasma paracetamol concentrations below this line are unlikely to develop liver damage. Patients with plasma concentrations above a similar parallel line joining 300 $mg.litre^{-1}$ at 4 h and 45 $mg.litre^{-1}$ at 15 h are at high risk with a 90% chance of severe liver damage. Values obtained earlier than 4 h after ingestion are unreliable as absorption may still be continuing (Prescott, 1983).

Paracetamol absorption following overdosage may be reduced by gastric lavage or induction of emesis within 4 h of ingestion. These measures are indicated in patients suspected of ingesting 100 $mg.kg^{-1}$ or more of paracetamol (Prescott, 1983). Activated charcoal or cholestyramine may also reduce absorption of paracetamol if taken within 1 h of ingestion (Dordoni et al, 1973). Haemoperfusion and haemodialysis have no place in the treatment of paracetamol poisoning (Prescott, 1983). Forced diuresis does not enhance paracetamol

elimination and the renal clearance of paracetamol is independent of urinary pH (Prescott and Wright, 1973).

Mitchell's work provided a rational approach to the treatment of paracetamol toxicity. As glutathione itself does not penetrate hepatocytes it was proposed that administration of sulphydryl compounds which are glutathione precursors may protect against paracetamol toxicity (Mitchell et al, 1974). Successful treatment of severe paracetamol overdosage with intravenous cysteamine was first described in 1974 (Prescott et al). Further reports of successful treatment using cysteamine followed (Hughes et al, 1976; James, 1976; Prescott et al, 1976; Hamlyn et al, 1981). The mechanism by which cysteamine acts is by preventing hepatic glutathione depletion and possibly inhibition of the metabolic activation of paracetamol (Buckpitt et al, 1979). Unfortunately cysteamine also produces unpleasant gastro-intestinal and central nervous system side effects therefore alternative treatments were sought.

Orally methionine is also effective and it has no serious side effects (Crome et al, 1976; Hamlyn et al, 1981; Vale et al, 1981). Its use for the treatment of paracetamol toxicity will be dealt with in detail in Section 2 of this chapter.

N-Acetylcysteine is another sulphydryl compound which is effective in animals (Piperno and Berssenbruegge, 1976) and its success in the treatment of paracetamol poisoning in man was reported by Prescott et al, 1977, 1979. N-Acetylcysteine administered within 10 h of paracetamol ingestion as a total intravenous dose of 300 $mg.kg^{-1}$ over

20 h is effective in preventing liver damage, renal failure and death and it is currently the treatment of choice (Prescott, 1983; Prescott and Critchley, 1983). There is no evidence that treatment beyond 15 h is beneficial and oral administration of N-acetylcysteine may be unreliable as vomitting may impair its absorption (Prescott, 1981).

N-Acetylcysteine primarily protects by stimulating hepatic glutathione synthesis. It greatly increases the concentrations of circulating cysteine, but glutathione synthesis is not actually increased unless the glutathione pool is stressed as would occur after paracetamol overdosage (Burgunder et al, 1989). N-Acetylcysteine may also conjugate directly with the reactive intermediate (Buckpitt et al, 1979) and stimulates sulphate conjugation (Prescott, 1980). These latter actions however, are unlikely to be relevant to the protective properties of N-acetylcysteine.

Mild allergic reactions to N-acetylcysteine have been reported and these include mild skin manifestations and occasional bronchospasm and hypotension. These reactions usually occur within 20-60 min after starting the infusion (Walton et al, 1979; Mant et al, 1984).

Dimercaptol and D-penicillanamine have little or no protective action in man (Hughes et al, 1976; Prescott et al, 1976).

SECTION 2

PARACETAMOL N-ACETYL-D-L-METHIONATE

a) Introduction

Paracetamol is a safe and effective analgesic and antipyretic agent when taken in therapeutic doses. When taken in overdose, however, paracetamol can cause severe and occasionally fatal liver damage. Treatment of paracetamol poisoning with sulphydryl compounds and glutathione precursors is very effective if these are administered within 10 h of ingestion (Prescott and Critchley, 1983).

One such treatment is 2.5 g of oral methionine repeated at 4 h intervals up to a total dose of 10 g. This regime has been shown to be effective in preventing severe liver damage and death after paracetamol overdosage with no serious side effects (Crome et al, 1976; Hamyln et al, 1981; Vale et al, 1981). Methionine administered intravenously or as a large oral dose may cause nausea and vomitting. Following intravenous administration drowsiness and irritability may also be encountered. Possible interactions include the reversal of levodopa effectiveness in parkinsonism and superimposed symptoms of intoxication in schizophrenic patients being treated with monoamine oxidase inhibitors (Martindale, 1982). The observation that methionine may exacerbate existing liver disease (Phear et al, 1956) is of particular importance as late administration of methionine following paracetamol overdosage may be harmful.

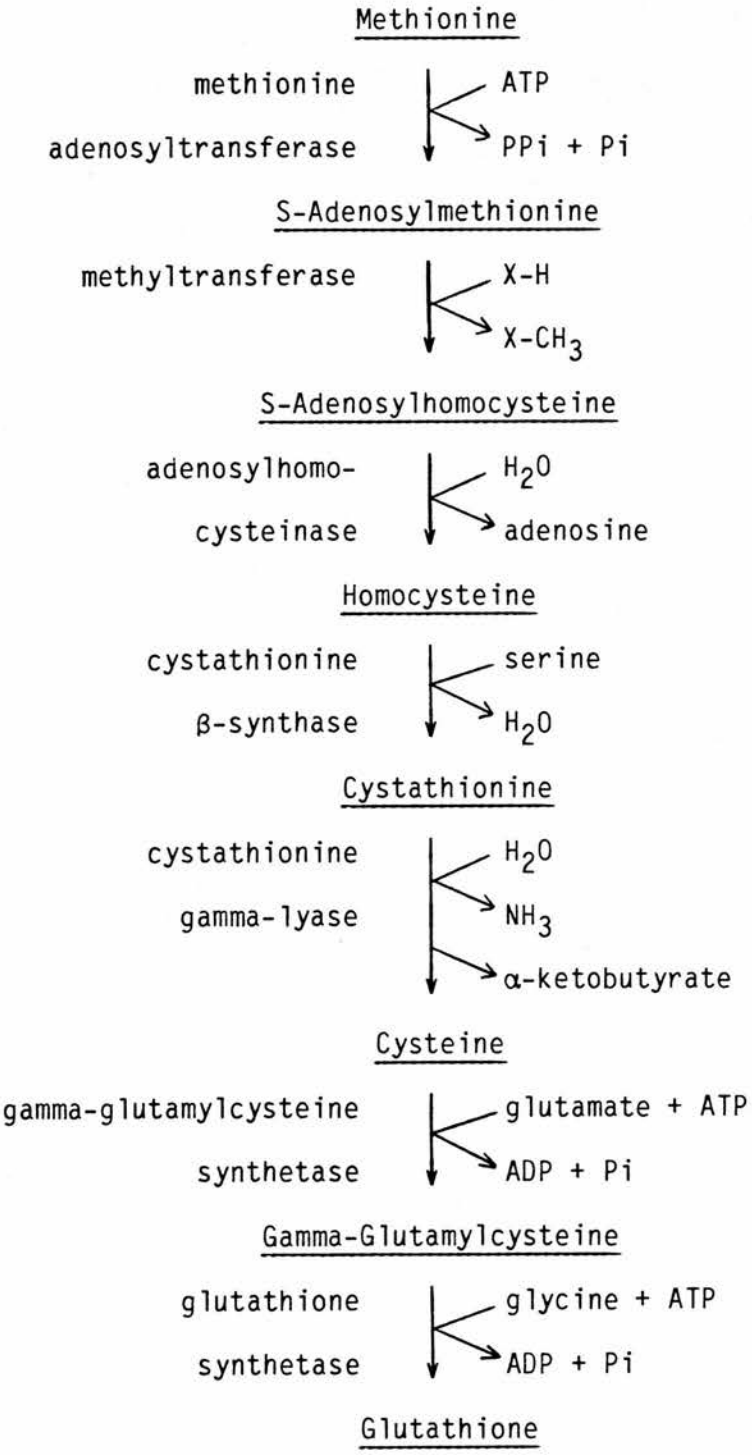
Methionine is a glutathione precursor as it is metabolised to cysteine via the transsulphuration pathway (Cooper, 1983). Cysteine is then incorporated into glutathione (Figure 1.2) enabling glutathione levels to be maintained following paracetamol overdosage (Miners et al, 1984; Pratt and Ioannides, 1985; Warrander et al, 1985). This is probably the main mechanism by which methionine exherts its protective effect as it does not bind directly to the reactive intermediate of paracetamol (Buckpitt et al, 1979; Huggett and Blair, 1983).

An alternative transaminative pathway of methionine catabolism also exists, but little is known of its importance (Benevenga, 1985). In addition, methionine exists in 2 isomeric forms designated -D and -L. L-methionine is an essential amino acid and is more readily utilised than the -D form in man (Stegink et al, 1986).

Even with effective methods of treatment, the number of deaths attributed to paracetamol poisoning in England and Wales has risen steadily since 1966 with 176 deaths due to paracetamol alone reported in 1984. Although this figure is probably an overestimate (Meredith et al, 1986) mortalities arising from paracetamol overdosage continue to be a serious problem.

Delay in initial presentation to hospital is probably the major reason why death following paracetamol overdosage still occurs (Read et al, 1986). A possible method of prevention would, therefore, be to incorporate methionine into paracetamol tablets. This would enable the rapid onset of action of the antidote and the dose of

FIGURE 1.2 The Role of Methionine in Glutathione Biosynthesis (Adapted from Lehninger, 1975 and Cooper, 1983



methionine would automatically increase with the amount of paracetamol ingested.

This concept was first described by McLean and Day in 1975. They showed that oral administration of 0.3 $g.kg^{-1}$ of methionine (30%) with 1 $g.kg^{-1}$ paracetamol prevented liver damage in rats. Fifteen percent and 10% provided some protection while 5% was ineffective. Similarly, oral administration of a paracetamol and methionine (4:1 w/w) mixture at increasing doses showed that most rats survived a dose of paracetamol of 7.5 $g.kg^{-1}$ whereas the LD_{50} of paracetamol alone is about 2 $g.kg^{-1}$ (McLean and Day, 1975).

More recently the analgesic effect and acute toxicity (LD_{50}) of orally administered paracetamol and L-methionine (5:1 w/w) has been studied in animals. The analgesic effect of paracetamol was not altered by the addition of methionine in rats, however, the addition of methionine did reduce the acute toxicity of paracetamol by 50% in non-fasted, fasted and phenobarbitone pretreated mice (Neuvonen et al, 1985).

The same investigators administered oral paracetamol (1.5 g) and L-methionine (0.3 g) to 10 healthy human subjects. Methionine did not affect the pharmacokinetics of paracetamol. In addition, peak plasma concentrations of methionine were observed at 30 min and were 3 to 4 times higher than after 1.5 g paracetamol alone. It was, therefore, concluded that the addition of L-methionine to paracetamol tablets should increase safety of paracetamol without loss of pharmacological activity (Neuvonen et al, 1985).

Currently, the only commercially available application of this concept is 'Pameton', which was introduced in December 1986. It contains paracetamol (500 mg) and D-L-methionine (250 mg) and is particularly expensive (£4.79 for 60 tablets compared to 45p for 60 paracetamol tablets). Pameton is available without prescription, but is not included in the limited list for National Health Service prescription in general practice (Drug and Therapeutic Bulletin, 1987).

Sterling Winthrop have now developed paracetamol N-acetyl-D-L-methionate in which paracetamol is linked by an ester bond to N-acetyl-D-L-methionine. This compound is tasteless and is hydrolysed in vivo to yield paracetamol and methionine in equimolar quantities. At present it is not available commercially.

Chemical data for paracetamol N-acetyl-D-L-methionate are shown in Appendix 1.2

b) Pharmacology

The analgesic activity of paracetamol N-acetyl-D-L-methionate and paracetamol have been compared using the Randall-Selitto test in rats (SWRD Report 205032, 1978). Inflammation was induced in the rear right foot by a subplanter injection of 0.1 ml of a 20% bakers yeast suspension in saline. The test compound was administered 2.5 h after the injection and the reaction threshold to pressure measured in both hind paws at timed intervals.

At doses ranging from 0.25-0.50 mmol.kg^{-1}, paracetamol N-acetyl-D-L-methionate was slightly, but consistently more effective than paracetamol in increasing the pain threshold of the inflamed paw. At higher doses of 0.7-2.0 mmol.kg^{-1} there were no quantitative differences in analgesic activity between the 2 compounds.

The peak analgesic effect of paracetamol N-acetyl-D-L-methionate was reached within 1-3 h and was maintained for 5-5.5 h after oral administration. Compared with paracetamol, paracetamol N-acetyl-D-L-methionate had a slower onset of action but a longer dose dependent duration of analgesia. An equimolar mixture of paracetamol N-acetyl-D-L-methionate and paracetamol combined the early onset of action of paracetamol with the prolonged duration of effect of paracetamol N-acetyl-D-L-methionate.

Similar comparative studies were also undertaken using the mouse writhing test (SWRD Report 205032, 1978). Writhing was induced by intraperitoneal administration of 3.5 mg.kg^{-1} acetylcholine chloride in saline and the ability of the test drugs to inhibit the reaction was tested at intervals following oral administration. Responses during the period 25-120 seconds following the injection were recorded and the results expressed as the percentage protection from writhing. At doses over the range of 0.25-2.8 mmol.kg^{-1} paracetamol, paracetamol N-acetyl-D-L-methionate and the equimolar combination had similar analgesic activity and duration of effect. With repeated administration of paracetamol N-acetyl-D-L-methionate or paracetamol over 4 days there was no evidence of tolerance to either compound in this test (SWRD Report 205032, 1978).

The comparative analgesic efficacy of the equimolar combination of (1) paracetamol N-acetyl-D-L-methionate and paracetamol and (2) paracetamol alone was investigated in patients following dental surgery (Skoglund and Skjelbred, 1984). Twenty seven patients received the equimolar combination equivalent of 2 g of paracetamol 3 and 9 h after operation and the equivalent of 1 g of paracetamol at 0800 and 1600 h on the following 2 days. Another group of 26 patients received 1 g of paracetamol 3, 6, 9 and 12 h after operation on the day of surgery and 0.5 g at 0800, 1200, 1600 and 2000 h on the following 2 days.

An analgesic effect was demonstrated within 0.5 h of administration in both groups, but it was not possible to assess the duration of effect. Following the first dose the equimolar combination was more effective than paracetamol alone over the period 0.5-3.0 h.

In a similar study using the same dosage regime the equimolar combination reduced pain significantly more than placebo during the first day (Skoglund, 1986).

The antipyretic activity of paracetamol N-acetyl-D-L-methionate was studied in rats with hyperthermia induced by subcutaneous injection of dried yeast. Oral paracetamol N-acetyl-D-L-methionate and paracetamol (0.5 and 1.0 $mmol.kg^{-1}$) had a dose dependent antipyretic activity for 4 h after medication. Initially, paracetamol N-acetyl-D-L-methionate was less effective than paracetamol (SWRD Report 203236, 1977). Oral paracetamol (100 $mg.kg^{-1}$) and mixtures in which

10, 20 and 40% of the total paracetamol dose was replaced by equimolar quantities of paracetamol N-acetyl-D-L-methionate all had similar antipyretic activity in hyperthermic rats (SWRD Report 203490).

The equimolar combination of paracetamol N-acetyl-D-L-methionate and paracetamol was no better than placebo at reducing post operative swelling in 30 patients following bilateral oral surgery (Skoglund, 1986).

c) Absorption, Distribution, Metabolism and Excretion

Absorption: Peak plasma paracetamol concentrations following oral administration of paracetamol N-acetyl-D-L-methionate in mice and rats were delayed and lower compared to those obtained following an equivalent equimolar dose of paracetamol (SWRD Reports 305212 and 305213, 1978).

Eight healthy subjects received 2 g of oral paracetamol as (1) paracetamol, (2) paracetamol and N-acetyl-D-L-methionine, (3) paracetamol and paracetamol N-acetyl-D-L-methionate, and (4) paracetamol N-acetyl-D-L-methionate. Mean peak plasma paracetamol concentrations were 26.6, 21.1, 17.3 and 13.3 $\mu g.ml^{-1}$ respectively and the mean time to peak plasma paracetamol concentration for paracetamol N-acetyl-D-L-methionate was 2 h compared to 0.5 h for the other formulations (SWRD Report 305510, 1978).

The mechanism and site of paracetamol N-acetyl-D-L-methionate absorption is not known.

Distribution: No information is available concerning the distribution of paracetamol N-acetyl-D-L-methionate.

Metabolism: Paracetamol N-acetyl-D-L-methionate is hydrolysed *in vivo* to paracetamol and N-acetyl-D-L-methionine. Esterase enzymes are ubiquitous. Orally administered ester prodrugs are mainly hydrolysed by intestinal, hepatic and plasma esterases (Williams, 1985).

Attempts to measure paracetamol N-acetyl-D-L-methionate in biological fluids have been unsuccessful. Using a non-specific enzymatic method and reverse phase high performance liquid chromatography paracetamol N-acetyl-D-L-methionate could not be detected in the plasma of mice (SWRD Report 305213, 1978), rats (SWRD Report 305212, 1978) or man (SWRD Report 304655, 1977; SWRD Report 305510, 1978).

Paracetamol N-acetyl-D-L-methionate was administered orally to 4 beagle dogs at 675 $mg.kg^{-1}$ daily for 29 days. The mean difference between serum 'total paracetamol' (paracetamol and its glucuronide and sulphate conjugates, paracetamol metabolites and paracetamol N-acetyl-D-L-methionate) and 'enzymatic paracetamol' (paracetamol and its glucuronide and sulphate conjugates) was 11 $\mu g.ml^{-1}$ at 4 h on Day 29. The precise form which accounts for this difference was, however, not identified (SWRD Report 304686, 1977).

The plasma concentrations of paracetamol and its glucuronide and sulphate conjugates following administration of 2 g of paracetamol and the equivalent dose of paracetamol N-acetyl-D-L-methionate were investigated in healthy volunteers. Peak concentrations of paracetamol conjugates occurred 1 h after the peak concentration of paracetamol following administration of paracetamol and 2 h after paracetamol N-acetyl-D-L-methionate. The glucuronide and sulphate conjugates were formed at similar rates and in similar quantities for each formulation. The elimination kinetics of paracetamol and its conjugates were also similar (SWRD Report 305510, 1978).

As methionine is a precursor of glutathione the effect of paracetamol N-acetyl-D-L-methionate administration on hepatic glutathione was investigated. Paracetamol N-acetyl-D-L-methionate was administered to mice at a dose level equivalent to 400 $mg.kg^{-1}$ of paracetamol. There was less depletion of hepatic glutathione than with the equivalent paracetamol dose (Skoglund et al, 1986). The maximum reduction with paracetamol N-acetyl-D-L-methionate was 54% at 1 h and this would probably protect against liver damage as this does not occur until glutathione is reduced to 20-30% of normal (Mitchell et al, 1973b). At 16 h hepatic glutathione concentrations were significantly greater than in control mice.

Similarly, enhanced glutathione synthesis was reported following administration of an equimolar combination of paracetamol and paracetamol N-acetyl-D-L-methionate equivalent to 400 and 800 $mg.kg^{-1}$ of paracetamol in mice (Skoglund et al, 1988).

Excretion: The excretion of paracetamol following administration of paracetamol N-acetyl-D-L-methionate was investigated in man following doses equivalent to 2 g of paracetamol. The mean 24 h total urinary recovery of paracetamol and metabolites was 62% for paracetamol N-acetyl-D-L-methionate and 74% for paracetamol. The fractional recovery of paracetamol and its glucuronide, sulphate, cysteine and mercapturic acid conjugates were similar to that with paracetamol (SWRD Report 305510, 1978).

d) Toxicology

Paracetamol administered orally to mice at 400 $mg.kg^{-1}$ produced a transitory rise in plasma ALAT (alanine aminotransferase) activity but this did not occur with the equivalent dose as paracetamol N-acetyl-D-L-methionate (Skoglund et al, 1986). Similar findings were reported with paracetamol in doses of 400 and 800 $mg.kg^{-1}$ administered to mice as the equimolar combination of paracetamol and paracetamol N-acetyl-D-L-methionate (Skoglund et al, 1988).

The Oral LD_{50} (7 days) was determined following administration of paracetamol, paracetamol N-acetyl-D-L-methionate (PNAM) and mixtures of both in mice. The LD_{50} values were expressed as paracetamol equivalents:

Compound	LD_{50} 7 Days ($mg.kg^{-1}$)
Paracetamol	690
Paracetamol:PNAM (450:100 w/w)	800
Paracetamol:PNAM (400:200 w/w)	930
Paracetamol:PNAM (300:400 w/w)	1500
Paracetamol N-acetyl-D-L-methionate	>4000

Slow absorption of paracetamol N-acetyl-D-L-methionate probably contributes to its low acute toxicity in addition to the protective action of the methionine (SWRD Report 403238, 1977).

Six month chronic toxicity studies of paracetamol N-acetyl-D-L-methionate were performed in rats and dogs. Thirty rats were given oral paracetamol N-acetyl-D-L-methionate at doses of 130, 390 and 1170 $mg.kg^{-1}$. Paracetamol N-acetyl-D-L-methionate was well tolerated and weight gain, food consumption, blood pressure and behaviour were normal. At the highest dose the fur was sticky and unclean, and water consumption was increased. After 6 months there was an increase in the erythrocyte sedimentation rate and lymphocyte count in males and a decrease in the reticulocyte, platelet and lymphocyte counts in females. Urine analysis showed no notable abnormalities and clinical biochemical tests were normal, except for an elevated cholesterol level in females at the highest dose. At the same dose level there was an increase in the liver weight in females, but microscopic examination showed no changes attributable to the drug (SWRD Report 505300, 1978).

Six dogs were given daily for 6 months paracetamol N-acetyl-D-L-methionate at 108 and 215 $mg.kg^{-1}$ and 8 dogs at 430 $mg.kg^{-1}$. It was well tolerated and weight gain, biochemistry, urine analysis, optical tests, and tests of kidney and adrenal function and behaviour were all normal. At 430 $mg.kg^{-1}$ there was a decrease in red blood cells and haematocrit, and an increase in reticulocytes. There was a slight biliary pigmentation of the Kupffer cells in the liver at the highest dose level. No microscopic changes were observed (SWRD Report 505263, 1978).

No serious toxic effects attributable to paracetamol N-acetyl-D-L-methionate were found in either acute or chronic toxicity studies (Investigators Handbook, SWRD).

The teratogenicity of paracetamol N-acetyl-D-L-methionate was studied in mice, rats and rabbits. Rats and mice received oral paracetamol N-acetyl-D-L-methionate daily at 130, 390 and 1170 $mg.kg^{-1}$ from Days 6-15 of gestation and rabbits daily at 130, 260 and 520 $mg.kg^{-1}$ from Days 6-18 of gestation. At the highest dose in rats (but not in mice or rabbits) there was an increase in the number of foetuses with 14 ribs and an extra lumbar vertebra (SWRD Report 607474, 1980).

In further studies in rats paracetamol N-acetyl-D-L-methionate was administered at 130, 260 and 1170 $mg.kg^{-1}$ daily to males for 63 days and to females for 14 days prior to mating. No effect on spermatogenesis or oogenesis were observed. Treatment was continued during pregnancy and no gross malformations were observed at 15 days of

gestation. At 21 days the number of foetuses with 14 ribs and an extra lumbar vertebrae was again increased. Dams were treated until 21 days post partum with no adverse effects on maternal lactation. There were no adverse effects from weaning to maturity in the second generation and fertility and reproductive performance at sexual maturity were normal. Pregnancy in dams of the second generation was normal at each dose and the progeny of the second generation were also normal (SWRD Report 605307, 1978).

Paracetamol N-acetyl-D-L-methionate was devoid of mutagenic activity in 6 strains of Salmonella typhimurium (Investigators Handbook, SWRD).

A single oral dose of 859.5 $mg.kg^{-1}$ of N-acetyl-D-L-methionine administered in mice produced no histological damage to the liver or kidneys or increase in plasma ALAT (Skoglund et al, 1986).

SECTION 3

STUDY OBJECTIVES

In an attempt to make a safer form of paracetamol Sterling Winthrop have linked paracetamol and N-acetyl-D-L-methionine as the N-acetyl-D-L-methionine ester of paracetamol. The objective of this study is to complement existing pharmacological and toxicity data on paracetamol N-acetyl-D-L-methionate by studying in detail its disposition in healthy subjects and patients with chronic renal failure. This will provide previously unpublished data in addition to furthering the development of this compound.

Firstly, existing methods for measurement of paracetamol and its metabolites in plasma and urine require to be validated in addition to development of a method for measurement of paracetamol N-acetyl-D-L-methionate. Having established suitable analytical methods the study of the disposition of paracetamol N-acetyl-D-L-methionate in healthy subjects will be undertaken in a double blind cross-over clinical trial. Each healthy subject will receive 1 g of paracetamol as (1) paracetamol and (2) paracetamol N-acetyl-D-L-methionate. The data generated from healthy subjects receiving paracetamol alone will provide control data with which the disposition of paracetamol following administration of paracetamol N-acetyl-D-L-methionate can be compared.

Following on, the same double blind cross-over clinical trial will then be performed in patients with chronic renal failure. The data generated from chronic renal failure patients following administration of 1 g of paracetamol alone will be compared with that obtained following administration of 1 g of paracetamol to healthy subjects. This will provide more detailed information of the disposition of paracetamol in patients with chronic renal failure than previously reported. The data obtained after administration of paracetamol N-acetyl-D-L-methionate in patients with chronic renal failure will then be compared with that obtained after administration of paracetamol alone to the same patients. This will provide information as to the disposition of paracetamol N-acetyl-D-L-methionate in patients with chronic renal failure.

CHAPTER II

Analytical Methods

SECTION 1

ESTIMATION OF PARACETAMOL AND ITS METABOLITES IN PLASMA AND URINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

a) Introduction

Many methods have been described for the estimation of paracetamol. These can be divided into 2 main areas of interest.

i) The Estimation of Paracetamol Concentrations in Plasma

These methods are generally used to aid diagnosis and management of overdose patients and therefore require to be specific, accurate and rapid. Early techniques, however, utilised non specific procedures whereby paracetamol was hydrolysed directly with acid to p-aminophenol. These methods over-estimate free paracetamol, as its conjugates were also hydrolysed to p-aminophenol (Stewart et al, 1979). Techniques where conjugates do not interfere include, u.v. spectrophotometry following solvent extraction (Routh et al, 1968) and phenolic ring nitration (Glynn and Kendal, 1975). These methods are however, susceptible to interference from unrelated compounds.

More recently, a paracetamol enzyme assay kit has been developed. This new approach uses a bacterial acyl amidase which specifically converts free paracetamol to p-aminophenol with quantitation by a

previously developed colorimetric technique. This method correlates well with existing methods (Widdop, 1984).

ii) The Estimation of Paracetamol and its Metabolites in Biological Fluids

These methods require to be sensitive in addition to being specifc and accurate and are generally used to measure therapeutic concentrations for research purposes. Methods include gel filtration (Jagenburg et al, 1968), gas-liquid chromatography (Prescott, 1971), ion exchange chromatography (Mrochek et al, 1974), and thin layer chromatography (Andrews et al, 1976).

Significant progress has been made since the introduction of High Performance Liquid Chromatography (HPLC). Separation of paracetamol and its major metabolites was achieved by Knox and Jurand in 1979 using a reverse phase support, however, quantitation was not attempted. Reverse phase HPLC utilises a polar mobile phase with a non-polar support. Polar components have a higher affinity for the eluent than for the support, and so are eluted first. Reverse phase HPLC therefore, is a very useful technique for the separation and quantitation of drugs and their metabolites. Estimation of paracetamol and its metabolites in urine (Howie et al, 1977; Adrianssens, 1980) and plasma (Adrianssens and Prescott, 1978) by HPLC provides a sensitive, specific, accurate and rapid technique with minimal sample preparation. The procedures used throughout this work for the esti-

mation of paracetamol and its metabolites are based on the methods of Howie et al, and Adrianssens et al.

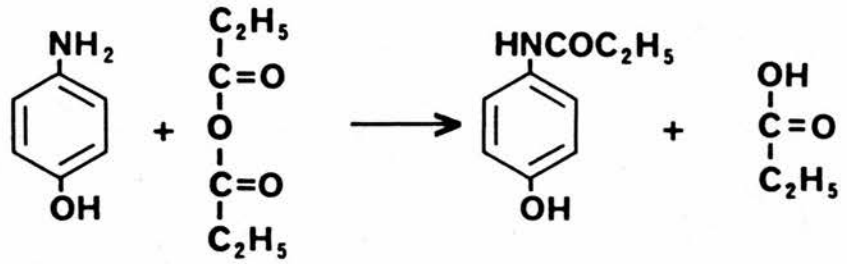
b) High Performance Liquid Chromatography Assays for the Estimation of Paracetamol and its Metabolites in Plasma and Urine

i) Materials

Paracetamol, paracetamol glucuronide, paracetamol sulphate, paracetamol cysteine and paracetamol mercapturic acid for reference standards, were kindly donated by Sterling-Winthrop Research and Development Division, Alnwick, England.

Potassium dihydrogen orthophosphate, formic acid, iso-propyl alcohol, and acetic acid were supplied by BDH Chemicals Limited, Poole, England, 60% perchloric acid and ethyl acetate were obtained from Fisons Scientific Apparatus, Loughborough, England, and helium from The British Oxygen Company.

The internal standard used was N-propionyl-4-aminophenol (NPA). It was synthesised from 4-aminophenol and propionic anhydride (BDH Chemicals Limited), as described by Adrianssens (1980), with comparable yield and purity.



4-aminophenol	propionic anhydride	N-propionyl-4-aminophenol	propionic acid

All other reagents used were of analytical grade.

ii) Instrumentation

The HPLC system consisted of an Altex 110A pump which delivered the mobile phase (degassed with helium prior to use) via a filter to the system.

The mobile phase then passed to an Altex 210 Manual Sample Injection Valve, and samples were injected into the loop via a needle port using a blunt 22 gauge Hamilton syringe. A Waters Intelligent Sample Processor 710B (WISP), an automatic sample injection module, was also used.

Prior to passing through the analytical column, the solvent was passed through a metal filter and a Waters Guard-PAK precolumn containing the same packing material as the column.

The columns used were 8 mm x 10 cm Waters Radial-PAK cartridges containing a reverse phase, 5 or 10 μ, C18 bonded spherical silica support. The cartridge was housed in a Waters RCM-100 Radial Compression Module (Figure 2.1).

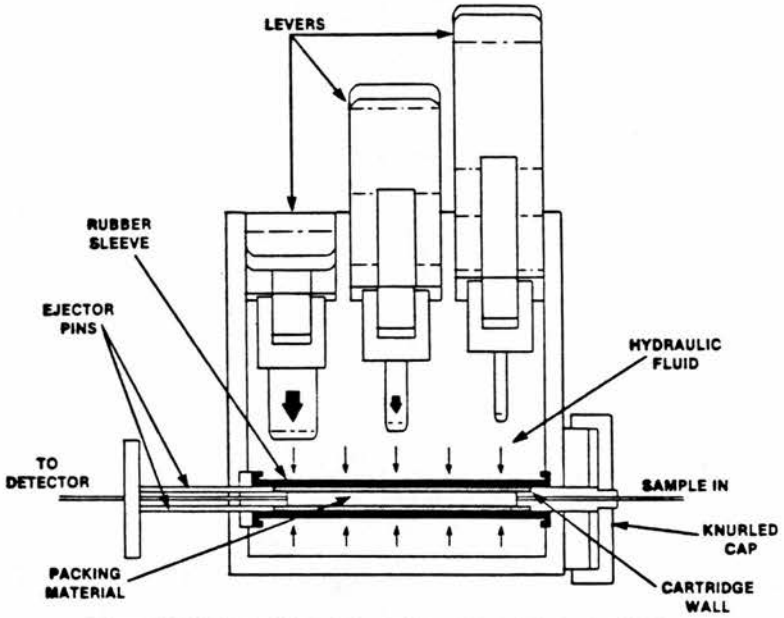
From the column, the eluate then passed into a ultraviolet (u.v.) absorbance detector (Perkin-Elmer Model LC-75 spectrophotometric detector or the Waters Series 441 absorbance detector).

The u.v. detector output was passed to a Hewlett-Packard 3390A Single Channel Plotting/Reporting Integrator to produce the chromatogram and peak areas. The HPLC system is shown in Figure 2.2.

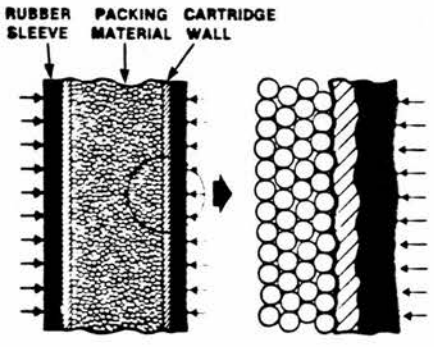
iii) Collection and Storage of Samples

Prior to analysis, all samples were stored at -20°C. Blood samples were collected into 10 ml heparinised tubes, centrifuged for 10 min at 1400 x g and the plasma separated for storage. Urine volumes and pH were recorded and a 50 ml aliquot stored. Paracetamol and paracetamol conjugates are stable under these conditions for a period of years (Adrianssens, 1980).

FIGURE 2.1 Copied from the Operators Manual for the Waters Radial Compression Separation System

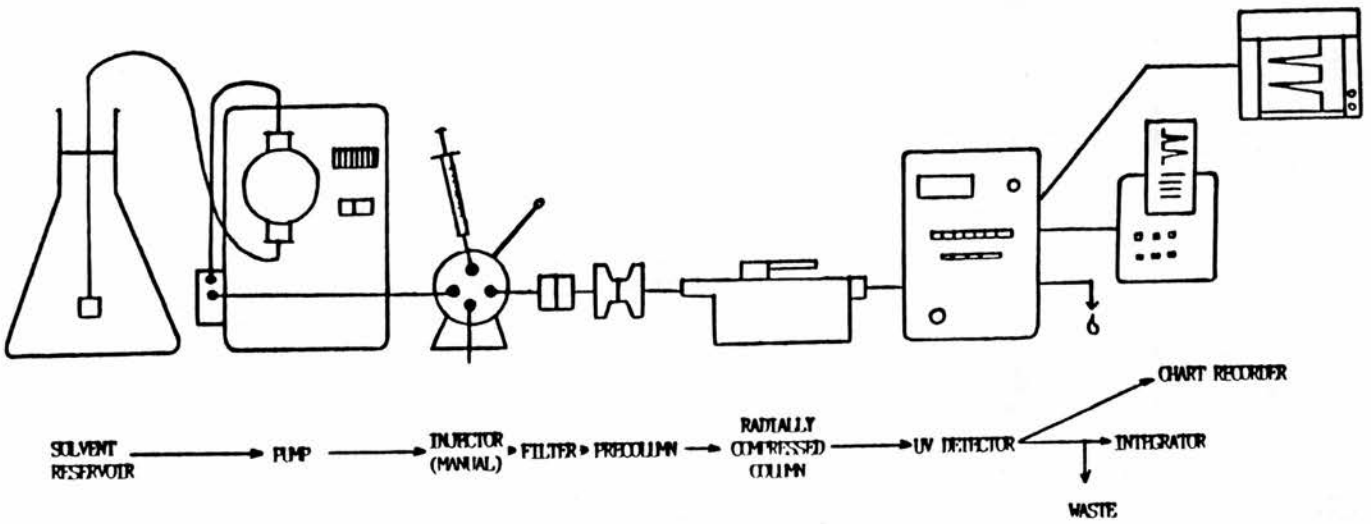


RADIAL COMPRESSION SEPARATION SYSTEM (CROSS SECTION DIAGRAM)



RADIALLY COMPRESSED CARTRIDGE (CROSS SECTION)

FIGURE 2.2 Schematic Representation of the HPLC System Used



iv) Plasma Assay

Chromatographic Conditions

For the estimation of plasma paracetamol, paracetamol glucuronide and paracetamol sulphate a Radial-PAK, C18, 5 µ cartridge was used under the following chromatographic conditions:

Mobile Phase:	0.05 M potassium dihydrogen orthophosphate containing 0.1% formic acid:isopropanol (295:5 v/v)
Flow Rate:	3 $ml.min^{-1}$
Pressure:	1000 psi
Detector Wavelength:	254 nm

Preparation of Standards and Test Samples for Plasma Assay

For estimating therapeutic concentrations of paracetamol and its metabolites, spiked plasma standards containing 5 and 25 $\mu g.ml^{-1}$ of paracetamol were prepared and run in duplicate with the test samples. To 0.5 ml of plasma in a plastic tube, 50 µl of 30 mg% NPA in 30% w/v aqueous perchloric acid was added with continuous mixing (Whirlimix, Jencons) to precipitate the plasma proteins. The tubes were centrifuged for 10 min at 1400 x g and up to 30 µl of the clear supernatant injected directly into the HPLC system.

Calculation of Results for Plasma Assay

Concentrations were calculated in paracetamol equivalents from the peak area ratios of paracetamol and paracetamol conjugates to the internal standard (NPA). The ratios were then multiplied by a factor (F) representing the reciprocal of the slope of the regression line for the standards, to give the paracetamol concentration in $\mu g.ml^{-1}$. The calculation of the factor (F) is shown in Appendix 2.1.

As only authentic paracetamol standards were used, values obtained are only accurate for paracetamol. The molar extinction coefficients of glucuronide and sulphate conjugates of paracetamol are not the same as paracetamol and correction factors of 0.835 and 1.027 respectively must be applied (Adrianssens and Prescott, 1978).

$$\text{Paracetamol} = \frac{Pa}{NPAa} \times F \times 1.000 = \text{paracetamol concentration } (\mu g.ml^{-1})$$

$$\text{Glucuronide} = \frac{Ga}{NPAa} \times F \times 0.835 = \text{paracetamol equivalents } (\mu g.ml^{-1})$$

$$\text{Sulphate} = \frac{Sa}{NPAa} \times F \times 1.027 = \text{paracetamol equivalents } (\mu g.ml^{-1})$$

Where: Pa, Ga, Sa and NPAa are the peak areas for paracetamol, glucuronide, sulphate and internal standard respectively. F is the factor representing the reciprocal of the slope of the regression line for the standards. For the mean value of F obtained see Appendix 2.1.

Results of Plasma Assay

Chromatograms for drug-free plasma and from plasma obtained 45 min following a dose of 1 g of paracetamol are shown in Figure 2.3. The retention times of paracetamol glucuronide, paracetamol sulphate, paracetamol and internal standard (NPA) were 2.7, 4.2, 6.3 and 12.0 min respectively. The system also separates the cysteine (5.3 min) and mercapturic acid (15.0 min) conjugates of paracetamol, but these metabolites were rarely detectable except after overdosage. A chromatogram of plasma obtained 15.25 h after a paracetamol overdose is shown in Figure 2.4. The limit of detection of each compound is less than 1 $\mu g.ml^{-1}$ and there is no interference from commonly used drugs (Adrianssens, 1980).

Validation of Plasma Assay

As authentic metabolite standards were not available in quantity, the accuracy of the assay could not be assessed. The accuracy has, however, been demonstrated previously whereby measured concentrations were within 6% of weighed-in values over a wide range of concentrations (Adrianssens, 1980). The linearity and the precision for the plasma assay was determined by repeated analysis of serially diluted overdose samples.

Plasma Paracetamol: Serial dilutions of a stock solution of paracetamol in blank plasma (Blood Transfusion Service) were prepared

FIGURE 2.3 Chromatograms Obtained from:

A Drug free plasma (30 µl; 0.05 AUFS)

B Plasma obtained 45 min following a dose of 1 g of paracetamol (30 µl; 0.05 AUFS)
The peaks are: 6.2 $\mu g.ml^{-1}$ paracetamol glucuronide (G)
3.4 $\mu g.ml^{-1}$ paracetamol sulphate (S)
11.2 $\mu g.ml^{-1}$ paracetamol (P) and internal standard (IS)

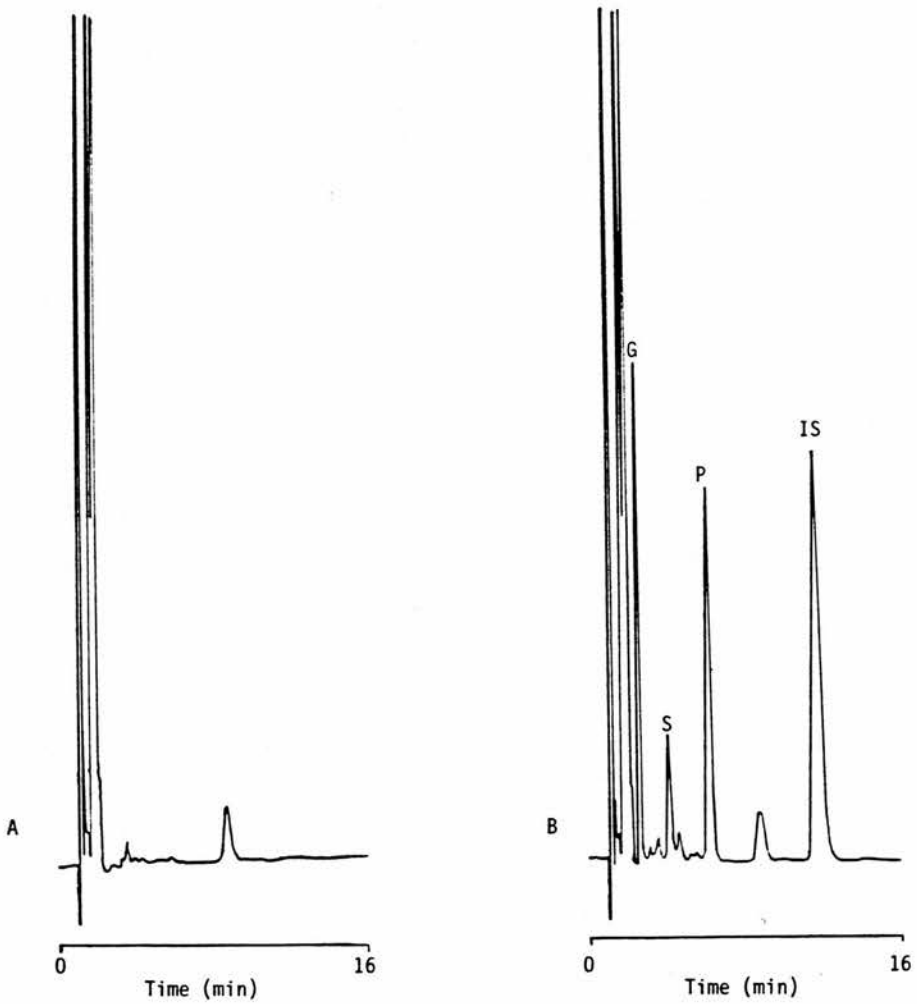
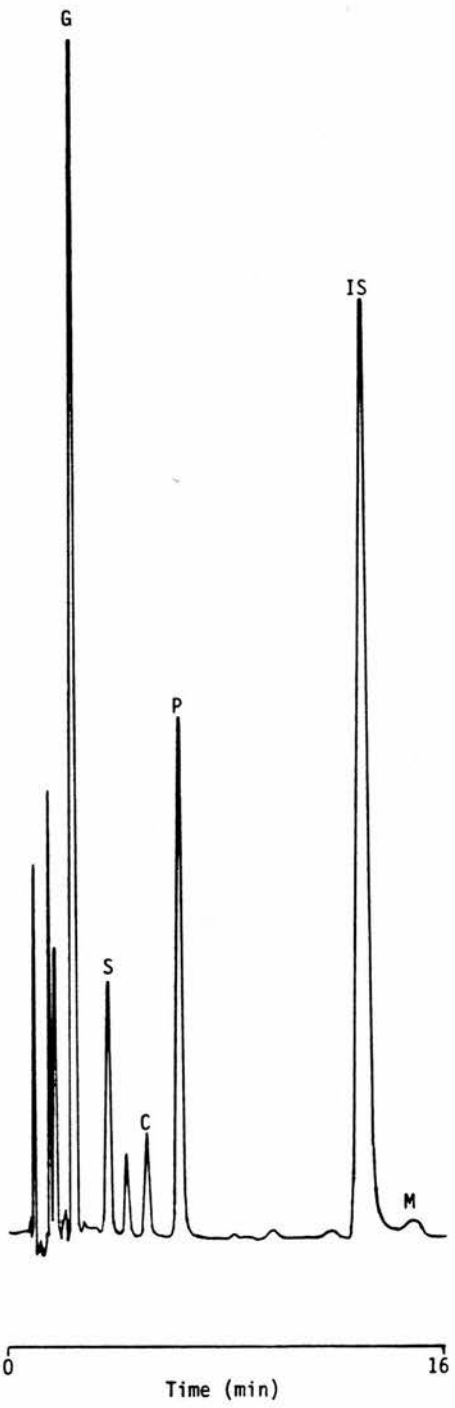


FIGURE 2.4 Chromatogram Obtained from Plasma Taken 15.25 h After Paracetamol Overdosage

The peaks are: 183.1 $\mu g.ml^{-1}$ paracetamol glucuronide (G)
40.4 $\mu g.ml^{-1}$ paracetamol sulphate (S)
19.3 $\mu g.ml^{-1}$ paracetamol cysteine (C)
104.5 $\mu g.ml^{-1}$ paracetamol (P)
5.4 $\mu g.ml^{-1}$ paracetamol mercapturic acid (M) and internal standard (IS)



to give concentrations ranging from 5-50 $\mu g.ml^{-1}$. Samples were assayed as described, and analysed 5 times over a period of 2 days.

The results are shown in Table 2.1 and Figure 2.5. The coefficients of variation ranged from 1.4-3.1% while the calibration plot was linear and passed through the origin.

Plasma Paracetamol Metabolites: Plasma obtained from an overdose patient was serially diluted with blank plasma to give concentrations of paracetamol, paracetamol glucuronide and paracetamol sulphate ranging from 9.9-118.5, 6.2-75.3 and 1.1-13.6 $\mu g.ml^{-1}$ respectively. Each sample was assayed 5 times over a period of 2 days.

The results are shown in Tables 2.2(i-iii) and Figure 2.6. The coefficients of variation for the determination of paracetamol and paracetamol glucuronide were within 5% over the range of concentrations investigated. The precision for paracetamol sulphate, however, ranged from 6.5-13.6%. This result is explained by the very much lower concentrations of the paracetamol sulphate conjugate. The paracetamol sulphate peak is also subject to interference at low concentrations. The calibration plots were linear for paracetamol and its glucuronide and sulphate conjugates.

TABLE 2.1 Precision of Plasma Paracetamol HPLC Assay (5-50 $\mu g.ml^{-1}$)

Paracetamol Concentration ($\mu g.ml^{-1}$)	Peak Area Ratio (Paracetamol/NPA)					Mean ± SD	Coefficient of Variation (%)
	Run 1	Run 2	Run 3	Run 4	Run 5		
5	0.178	0.181	0.182	0.192	0.188	0.184 ± 0.006	3.1
10	0.380	0.367	0.378	0.376	0.373	0.375 ± 0.005	1.4
20	0.762	0.768	0.780	0.766	0.749	0.765 ± 0.011	1.5
30	1.101	1.134	1.146	1.127	1.137	1.129 ± 0.017	1.5
40	1.456	1.473	1.547	1.543	1.520	1.508 ± 0.041	2.7
50	1.838	1.871	1.883	1.918	1.875	1.877 ± 0.029	1.5

FIGURE 2.5 Linearity of HPLC Plasma Assay: Calibration Plot of the Mean Peak Area Ratio Obtained Following Repeated Analysis (n = 5) of Plasma Containing Paracetamol Concentrations in the Range 5-50 $\mu g.ml^{-1}$

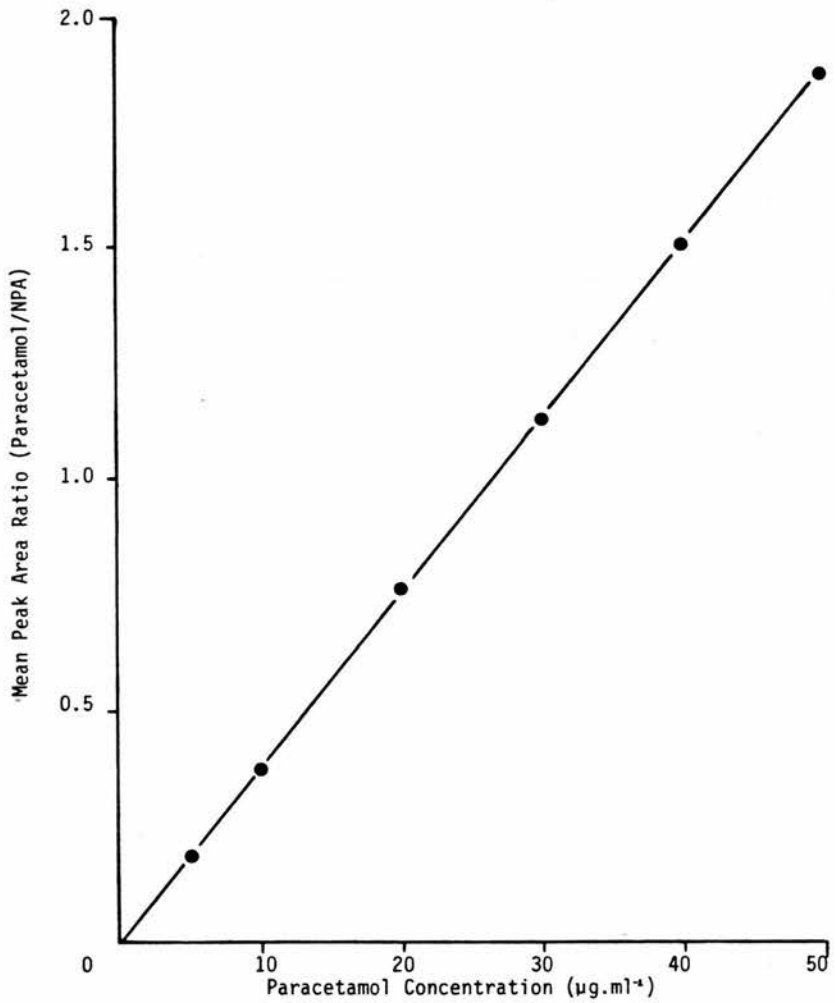


TABLE 2.2(i) Precision of Plasma HPLC Assay - Paracetamol

Sample Dilution	Paracetamol Concentration ($\mu g.ml^{-1}$)					Mean ± SD	Coefficient of Variation (%)
	Run 1	Run 2	Run 3	Run 4	Run 5		
0.083	9.7	10.4	10.1	9.5	9.6	9.9 ± 0.4	3.8
0.167	19.3	20.9	19.3	19.1	19.5	19.6 ± 0.7	3.7
0.500	57.9	61.3	58.7	57.5	57.4	58.6 ± 1.6	2.8
0.833	95.0	100.2	96.7	95.4	95.7	96.6 ± 2.1	2.2
1.000	118.1	123.8	117.1	115.3	118.2	118.5 ± 3.2	2.7

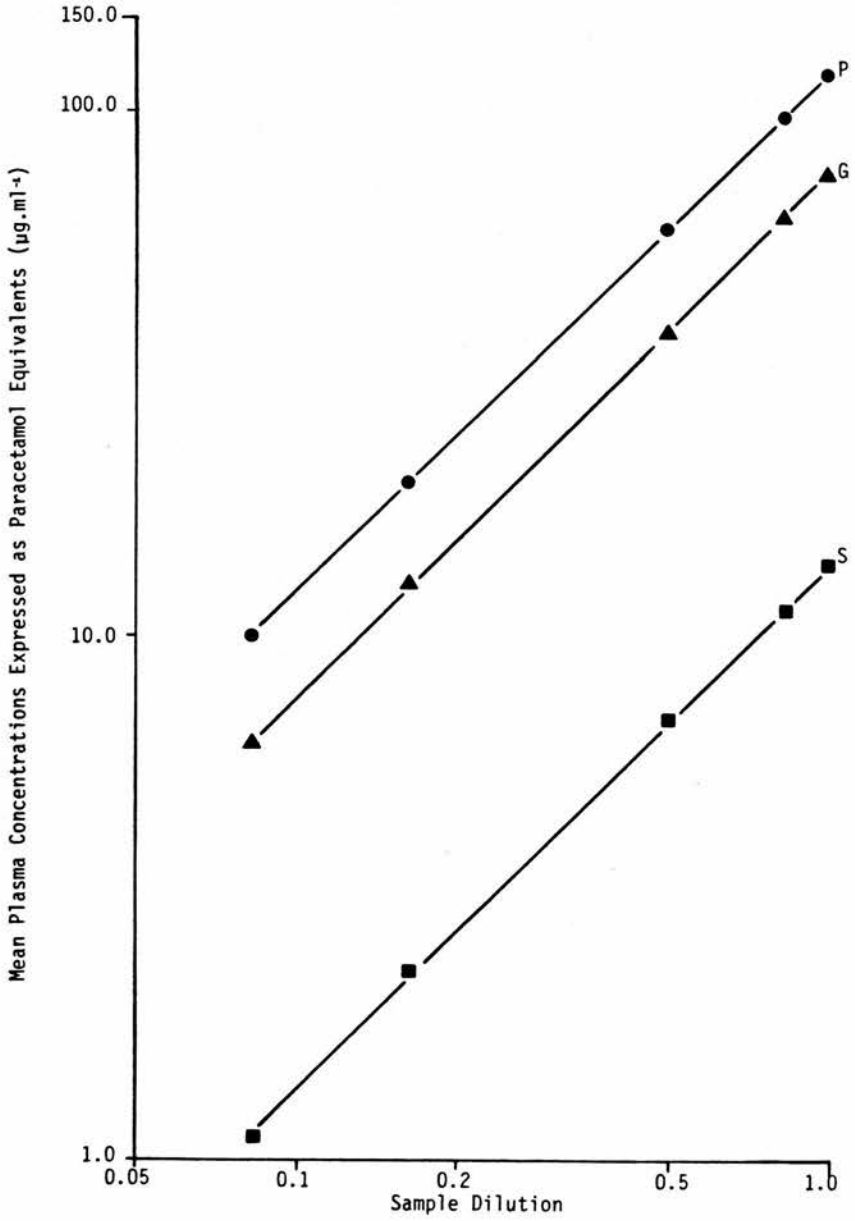
TABLE 2.2(ii) Precision of Plasma HPLC Assay - Paracetamol Glucuronide

Sample Dilution	Paracetamol Glucuronide Concentration ($\mu g.ml^{-1}$)					Mean ± SD	Coefficient of Variation (%)
	Run 1	Run 2	Run 3	Run 4	Run 5		
0.083	5.9	6.2	6.3	6.1	6.3	6.2 ± 0.2	2.7
0.167	11.7	12.8	13.2	12.8	12.4	12.6 ± 0.6	4.5
0.500	36.5	39.1	40.0	36.8	36.6	37.8 ± 1.6	4.3
0.833	59.8	64.5	63.5	61.5	62.0	62.3 ± 1.8	2.9
1.000	72.1	77.2	75.5	75.3	76.3	75.3 ± 1.9	2.5

TABLE 2.2(iii) Precision of Plasma HPLC Assay - Paracetamol Sulphate

Sample Dilution	Paracetamol Sulphate Concentration ($\mu g.ml^{-1}$)					Mean ± SD	Coefficient of Variation (%)
	Run 1	Run 2	Run 3	Run 4	Run 5		
0.083	1.2	0.9	1.1	1.3	1.2	1.1 ± 0.2	13.6
0.167	2.1	1.9	2.5	2.4	2.3	2.3 ± 0.3	10.9
0.500	6.3	6.5	7.4	7.0	7.1	6.9 ± 0.5	6.5
0.833	10.0	10.7	12.0	11.8	11.6	11.2 ± 0.8	7.5
1.000	11.9	13.1	14.6	14.2	14.3	13.6 ± 1.1	8.2

FIGURE 2.6 Linearity of HPLC Plasma Paracetamol Metabolite Assay: Calibration Plot of the Mean Paracetamol (P), Glucuronide (G) and Sulphate (S) Concentrations Obtained Following Repeated Analysis (n = 5) of Serially Diluted Overdose Plasma



v) Urine Assay

Chromatographic Conditions

For the estimation of paracetamol, paracetamol glucuronide, paracetamol sulphate, paracetamol mercapturic acid and paracetamol cysteine in urine, a Radial-PAK, 10 µ cartridge was used under the following chromatographic conditions:

Mobile Phase:	1% acetic acid:ethyl acetate (99:0.5 v/v)
Flow Rate:	3 $ml.min^{-1}$
Pressure:	1000 psi
Detector Wavelength:	254 nm

Preparation of Standards and Test Samples for Urine Assay

For estimating paracetamol and its metabolites in urine, aqueous standard solution of paracetamol (1 $mg.ml^{-1}$) and NPA (2 $mg.ml^{-1}$) were prepared. Three standards were set up by combining the paracetamol and NPA solutions in the following proportions: 1:1, 1:2 and 2:1 (v/v). These standards were then run with the test samples.

The urine was mixed with NPA standard in different proportions according to the concentration of paracetamol and its metabolites present. The ratios of urine to NPA solution ranged from 1:1 to 20:1 for samples obtained after a therapeutic paracetamol dose. Volumes up to 10 µl were injected directly into the HPLC system.

Calculation of Results for Urine Assay

As described for the plasma assay the factor (F) representing the reciprocal of the slope of the regression line for the standards was calculated (see Appendix 2.1). Concentrations of paracetamol and its conjugates were calculated as described on Page 46, using correction factors of 0.945 and 0.909 for paracetamol sulphate and paracetamol glucuronide conjugates respectively. No correction factor was used for paracetamol cysteine and paracetamol mercapturic acid conjugates (Adriaenssens, 1980).

$$\text{Paracetamol} = \frac{Pa}{NPAa} \times F \times \frac{NPAv}{Uv} \times 1.000 = \text{paracetamol concentration } (mg.ml^{-1})$$

$$\text{Cysteine} = \frac{Ca}{NPAa} \times F \times \frac{NPAv}{Uv} \times 1.000 = \text{paracetamol equivalents } (mg.ml^{-1})$$

$$\text{Mercapturate} = \frac{Ma}{NPAa} \times F \times \frac{NPAv}{Uv} \times 1.000 = \text{paracetamol equivalents } (mg.ml^{-1})$$

$$\text{Sulphate} = \frac{Sa}{NPAa} \times F \times \frac{NPAv}{Uv} \times 0.945 = \text{paracetamol equivalents } (mg.ml^{-1})$$

$$\text{Glucuronide} = \frac{Ga}{NPAa} \times F \times \frac{NPAv}{Uv} \times 0.909 = \text{paracetamol equivalents } (mg.ml^{-1})$$

(Note: $mg.ml^{-1} \times 1000 = \mu g.ml^{-1}$)

Where: Pa, Ca, Ma, Sa, Ga and NPAa are the peak areas for paracetamol, cysteine, mercapturic acid, sulphate, glucuronide and internal standard respectively. F is the factor representing the reciprocal

of the slope of the regression line for the standards. NPAa and Uv are the volume of internal standard and urine present in the sample mixture respectively. For the mean value of F obtained see Appendix 2.1.

Results of Urine Assay

Chromatograms obtained from drug free urine and from urine collected 2-4 h following a dose of 1 g of paracetamol are shown in Figure 2.7. The retention times of paracetamol sulphate, paracetamol glucuronide, paracetamol, internal standard (NPA), paracetamol cysteine and paracetamol mercapturic acid were 0.9, 2.1, 4.8, 9.7, 11.1 and 12.5 min respectively. The order of elution of the cysteine and mercapturic acid conjugates was reversed compared to the original method. The limit of detection for the assay was dependent on the presence of interfering peaks from endogenous material present in the urine.

Validation of Urine Assay

Urinary Paracetamol: A stock solution of paracetamol in blank urine was serially diluted to give concentrations ranging from 25-500 $\mu g.ml^{-1}$. Samples were assayed as described and analysed 5 times over a period of 2 days.

The results are shown in Table 2.3 and Figure 2.8. The coefficient of variation was less than 3% for concentrations in the range 50-500 $\mu g.ml^{-1}$. At 25 $\mu g.ml^{-1}$ the coefficient of variation was 12%. The

FIGURE 2.7 Chromatograms Obtained from:

A Drug free urine (5 µl; 0.2 AUFS)
B Urine collected 2-4 h following a dose of 1 g of paracetamol (3 µl; 0.2 AUFS)
The peaks are: 674 $\mu g.ml^{-1}$ paracetamol sulphate (S)
952 $\mu g.ml^{-1}$ paracetamol glucuronide (G)
65 $\mu g.ml^{-1}$ paracetamol (P)
66 $\mu g.ml^{-1}$ paracetamol cysteine (C)
111 $\mu g.ml^{-1}$ paracetamol mercapturic acid (M) and internal standard (IS)

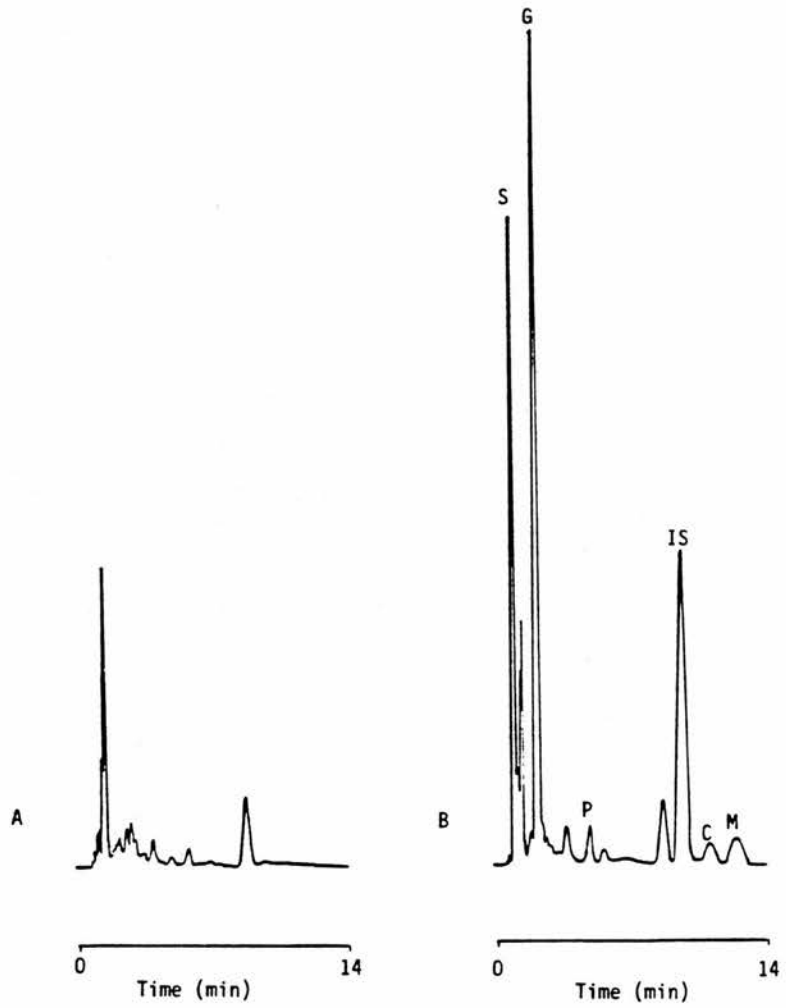
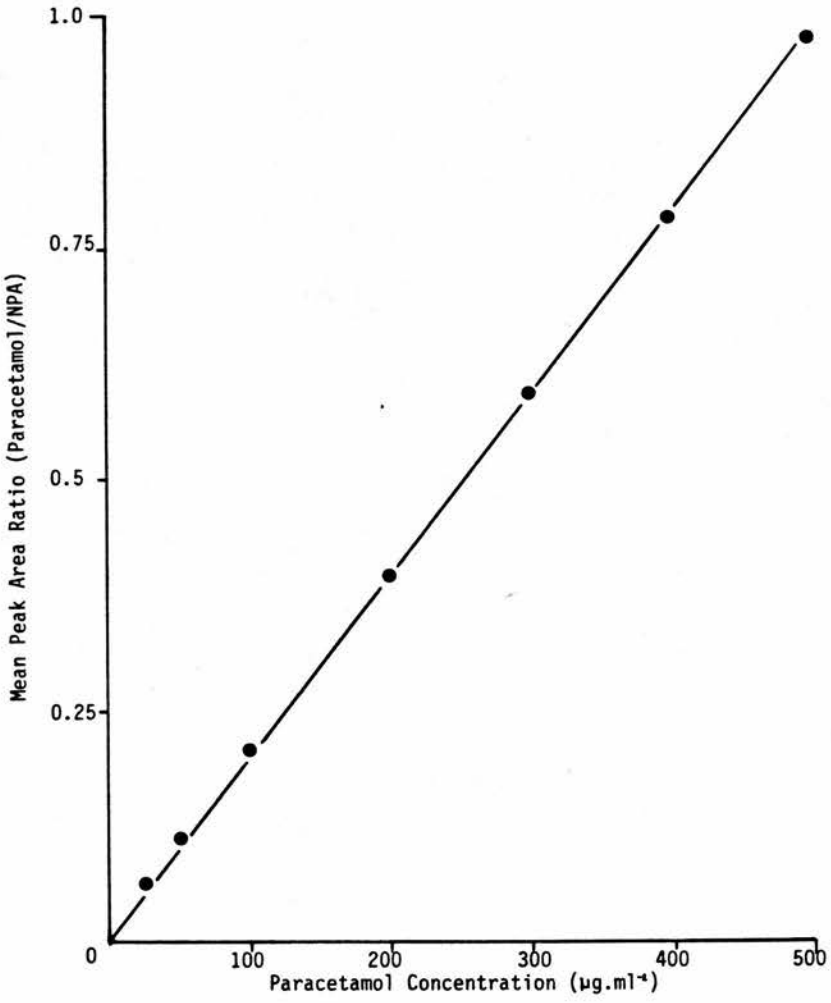


TABLE 2.3 Precision of Urine Paracetamol HPLC Assay - (25-500 $\mu g.ml^{-1}$)

Paracetamol Concentration ($\mu g.ml^{-1}$)	Peak Area Ratio (Paracetamol/NPA)					Mean ± SD	Coefficient of Variation (%)
	Run 1	Run 2	Run 3	Run 4	Run 5		
25	0.053	0.059	0.063	0.071	0.070	0.063 ± 0.008	12.0
50	0.112	0.108	0.116	0.110	0.113	0.112 ± 0.003	2.7
100	0.214	0.202	0.204	0.204	0.204	0.206 ± 0.005	2.3
200	0.411	0.387	0.386	0.389	0.393	0.395 ± 0.010	2.6
300	0.603	0.582	0.581	0.593	0.591	0.590 ± 0.009	1.5
400	0.818	0.762	0.765	0.780	0.782	0.781 ± 0.022	2.9
500	1.019	0.957	0.947	0.987	0.977	0.977 ± 0.028	2.9

FIGURE 2.8 Linearity of HPLC Urine Paracetamol Assay: Calibration Plot of the Mean Peak Area Ratio Obtained Following Repeated Analysis (n = 5) of Urine Containing Paracetamol Concentrations in the Range 50–500 $\mu g.ml^{-1}$



plot of mean peak area ratio against concentration was linear and passed through the origin.

Urinary Paracetamol Metabolites: Urine obtained from an overdose patient was serially diluted with distilled water to give concentrations of paracetamol, paracetamol glucuronide, paracetamol sulphate, paracetamol cysteine and paracetamol mercapturic acid ranging from 53-3383, 210-13078, 21-1309, 4-289 and 4-242 $\mu g.ml^{-1}$ respectively. Each sample was analysed 5 times over a period of 2 days.

The results are shown in Tables 2.4(i-iii) and 2.5(i-ii) and Figures 2.9 and 2.10.

The coefficients of variation for the determination of paracetamol, paracetamol glucuronide, paracetamol sulphate and paracetamol cysteine ranged from 1.6-7.0, 1.1-5.5, 1.1-6.7 and 0-17.7% respectively. In each case the greater variation in the range was associated with the lowest concentration. The calibration plots were linear for paracetamol and its glucuronide, sulphate and cysteine conjugates.

The coefficient of variation for the determination of paracetamol mercapturate ranged from 2.5-10.6%. At concentrations below 60 $\mu g.ml^{-1}$ the coefficient of variation exceeded 5% and there was a corresponding loss of linearity at the lowest concentrations.

TABLE 2.4(i) Precision of Urine HPLC Assay - Paracetamol

Sample Dilution	Paracetamol Concentration ($\mu g.ml^{-1}$)					Mean ± SD	Coefficient of Variation (%)
	Run 1	Run 2	Run 3	Run 4	Run 5		
0.016	60	52	52	52	51	53 ± 3.7	7.0
0.031	108	105	103	101	103	104 ± 2.6	2.5
0.063	211	206	206	207	202	206 ± 3.2	1.6
0.125	429	395	404	391	417	407 ± 15.8	3.9
0.250	836	879	859	885	782	848 ± 41.7	4.9
0.500	1682	1784	1711	1660	1578	1683 ± 75.1	4.5
1.000	3469	3616	3250	3379	3202	3383 ± 167.4	4.9

TABLE 2.4(ii) Precision of Urine HPLC Assay - Paracetamol Glucuronide

Sample Dilution	Paracetamol Glucuronide Concentration ($\mu g.ml^{-1}$)					Mean ± SD	Coefficient of Variation (%)
	Run 1	Run 2	Run 3	Run 4	Run 5		
0.016	230	206	203	202	209	210 ± 11.5	5.5
0.031	419	408	406	396	405	407 ± 8.2	2.0
0.063	819	802	802	811	798	806 ± 8.5	1.1
0.125	1672	1547	1587	1525	1650	1596 ± 63.7	4.0
0.250	3204	3398	3341	3447	3184	3315 ± 116.7	3.5
0.500	6515	6972	6581	6405	6391	6573 ± 236.6	3.6
1.000	13206	13738	12450	12995	12999	13078 ± 463.5	3.5

TABLE 2.4(iii) Precision of Urine HPLC Assay - Paracetamol Sulphate

Sample Dilution	Paracetamol Sulphate Concentration ($\mu g.ml^{-1}$)					Mean ± SD	Coefficient of Variation (%)
	Run 1	Run 2	Run 3	Run 4	Run 5		
0.016	22	20	20	20	23	21 ± 1.4	6.7
0.031	41	41	41	39	41	40 ± 0.9	2.1
0.063	80	78	78	79	78	79 ± 0.9	1.1
0.125	162	150	154	149	162	155 ± 6.1	4.1
0.250	310	332	326	335	346	330 ± 13.2	4.0
0.500	635	683	647	629	629	657 ± 22.7	4.3
1.000	1275	1352	1222	1284	1411	1309 ± 73.5	5.6

FIGURE 2.9 Linearity of HPLC Urine Paracetamol Metabolite Assay: Calibration Plot of the Mean Paracetamol (P), Glucuronide (G) and Sulphate (S) Concentrations Obtained Following Repeated Analysis (n = 5) of Serially Diluted Overdose Urine

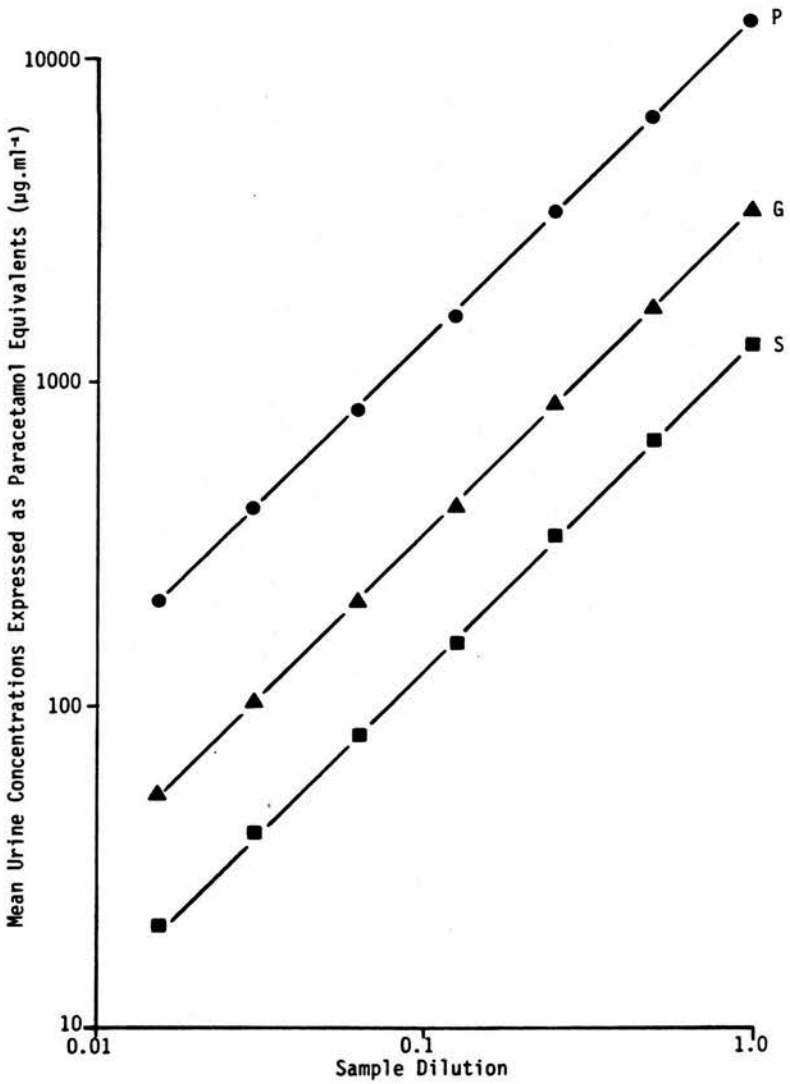


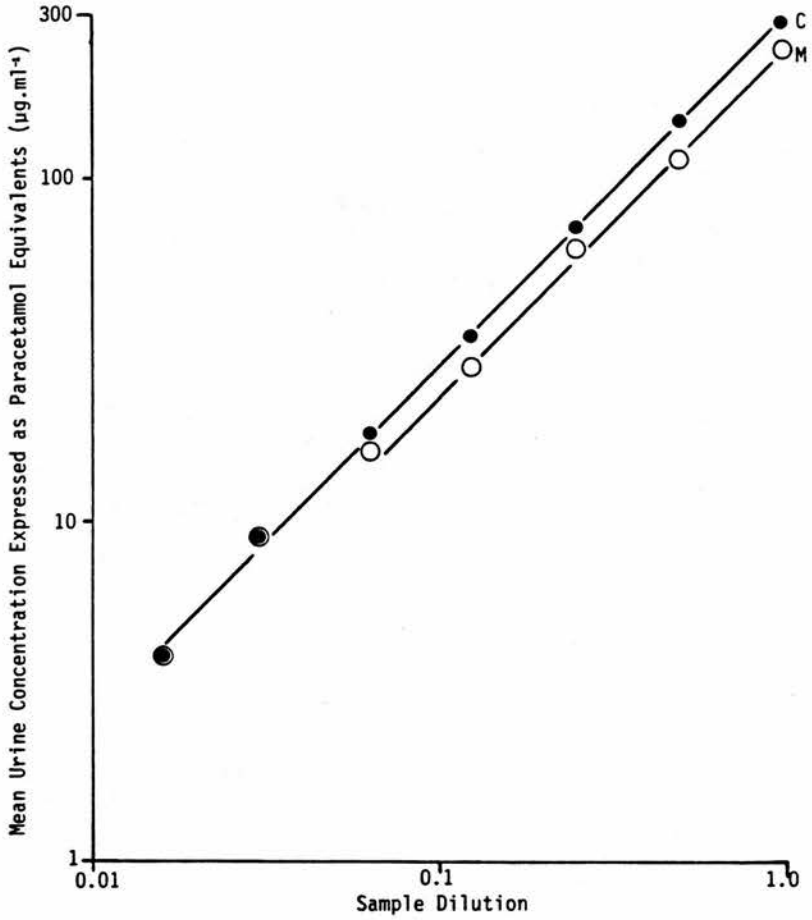
TABLE 2.5(i) Precision of Urine HPLC Assay - Paracetamol Cysteine

Sample Dilution	Paracetamol Cysteine Concentration ($\mu g.ml^{-1}$)					Mean ± SD	Coefficient of Variation (%)
	Run 1	Run 2	Run 3	Run 4	Run 5		
0.016	3	4	4	4	5	4 ± 0.7	17.7
0.031	10	9	9	9	9	9 ± 0.4	4.9
0.063	18	18	18	18	18	18 ± 0.0	0.0
0.125	36	33	33	32	35	34 ± 1.6	4.9
0.250	68	76	73	76	72	73 ± 3.3	4.5
0.500	143	152	149	146	147	147 ± 3.4	2.3
1.000	285	300	276	290	294	289 ± 9.1	3.2

TABLE 2.5(ii) Precision of Urine HPLC Assay - Paracetamol Mercapturic Acid

Sample Dilution	Paracetamol Mercapturic Acid Concentration ($\mu g.ml^{-1}$)					Mean ± SD	Coefficient of Variation (%)
	Run 1	Run 2	Run 3	Run 4	Run 5		
0.016	5	4	4	4	4	4 ± 0.4	10.6
0.031	9	9	8	9	8	9 ± 0.5	6.4
0.063	17	15	17	16	17	16 ± 0.9	5.5
0.125	30	27	26	26	29	28 ± 1.8	6.6
0.250	62	63	62	67	63	63 ± 2.1	3.3
0.500	124	133	130	130	129	129 ± 3.3	2.5
1.000	242	252	228	242	246	242 ± 8.8	3.6

FIGURE 2.10 Linearity of HPLC Urine Paracetamol Metabolite Assay: Calibration Plot of the Mean Cysteine (C) and Mercapturic Acid (M) Concentrations Obtained Following Repeated Analysis (n = 5) of Serially Diluted Overdose Urine



c) Discussion and Summary

The ability to measure drugs and their metabolites has been significantly enhanced by the advent of HPLC. Methods based on established HPLC assays for measuring paracetamol and its metabolites in plasma and urine, using N-propionyl-4-aminophenol (NPA) as the internal standard have been described. Standard HPLC equipment and materials were used in conjunction with radially compressed columns.

For paracetamol and metabolite concentrations in the therapeutic range in plasma and urine, the chromatograms obtained were similar to those described in the original method. By introducing radially compressed cartridges, the order of elution of the cysteine and mercapturic acid conjugates was reversed when compared to the original urine assay. A relatively short run time, minimal sample preparation and calibration using only paracetamol standards enabled rapid analysis.

Validation of both plasma and urine assays was carried out using serially diluted samples from patients who had taken paracetamol in overdosage.

SECTION 2

ESTIMATION OF PARACETAMOL N-ACETYL-D-METHIONATE IN PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

a) Introduction

A reverse phase HPLC method for the simultaneous determination of paracetamol and paracetamol N-acetyl-D-L-methionate in plasma has previously been described (SWRD Report 16/77, 1977). The method used N-butyryl-p-aminophenol as the internal standard with a mobile phase of methanol:water, 40:60 v/v at a flow rate of 2 $ml.min^{-1}$, detection was by u.v. at 254 nm. Aliquots of plasma (2 ml) were washed with iso-octane prior to extraction into ether. The ether extract was then evaporated to dryness, reconstituted in methanol and 25 µl injected into the HPLC system. The range of concentration for both paracetamol and paracetamol N-acetyl-D-L-methionate was 0.5-10.0 $\mu g.ml^{-1}$.

The disadvantages of this method included the elution of paracetamol very close to the solvent front, a lengthy extraction process and loss of precision at lower concentrations.

Attempts to increase the retention time of paracetamol while maintaining the quality of chromatography required for accurate deter-

mination of paracetamol N-acetyl-D-L-methionate under isocratic conditions were unsuccessful. Therefore, an HPLC method and extraction technique for measuring plasma paracetamol N-acetyl-D-L-methionate alone was developed.

b) Development of a High Performance Liquid Chromatography Assay for the Estimation of Paracetamol N-acetyl-D-L-methionate in Plasma

i) Materials

Paracetamol N-acetyl-D-L-methionate was kindly donated by Sterling-Winthrop Research and Development Division, Alnwick, England.

Acet-p-toluidide was supplied by BDH Chemicals Limited, Poole, England, acetonitrile and methanol were obtained from Fisons Scientific Apparatus, Loughborough, and helium from The British Oxygen Company.

Other reagents were of analytical grade.

ii) Instrumentation

The HPLC equipment for measuring paracetamol N-acetyl-D-L-methionate was as described on Pages 41-42, for measuring paracetamol.

Paracetamol N-acetyl-D-L-methionate was extracted from plasma using 'Bond Elut' (Analytichem International) solid phase extraction columns. The columns consisted of a polypropylene tube of 1 ml capacity with a Luer fitting at one end containing 100 mg of end capped, 40 µ C18 bonded silica between 2 porous polypropylene frits (20 µ). A diagram of a 'Bond Elut' solid phase extraction column is shown in Figure 2.11.

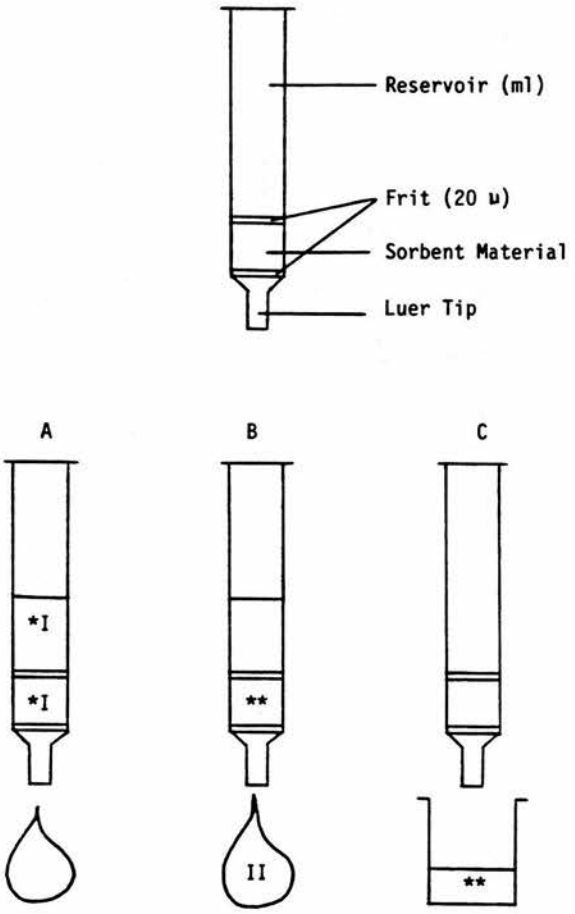
Samples can be prepared as follows: methanol is passed through the column to wet the sorbent bed followed by a conditioning flush of distilled water or buffer. The sample is then passed through the column. According to the principles of reverse phase HPLC, lipid soluble components are retained by the non-polar support, whereas polar material passes straight through. Potentially interfering material may, however, also be retained but can be removed by flushing the column with distilled water, buffer or solvent. The component of interest is then selectively eluted from the column with 300-500 µl of an appropriate solvent. The extract is collected, and either concentrated or injected directly into the chromatograph (Figure 2.11).

This procedure provides a fast, simple sample preparation with selective retention of the component of interest and economy of solvent.

'Bond Elut' columns used with a 'Vac Elut' (Analytichem International) processing station enabled up to 10 extractions to be

FIGURE 2.11 Diagram of a 'Bond Elut' Solid Phase Extraction Column and the Procedure Used for its Use

A Sample applied to conditioned column
B Cleaning step to elute off interfering (I) material
C Elution and collection of the component of interest (*)



processed at one time. The 'Vac Elut' comprised of a solvent resistant basin with a detachable moulded cover which received the Luer tips of the 'Bond Elut' columns. A drain in the base of the basin was fitted with a vacuum guage and release ring. The vacuum controller/release was connected to a glass side armed flask which in turn was linked to a vacuum pump (Millipore Corporation). A diagram representing the 'Bond Elut'/'Vac Elu' system is shown in Figure 2.12.

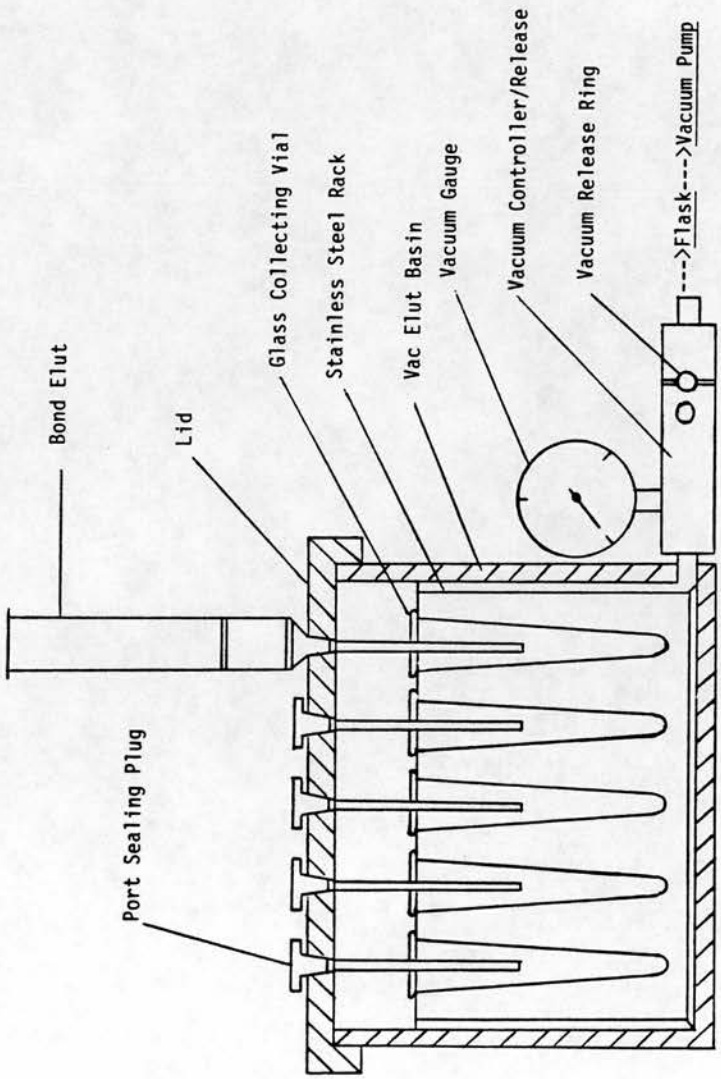
This system was capable of undertaking all the steps previously described for 'Bond Elut' extraction. The vacuum applied was generally between 10 and 20 inches of mercury and the vaccuum release ring enabled rapid application or release of the vacuum whenever appropriate. When the eluent was not required, liquids applied to the column passed directly through the 'Vac Elut' into the collection flask for disposal. When the eluent was to be collected, the vacuum was released and the lid removed. A stainless steel rack containing appropriately positioned glass collection vials was then placed into the basin, the lid was replaced and the eluting solvent added to the column. On re-application of the vacuum, the solvent passed through the column and into the collection vials positioned below.

iii) Chromatographic Conditions

Detection

Solutions of paracetamol N-acetyl-D-L-methionate and acet-p-toluidide (internal standard) were scanned from 210-260 nm using a Pye Unicam

FIGURE 2.12 'Bond Elut/Vac Elut' Separation System



SP1750 dual beam u.v. spectrophotometer (Figure 2.13). The absorbance maxima for paracetamol N-acetyl-D-L-methionate and act-p-toluidide was approximately 243 nm, however, fixed wavelength detectors (254 nm) were used.

Mobile Phase

The flow rate was 1.5 $ml.min^{-1}$ and the separation using different proportions of acetonitrile to water is shown in Figure 2.14.

Results

The optimum chromatographic conditions were as follows:

Mobile Phase:	Acetonitrile:water (35:65 v/v)
Flow Rate:	1.5 $ml.min^{-1}$
Pressure:	1000 psi
Wavelength:	254 nm

Under these conditions the retention time of paracetamol N-acetyl-D-L-methionate and acet-p-toluidide were 4.0 and 6.2 min respectively (Figure 2.15).

The chromatography is simple and fast with sharp, well resolved peaks. The number of theoretical plates ($N = 5.54\ R/W_{\frac{1}{2}}$, where R is the peak retention time and $W_{\frac{1}{2}}$ is the peak width at half height) was 2200 for paracetamol N-acetyl-D-L-methionate and 2300 for acet-p-toluidide using a 5 µ radial compression cartridge.

FIGURE 2.13 Ultraviolet Scan of 10 $\mu g.ml^{-1}$ Paracetamol N-acetyl-D-L-methionate (———) and Acet-p-toluidide (-----) from 210–260 nm

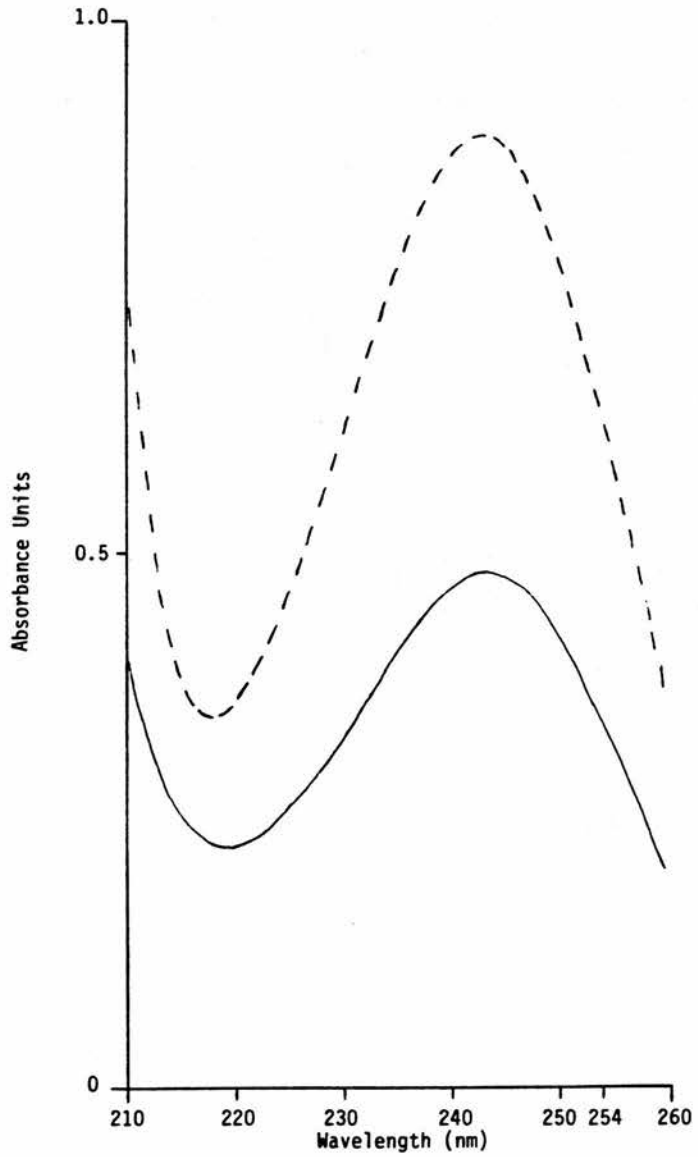


FIGURE 2.14 The Retention Times of the Solvent Front (SF), Paracetamol N-Acetyl-D-L-methionate (PNAM) and Acet-p-toluidide (APT) Obtained Using Different Proportions of Acetonitrile to Water in the Mobile Phase at a Flow Rate of 1.5 $ml \cdot min^{-1}$

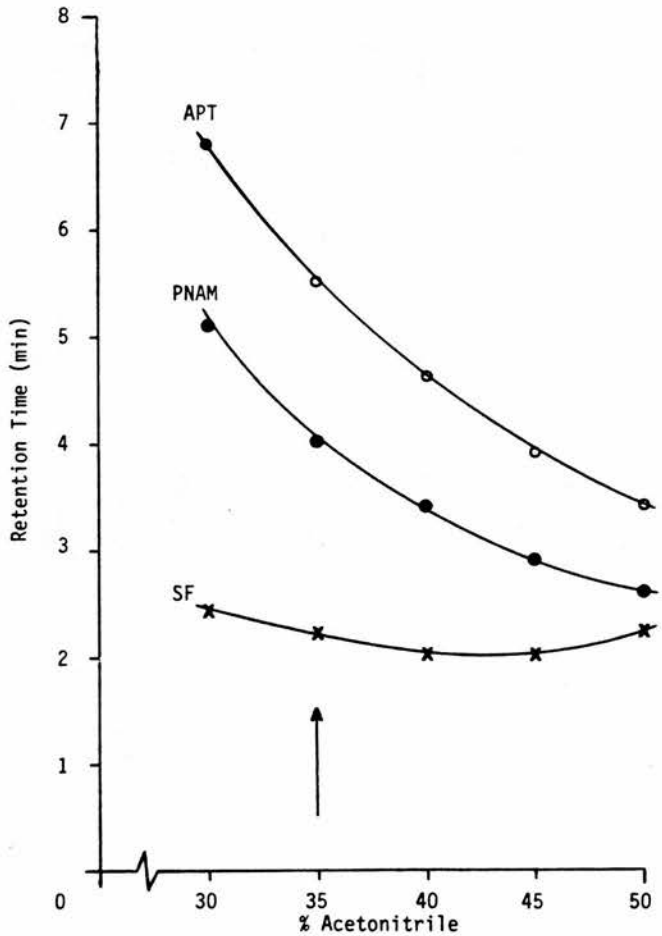
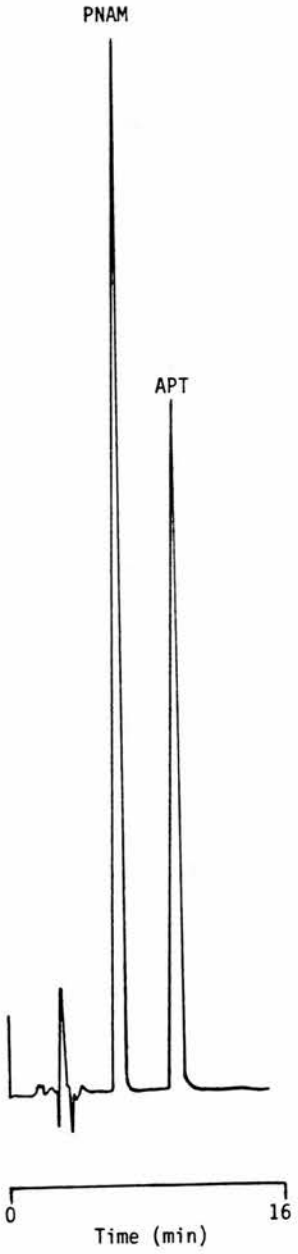


FIGURE 2.15 Chromatogram Obtained from an Aqueous Mix of Paracetamol N-Acetyl-D-L-methionate (PNAM) and Acet-p-toluidide (APT)



iv) Extraction from Aqueous Solutions

Determination of Optimal Extraction Conditions

'Bond Elut' (C18) columns were first conditioned with 1 ml of methanol followed by distilled water (2 x 1 ml). A 1 ml aliquot containing aqueous paracetamol N-acetyl-D-L-methionate (10 $\mu g.ml^{-1}$) and acet-p-toluidide (5 $\mu g.ml^{-1}$) was then passed through the column, followed by a 1 ml distilled water wash. The eluents from each step were collected and analysed by HPLC using the method described in (iii). By comparing the peak areas obtained for paracetamol N-acetyl-D-L-methionate and acet-p-toluidide with that of the non extracted mixture, the percentage recovery of each component could be calculated. It was observed that all the paracetamol N-acetyl-D-methionate and acet-p-toluidide was retained on the column after the distilled water wash. To determine the optimal strength of methanol for sample clean up and elution of the compounds, 1 ml washes of 15, 20, 25 and 30% methanol in water were passed through followed by 2 x 200 µl of 100% methanol. The percentage recoveries of paracetamol N-acetyl-D-L-methionate and acet-p-toluidide in each fraction were calculated and the results are shown in Table 2.6. A 25% methanol wash followed by elution with 300 µl of 100% methanol appeared to provide a suitable extraction method. However, repeated extractions revealed that small percentages of acet-p-toluidide were detected in the eluent following the 25% methanol wash. This was prevented by using a 20% methanol wash, followed by elution with 300 µl of 100% methanol. The final procedure is summarised as follows:

TABLE 2.6 The Percentage of 10 µg Paracetamol N-acetyl-D-L-methionate (PNAM) and 5 µg Acet-p-toluidide (APT) Recovered In Each Bond Elut Fraction Using Different Percentages of Methanol Wash Following Application of an Aqueous Sample

	Percentage Methanol Wash							
	15%		20%		25%		30%	
Bond Elut Fraction Samples	% PNAM Recovered	% APT Recovered	% PNAM Recovered	% APT Recovered	% PNAM Recovered	% APT Recovered	% PNAM Recovered	% APT Recovered
Sample Eluent	0	0	0	0	0	0	0	0
1 ml Distilled Water Wash	0	0	0	0	0	0	0	0
1 ml % Methanol Wash	0	0	0	0	0	0	0	0
First 200 µl Methanol Step	103	103	101	100	103	102	102	98
Second 200 µl Methanol Step	5	5	3	3	4	4	3	3

1. Column Conditioned: a) 1 ml methanol
   b) 2 x 1 ml distilled water
2. Aqueous Mixture Applied to Column (1 ml)
3. Sample Clean Up: a) 1 ml distilled water
   b) 1 ml 20% methanol
4. Selective Elution: 300 µl 100% methanol

Assessment of Extraction Procedure Using Aqueous Paracetamol N-acetyl-D-L-methionate Solutions (Upper Range 2-10 $\mu g.ml^{-1}$)

Aqueous solutions of paracetamol N-acetyl-D-L-methionate (2-10 $\mu g.ml^{-1}$) and acet-p-toluidide (2.5 $\mu g.ml^{-1}$) were extracted 4 times at each paracetamol N-acetyl-D-L-methionate concentration. The peak areas and peak area ratios were compared to the appropriate non-extracted aqueous mixture.

Recovery: The apparent mean percentage recovery of paracetamol N-acetyl-D-L-methionate and acet-p-toluidide ranged from 109-118% with coefficients of variation of less than 9% (Table 2.7). The spuriously high recoveries were attributed to evaporation of the final methanol extract in the 'Vac Elut' processing station, since methanol solutions containing the compound placed under vacuum for 1 min also showed an increase in peak area of up to 28% compared to solutions left on the bench.

Aqueous solutions containing paracetamol N-acetyl-D-l-methionate (10 $\mu g.ml^{-1}$) and acet-p-toluidide (5 $\mu g.ml^{-1}$) were extracted 4 times.

TABLE 2.7 Percentage Recovery of Paracetamol N-acetyl-D-L-methionate (PNAM) and Acet-p-toluidide (APT) Following Bond Elut Extraction of Aqueous Solutions Containing PNAM in the Range 2-10 $\mu g.ml^{-1}$. The Concentration of APT in Each Sample was 2.5 $\mu g.ml^{-1}$

Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Run Number								Mean		Coefficient of Variation (%)	
	1		2		3		4					
	% PNAM Recovered	% APT Recovered	% PNAM Recovered	% APT Recovered	% PNAM Recovered	% APT Recovered	% PNAM Recovered	% APT Recovered	% PNAM Recovered	% APT Recovered	PNAM	APT
2	117	117	120	121	112	109	115	115	116	116	2.9	4.3
4	124	123	116	112	112	110	114	109	117	114	4.5	5.7
6	128	127	118	117	113	110	114	113	118	117	5.8	6.3
8	126	125	106	102	111	111	113	110	114	112	7.5	8.5
10	110	108	110	110	111	108	109	110	110	109	0.7	0.9

The percentage recoveries for each component in the first methanol elution were again high (108-116%). However, neither component was detected in the 20% methanol wash nor the second methanol elution (Table 2.8). It was therefore reasonable to assume that the recoveries of both paracetamol N-acetyl-D-L-methionate and acet-p-toluidide were probably quantitative with the spuriously high recoveries obtained due to evaporation of the final methanol extract.

Peak Area Ratios: Mean peak area ratios with and without extraction varied by less than 3% (Table 2.9). The calibration plot was linear following 'Bond Elut' extraction and passed through the origin (Figure 2.16). The coefficient of variation for the peak area ratios following 4 'Bond Elut' extractions at each concentration was less than 2%.

Assessment of Extraction Procedure Using Aqueous Paracetamol N-acetyl-D-L-methionate Solution (Lower Range 0.2-1.0 $\mu g.ml^{-1}$)

Aqueous solutions containing paracetamol N-acetyl-D-L-methionate (0.2-1.0 $\mu g.ml^{-1}$) and acet-p-toluidide (0.25 $\mu g.ml^{-1}$) were prepared and extracted as above.

The results are shown in Table 2.10 and Figure 2.17. The percentage recoveries of paracetamol N-acetyl-D-L-methionate and acet-p-toluidide were again quantitative. The coefficient of variation for the peak area ratios of paracetamol N-acetyl-D-L-methionate/acet-p-toluidide following 4 'Bond Elut' extractions at paracetamol N-acetyl-D-L-methionate concentrations in the range 0.4-1.0 $\mu g.ml^{-1}$ was less than 2%. At 0.2 $\mu g.ml^{-1}$ it was 6.5%. The calibration plot was linear.

TABLE 2.8 The Percentage of 10 µg Paracetamol N-acetyl-D-L-methionate (PNAM) and 5 µg Acet-p-toluidide (APT) Recovered in Bond Elut Fractions Following Application of Aqueous Samples

Run	20% Methanol Wash		First 300 µl Methanol Elution		Second 300 µl Methanol Elution	
	% PNAM Recovered	% APT Recovered	% PNAM Recovered	% APT Recovered	% PNAM Recovered	% APT Recovered
1	0	0	109	109	0	0
2	0	0	114	110	0	0
3	0	0	116	112	0	0
4	0	0	110	108	0	0

TABLE 2.9 Peak Area Ratios of Paracetamol N-acetyl-D-L-methionate (PNAM)/Acet-p-toluidide (APT) With and Without Bond Elut Extraction of Aqueous Solutions Containing PNAM in the Range 2-10 $\mu g.ml^{-1}$. The Concentration of APT in Each Sample was 2.5 $\mu g.ml^{-1}$

Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Mean PNAM/APT Ratio Without Extraction	Peak Area Ratio PNAM/APT Following Bond Elut Extraction				Mean	Coefficient of Variation (%)	Mean Extracted Ratio / Unextracted Ratio (%)
		Run 1	Run 2	Run 3	Run 4			
2	0.413	0.411	0.410	0.422	0.416	0.415	1.3	100
4	0.829	0.836	0.857	0.843	0.866	0.851	1.6	103
6	1.258	1.267	1.263	1.296	1.276	1.276	1.3	101
8	1.655	1.670	1.720	1.654	1.702	1.687	1.8	102
10	2.124	2.152	2.132	2.169	2.092	2.136	1.6	101

FIGURE 2.16 Mean Paracetamol N-Acetyl-D-L-methionate/Acet-p-toluidide Peak Area Ratios Obtained from Aqueous Solutions Extracted Using 'Bond Elut' Columns (n = 4). Paracetamol N-Acetyl-D-L-methionate Concentrations Ranged from 2-10 $\mu g.ml^{-1}$. The concentration of Acet-p-toluidide was 2.5 $\mu g.ml^{-1}$ in Each Sample

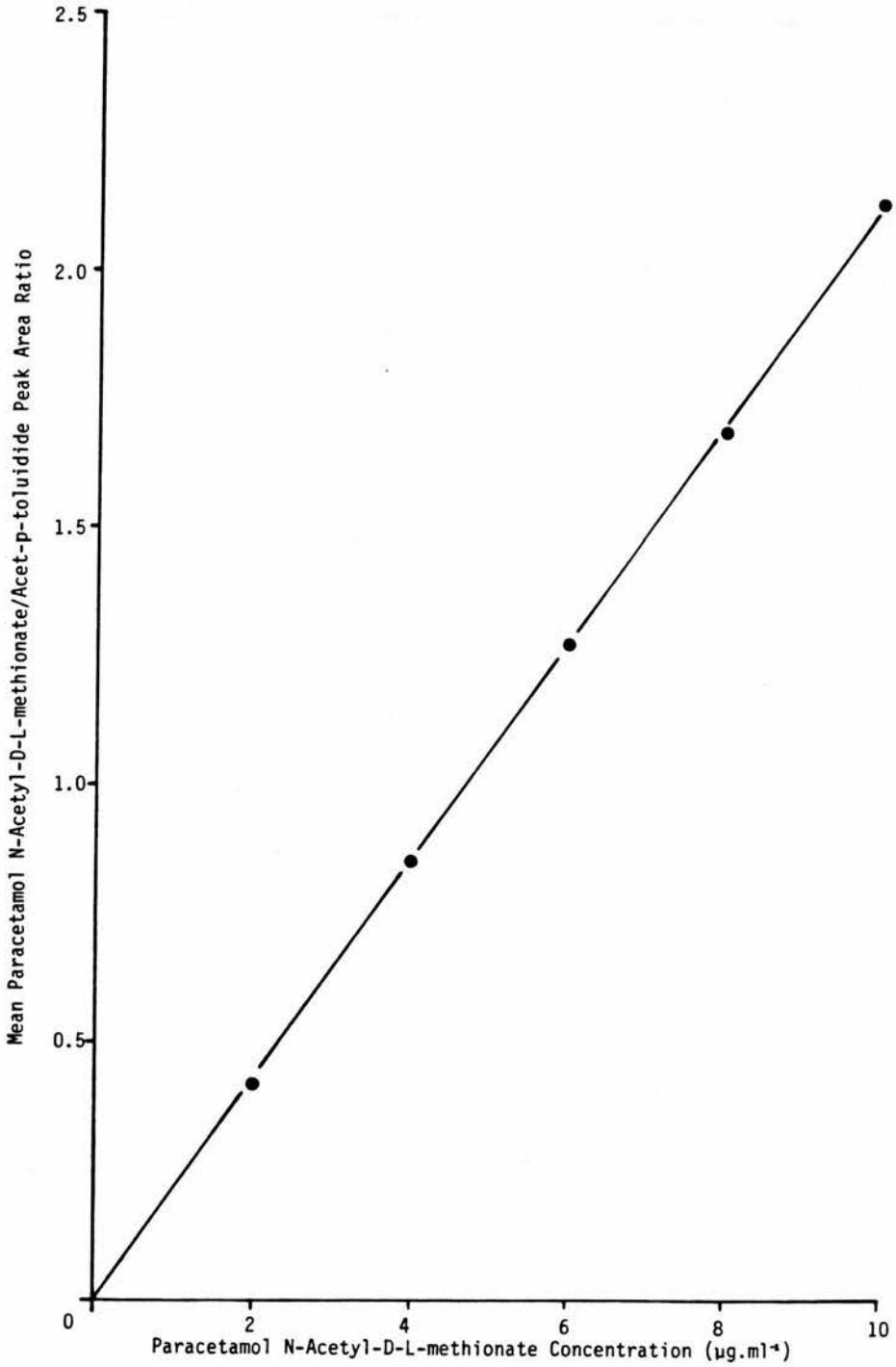
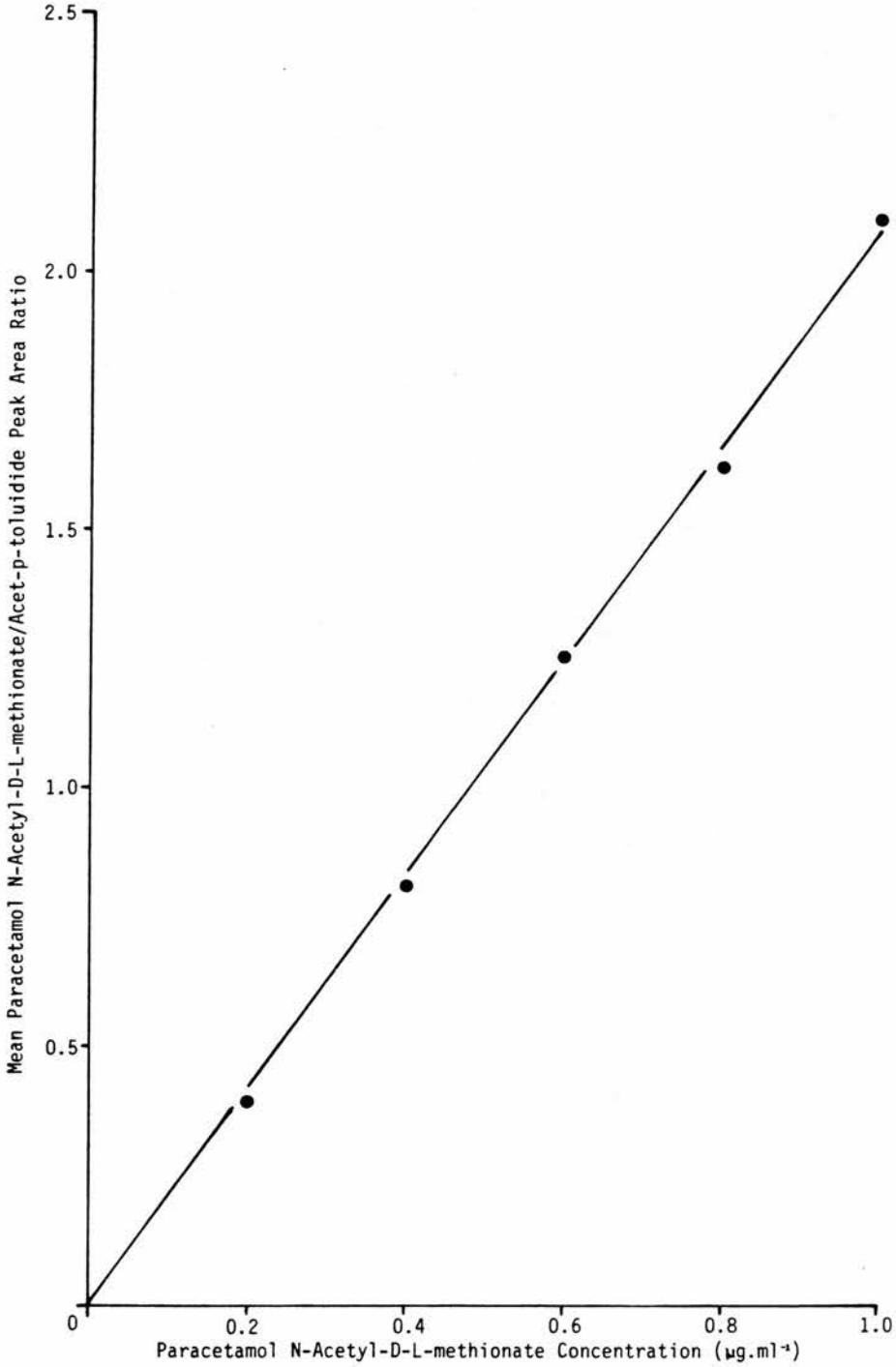


TABLE 2.10 Peak Area Ratios of Paracetamol N-acetyl-D-L-methionate (PNAM)/Acet-p-toluidide (APT) With and Without Bond Elut Extraction of Aqueous Solution Containing PNAM Concentrations in the Range 0.2-1.0 $\mu g.ml^{-1}$. The Concentration of APT in Each Sample was 0.25 $\mu g.ml^{-1}$

Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Mean PNAM/APT Ratio Without Extraction	Peak Area Ratio PNAM/APT Following Bond Elut Extraction				Mean	Coefficient of Variation (%)	Mean Extracted Ratio / Unextracted Ratio (%)
		Run 1	Run 2	Run 3	Run 4			
0.2	0.394	0.362	0.411	0.373	0.410	0.389	6.5	99
0.4	0.831	0.794	0.815	0.810	0.825	0.811	1.6	98
0.6	1.271	1.277	1.246	1.254	1.228	1.251	1.6	98
0.8	1.643	1.581	1.617	1.641	1.641	1.620	1.8	99
1.0	2.100	2.162	2.112	2.098	2.136	2.127	1.3	101

FIGURE 2.17 Mean Paracetamol N-Acetyl-D-L-methionate/Acet-p-toluidide Peak Area Ratios Obtained from Aqueous Solutions Extracted Using 'Bond Elut' Columns (n = 4). Paracetamol N-Acetyl-D-L-methionate Concentrations Ranged from 0.2-1.0 $\mu g.ml^{-1}$. The Concentration of Acet-p-toluidide was 0.25 $\mu g.ml^{-1}$ in Each Sample



v) Extraction from Plasma

Determination of Optimal Extraction Conditions

The extraction of plasma spiked with paracetamol N-acetyl-D-L-methionate, revealed rapid hydrolysis of the compound. Plasma was therefore spiked and analysed immediately to minimise this loss.

A small percentage (<6%) of paracetamol N-acetyl-D-L-methionate and acet-p-toluidide was found to be retained after elution with 300 µl of 100% methanol. Recovery was quantitative when the elution volume was increased to 350 µl. The optimum extraction regime for plasma was found to be as follows:

1.	Column Conditioned:	a) 1 ml methanol
		b) 2 x 1 ml distilled water
2.	Plasma Sample Applied to Column (1 ml)	
3.	Sample Clean Up:	a) 1 ml distilled water
		b) 1 ml 20% methanol
4.	Selective Elution:	350 µl 100% methanol

Assessment of Extraction Procedure Using Plasma Containing Paracetamol N-acetyl-D-L-methionate (Upper Range 2-10 $\mu g.ml^{-1}$)

The extraction of plasma and equivalent aqueous solutions were compared. Stock aqueous solutions of paracetamol N-acetyl-D-L-methionate (1 $mg.ml^{-1}$) and acet-p-toluidide (25 $\mu g.ml^{-1}$) were prepared. An

aqueous solution of 10 $\mu g.ml^{-1}$ paracetamol N-acetyl-D-L-methionate was made by diluting 100 µl of the stock solution in 10 ml of distilled water. Duplicate extractions were made after mixing 1 ml aliquots with 100 µl of the 25 $\mu g.ml^{-1}$ acet-p-toluidide (internal standard) solution in plastic tubes. The extracts were then analysed directly by HPLC. The same procedure was then carried out using blank plasma. Four aliquots of spiked plasma (10 $\mu g.ml^{-1}$) were extracted immediately. The 20% methanol wash in addition to the eluates after application of 2 x 350 µl of methanol were analysed by HPLC.

Aqueous and plasma samples containing paracetamol N-acetyl-D-L-methionate at 2, 4, 6 and 8 $\mu g.ml^{-1}$ were prepared similarly and run through the procedure. Only the first 350 µl methanol elution was analysed at these concentrations.

Recovery: By comparing the peak areas of paracetamol N-acetyl-D-L-methionate and acet-p-toluidide obtained from the plasma and aqueous extracts the percentage recovery of each from plasma was calculated.

The recovery of paracetamol N-acetyl-D-L-methionate (10 $\mu g.ml^{-1}$) and acet-p-toluidide in the first 350 µl methanol elution was in the range 92-97%. Analysis of the other fractions showed neither paracetamol N-acetyl-D-L-methionate nor acet-p-toluidide to be present (Table 2.11).

TABLE 2.11 The Percentage Paracetamol N-acetyl-D-L-methionate (PNAM) and Acet-p-toluidide (APT) Recovered In Bond Elut Fractions Collected Following Applications of a Spiked 10 $\mu g.ml^{-1}$ PNAM Plasma Sample

Run	20% Methanol Wash		First 350 µl Methanol Elution		Second 350 µl Methanol Elution	
	% PNAM Recovered	% APT Recovered	% PNAM Recovered	% APT Recovered	% PNAM Recovered	% APT Recovered
1	0	0	95	97	0	0
2	0	0	95	97	0	0
3	0	0	92	93	0	0
4	0	0	92	93	0	0

Chromatograms obtained from the extract of aqueous and plasma samples were similar. In each case, both peaks were well resolved and free from any interference. Analysis of the 20% methanol wash following application of a plasma sample (10 $\mu g.ml^{-1}$) demonstrated the degree of clean up achieved (Figure 2.18). This step was particularly useful as it removed a potentially interfering peak eluting close to the paracetamol N-acetyl-D-L-methionate peak.

The mean percentage recovery of paracetamol N-acetyl-D-L-methionate (2-10 $\mu g.ml^{-1}$) and acet-p-toluidide ranged from 91-100% with the coefficient of variation for each component less than 5% (Table 2.12).

Peak Area Ratios: Peak area ratios of paracetamol N-acetyl-D-L-methionate/acet-p-toluidide obtained from aqueous and plasma sample extractions are shown in Table 2.13. Comparing the mean peak area ratios of paracetamol N-acetyl-D-L-methionate/acet-p-toluidide following plasma extraction, with that following aqueous extraction demonstrated that no significant change to the proportions of the 2 components was taking place. The coefficients of variation for 4 plasma extractions at each concentration was less than 5%.

Assessment of Extraction Procedure Using Plasma Containing Paracetamol N-acetyl-D-L-methionate (Lower Range 0.2-1.0 $\mu g.ml^{-1}$)

The same experiments were carried out with spiked plasma containing paracetamol N-acetyl-D-L-methionate in the concentration range 0.2-1.0 $\mu g.ml^{-1}$. Aqueous solutions of paracetamol N-acetyl-D-L-methionate

FIGURE 2.18 Chromatograms of:

A Aqueous paracetamol N-acetyl-D-L-methionate (10 $\mu g.ml^{-1}$) 'Bond Elut' extract (20 µl; 0.04 AUFS)

B 20% Methanol wash following extraction of plasma containing 10 $\mu g.ml^{-1}$ paracetamol N-acetyl-D-L-methionate (20 µl; 0.02 AUFS)

C Plasma containing paracetamol N-acetyl-D-L-methionate (10 $\mu g.ml^{-1}$), 'Bond Elut' extract (20 µl; 0.04 AUFS)

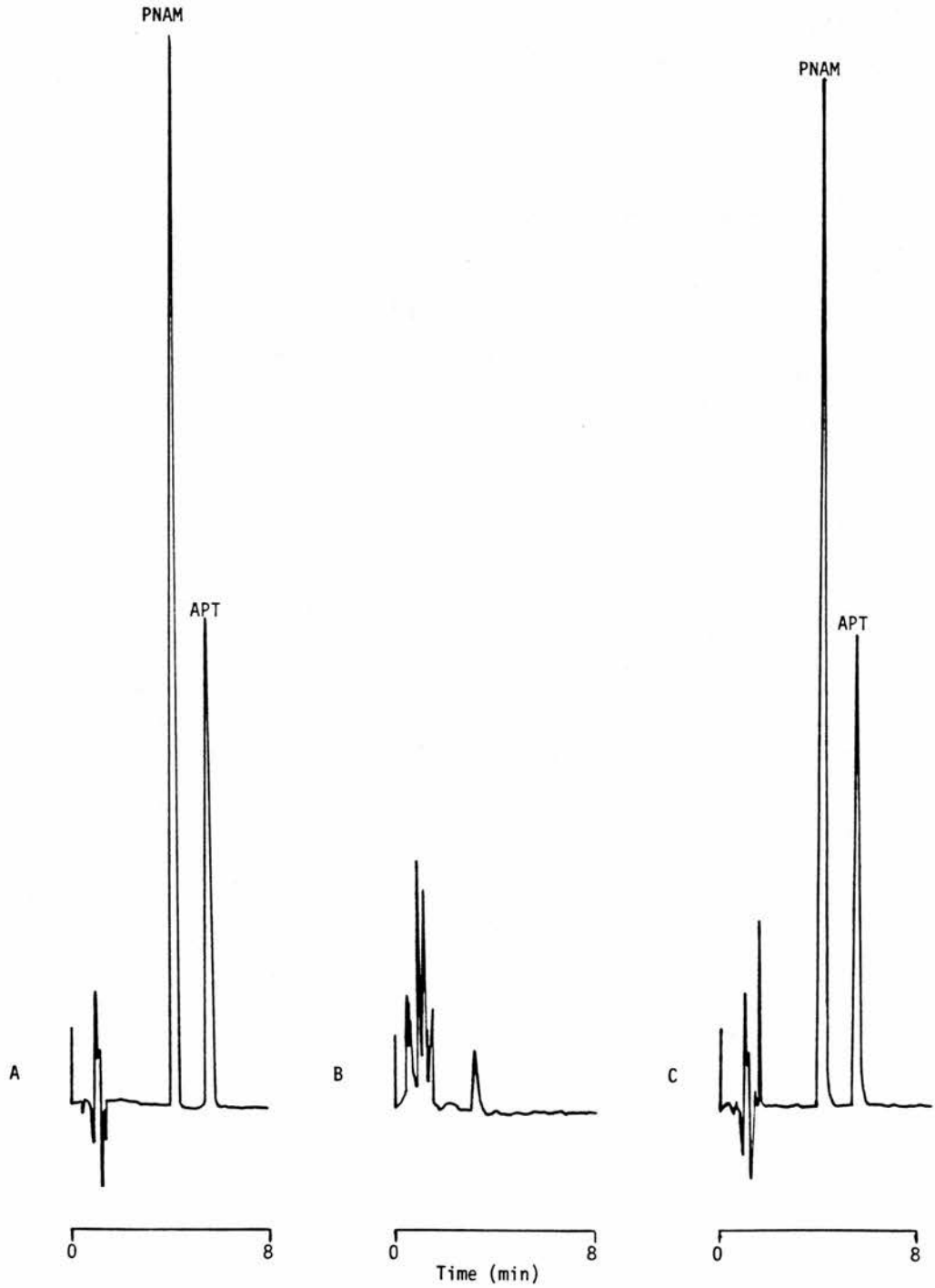


TABLE 2.12 Percentage Recovery of Paracetamol N-acetyl-D-L-methionate (PNAM) and Acet-p-toluidide (APT) from Spiked Plasma Samples Relative to Aqueous Standards Following Bond Elut Extraction. Paracetamol N-acetyl-D-L-methionate Concentrations Ranged from 2-10 $\mu g.ml^{-1}$

Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Run Number								Mean		Coefficient of Variation (%)	
	1		2		3		4					
	% PNAM Recovered	% APT Recovered	% PNAM Recovered	% APT Recovered	% PNAM Recovered	% APT Recovered	% PNAM Recovered	% APT Recovered	% PNAM Recovered	% APT Recovered	PNAM	APT
2	87	98	94	101	92	99	91	99	91	99	3.2	1.3
4	92	101	88	99	93	100	89	98	91	100	2.6	1.3
6	99	99	100	96	98	96	96	96	98	97	1.7	1.6
8	95	92	96	98	100	97	96	102	97	97	2.3	4.2
10	95	97	95	97	92	93	92	93	94	95	1.9	2.4

TABLE 2.13 Peak Area Ratios of Paracetamol N-acetyl-D-L-methionate (PNAM)/Acet-p-toluidide (APT) in Spiked Aqueous and Plasma Samples Following Bond Elut Extraction. Paracetamol N-acetyl-D-L-methionate Concentrations Ranged from 2-10 $\mu g.ml^{-1}$

Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Mean Aqueous PNAM/APT Ratio	Plasma Peak Area Ratio PNAM/APT				Mean	Coefficient of Variation (%)	Mean Aqueous Ratio / Plasma Ratio (%)
		Run 1	Run 2	Run 3	Run 4			
2	0.414	0.370	0.384	0.386	0.379	0.380	1.9	92
4	0.912	0.834	0.818	0.854	0.835	0.835	1.8	92
6	1.083	1.080	1.124	1.098	1.079	1.095	1.9	101
8	1.574	1.611	1.547	1.623	1.480	1.565	4.2	99
10	1.925	1.877	1.883	1.895	1.897	1.888	0.5	98

(0.1 $mg.ml^{-1}$) and acet-p-toluidide (2.5 $\mu g.ml^{-1}$) were prepared and used as before.

The mean percentage recoveries of paracetamol N-acetyl-D-L-methionate and acet-p-toluidide from plasma ranged from 94-101%, with coefficients of variation of less than 5% in the range 0.4-1.0 $\mu g.ml^{-1}$. At 0.2 $\mu g.ml^{-1}$ the coefficient of variation for paracetamol N-acetyl-D-L-methionate was 6.1% (Table 2.14).

Peak area ratios of paracetamol N-acetyl-D-L-methionate/acet-p-toluidide obtained from aqueous and plasma extracts were within 5% of each other, and the coefficient of variation following 4 plasma extractions was less than 4% (Table 2.15).

Chromatograms obtained from blank plasma and plasma containing 0.2 $\mu g.ml^{-1}$ paracetamol N-acetyl-D-L-methionate are shown in Figure 2.19.

At plasma paracetamol N-acetyl-D-L-methionate concentrations as low as 0.2 $\mu g.ml^{-1}$ peaks were easily detected and well resolved with minimal interference.

vi) Validation of the Assay for the Estimation of Paracetamol N-acetyl-D-L-methionate in Plasma

As paracetamol N-acetyl-D-L-methionate is unstable in plasma, it was not feasible to calibrate the assay using plasma standards. Previously in v) it was shown that spiked plasma samples extracted

TABLE 2.14 Percentage Recovery of Paracetamol N-acetyl-D-L-methionate (PNAM) and Acet-p-toluidide (APT) from Spiked Plasma Samples Relative to Aqueous Standards Following Bond Elut Extraction. Paracetamol N-acetyl-D-L-methionate Concentrations Ranged from 0.2-1.0 $\mu g.ml^{-1}$

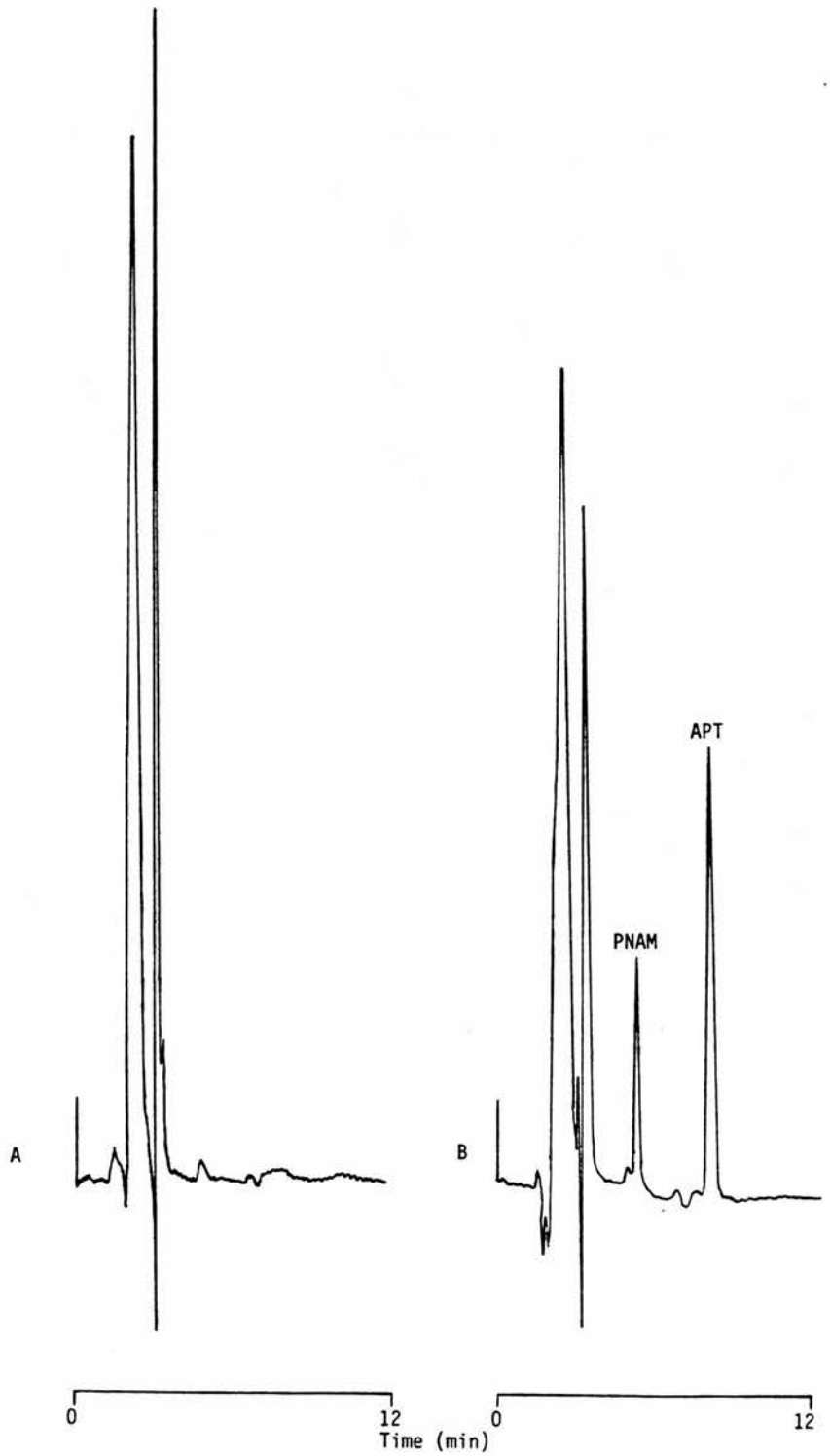
Paracetamol N-acetyl-D-L-methionate	Run Number								Mean		Coefficient of Variation (%)	
	1		2		3		4					
Concentration ($\mu g.ml^{-1}$)	% PNAM Recovered	% APT Recovered	% PNAM Recovered	% APT Recovered	% PNAM Recovered	% APT Recovered	% PNAM Recovered	% APT Recovered	% PNAM Recovered	% APT Recovered	PNAM	APT
0.2	102	105	88	97	94	99	93	95	94	99	6.1	4.4
0.4	96	99	96	96	95	94	94	97	95	97	1.0	2.2
0.6	96	102	103	101	98	96	100	99	99	100	3.0	2.7
0.8	98	101	98	101	95	95	96	99	97	99	1.6	2.9
1.0	97	96	102	101	105	106	99	100	101	101	3.5	4.1

TABLE 2.15 Peak Area Ratios of Paracetamol N-acetyl-D-L-methionate (PNAM)/Acet-p-toluidide (APT) In Spiked Aqueous and Plasma Samples Following Bond Elut Extraction. Paracetamol N-acetyl-D-L-methionate Concentrations Ranged from 0.2-1.0 $\mu g.ml^{-1}$

Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Mean Aqueous PNAM/APT Ratio	Plasma Peak Area Ratio PNAM/APT				Mean	Coefficient of Variation (%)	Mean Aqueous Ratio / Plasma Ratio (%)
		Run 1	Run 2	Run 3	Run 4			
0.2	0.363	0.354	0.330	0.345	0.354	0.346	3.3	95
0.4	0.730	0.711	0.725	0.735	0.705	0.719	1.9	98
0.6	1.055	0.993	1.079	1.077	1.072	1.055	3.9	100
0.8	1.660	1.560	1.566	1.620	1.579	1.581	1.7	95
1.0	2.020	2.046	2.055	1.998	2.005	2.026	1.4	100

FIGURE 2.19 Chromatograms of 'Bond Elut' Extractions Containing:

A Blank plasma (30 µl; 0.01 AUFS)
B Plasma containing 0.2 $\mu g.ml^{-1}$ paracetamol N-acetyl-D-L-methionate (50 µl; 0.02 AUFS)



immediately, gave comparable results to those obtained with equivalent aqueous samples. Aqueous standards were therefore used to calibrate the assay.

Precision and Linearity (5-25 $\mu g.ml^{-1}$)

An aqueous solution was serially diluted to provide paracetamol N-acetyl-D-L-methionate concentrations in the range 5-25 $\mu g.ml^{-1}$. The concentration of acet-p-toluidide (internal standard) was 50 $\mu g.ml^{-1}$. Prior to extraction, 100 μl of internal standard solution was mixed with the test sample (1 ml). Each concentration in the range was analysed 5 times on 5 separate occasions.

The results are shown in Table 2.16 and Figure 2.20. Coefficients of variation ranged from 0.6-1.7%. The plot of mean peak area ratio against concentration was linear and passed through the origin.

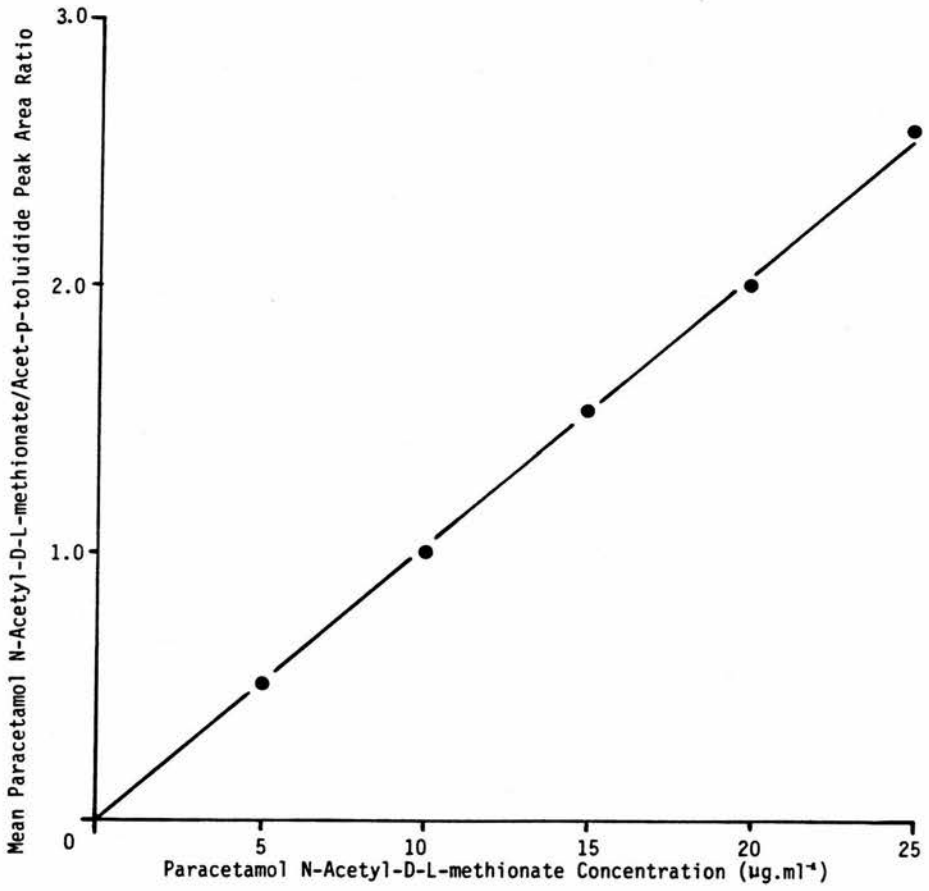
Precision and Linearity (0.1-1.0 $\mu g.ml^{-1}$)

An aqueous solution was serially diluted to give paracetamol N-acetyl-D-L-methionate concentrations in the range 0.1-1.0 $\mu g.ml^{-1}$ and the concentration of acet-p-toluidide (internal standard) was 2.5 $\mu g.ml^{-1}$. Prior to extraction, 100 μl of internal standard solution was mixed with the test sample (1 ml). Each concentration in the range was analysed 5 times over a period of 2 days.

TABLE 2.16 Precision of Paracetamol N-acetyl-D-L-methionate Assay - Aqueous Standards 5-25 $\mu g.ml^{-1}$

Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Peak Area Ratio PNAM/APT					Mean ± SD	Coefficient of Variation (%)
	Run 1	Run 2	Run 3	Run 4	Run 5		
5	0.512	0.507	0.503	0.508	0.504	0.507 ± 0.004	0.7
10	1.026	1.029	1.033	1.019	1.035	1.028 ± 0.006	0.6
15	1.555	1.548	1.532	1.538	1.574	1.549 ± 0.016	1.1
20	2.048	2.089	2.112	2.045	2.104	2.080 ± 0.031	1.5
25	2.557	2.600	2.587	2.522	2.636	2.580 ± 0.043	1.7

FIGURE 2.20 Linearity of Paracetamol N-Acetyl-D-L-methionate HPLC Assay: Calibration Plot of the Mean Peak Area Ratio Obtained Following Repeated Analysis (n = 5) of Aqueous Solutions Containing Paracetamol N-Acetyl-D-L-methionate in the Range 5-25 $\mu g.ml^{-1}$



The results are shown in Table 2.17 and Figure 2.21. Coefficients of variation ranged from 0.7-2.6% for paracetamol N-acetyl-D-L-methionate concentrations in the range 0.2-1.0 $\mu g.ml^{-1}$. At 0.1 $\mu g.ml^{-1}$ it was 6.6%. The plot of mean peak area ratio against concentration was linear and passed through the origin.

Quantitation

For plasma concentrations in the range 5-25 $\mu g.ml^{-1}$, aqueous standards of 5 and 25 $\mu g.ml^{-1}$ were prepared and processed in duplicate with the samples. From the paracetamol N-acetyl-D-L-methionate/acet-p-toluidide peak area ratios for the standards, a factor (F) representing the reciprocal of the slope of the regression line for the standards was calculated. The factor multiplied by the paracetamol N-acetyl-D-L-methionate/acet-p-toluidide peak area ratio for an unknown sample provided the paracetamol N-acetyl-D-L-methionate concentration in $\mu g.ml^{-1}$. The method of calculation of the factor (F) and the mean value obtained is shown in Appendix 2.1.

Accuracy (0.1, 1.0 and 10.0 $\mu g.ml^{-1}$)

Aqueous solutions of paracetamol N-acetyl-D-L-methionate at concentrations of 0.1, 1.0 and 10.0 $\mu g.ml^{-1}$ were run through the procedure 5 times. The percentage accuracy was determined by dividing the calculated concentration by the actual concentration.

The results are shown in Table 2.18. At 1.0 and 10 $\mu g.ml^{-1}$, there was good agreement between calculated and actual concentrations. At 0.1 $\mu g.ml^{-1}$ the percentage accuracy ranged from 90.5-106.3% with a coefficient of variation 6.5%.

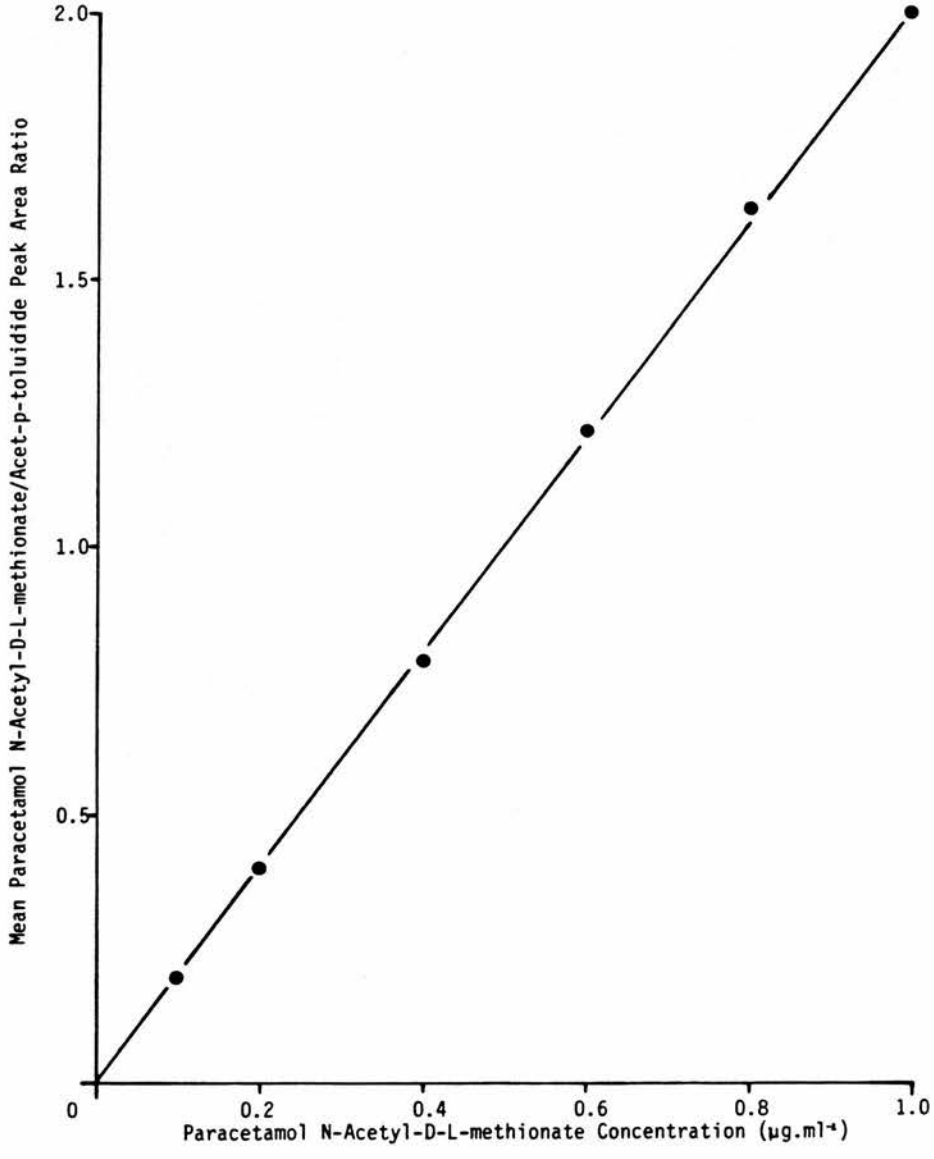
TABLE 2.17 Precision of Paracetamol N-acetyl-D-L-methionate Assay - Aqueous Standards 0.1-1.0 $\mu g.ml^{-1}$

Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Peak Area Ratio PNAM/APT					Mean ± SD	Coefficient of Variation (%)
	Run 1	Run 2	Run 3	Run 4	Run 5		
0.1	0.203	0.212	0.205	0.191	0.179	0.198 ± 0.013	6.6
0.2	0.402	0.393	0.403	0.407	0.389	0.399 ± 0.007	1.9
0.4	0.779	0.788	0.776	0.813	0.757	0.783 ± 0.020	2.6
0.6	1.224	1.219	1.232	1.211	1.198	1.217 ± 0.013	1.1
0.8	1.610	1.658	1.655	1.608	1.645	1.635 ± 0.024	1.5
1.0	2.014	2.022	2.008	2.041	2.009	2.019 ± 0.014	0.7

TABLE 2.18 Accuracy of Paracetamol N-acetyl-D-L-methionate Assay at 10.0, 1.0 and 0.1 $\mu g.ml^{-1}$

Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	% Accuracy					Mean	Coefficient of Variation (%)
	Run 1	Run 2	Run 3	Run 4	Run 5		
10.0	100.3	100.2	101.2	100.7	100.4	100.6	0.4
1.0	100.1	101.4	99.8	100.1	101.6	100.6	0.8
0.1	100.9	106.3	101.9	93.7	90.5	98.7	6.5

FIGURE 2.21 Linearity of Paracetamol N-Acetyl-D-L-methionate HPLC Assay: Calibration Plot of the Mean Peak Area Ratio Obtained Following Repeated Analysis (n = 5) of Aqueous Solutions Containing Paracetamol N-Acetyl-D-L-methionate Concentrations in the Range 0.1-1.0 $\mu g.ml^{-1}$



c) Discussion and Summary

An HPLC method and extraction technique for the estimation of paracetamol N-acetyl-D-L-methionate in plasma has been described. The chromatographic system used acet-p-toluidide as the internal standard with a mobile phase of acetonitrile:water 35:65 v/v at a flow rate of 1.5 $ml.min^{-1}$, detection was by u.v. at 254 nm. The chromatography was simple and fast with sharp, well resolved peaks.

The extraction of paracetamol N-acetyl-D-L-methionate from plasma was carried out using 'Bond Elut' C18 solid phase extraction columns. The method was developed using aqueous solutions and adapted for extraction from plasma. Plasma samples (1 ml) containing the internal standard were passed through a pre-conditioned column. The column was then washed using 1 ml aliquots of distilled water and 20% methanol. Quantitative recoveries of paracetamol N-acetyl-D-L-methionate and internal standard were obtained by selective elution from the column using 350 µl of 100% methanol. Plasma paracetamol N-acetyl-D-L-methionate levels as low as 0.1 $\mu g.ml^{-1}$ were detected with minimal interference. Sample preparation was rapid and easy to perform.

The method was validated by assessment of precision, linearity and accuracy.

SECTION 3

STABILITY OF PARACETAMOL N-ACETYL-D-L-METHIONATE

a) Introduction

Paracetamol N-acetyl-D-L-methionate is an ester prodrug where paracetamol is chemically linked by an ester bond to N-acetyl-D-L-methionine. Little is known regarding its stability, and the effects of pH and temperature were investigated. Using both the paracetamol N-acetyl-D-L-methionate and paracetamol HPLC assays, the disappearance of paracetamol N-acetyl-D-L-methionate and the simultaneous appearance of paracetamol was monitored.

Many ester prodrugs are hydrolysed by ubiquitous esterases (Sinkula and Yalkowsky; 1975 and Williams, 1985). The rate of paracetamol N-acetyl-D-L-methionate hydrolysis in the presence of simulated gastric and intestinal fluid, hog liver esterase and serum has previously been studied (SWRD Report 303944, 1977). Hydrolysis was slow in the presence of simulated gastric and intestinal fluid, negligible with hog liver esterase and rapid in the presence of serum. The stability of paracetamol N-acetyl-D-L-methionate in human blood samples, however, was not investigated. Its stability in plasma and whole blood was therefore studied.

b) Assessment of Paracetamol N-acetyl-D-L-methionate Stability

Methods

The stability of paracetamol N-acetyl-D-L-methionate at 20 $\mu g.ml^{-1}$ was investigated. Paracetamol N-acetyl-D-L-methionate concentrations were estimated using the HPLC method developed (Section 2).

Complete hydrolysis of 20 µg paracetamol N-acetyl-D-L-methionate yields 9.4 µg of paracetamol. The plasma paracetamol HPLC assay was therefore adapted to estimate paracetamol concentrations in the range 0.5-10 $\mu g.ml^{-1}$. Peaks were quantitated by measuring peak heights.

Process controls to assess the hydrolysis of paracetamol N-acetyl-D-L-methionate during sample preparation for the estimation of paracetamol were carried out. Hydrolysis to paracetamol did not occur if samples were analysed within 20 min after the addition of perchloric acid.

Hydrolysis of Paracetamol N-acetyl-D-L-methionate in Acetate Buffer, pH 4.0 at 0, 20 and 37°C

Duplicate solutions of paracetamol N-acetyl-D-L-methionate (20 $\mu g.ml^{-1}$) in 0.1 N acetate buffer, pH 4.0 were left to stand in ice (0°C), at room temperature (20°C) and in a heated water bath (37°C). Aliquots were taken from each solution and simultaneous estimations

of paracetamol N-acetyl-D-L-methionate and paracetamol were made at 0, 2, 4, 24 and 51 h.

The results are shown in Table 2.19 and Figure 2.22. The semilog plot of mean paracetamol N-acetyl-D-L-methionate concentration against time gave a linear response indicative of a first order process. The half life decreased as temperature increased, being greater than 100 h at 0 and 20°C and 72 h at 37°C.

The total paracetamol N-acetyl-D-L-methionate equivalents plus paracetamol N-acetyl-D-L-methionate in each sample in every case was close to 20 $\mu g.ml^{-1}$ and there was good agreement between the duplicate samples.

Hydrolysis of Paracetamol N-acetyl-D-L-methionate in Distilled Water, pH 6.1 at 0, 20 and 37°C

Duplicate solutions of paracetamol N-acetyl-D-L-methionate (20 $\mu g.ml^{-1}$) in distilled water, pH 6.1 were left to stand at 0, 20 and 37°C. Aliquots were taken from each solution and simultaneous estimations of paracetamol N-acetyl-D-L-methionate and paracetamol were made at 0, 2, 4, 24 and 96 h.

The results are shown in Table 2.20 and Figure 2.23. The semilog plot of mean paracetamol N-acetyl-D-L-methionate concentration against time was linear with the half life at 0 and 20°C greater than 100 h and 40 h at 37°C. All the paracetamol N-acetyl-D-L-methionate

TABLE 2.19 Stability of Aqueous Paracetamol N-acetyl-D-L-methionate (20 $\mu g.ml^{-1}$, pH 4.0) at 0, 20 and 37°C

Time (h)	Temperature (0°C)	1			2			Mean	
		Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)	Total Accounted for ($\mu g.ml^{-1}$)*	Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)	Total Accounted for ($\mu g.ml^{-1}$)*	Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)
0	0	20.2	ND	20.2	19.9	ND	19.9	20.1	ND
	20	19.9	ND	19.9	20.1	ND	20.1	20.0	ND
	37	20.4	ND	20.4	20.0	ND	20.0	20.2	ND
2	0	19.6	ND	19.6	19.3	ND	19.3	19.5	ND
	20	19.8	ND	19.8	19.7	ND	19.7	19.8	ND
	37	20.0	ND	20.0	19.7	0.5	20.8	19.9	0.3
4	0	19.4	ND	19.4	19.2	ND	19.2	19.3	ND
	20	19.2	ND	19.2	19.7	ND	19.7	19.5	ND
	37	19.3	0.5	20.4	18.9	0.6	20.2	19.1	0.6

ND = Not detected * = as paracetamol N-acetyl-D-L-methionate

TABLE 2.19 (continued)

Time (h)	Temperature (0°C)	1			2			Mean	
		Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)	Total Accounted for ($\mu g.ml^{-1}$)*	Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)	Total Accounted for ($\mu g.ml^{-1}$)*	Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)
24	0	19.1	0.5	20.2	18.8	0.5	19.9	19.0	0.5
	20	18.1	0.8	19.8	18.2	0.9	20.1	18.2	0.9
	37	16.1	1.9	20.2	16.1	1.9	20.2	16.1	1.9
51	0	18.1	ND	18.1	18.0	0.5	19.1	18.1	0.3
	20	16.9	1.4	19.9	17.4	1.4	20.4	17.2	1.4
	37	12.9	3.5	20.4	12.6	3.5	20.1	12.8	3.5

ND = Not detected * = as paracetamol N-acetyl-D-L-methionate

FIGURE 2.22 Stability of Aqueous Paracetamol N-Acetyl-D-L-methionate (20 µg.ml^{-1}, pH 4.0) at 0, 20 and 37°C

Results expressed as mean (n = 2) paracetamol N-acetyl-D-L-methionate concentration (µg.ml^{-1})

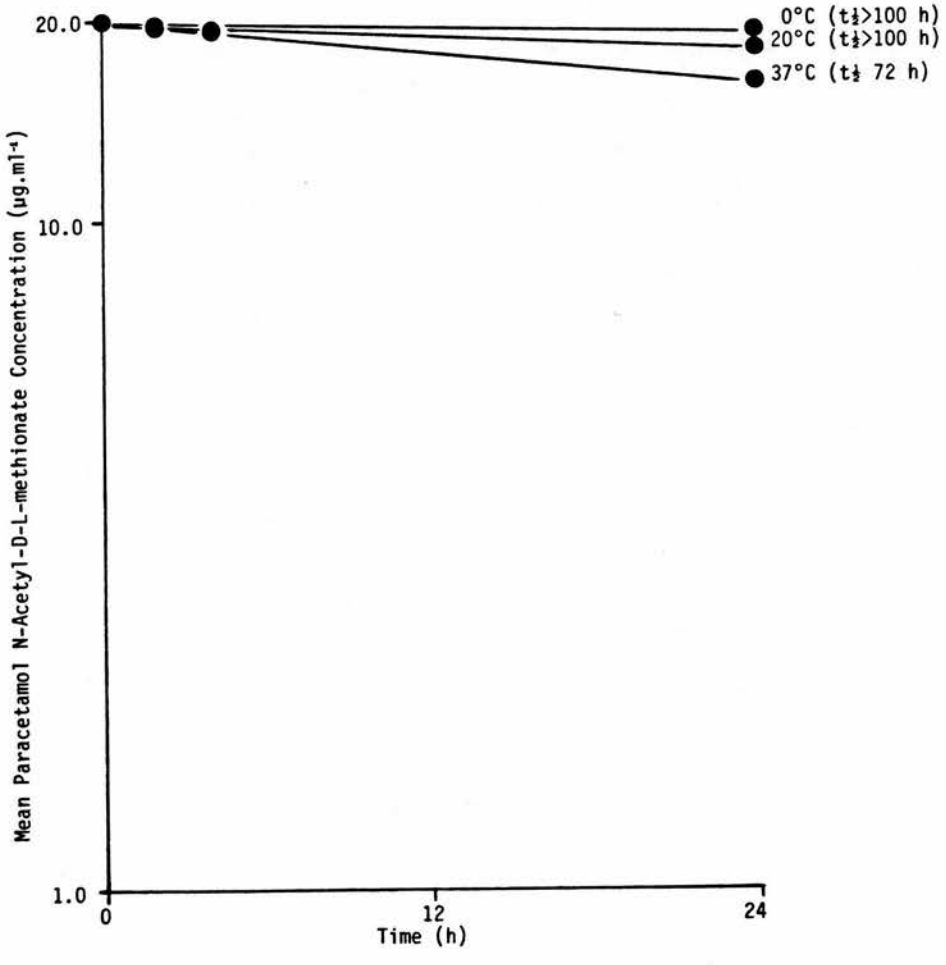


TABLE 2.20 Stability of Aqueous Paracetamol N-acetyl-D-L-methionate (20 $\mu g.ml^{-1}$, pH 6.1) at 0, 20 and 37°C

Time (h)	Temperature (0°C)	1			2			Mean	
		Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)	Total Accounted for ($\mu g.ml^{-1}$)*	Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)	Total Accounted for ($\mu g.ml^{-1}$)*	Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)
0	0	20.3	ND	20.3	20.2	ND	20.2	20.3	ND
	20	20.1	ND	20.1	20.0	ND	20.0	20.1	ND
	37	20.1	ND	20.1	20.2	ND	20.2	20.2	ND
2	0	20.1	ND	20.1	20.5	ND	20.5	20.3	ND
	20	20.0	ND	20.0	20.4	ND	20.4	20.2	ND
	37	19.1	0.8	20.9	19.6	0.7	21.1	19.4	0.8
4	0	19.8	ND	19.8	20.1	ND	20.1	20.0	ND
	20	20.4	ND	20.4	20.2	ND	20.2	20.3	ND
	37	18.1	1.4	21.0	18.4	1.2	21.1	18.3	1.3

ND = Not detected * = as paracetamol N-acetyl-D-L-methionate

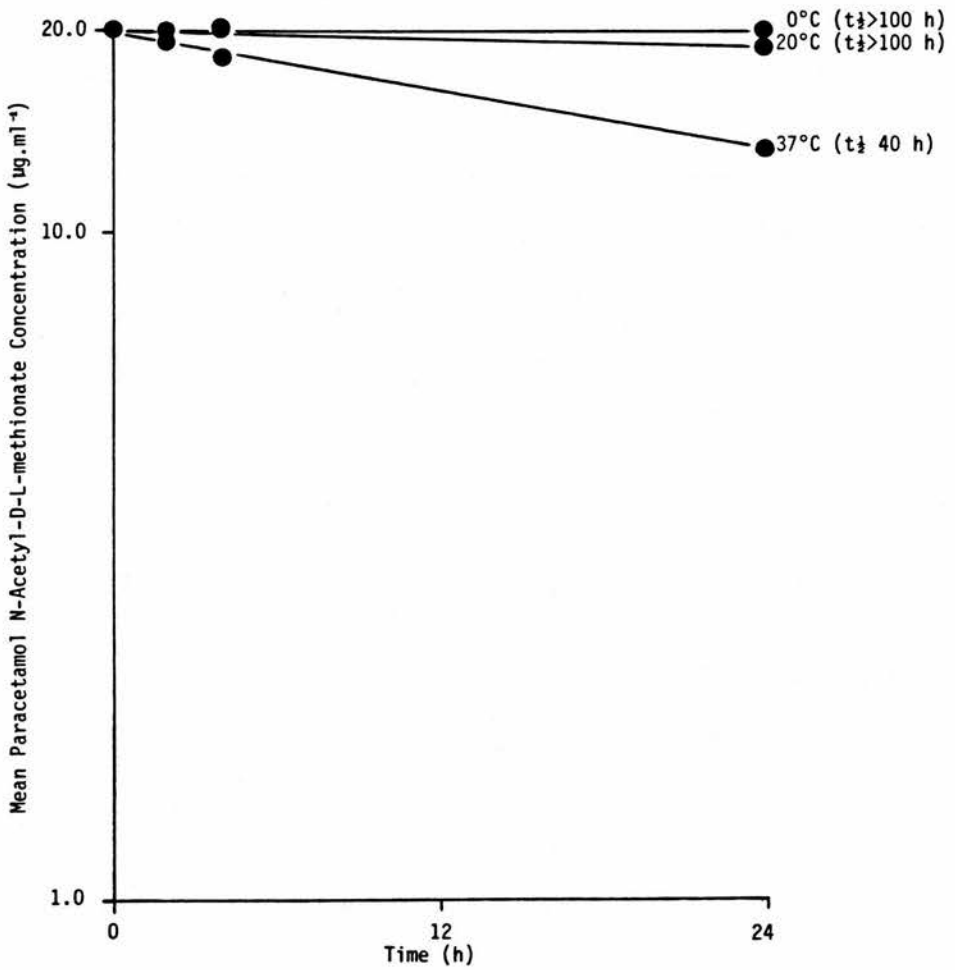
TABLE 2.20 (continued)

Time (h)	Temperature (0°C)	1			2			Mean	
		Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)	Total Accounted for ($\mu g.ml^{-1}$)*	Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)	Total Accounted for ($\mu g.ml^{-1}$)*	Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)
24	0	19.9	ND	20.4	20.0	ND	20.0	20.0	ND
	20	18.7	0.8	20.4	18.7	1.0	20.8	18.7	0.9
	37	13.4	3.6	21.1	13.0	3.9	21.3	13.2	3.8
96	0	19.3	0.7	20.8	19.3	0.5	20.4	19.3	0.6
	20	16.1	2.4	21.2	15.8	2.4	20.9	16.0	2.4
	37	6.7	6.9	21.5	5.8	7.5	21.2	6.3	7.2

ND = Not detected * = as paracetamol N-acetyl-D-L-methionate

FIGURE 2.23 Stability of Aqueous Paracetamol N-Acetyl-D-L-methionate (20 µg.ml^{-1}, pH 6.1) at 0, 20 and 37°C

Results expressed as mean (n = 2) paracetamol N-acetyl-D-L-methionate concentration (µg.ml^{-1})



was accounted for in each sample and there was good agreement between duplicate samples.

Hydrolysis of Paracetamol N-acetyl-D-L-methionate in Phosphate Buffer, pH 7.4 at 0, 20 and 37°C

Duplicate solutions of paracetamol N-acetyl-D-L-methionate (20 μg. ml^{-1}) in 0.07 M phosphate buffer, pH 7.4 were left to stand at 0, 20 and 37°C. Aliquots were taken from each solution and simultaneous estimations of paracetamol N-acetyl-D-L-methionate and paracetamol were made at 0, 2, 4, 6 and 24 h.

The results are shown in Table 2.21 and Figure 2.24. The semilog plot of mean paracetamol N-acetyl-D-L-methionate concentration against time was linear with the half lives at 0, 20 and 37°C being 95.0, 6.2 and 1.3 h respectively. At 24 h the values obtained for paracetamol N-acetyl-D-L-methionate and paracetamol in each sample at 20°C deviated from linearity. This may be explained by a fall in room temperature overnight. All the paracetamol N-acetyl-D-L-methionate was accounted for in each sample and there was good agreement between duplicate samples.

The hydrolysis of paracetamol N-acetyl-D-L-methionate at pH 7.4 appeared to be a first order process and a linear relationship was obtained by plotting:

$$\log \frac{P\infty}{P\infty - Pt} \text{ against time}$$

where $P\infty$ = Paracetamol concentration at infinity

Pt = Paracetamol concentration at time (t)

At each temperature, the process was linear and the half lives were similar to those obtained for the disappearance of paracetamol N-acetyl-D-L-methionate (Figure 2.25).

Hydrolysis of Paracetamol N-acetyl-D-L-methionate in Tris Buffer, pH 8.0 at 0, 20 and 37°C

Duplicate solutions of paracetamol N-acetyl-D-L-methionate (20 µg. ml^{-1}) in 0.2 M Tris buffer, pH 8.0 were left to stand at 0, 20 and 37°C. Aliquots were taken from each solution and simultaneous estimations of paracetamol N-acetyl-D-L-methionate and paracetamol were made at 0, 1, 2 and 4 h.

The results are shown in Table 2.22 and Figures 2.26 and 2.27. The semilog plot of mean paracetamol N-acetyl-D-L-methionate concentration against time was linear with the half lives at 0, 20 and 37°C being 5.0, 1.4 and 0.6 h respectively. Similar results were obtained from the semilog plot for the rate of appearance of paracetamol. All the paracetamol N-acetyl-D-L-methionate was accounted for in each sample and there was good agreement between duplicate samples. At pH 10.0 the hydrolysis of paracetamol N-acetyl-D-L-methionate was almost instantaneous.

TABLE 2.21 Stability of Aqueous Paracetamol N-acetyl-D-L-methionate (20 $\mu g.ml^{-1}$, pH 7.4) at 0, 20 and 37°C

Time (h)	Temperature (0°C)	1			2			Mean	
		Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)	Total Accounted for ($\mu g.ml^{-1}$)*	Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)	Total Accounted for ($\mu g.ml^{-1}$)*	Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)
0	0	20.0	0.5	21.1	19.4	0.5	20.6	19.7	0.5
	20	20.4	0.5	21.5	19.7	0.5	20.8	20.1	0.5
	37	19.4	ND	19.4	19.2	ND	19.2	19.3	ND
2	0	19.7	ND	19.7	19.8	0.5	20.9	19.8	0.3
	20	16.5	1.9	20.6	16.2	2.0	20.5	16.4	2.0
	37	6.8	6.2	20.1	6.7	5.9	19.4	6.8	6.1
4	0	19.6	0.6	20.9	18.7	0.6	20.0	19.2	0.6
	20	12.8	3.0	19.2	12.8	3.1	19.5	12.8	3.1
	37	2.5	7.9	19.4	2.3	7.8	19.0	2.4	7.9

ND = Not detected * = as paracetamol N-acetyl-D-L-methionate

TABLE 2.21 (continued)

Time (h)	Temperature (0°C)	1			2			Mean	
		Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)	Total Accounted for ($\mu g.ml^{-1}$)*	Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)	Total Accounted for ($\mu g.ml^{-1}$)*	Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)
6	0	18.6	0.7	20.1	18.2	0.8	19.9	18.4	0.8
	20	10.5	4.3	19.7	9.9	4.4	19.3	10.2	4.4
	37	1.0	9.3	21.0	0.9	8.7	19.6	1.0	9.0
24	0	17.3	1.5	20.5	16.5	1.5	19.7	16.9	1.5
	20	2.9	8.7	21.6	2.7	7.8	19.4	2.8	8.3
	37	ND	9.9	21.2	ND	9.8	21.0	ND	9.9

ND = Not detected * = as paracetamol N-acetyl-D-L-methionate

FIGURE 2.24 Stability of Aqueous Paracetamol N-Acetyl-D-L-methionate (20 $\mu g \cdot ml^{-1}$, pH 7.4) at 0, 20 and 37°C

Results expressed as mean (n = 2) paracetamol N-acetyl-D-L-methionate concentration ($\mu g \cdot ml^{-1}$)

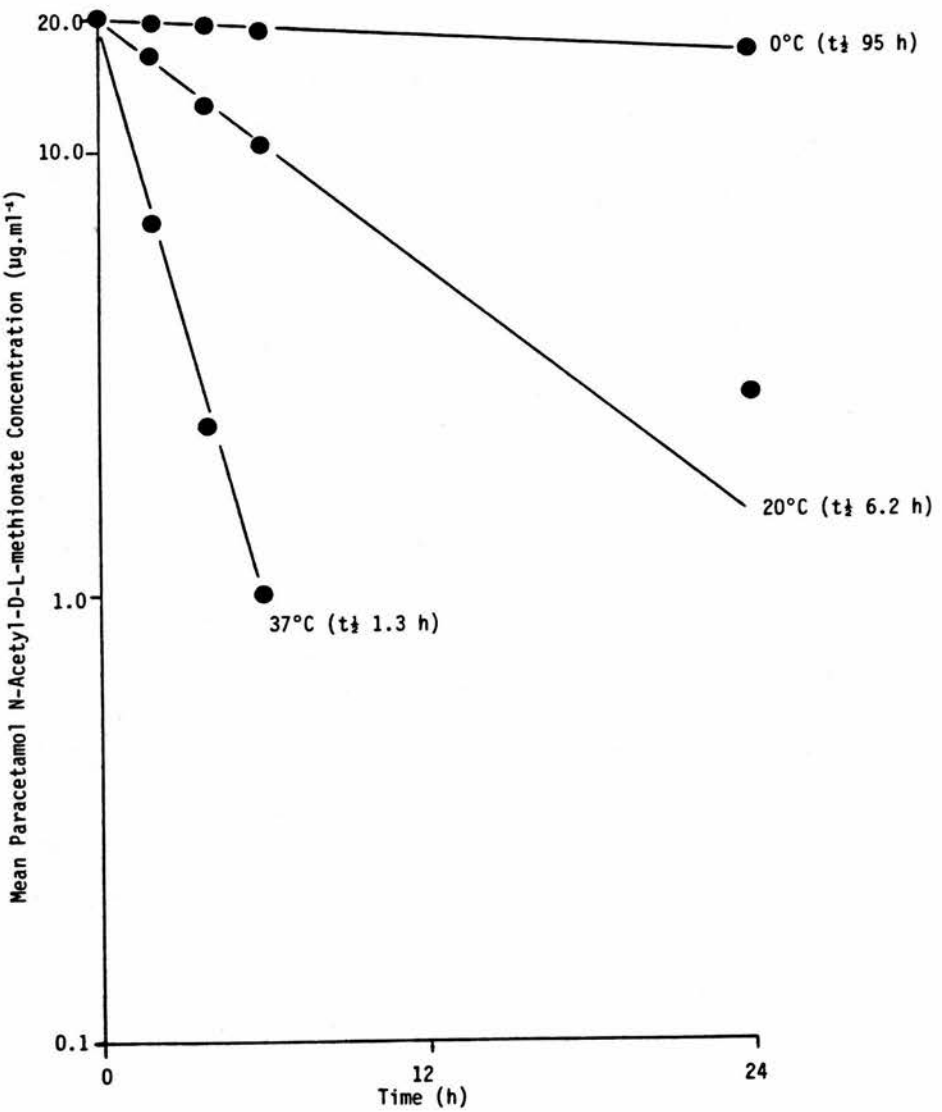


FIGURE 2.25 Stability of Aqueous Paracetamol N-Acetyl-D-L-methionate (20 $\mu g.ml^{-1}$, pH 7.4) at 0, 20 and 37°C

Results expressed as the mean appearance of paracetamol (n = 2)

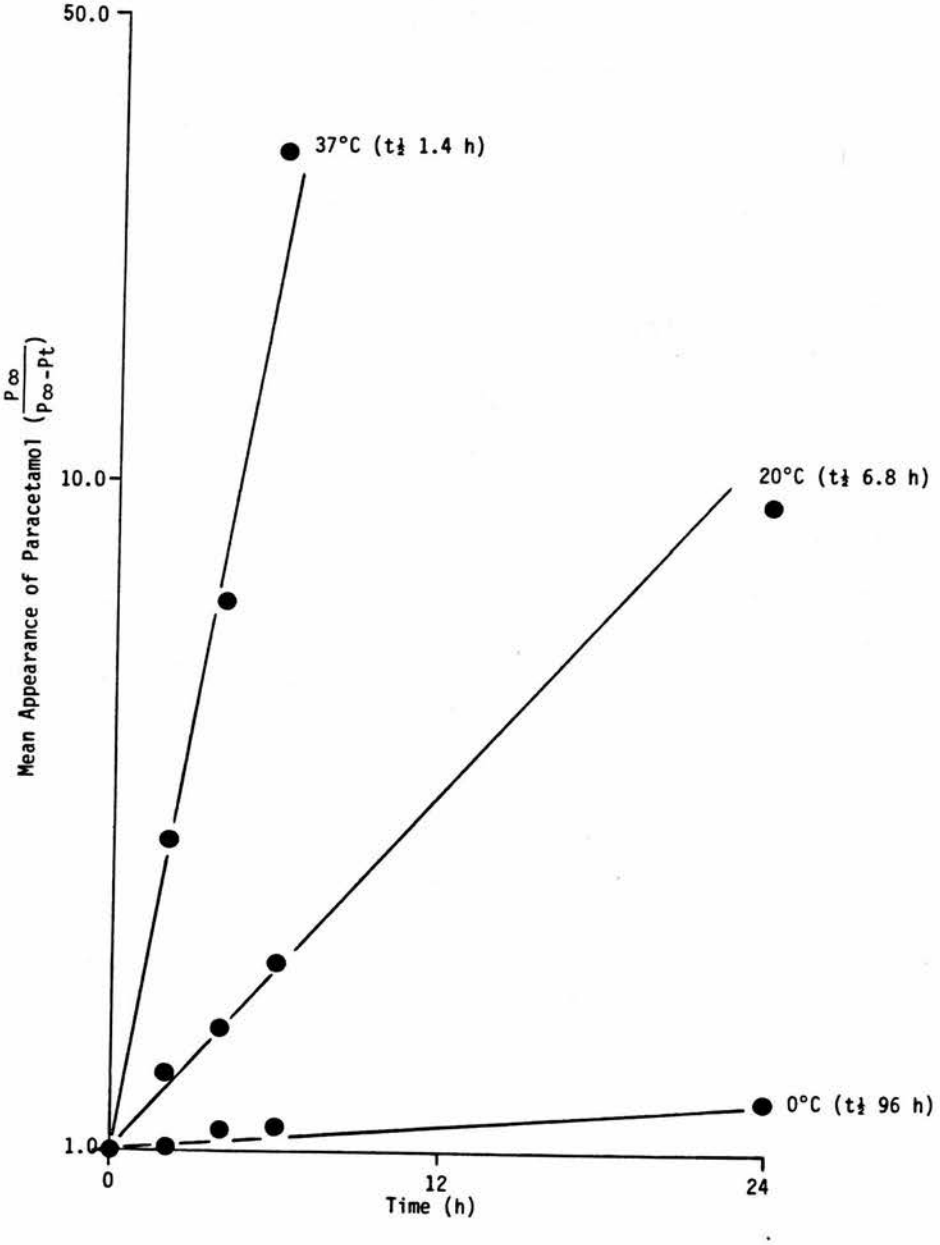


TABLE 2.22 Stability of Aqueous Paracetamol N-acetyl-D-L-methionate (20 $\mu g.ml^{-1}$, pH 8.0) at 0, 20 and 37°C

Time (h)	Temperature (0°C)	1			2			Mean	
		Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)	Total Accounted for ($\mu g.ml^{-1}$)*	Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)	Total Accounted for ($\mu g.ml^{-1}$)*	Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)	Paracetamol Concentration ($\mu g.ml^{-1}$)
0	0	19.3	ND	19.3	18.7	ND	18.7	19.0	ND
	20	18.8	ND	18.8	18.2	ND	18.2	18.5	ND
	37	18.7	ND	18.7	18.3	ND	18.3	18.5	ND
1	0	17.2	1.2	19.8	17.0	1.4	20.0	17.1	1.3
	20	12.3	3.7	20.2	12.1	3.7	20.0	12.2	3.7
	37	6.3	6.8	20.9	6.1	6.7	20.5	6.2	6.8
2	0	14.8	2.3	19.7	14.6	2.6	20.2	14.7	2.5
	20	7.3	6.3	20.8	7.4	6.4	21.4	7.4	6.4
	37	1.8	9.7	22.6	1.8	9.6	22.4	1.8	9.7
4	0	11.9	3.8	20.1	11.6	3.8	19.8	11.8	3.8
	20	2.7	8.8	21.6	2.8	8.6	21.3	2.8	8.7

ND = Not detected * = as paracetamol N-acetyl-D-L-methionate

FIGURE 2.26 Stability of Aqueous Paracetamol N-Acetyl-D-L-methionate (20 $\mu g.ml^{-1}$, pH 8.0) at 0, 20 and 37°C

Results expressed as the mean (n = 2) paracetamol N-acetyl-D-L-methionate concentration ($\mu g.ml^{-1}$)

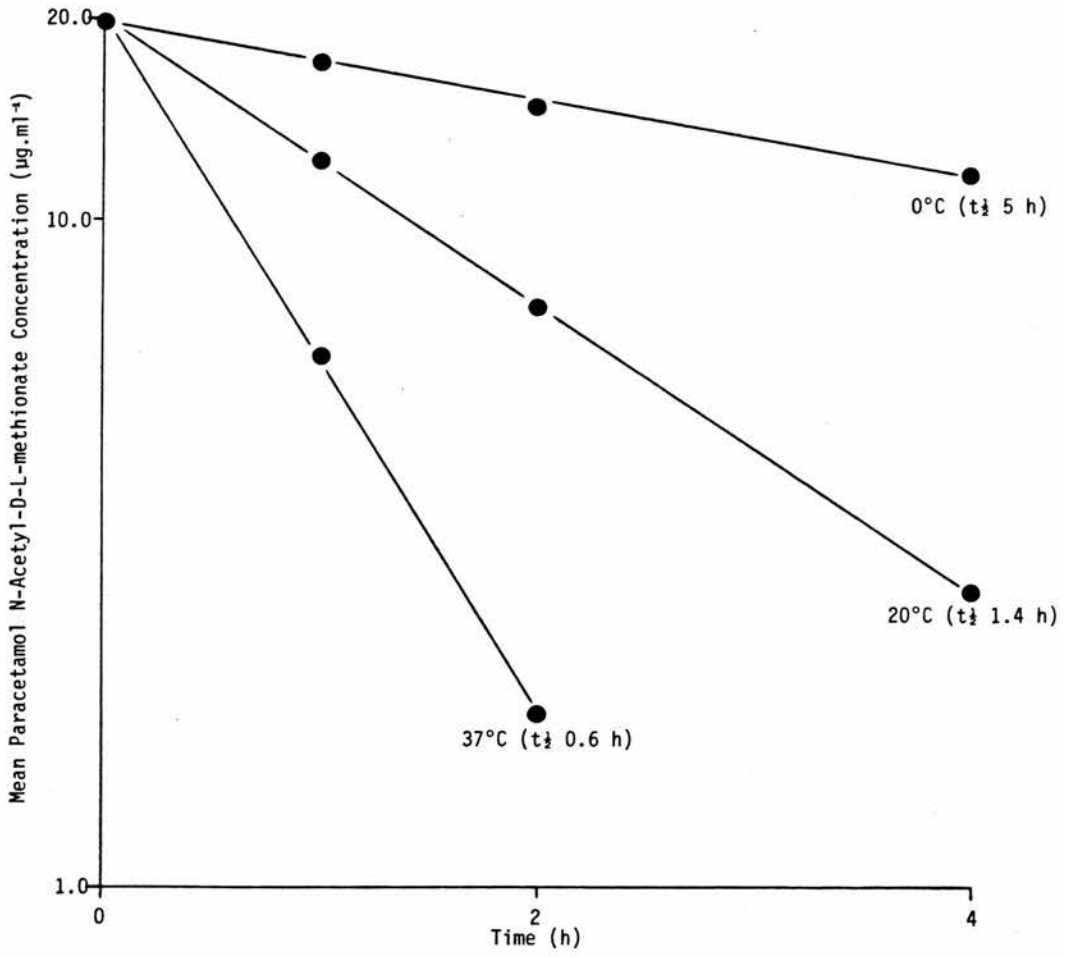
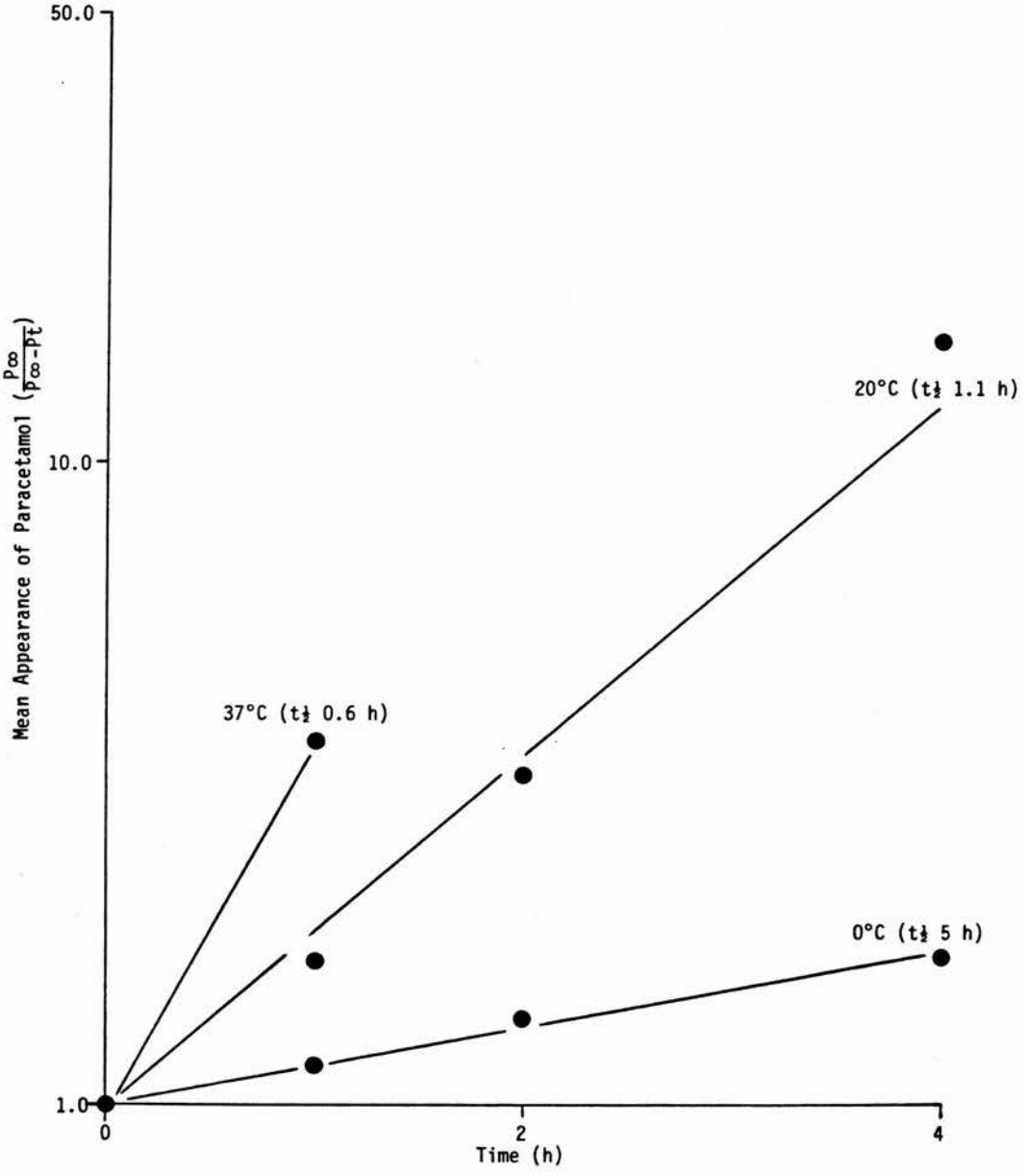


FIGURE 2.27 Stability of Aqueous Paracetamol N-Acetyl-D-L-methionate (20 $\mu g.ml^{-1}$, pH 8.0) at 0, 20 and 37°C

Results expressed as the mean appearance of paracetamol (n = 2)



Hydrolysis of Paracetamol N-acetyl-D-L-methionate in Plasma at 37°C

Four freshly prepared plasma samples containing 20 $\mu g.ml^{-1}$ paracetamol N-acetyl-D-L-methionate were incubated at 37°C. Aliquots were taken for the simultaneous estimation of paracetamol N-acetyl-D-L-methionate and paracetamol at 0, 15, 30, 45 and 60 min. The experiment was repeated 4 times.

The results are shown in Table 2.23 (i-iii) and Figures 2.28 and 2.29. The semilog plot of mean paracetamol N-acetyl-D-L-methionate concentration against time was linear and the mean half life was 24 min. Similar results were obtained from the semilog plot of the rate of appearance of paracetamol. All the paracetamol N-acetyl-D-L-methionate was accounted for in each sample and there was good agreement between replicate samples.

Hydrolysis of Paracetamol N-acetyl-D-L-methionate in Whole Blood at 37°C

Fresh whole blood taken into lithium-heparin tubes and containing 20 $\mu g.ml^{-1}$ paracetamol N-acetyl-D-L-methionate was incubated at 37°C. Three samples were prepared and analysed at intervals of 1, 2, 3, 4 and 5 min. Paracetamol N-acetyl-D-L-methionate was estimated by adapting the 'Bond Elut' extraction method (Page 91).

With whole blood the 'Bond Elut' columns were susceptible to blockage and sample clean up was inferior. However, the peaks were free from

TABLE 2.23(i) Stability of Paracetamol N-acetyl-D-L-methionate (20 µg.ml^{-1}) in Plasma at 37°C - Paracetamol N-acetyl-D-L-methionate Estimation

Time (mins)	Paracetamol N-acetyl-D-L-methionate Concentration (µg.ml^{-1})				Mean ± SD
	1	2	3	4	
0	18.9	19.8	20.0	18.2	19.2 ± 0.8
15	13.8	13.5	12.6	12.2	13.0 ± 0.8
30	8.2	9.4	8.0	8.4	8.5 ± 0.6
45	5.7	6.1	4.9	5.0	5.4 ± 0.6
60	3.4	4.3	2.5	3.1	3.3 ± 0.8

TABLE 2.23(ii) Stability of Paracetamol N-acetyl-D-L-methionate (20 µg.ml^{-1}) in Plasma at 37°C - Paracetamol Estimation

Time (mins)	Paracetamol Concentration (µg.ml^{-1})				Mean ± SD
	1	2	3	4	
0	ND	ND	ND	0.7	ND
15	2.7	3.1	3.4	3.3	3.1 ± 0.3
30	5.2	4.6	5.8	5.6	5.3 ± 0.5
45	7.1	5.7	7.2	7.1	6.8 ± 0.5
60	8.0	7.0	8.0	8.0	7.8 ± 0.5

ND = Not detected

TABLE 2.23(iii) Stability of Paracetamol N-acetyl-D-L-methionate (20 $\mu g.ml^{-1}$) in Plasma at 37°C - Total Accounted for as Paracetamol-N-acetyl-D-L-methionate

Time (mins)	Total Accounted for as Paracetamol N-acetyl-D-L-methionate ($\mu g.ml^{-1}$)				Mean ± SD
	1	2	3	4	
0	18.9	19.8	20.0	19.7	19.6 ± 0.5
15	19.6	20.2	19.9	19.3	19.8 ± 0.4
30	19.4	19.3	20.5	20.4	19.9 ± 0.6
45	21.0	18.4	20.4	20.3	20.0 ± 1.1
60	20.6	19.4	19.7	20.3	20.0 ± 0.5

FIGURE 2.28 Stability of Paracetamol N-Acetyl-D-L-methionate (20 µg.ml^{-1}) in Plasma at 37°C

Results expressed as the mean (n = 4) paracetamol N-acetyl-D-L-methionate concentration (µg.ml^{-1})

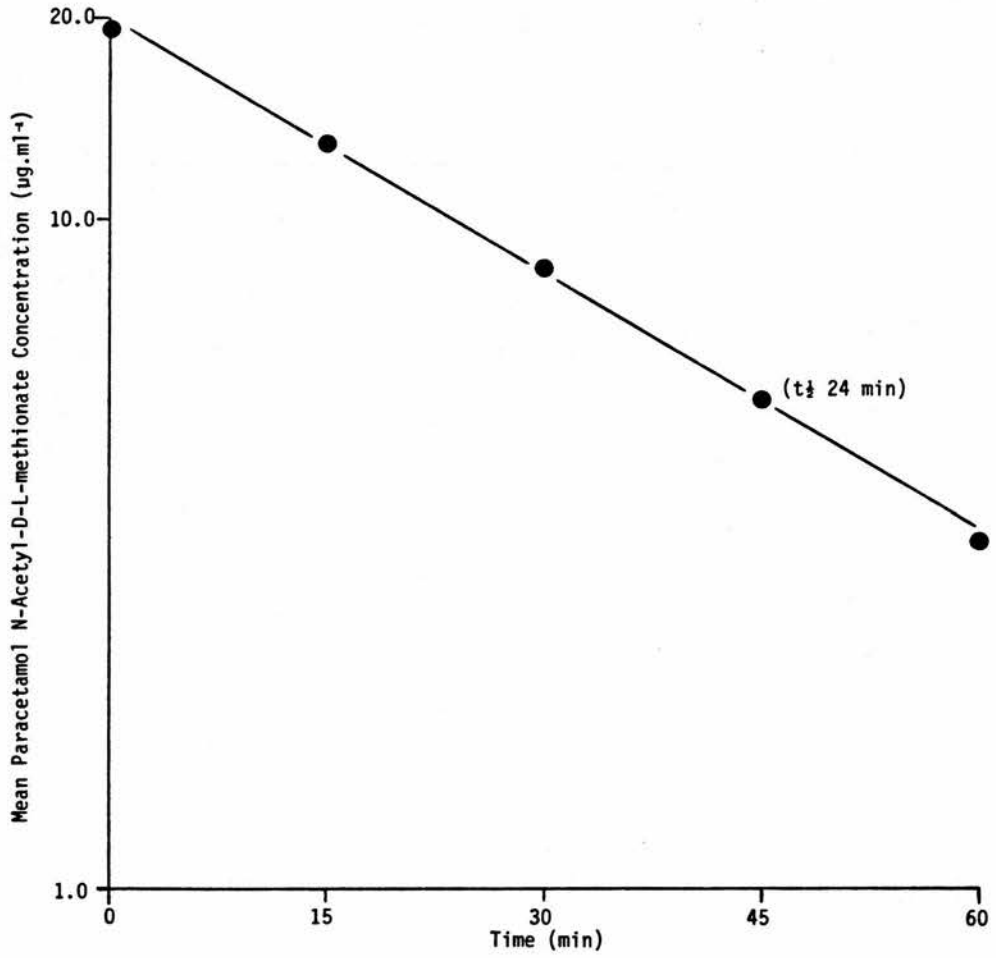
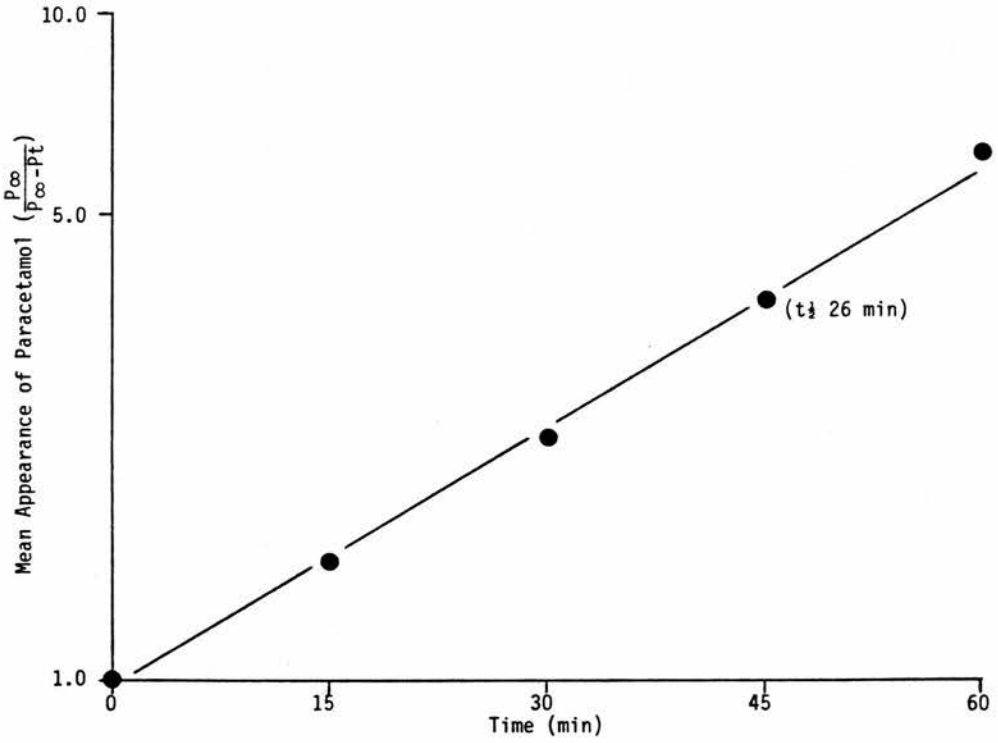


FIGURE 2.29 Stability of Paracetamol N-Acetyl-D-L-methionate (20 µg.ml^{-1}) in Plasma at 37°C

Results expressed as the mean appearance of paracetamol (n = 4)



interference and the recovery of acet-p-toluidide was greater than 80%. Recovery of paracetamol N-acetyl-D-L-methionate was always slightly less probably due to hydrolysis. This method was used to provide an indication of the rate of paracetamol N-acetyl-D-L-methionate hydrolysis in whole blood at 37°C. Paracetamol was not estimated.

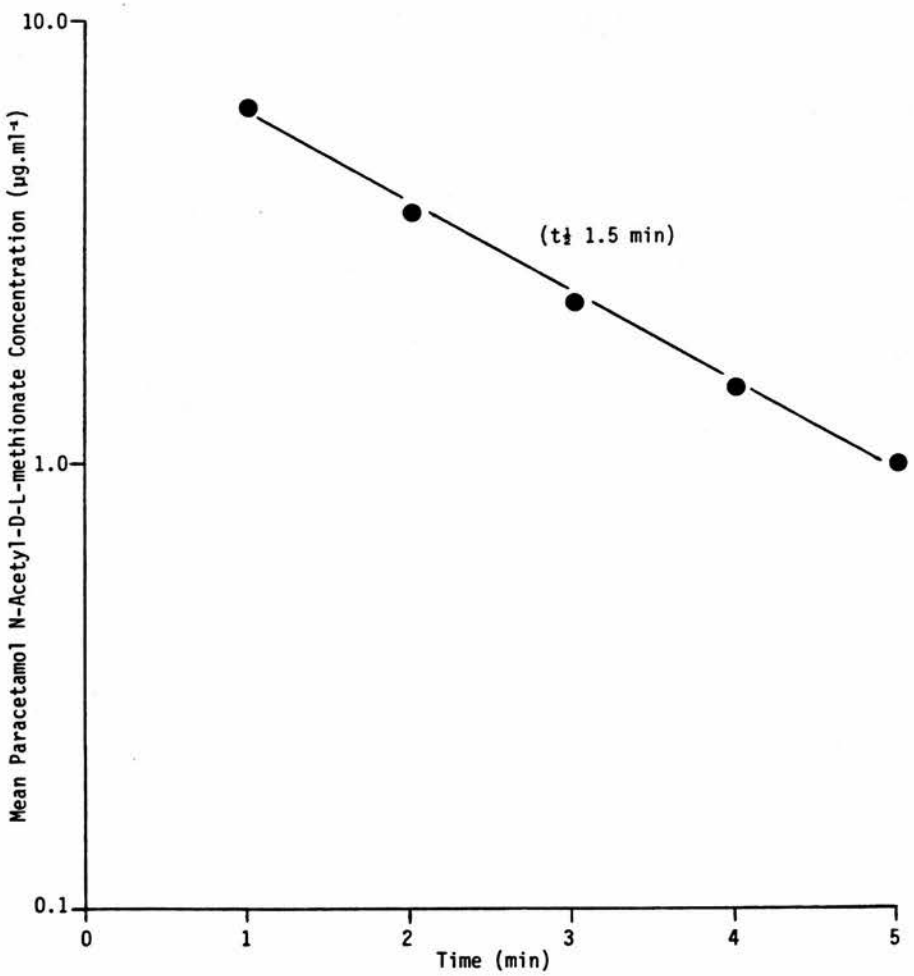
The results are shown in Table 2.24 and Figure 2.30. The semilog plot of mean paracetamol N-acetyl-D-L-methionate concentration against time was linear with a half life of 1.5 min.

TABLE 2.24 Stability of Paracetamol N-acetyl-D-L-methionate (20 $\mu g.ml^{-1}$) in Whole Blood at 37°C

Time (mins)	Paracetamol N-acetyl-D-L-methionate Concentration ($\mu g.ml^{-1}$)			Mean
	1	2	3	
1	6.1	6.3	6.5	6.3
2	4.3	3.8	3.1	3.7
3	2.2	2.3	2.3	2.3
4	-	1.5	1.5	1.5
5	-	1.1	0.8	1.0

FIGURE 2.30 Stability of Paracetamol N-Acetyl-D-L-methionate (20 $\mu g.ml^{-1}$) in Whole Blood at 37°C

Results expressed as mean (n = 3) paracetamol N-acetyl-D-L-methionate concentration ($\mu g.ml^{-1}$)



c) Discussion and Summary

The stability of aqueous paracetamol N-acetyl-D-L-methionate (20 μg. ml^{-1}) at different temperatures and pH was investigated. Stability was assessed by the simultaneous estimation of the disappearance of paracetamol N-acetyl-D-L-methionate and the appearance of paracetamol by HPLC. All the paracetamol N-acetyl-D-L-methionate could be accounted for as the parent compound and free paracetamol. The process obeyed first order kinetics and the rate of hydrolysis was accelerated by increased pH and temperature.

Similarly, the stability of paracetamol N-acetyl-D-L-methionate (20 $\mu g.ml^{-1}$) in plasma and whole blood at 37°C was investigated. The rate of hydrolysis in these biological fluids was much greater, especially in whole blood.

The results are summarised in Table 2.25.

TABLE 2.25 Summary of Results

Paracetamol N-acetyl-D-L-methionate (20 $\mu g.ml^{-1}$) Stability in Aqueous Solutions

Temperature (0°C)	Half Life (h)			
	pH 4.0	pH 6.1	pH 7.4	pH 8.0
0	>100	>100	95	5
20	>100	>100	6.2	1.4
37	72	40	1.3	0.6

Paracetamol N-acetyl-D-L-methionate (20 $\mu g.ml^{-1}$) Stability in Plasma and Whole Blood

Sample	Temperature (°C)	pH	Half Life (mins)
Plasma	37	7.4	24
Whole Blood	37	7.4	1.5

CHAPTER III

Paracetamol Disposition in Healthy Male Subjects

PARACETAMOL DISPOSITION IN HEALTHY MALE SUBJECTS

a) Introduction

Paracetamol metabolism in healthy subjects has been reported previously (Davis et al, 1976; Adrienssens, 1980; Prescott, 1980; Forrest et al, 1982; Critchley et al, 1986). In order to provide appropriate control data further studies were carried out in healthy male subjects.

b) Methods

Subjects

Ten healthy males with a mean age of 28 ± 6 years, body weight 72 ± 11 kg and height 174 ± 6 cm were entered into the study. Each was given a physical examination and screening tests for plasma biochemistry, haematology were performed together with urinalysis. The results in all subjects were normal and each subject had a creatinine clearance value greater than 100 $ml.min^{-1}$.

Individual details of age, body weight and height are shown in Table 3.1 and mean plasma biochemistry and haematology screen data are shown in Tables 3.2 and 3.3.

No other drugs were taken for one week and no alcohol for 3 days prior to and during the study. None of the subjects regularly consumed excessive quantities of alcohol and one subject smoked.

TABLE 3.1 Details of the Healthy Male Subjects

Subject Number	Age (Years)	Body Weight (kg)	Height (cm)
1	30	66.8	171
2	27	56.8	165
3	25	73.2	177
4	35	77.1	175
5	20	64.2	171
6	29	66.6	168
7	32	88.0	179
8	39	87.0	180
9	23	77.8	181
10	23	60.9	170
Mean $\pm$ SD	28.3 $\pm$ 5.9	71.8 $\pm$ 10.6	173.7 $\pm$ 5.5

TABLE 3.2 Plasma Biochemistry Screen in 10 Healthy Male Subjects

	Mean	Range	Normal Range+
Total Protein ($g.litre^{-1}$)	71	66-77	60-80
Albumin ($g.litre^{-1}$)	45	42-48	36-47
Total Bilirubin ($\mu mol.litre^{-1}$)	11	7-25	2-17
Alkaline Phosphatase ($U.litre^{-1}$)	67	48-94	40-100
ALT ($U.litre^{-1}$)	20	<10-26	10-40
Gamma GT ($U.litre^{-1}$)	16	9-27	10-55
Fasting Glucose ($mmol.litre^{-1}$)	4.9	4.0-6.8	3.6-5.8
Sodium ($mmol.litre^{-1}$)	139	136-143	132-144
Potassium ($mmol.litre^{-1}$)	3.6	3.5-3.8	3.3-4.7
Calcium ($mmol.litre^{-1}$)	2.33	2.21-2.44	2.12-2.62
Bicarbonate ($mmol.litre^{-1}$)	25	23-27	24-30
Creatinine ($\mu mol.litre^{-1}$)	93	82-137	55-150
Urea ($mmol.litre^{-1}$)	4.8	3.4-5.6	2.5-6.6

+ = Department of Clinical Chemistry, Royal Infirmary, Edinburgh

TABLE 3.3 Haematology Screen in 10 Healthy Male Subjects

	Mean	Range	Normal Range+
Haemoglobin ($g.dl^{-1}$)	15.1	13.2-16.9	13.5-18.0
Haematocrit	0.44	0.38-0.49	0.40-0.54
RBC ($x\ 10^{12}.litre^{-1}$)	4.8	4.1-5.1	4.5-6.5
WBC $x\ 10^{9}.litre^{-1}$)	6.6	4.9-10.2	4.0-11.0
Neutrophils (%)	65	52-77	40-75
Lymphocytes (%)	29	19-38	20-45
Monocytes (%)	4	2-8	2-10
Eosinophils (%)	2	0-5	1-6
Basophils (%)	0	0-0	<1
MCHC ($g.dl^{-1}$)	32	32-36	30-35
MCH (pg)	31	30-33	27-32
MCV (fl)	92	87-96	76-96
Platelets ($x\ 10^{9}.litre^{-1}$)	243	167-392	150-400
ESR ($mm.h^{-1}$)	4	1-17	0-10

+ = South Lothian Districs Haematology Departments

Informed consent was given by each subject and the study was approved by the local Ethics Committee.

Drug Administration and Sampling

Subjects attended the University Department of Clinical Pharmacology Patient Investigation Room in the morning after fasting overnight and an in-dwelling cannula was inserted into a forearm vein. Sachets containing 1 g of an effervescent formulation of paracetamol were supplied by Sterling Winthrop, Alnwick. Each subject received the contents of one sachet orally in 200 ml of water. The subjects then remained recumbent for 3 h and lunch and supper were allowed at 4 and 8 h respectively. They received 200 ml of water every 2 h up to 12 h and, thereafter, fluid and food intake were unrestricted.

10 ml venous blood samples were taken into heparinised tubes at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 24 h after dosing. The blood was centrifuged at 1400 x g for 10 min and the plasma separated and stored at -20°C prior to analysis. Urine samples were collected immediately before drug administration and from 0-2, 2-4, 4-6, 6-8, 8-10, 10-12 and 12-24 h after dosing. The total urine volume and pH were recorded and 50 ml aliquots were stored at -20°C prior to analysis. Individual urine volumes and pH values are shown in Appendix 3.9.

Drug Analysis

Plasma concentrations of paracetamol, paracetamol sulphate and paracetamol glucuronide, and urine concentrations of paracetamol, paracetamol sulphate, paracetamol glucuronide, paracetamol cysteine and paracetamol mercapturic acid were measured by HPLC as described in Chapter II, Section 1. Metabolite concentrations are expressed as paracetamol equivalents.

Data Analysis

i) Plasma Concentrations of Paracetamol and its Metabolites

Mean plasma concentrations of paracetamol and its sulphate and glucuronide conjugates were plotted on a semilogarithmic graph against time and individual peak plasma concentrations (Cmax) and time to reach peak plasma concentration (Tmax) determined.

ii) Plasma Elimination Half Life

After absorption and distribution are complete the elimination of paracetamol is a first order process (Nelson and Moriska, 1963). The plasma concentration (Cp) at any time (t) can be determined from the following equation which describes a first order elimination process:

$$Cp = Co e^{-kel.t}$$

where : Co = plasma concentration at zero time

or

$$\ln Cp = \ln Co - kel.t$$

kel = elimination rate constant

or

$$\log Cp = \log Co \frac{-kel.t}{2.303}$$

Therefore, a semilogarithmic plot of plasma concentration against time exhibits a linear elimination phase with the slope = $\frac{-kel}{2.303}$ As the half life is defined as the time taken for the concentration of drug in plasma to decline to half its original value, the above equation can be rearranged to determine the elimination half life ($t_{\frac{1}{2}}$):

$$\log \left(\frac{Cp}{Co}\right) = \frac{-kel.t}{2.303}$$

when $\log \left(\frac{Cp}{Co}\right) = 0.5$ and $t = t_{\frac{1}{2}}$

then $t_{\frac{1}{2}} = \frac{\log 0.5}{\frac{-kel}{2.303}} = \frac{0.301}{Slope}$

The plasma elimination half life for paracetamol and the apparent plasma elimination half life for paracetamol sulphate and paracetamol glucuronide were determined for each subject as described above. The slope was determined by direct linear regression from semilogarithmic plots of plasma concentration against time, over the periods 2-8 h for paracetamol, 3-8 h for paracetamol sulphate and 5-8 h for paracetamol glucuronide. Formal model dependent curve fitting and compartmental analysis was not performed.

The plasma paracetamol half life from 8-24 h was also determined as described above.

iii) Recovery of Paracetamol and its Metabolites in Urine

The percentage of the dose recovered in urine in 24 h as paracetamol and its sulphate, glucuronide, cysteine and mercapturic acid conjugates was determined and the mean cummulative urinary recovery of paracetamol and its metabolites plotted against time. The fractional urinary recovery of paracetamol and its metabolites was also determined in divided urine collections.

iv) Urinary Excretion

The urinary excretion rate ($mg.h^{-1}$) of paracetamol and its metabolites was determined for each of the divided urine collections up to 12 h. The mean urinary excretion rate was plotted on a semilogarithmic graph against time where the time taken was the midpoint of each urine collection.

Plots of the log of urinary excretion rate of paracetamol and its metabolites against time eventually become linear (Cummings et al, 1967). Therefore, the urinary elimination half life for paracetamol and the apparent urinary elimination half life for its conjugates were determined from the slopes of the linear elimination phase as described in (ii). The slope was determined by linear regression using data points from 0-8 h for paracetamol, 2-12 h for paracetamol

sulphate and glucuronide and 4-12 h for paracetamol cysteine and mercapturic acid.

v) Renal Clearance

The renal clearance is defined as the volume of plasma completely cleared of drug as it passes through the kidney in unit time. The renal clearance of paracetamol and its glucuronide and sulphate conjugates was calculated using the following equation:

$$\text{Renal Clearance} = \frac{\text{Amount excreted in urine}}{\text{Area under the plasma concentration time curve over the same time period}}$$

The area under the plasma concentration time curve was calculated by the trapezoidal method. The renal clearances of paracetamol and its sulphate and glucuronide conjugates were determined over the period 0-8 h after dosing.

vi) Statistical Analysis

Mean values $\pm$ standard deviation (SD) are presented. The relationship between quantitative observations on each subject (bivariate data) was determined by calculation of the correlation coefficient (r). The correlation coefficient was tested for statistical significance using tabulated values (Geigy Scientific Tables, page 61).

The Student t-test for paired data was used to test for statistical significance.

c) Results

i) Plasma Concentrations of Paracetamol and its Sulphate and Glucuronide Conjugates

Mean plasma concentrations of paracetamol and its sulphate and glucuronide conjugates following oral administration of 1 g of paracetamol to 10 healthy male subjects are shown in Table 3.4 and Figure 3.1. Plasma concentrations of the cysteine and mercapturic acid conjugates of paracetamol were below the limit of detection. Individual values for peak concentration (Cmax), time to reach peak concentration (Tmax) and apparent elimination half life are shown in Tables 3.5 and 3.6 respectively. Plasma concentrations for each subject are shown in Appendices 3.1-3.3

Paracetamol

Paracetamol absorption was rapid with a mean individual peak plasma paracetamol concentration of 20.0 $\pm$ 8.4 $\mu g.ml^{-1}$ (range 12.9-40.7) occurring at 0.35 $\pm$ 0.17 h (range 0.25-0.75) after administration. In most subjects the peak plasma paracetamol concentration occurred at or before the time of the first sample at 15 min. Following distribution, plasma paracetamol concentrations declined in a linear fashion from 2-8 h when plotted against time on a semilogarithmic

TABLE 3.4 Mean Plasma Concentrations of Paracetamol and its Sulphate and Glucuronide Conjugates in 10 Healthy Male Subjects Following Ingestion of 1 g of Paracetamol

Time After Ingestion (h)	Plasma Concentration ($\mu g.ml^{-1}$)		
	Paracetamol (Mean ± SD)	Paracetamol Sulphate (Mean ± SD)	Paracetamol Glucuronide (Mean ± SD)
0.25	18.7 ± 9.3	2.0 ± 0.6	1.7 ± 0.7
0.5	13.8 ± 3.2	3.0 ± 0.6	4.2 ± 1.5
0.75	11.0 ± 1.9	3.3 ± 0.7	6.4 ± 2.0
1.0	9.7 ± 1.7	3.4 ± 0.8	7.6 ± 2.3
1.5	8.1 ± 1.5	3.3 ± 0.7	9.0 ± 2.7
2.0	6.5 ± 1.4	3.0 ± 0.6	9.2 ± 2.3
3.0	5.0 ± 1.2	2.7 ± 0.6	9.1 ± 2.5
4.0	3.6 ± 1.0	2.1 ± 0.5	7.6 ± 2.3
5.0	2.6 ± 0.8	1.8 ± 0.4	6.0 ± 1.7
6.0	1.9 ± 0.7	1.4 ± 0.3	4.9 ± 1.3
7.0	1.4 ± 0.6	1.1 ± 0.2	3.8 ± 1.2
8.0	1.1 ± 0.5	0.9 ± 0.2	2.9 ± 0.7
9.0	0.9 ± 0.5	0.8 ± 0.2	2.3 ± 0.4
10.0	0.7 ± 0.3	0.5 ± 0.3	1.9 ± 0.5
12.0	0.5 ± 0.3	0.4 ± 0.4	1.1 ± 0.3+
24.0	0.1 ± 0.2	0.1 ± 0.2	0.3 ± 0.3

+ = Mean of 9

FIGURE 3.1 Mean Plasma Concentrations of Paracetamol and its Sulphate and Glucuronide Conjugates in 10 Healthy Male Subjects Following Ingestion of 1 g of Paracetamol

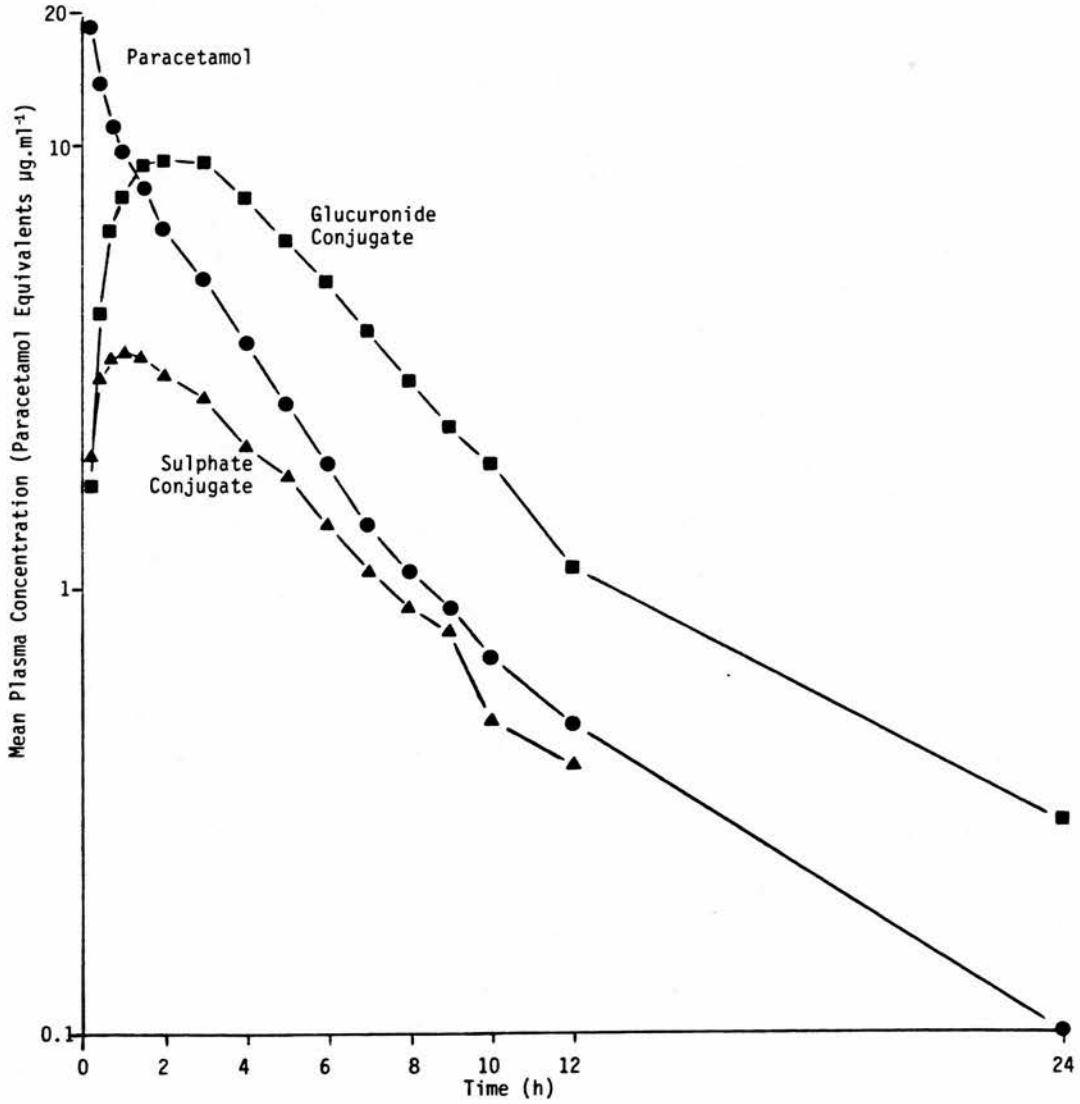


TABLE 3.5 Peak Plasma Concentrations (Cmax) and Time to Reach Peak Plasma Concentrations (Tmax) of Paracetamol and its Sulphate and Glucuronide Conjugates in 10 Healthy Male Subjects Following Ingestion of 1 g of Paracetamol

Subject Number	Paracetamol		Paracetamol Sulphate		Paracetamol Glucuronide	
	Cmax ($\mu g.ml^{-1}$)	Tmax (h)	Cmax ($\mu g.ml^{-1}$)	Tmax (h)	Cmax ($\mu g.ml^{-1}$)	Tmax (h)
1	14.9	0.25	2.1	1.50	7.3	1.50
2	22.8	0.25	3.2	0.50	9.8	1.50
3	20.3	0.25	3.5	0.50	11.1	2.00
4	12.9	0.75	4.0	2.00	5.8	2.00
5	40.7	0.25	3.1	1.50	13.3	3.00
6	24.7	0.25	4.2	1.00	9.9	2.00
7	15.2	0.25	4.0	1.00	5.8	2.00
8	15.1	0.25	4.5	0.75	13.0	1.50
9	12.9	0.50	3.1	0.50	9.3	2.00
10	20.7	0.50	3.4	1.50	9.0	2.00
Mean $\pm$ SD	20.0 $\pm$ 8.4	0.35 $\pm$ 0.17	3.5 $\pm$ 0.7	1.08 $\pm$ 0.53	9.4 $\pm$ 2.6	2.00 $\pm$ 0.44

TABLE 3.6 Apparent Plasma Elimination Half Life of Paracetamol and Its Sulphate and Glucuronide Conjugates In 10 Healthy Male Subjects Following Ingestion of 1 g of Paracetamol

Subject Number	Apparent Plasma Elimination Half Life (h)		
	Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide
1	2.7	3.5	2.8
2	2.6	3.4	3.0
3	2.2	2.8	2.8
4	2.7	2.9	3.2
5	2.2	3.1	2.4
6	2.2	3.1	2.9
7	2.0	3.2	3.3
8	1.9	2.9	3.0
9	1.9	2.8	2.7
10	2.5	3.9	3.4
Mean ± SD	2.3 ± 0.3	3.2 ± 0.4	3.0 ± 0.3

Half life calculated over: 2-8 h for paracetamol
3-8 h for paracetamol sulphate
5-12 h for paracetamol glucuronide

graph. The mean elimination half life over this period was 2.3 ± 0.3 h (range 1.9-2.7). From 8-24 h the paracetamol disappeared more slowly with a mean half life of 5.4 ± 2.4 h. Little or no paracetamol was detected after 24 h.

Paracetamol Sulphate

Paracetamol sulphate was detected in plasma at 15 min and the mean individual peak concentration was 3.5 ± 0.7 $\mu g.ml^{-1}$ (range 2.1-4.5) at 1.08 ± 0.53 h (range 0.5-2.0). The mean concentrations of sulphate conjugate were lower than those of the parent drug at all times and the mean half life from 3-8 h was 3.2 ± 0.4 h (range 2.8-3.9). Little or no paracetamol sulphate was detected after 24 h.

Paracetamol Glucuronide

Plasma paracetamol glucuronide concentrations rose steadily after 15 min, exceeding the plasma concentrations of paracetamol in most subjects after 1 to 2 h. The mean individual peak concentration was 9.4 ± 2.6 $\mu g.ml^{-1}$ (range 5.8-13.3) at 2.00 ± 0.44 h (range 1.5-3.0) and the mean half life from 5-12 h was 3.0 ± 0.3 h (range 2.4-3.4). The mean individual time to reach peak paracetamol glucuronide concentration differed significantly from that of the sulphate conjugate ($t = 4.869$, $P<0.001$). After 24 h only trace amounts of the glucuronide conjugate were detected in plasma in 6 of the 10 subjects.

ii) Urine Recovery of Paracetamol and its Sulphate, Glucuronide Cysteine and Mercapturic Acid Conjugates

The percentages of the dose recovered in the urine in 24 h as paracetamol and its metabolites are shown in Table 3.7 and the mean cumulative urinary recoveries in Figure 3.2. Individual urinary excretion data are shown in Appendices 3.4-3.8.

Most of the administered dose was recovered in urine as the sulphate and glucuronide conjugates of paracetamol. In all subjects the 24 h urinary recovery of the glucuronide conjugate was greater than that of the sulphate conjugate. The mean 24 h urinary recovery of paracetamol sulphate and paracetamol glucuronide was 23.7 $\pm$ 4.5 (range 18.1-31.2) and 49.8 $\pm$ 6.8% of the dose (range 39.2-63.7) respectively. Approximately half of the total glucuronide and sulphate conjugates recovered was excreted within 4 h.

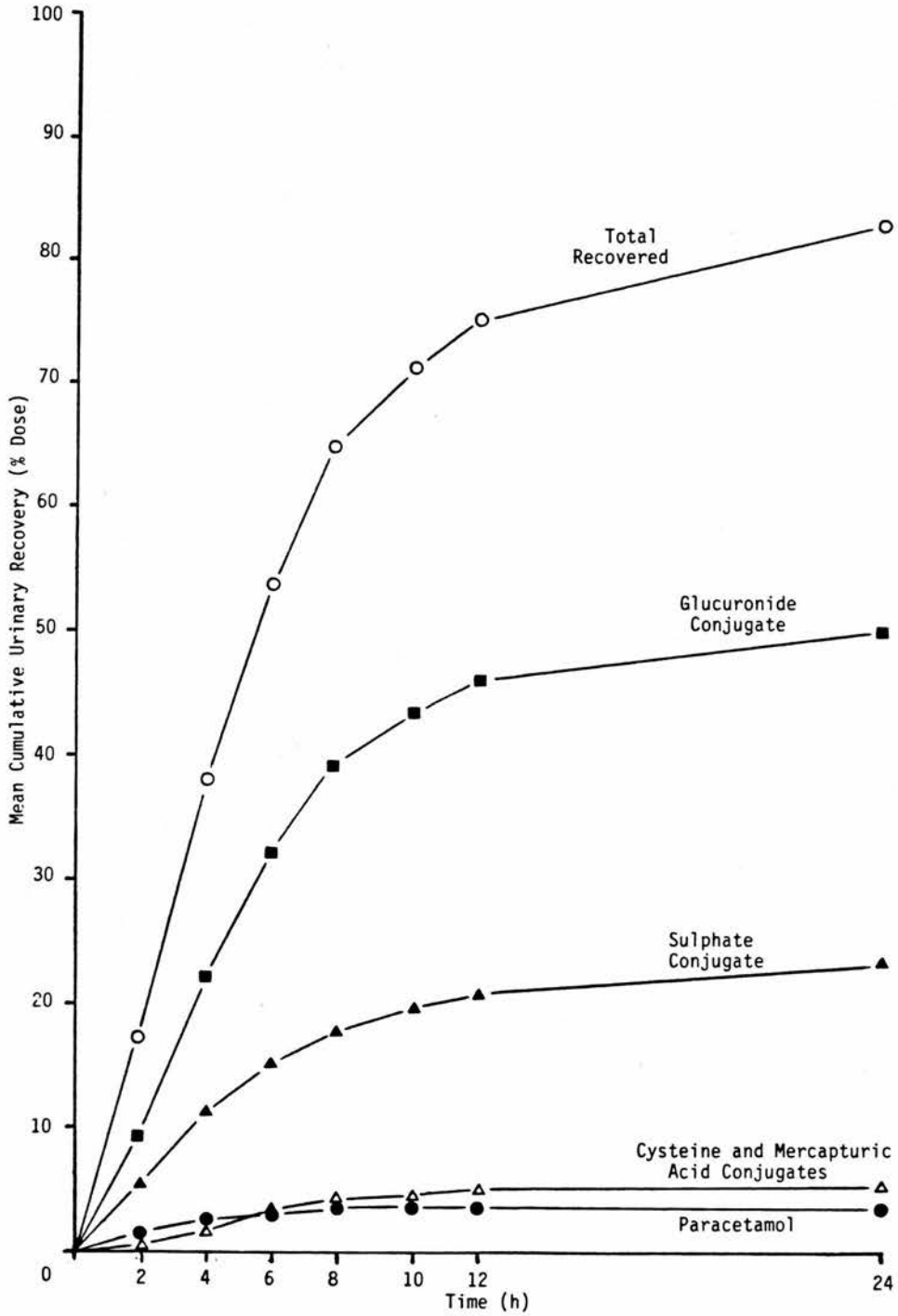
The mean 24 h urinary recovery of paracetamol was 3.6 $\pm$ 1.5% of the dose (range 1.5-6.0), of which approximately half was recovered after 2 h.

After 24 h, 5.6 $\pm$ 2.5% of the dose was recovered as the cysteine and mercapturic acid conjugates of paracetamol. The cysteine and mercapturic acid conjugates combined represented 1.8 $\pm$ 0.8 after 4 h and 4.2 $\pm$ 1.7% of the dose after 8 h. More mercapturic acid than cysteine conjugate was recovered in all subjects. The mean 24 h

TABLE 3.7 Percentage of Dose Recovered in Urine as Paracetamol and its Sulphate, Glucuronide, Cysteine and Mercapturic Acid Conjugates in 24 h Following Ingestion of 1 g of Paracetamol in 10 Healthy Male Subjects

Subject Number	Percentage of Dose Recovered in Urine Over 24 h						
	Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide	Paracetamol Cysteine	Paracetamol Mercapturic Acid	Cysteine + Mercapturic Acid	Total
1	6.0	26.1	47.8	3.1	4.8	7.9	87.8
2	1.5	18.7	51.2	2.1	3.3	5.4	76.8
3	3.2	18.5	50.0	1.9	2.0	3.9	75.3
4	4.6	31.2	39.2	4.4	5.1	9.5	84.5
5	2.7	18.1	54.0	2.1	3.0	5.1	79.9
6	2.7	23.6	53.4	1.3	2.3	3.6	83.4
7	3.9	29.7	42.6	3.7	5.6	9.3	85.5
8	1.6	23.9	50.3	0.8	1.5	2.3	78.1
9	5.6	22.4	63.7	1.0	3.4	4.4	96.1
10	3.7	24.3	45.7	2.1	2.8	4.9	78.6
Mean ± SD	3.6 ± 1.5	23.7 ± 4.5	49.8 ± 6.8	2.3 ± 1.2	3.4 ± 1.4	5.6 ± 2.5	82.6 ± 6.3

FIGURE 3.2 Mean Cumulative Urinary Recovery of Paracetamol and its Metabolites up to 24 h in 10 Healthy Male Subjects Following Ingestion of 1 g of Paracetamol



urinary recoveries of paracetamol cysteine and paracetamol mercapturic acid were 2.3 ± 1.2 (range 0.8-4.4) and 3.4 ± 1.2% of the dose (range 1.5-5.6) respectively.

The mean total amounts recovered in urine after 6, 12 and 24 h were 53.9%, 75.9% and 82.6% of the dose respectively.

iii) Fractional Excretion of Paracetamol and its Sulphate, Glucuronide, Cysteine and Mercapturic Acid Conjugates

The mean percentages of total drug excreted in divided urine collections as paracetamol and its metabolites following ingestion of 1 g of paracetamol in 10 healthy male subjects are shown in Table 3.8 and Figure 3.3. Individual data are shown in Appendices 3.4-3.8.

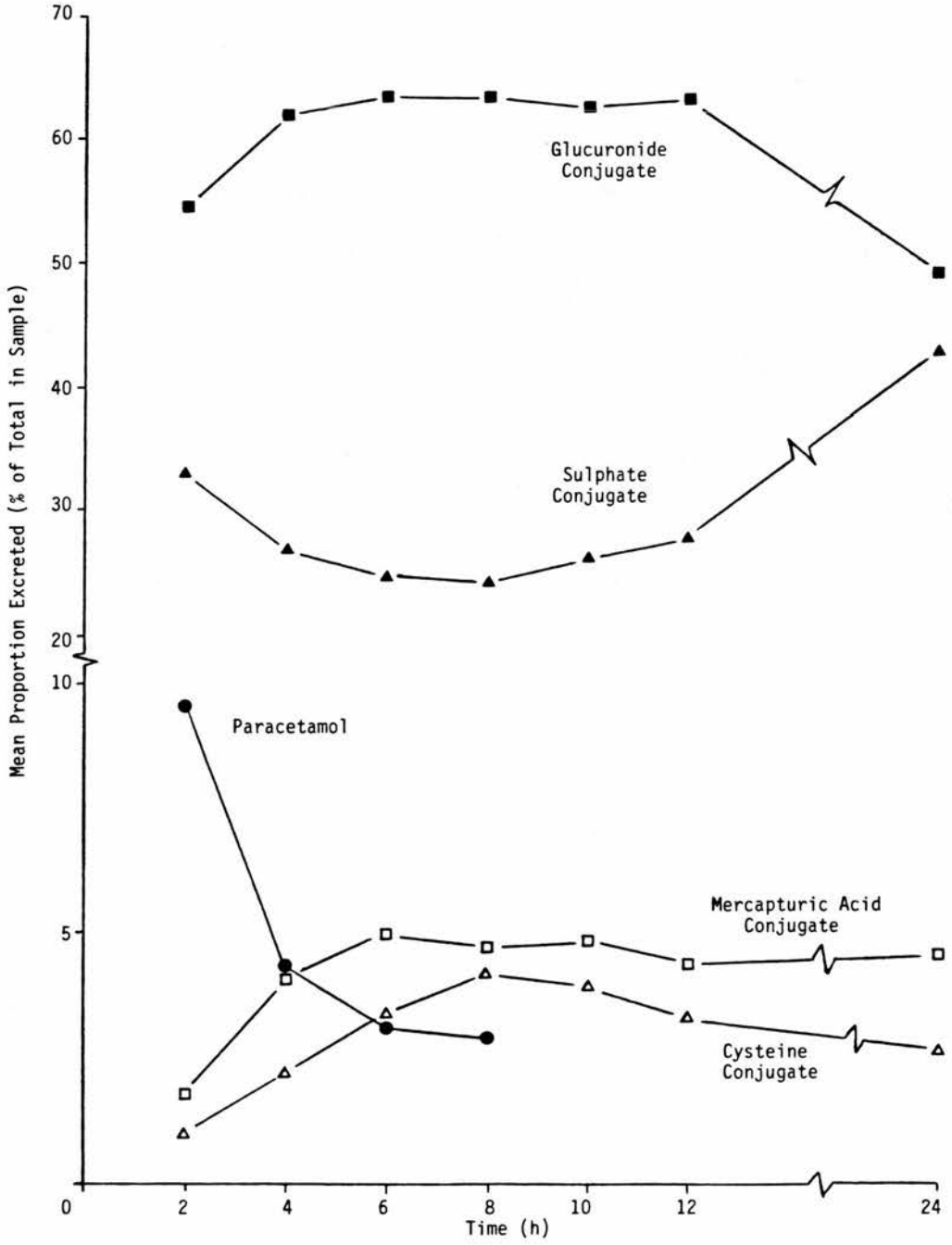
The glucuronide conjugate of paracetamol accounted for the greatest proportion of the total excreted. Over the first 2 h the mean fraction excreted as the glucuronide conjugate was 54.8 ± 8.4%, increasing to 62.4 ± 7.1% by 4 h. For the next 12 h the mean fraction excreted as the glucuronide conjugate remained constant, after which it declined to 49.5 ± 27.9% from 12 to 24 h.

The fraction excreted as paracetamol sulphate followed the opposite pattern to that of the glucuronide conjugate, accounting for 32.9 ± 6.6% up to 2 h, then falling to 27.1 ± 4.7% by 4 h. The fraction excreted as sulphate conjugate remained relatively constant until 12 h, after which it increased to 43.2 ± 23.3% from 12 to 24 h.

TABLE 3.8 Mean Percentage of Total Drug Excreted in Divided Urine Collections as Paracetamol and its Metabolites Following Ingestion of 1 g of Paracetamol in 10 Healthy Male Subjects

Time After Ingestion (h)	Mean Proportion Excreted (as Percentage of Total in Sample) ± SD				
	Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide	Paracetamol Cysteine	Paracetamol Mercapturic Acid
0-2	9.6 ± 2.8	32.9 ± 6.6	54.8 ± 8.4	1.0 ± 0.8	1.8 ± 1.1
2-4	4.3 ± 2.3	27.1 ± 4.7	62.4 ± 7.1	2.2 ± 0.8	4.1 ± 1.4
4-6	3.1 ± 1.8	24.8 ± 4.7	63.6 ± 7.7	3.4 ± 2.0	5.0 ± 2.2
6-8	2.9 ± 2.9	24.5 ± 5.2	63.7 ± 7.8	4.2 ± 2.2	4.8 ± 1.8
8-10	1.2 ± 2.1	26.6 ± 5.3	63.3 ± 8.2	4.0 ± 2.5	4.9 ± 1.9
10-12	0.3 ± 1.0	28.0 ± 4.5	63.8 ± 7.5	3.4 ± 2.3	4.5 ± 1.4
12-24	-	43.2 ± 23.3	49.5 ± 27.9	2.7 ± 3.6	4.7 ± 5.7

FIGURE 3.3 Changes in the Mean Proportional Urinary Excretion of Paracetamol and its Sulphate, Glucuronide, Cysteine and Mercapturic Acid Conjugates with Time Following Ingestion of 1 g of Paracetamol in 10 Healthy Male Subjects



Paracetamol accounted for 9.6 ± 2.8% of the total excreted over the first 2 h. Thereafter, the fraction excreted unchanged decreased. After 10 h urinary concentrations of paracetamol were below the limit of detection in most subjects.

The patterns of excretion of the cysteine and mercapturic acid conjugates of paracetamol were similar with greater recovery as mercapturate than the cysteine conjugate. Over the first 2 h less than 2% of the total was excreted as the cysteine or the mercapturate conjugate. The fraction increased to 4.2 ± 2.2 and 4.8 ± 1.8% respectively by 8 h. Thereafter the fractional excretion of these metabolites gradually declined.

iv) Urinary Excretion Rate of Paracetamol and its Sulphate, Glucuronide, Cysteine and Mercapturic Acid Conjugates

The mean urinary excretion rates of paracetamol and its metabolites in 10 healthy male subjects following ingestion of 1 g of paracetamol are plotted on a semilogarithmic graph in Figure 3.4. The apparent urinary elimination half life values of paracetamol and its metabolites calculated from plots of individual urinary excretion rates are shown in Table 3.9. Individual data are shown in Appendices 3.4-3.8.

FIGURE 3.4 Mean Urinary Excretion Rates of Paracetamol and its Metabolites in 10 Healthy Male Subjects Following Ingestion of 1 g of Paracetamol

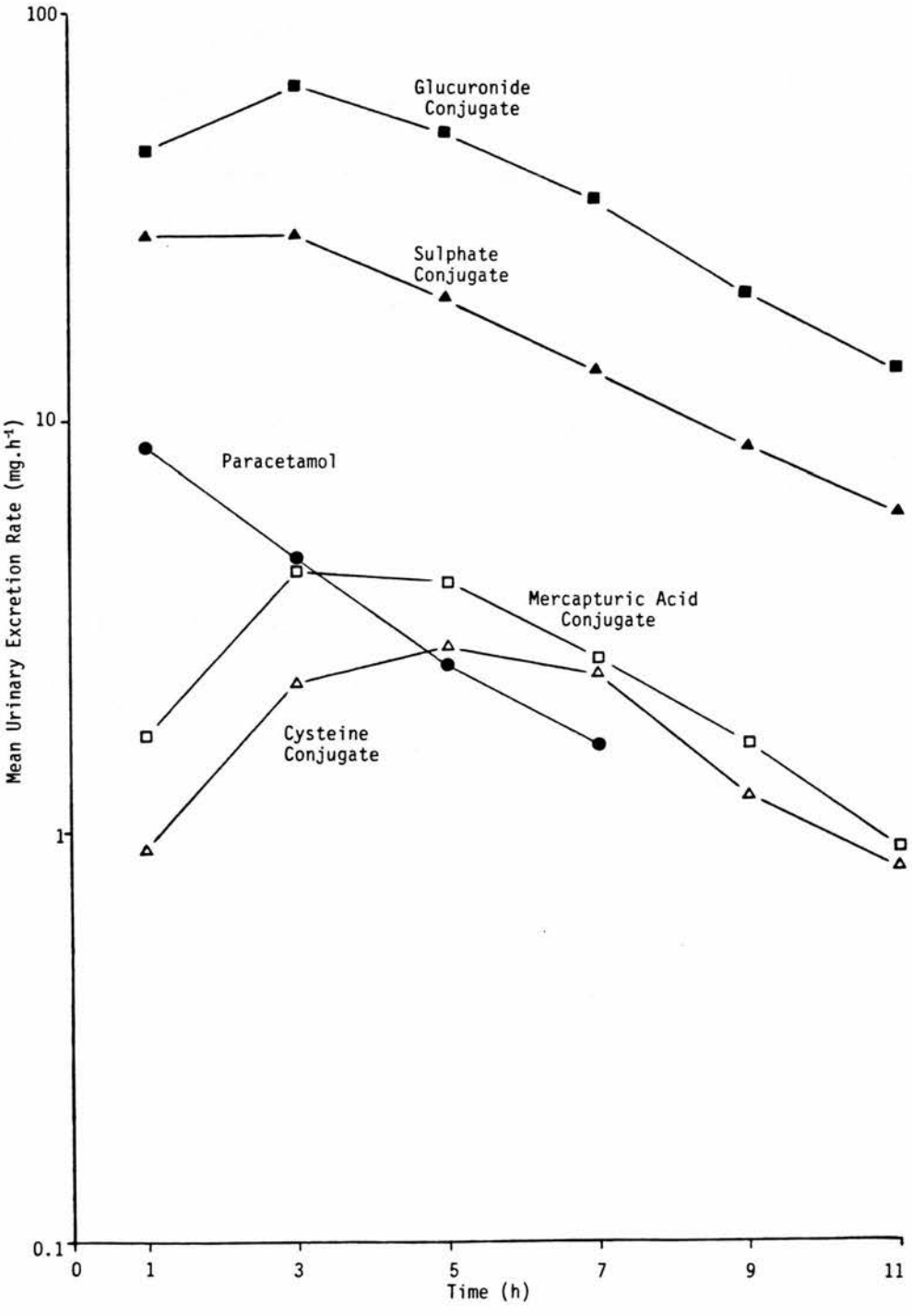


TABLE 3.9 Apparent Urinary Elimination Half Life of Paracetamol and its Metabolites in 10 Healthy Male Subjects Following Ingestion of 1 g of Paracetamol

Subject Number	Apparent Urinary Elimination Half Life (h)				
	Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide	Paracetamol Cysteine	Paracetamol Mercapturic Acid
1	3.7	4.0	3.6	4.9	3.5
2	-	2.4	2.9	2.2	2.3
3	+	+	+	+	+
4	2.4	3.7	3.4	4.2	3.4
5	1.8	3.0	2.6	2.7	2.9
6	2.1	3.4	2.8	3.4	2.8
7	2.4	3.5	3.5	1.8	2.1
8	+	3.1	3.4	3.8	3.4
9	2.0	3.2	2.8	2.4	2.4
10	2.2	3.4	3.0	3.7	3.4
Mean ± SD	2.4 ± 0.6 (n = 7)	3.3 ± 0.5 (n = 9)	3.1 ± 0.4 (n = 9)	3.2 ± 1.0 (n = 9)	2.9 ± 0.5 (n = 9)

+ = Accurate determination not possible due to fluctuation in excretion rate

Where enough data points were available, half life was calculated over:
0-8 h for paracetamol
2-12 h for paracetamol sulphate and paracetamol glucuronide
4-12 h for paracetamol cysteine and paracetamol mercapturic acid

Paracetamol

The mean urinary excretion rate of paracetamol was 8.6 ± 4.0 $mg.h^{-1}$ over the first 2 h and after 4 h the rate was less than that of its metabolites. A plot of the log of the mean urinary excretion rate of paracetamol against time was linear from 0-8 h and the mean urinary elimination half life over this time was 2.4 ± 0.6 h (range 1.8-3.7).

Individual excretion rates fluctuated and accurate determination of the apparent urinary elimination half life was not possible for paracetamol in Subject 8 or for paracetamol and its metabolites in Subject 3. The urinary half life for paracetamol was not determined in Subject 2 as there were too few data points.

Paracetamol Sulphate

The mean urinary excretion rate of paracetamol sulphate peaked during the first 4 h. Mean urinary excretion rates from 0-2 and 2-4 h were 28.2 ± 10.4 and 28.3 ± 5.9 $mg.h^{-1}$ respectively and, thereafter, the mean urinary excretion declined. Semilog plots of paracetamol sulphate excretion rate against time were linear from 2 to 12 h. The mean apparent urinary elimination half life for paracetamol sulphate from 2-12 h was 3.3 ± 0.5 h (range 2.4-4.0).

Individual paracetamol sulphate urinary excretion rates were generally lower than those of the glucuronide conjugate throughout. In

Subjects 4 and 7, however, the excretion rate of the sulphate conjugate from 0-2 h was marginally higher than that of the glucuronide conjugate.

Paracetamol Glucuronide

At each time point the mean urinary excretion rates of the glucuronide conjugate exceeded those of the parent drug and the other metabolites. The mean paracetamol glucuronide excretion rate increased from 46.5 ± 18.8 $mg.h^{-1}$ over the first 2 h to peak at 66.2 ± 10.5 mg. h^{-1} from 2-4 h. Thereafter, a plot of log of the mean urinary excretion rate of the glucuronide conjugate against time was linear. The mean urinary elimination half life from 2-12 h was 3.1 ± 0.4 h (range 2.6-3.6).

Paracetamol Cysteine and Mercapturic Acid

Mean urinary excretion rates of the cysteine and mercapturic acid conjugates were initially low, increasing to 2.8 ± 1.6 $mg.h^{-1}$ from 4-6 h and 4.3 ± 1.6 $mg.h^{-1}$ from 2-4 h respectively. The mean urinary excretion rates of the mercapturic conjugate were slightly higher than those of the cysteine conjugate at each time point. The mean apparent urinary elimination half life from 4-12 h was 3.2 ± 1.0 (range 1.8-4.9) and 2.9 ± 0.5 h (range 2.1-3.5) for the cysteine and mercapturic acid conjugates respectively.

v) Renal Clearances of Paracetamol and its Sulphate and Glucuronide Conjugates

The mean renal clearances of paracetamol, paracetamol sulphate and paracetamol glucuronide calculated over 0-8 h were 15.2 $\pm$ 7.0 (range 6.2-28.2), 180.5 $\pm$ 31.6 (range 143.0-247.0) and 136.9 $\pm$ 32.9 $ml.min^{-1}$ (range 100.3-191.9) respectively. The paracetamol renal clearance (0-8 h) was positively correlated with urine flow rate ($r = 0.81$, $P<0.01$), but no such correlation existed for the sulphate or the glucuronide conjugates. There was no significant correlation between the 0-8 h renal clearance of paracetamol or its glucuronide or sulphate conjugates and urinary pH.

d) Discussion

The absorption of paracetamol in healthy male subjects was rapid and peak plasma concentration occurred in most subjects before the first blood sample was taken at 0.25 h. Paracetamol is predominantly unionised at physiological pH and is absorbed by passive diffusion from the upper gastro-intestinal tract. As paracetamol was administered in solution and all subjects had fasted overnight, factors likely to delay absorption were minimised. The mean peak plasma paracetamol concentrations and the times to reach the peak were comparable to those reported in other studies where 1 g of paracetamol was administered under similar conditions (McGilvary and Mattock, 1972; Rawlins et al, 1977; Perucca and Richens, 1979).

Following absorption, there is a distribution phase during which in addition to elimination, paracetamol leaves the circulation to be taken up by peripheral tissues. The distribution phase ends with equilibrium between the tissues and the circulation and this occurred at approximately 1.5 h. Paracetamol is distributed throughout most tissues but not fat (Gwilt et al, 1963).

From about 2-8 h the fall in plasma paracetamol concentrations represents removal of paracetamol by hepatic metabolism. The mean plasma paracetamol elimination half life over this period was 2.3 $\pm$ 0.3 h (range 1.5-2.5) in good agreement with other published data (Prescott, 1980; Forrest et al, 1982).

A third phase from 8-24 h was also observed in which the mean half life was 5.4 ± 2.4 h. This is consistent with a 3 compartment model and was similar to the value of 4.2 h observed after sampling for 12 h (Clements and Prescott, 1976).

Metabolism of paracetamol by the gastro-intestinal tract is insignificant in man (Clements et al, 1984; Rogers et al, 1987), however, once absorption has taken place paracetamol reaches the systemic circulation via the portal vein and the liver. Paracetamol is extensively metabolised on its first pass through the liver and the amount of parent drug reaching the systemic circulation is significantly reduced. The oral bioavailability in man after 1 g of paracetamol is 89% (Rawlins et al, 1977; Perucca and Richens, 1979) and is independent of dose (Clements et al, 1984).

The extent and rate of paracetamol metabolism is reflected in the mean recovery of only 3.6% of the dose unchanged in urine in 24 h, 72% of which was recovered in the first 4 h.

As expected, the mean elimination half life values of paracetamol in urine and plasma were similar. The pattern of excretion of paracetamol and its metabolites in the present study was comparable to that reported in similar studies in healthy subjects (Adrienssens, 1980; Prescott, 1980; Forrest et al, 1982).

The sulphate and glucuronide conjugates are the major metabolites of paracetamol and their combined mean 24 h urinary recovery represented

73.4% of the dose. The sulphate conjugate appeared more rapidly than the other metabolites of paracetamol. At 0.25 h the mean plasma paracetamol sulphate concentration was greater than that of any of the other metabolites and the mean individual peak plasma concentration occurred at 1.1 $\pm$ 0.5 h. In addition, the fractional urinary excretion of paracetamol sulphate and the maximum urinary excretion rates were greatest at early time points. Sulphate conjugation is, however, susceptible to dose dependent saturation within the therapeutic dose range (Prescott, 1984), and in keeping with this the mean paracetamol sulphate excretion rate was relatively constant over the first 4 h. This has been reported previously and was thought to be consistent with capacity limited formation of paracetamol sulphate (Levy and Yamada, 1971). Following rapid absorption of paracetamol, inorganic sulphate (a precursor of 3'-phosphoadenosine-5'-phosphosulphate) may become depleted, thus reducing the capacity for sulphation.

Plasma paracetamol glucuronide concentrations were higher than those of the parent drug after 1-2 h and they continued to rise after the peak plasma paracetamol sulphate concentration had been attained. The mean of the individual peak plasma paracetamol glucuronide concentrations was 9.4 $\pm$ 2.6 $\mu g.ml^{-1}$ at 2 h.

The mean apparent plasma elimination half life values for paracetamol sulphate and paracetamol glucuronide were greater than that for the parent drug (3.2 $\pm$ 0.4 and 3.0 $\pm$ 0.3 h respectively). Similar values were obtained for the mean urinary elimination half life for the

sulphate and glucuronide conjugates of paracetamol. Comparable findings and values were reported by Adrienssens (1980).

A minor fraction of paracetamol is metabolised to a reactive intermediate which is probably N-acetyl-p-benzoquinone-imine (Miner and Kissinger, 1979). This reactive intermediate is normally deactivated by conjugation with hepatic glutathione (Mitchell et al, 1973b, 1974) and is metabolised further to form the cysteine and mercapturic acid conjugates of paracetamol (Prescott, 1980). The plasma concentrations of these conjugates of paracetamol were below the limits of detection but both were detected in the urine of most subjects within 2 h. The mean 24 h urinary recoveries of the cysteine and mercapturic acid conjugates were 2.3 $\pm$ 1.2 and 3.4 $\pm$ 1.4% of the dose respectively. The maximum excretion rates of these conjugates were delayed relative to the other metabolites. The mean apparent urinary elimination half life of the cysteine and mercapturic acid conjugates were similar to those for sulphate and glucuronide conjugates.

In young healthy subjects approximately 85-95% of a therapeutic dose of paracetamol is excreted in the urine within 24 h (Forrest et al, 1982). In the present study the mean total 24 h urinary recovery was 82.6 $\pm$ 6.3% of the dose (range 75.3-96.1) which is slightly lower. This is likely to be associated with collection of urine samples and compliance with the protocol.

The inter-individual variation in paracetamol metabolism previously reported (Caldwell et al, 1980; Clements et al, 1984; Critchley et

al, 1986) was observed in the present study. In particular, Subjects 4 and 7 produced less paracetamol glucuronide and more paracetamol sulphate, cysteine and mercapturic acid conjugates than the other subjects. Factors such as age, alcohol, drugs and diet may influence paracetamol metabolism.

The mean renal clearance of paracetamol was low (15.2 $\pm$ 7.0 $ml.min^{-1}$) and positively correlated with urine flow rate but not urinary pH. This is in keeping with filtration of paracetamol at the glomerulus followed by passive reabsorption. The renal clearances of the sulphate and glucuronide conjugates of paracetamol were greater than the glomerular filtration rate (180.5 $\pm$ 31.6 and 136.9 $\pm$ 32.9 $ml.min^{-1}$ respectively). The renal clearances of these metabolites were not correlated with urine flow rate or pH suggesting active tubular secretion with negligible reabsorption. These results are consistent with other data obtained in healthy subjects where the mean renal clearances of paracetamol, paracetamol sulphate and paracetamol glucuronide were 13, 170 and 130 $ml.min^{-1}$ respectively (Prescott, 1980; Forrest et al, 1982; Morris and Levy, 1984).

e) Summary

The absorption, distribution, metabolism and elimination of paracetamol were studied in 10 healthy male subjects after oral administration of 1 g of an effervescent formulation of paracetamol.

Paracetamol was rapidly absorbed, distributed and extensively metabolised with only 3.6% of the dose recovered unchanged in urine. The mean individual peak plasma paracetamol concentration was 20 $\mu g.ml^{-1}$ at 0.35 h and the mean elimination half life was 2.3 h. The major metabolite of paracetamol was paracetamol glucuronide representing 49.8% of the dose with 23.7% of the dose excreted as paracetamol sulphate. Conversion to paracetamol sulphate, was however, initially more rapid. The capacity for sulphate conjugation was probably reduced because of saturation of the pathway.

A minor fraction of the dose was recovered as the cysteine (2.3%) and mercapturic acid (3.4%) conjugates of paracetamol. The appearance of these metabolites in urine was initially slow. They represent the proportion of the dose metabolised to the reactive intermediate which is then conjugated with glutathione.

The renal clearance of paracetamol was 15.2 $ml.min^{-1}$ with paracetamol filtered freely at the glomerulus and then passively reabsorbed. The glucuronide and sulphate conjugates were actively secreted by the tubule as their clearances were greater than the normal glomerular filtration rate, being 137 and 181 $ml.min^{-1}$ respectively. The mean

total 24 h urinary recovery ranged from 75.3-96.1% of the administered dose.

CHAPTER IV

Paracetamol N-Acetyl-D-L-methionate Dispostion in Healthy Male Subjects

PARACETAMOL N-ACETYL-D-L-METHIONATE DISPOSITION IN HEALTHY MALE SUBJECTS

a) Introduction

Oral administration of the glutathione precursor methionine, is an effective method of preventing severe liver damage and death after paracetamol overdosage (Crome et al, 1976; Hamlyn et al, 1981, Vale et al, 1981). Initial delay in presentation to hospital probably accounts for the increasing mortality rate attributed to paracetamol poisoning, as to be effective, methionine must be given within 10 h of paracetamol ingestion.

It was first suggested in 1975 by McLean and Day that the addition of methionine to paracetamol tablets may make paracetamol safer when taken in overdosage. These suggestions have been reiterated more recently (Neuvonen et al, 1985). In late 1986, 'Pameton' which contains paracetamol (500 mg) and D-L-methionine (250 mg) was introduced. However, it is expensive and was not included in the National Health Service Limited List.

Continuing along these lines, Sterling Winthrop have linked paracetamol and N-acetyl-D-L-methionine as the N-acetyl-D-L-methionine ester of paracetamol. This compound is tasteless and on hydrolysis yields equimolar quantities of paracetamol and methionine.

As part of the evaluation and development of this ester, healthy male subjects received orally, 1 g of paracetamol as (1) paracetamol and (2) paracetamol N-acetyl-D-L-methionate. At least one week was allowed between each administration. This chapter describes the disposition of paracetamol following administration of paracetamol N-acetyl-D-L-methionate alone. Data obtained from healthy male subjects who received paracetamol alone (Chapter III) were used for comparison.

b) Methods

Subjects

The same 10 healthy male subjects as previously described in Chapter III with a mean age of 28 ± 6 years and body weight 72 ± 11 kg were entered into the study. Screening tests for plasma biochemistry, haematology, urinalysis and creatinine clearance were normal. Individual details of age, body weight and height are shown in Table 3.1 and mean plasma biochemistry and haematology screen data are shown in Tables 3.2 and 3.3

No other drugs were taken for one week and no alcohol for 3 days prior to and during the study. None of the subjects regularly consumed excessive quantities of alcohol and one subject smoked. Informed consent was given by each subject and the study was approved by the local Ethics Committee.

Drug Administration and Sampling

Subjects attended the University Department of Clinical Pharmacology Patient Investigation Room in the morning after fasting overnight, and an in-dwelling cannula was inserted into a forearm vein. Sachets containing 2.146 g of an effercescent formulation of paracetamol N-acetyl-D-L-methionate (equivalent to 1 g paracetamol) were supplied by Sterling Winthrop, Alnwick. Each subject received the contents of one sachet orally in 200 ml of water. The subjects remained recumbent for 3 h and lunch and supper were allowed at 4 and 8 h respectively. They received 200 ml of water every 2 h up to 12 h, and thereafter fluid and food intake were unrestricted.

10 ml venous blood samples were taken into heparinised tubes at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 24 h after dosing. The blood was centrifuged at 1400 xg for 10 min and the plasma separated and stored at -20°C prior to analysis. Urine samples were collected immediately before drug administration and from 0-2, 2-4, 4-6, 6-8, 8-10, 10-12 and 12-24 h after dosing. The total urine volume and pH were recorded and 50 ml aliquots were stored at -20°C prior to analysis. Individual urine volumes and pH values are shown in Appendix 4.9.

Drug Analysis

In addition to measurement of plasma and urine concentrations of paracetamol and its metabolites as described in Chapter II, Section 1 measurement of plasma concentrations of paracetamol N-acetyl-D-L-

methionate was attempted by HPLC as described in Chapter II, Section 2.

10 ml venous blood samples from 4 subjects were taken into cooled heparinised tubes in ice (0°C) at times ranging from 0.75-3 h after dosing. The blood was centrifuged at 1400 x g at 0°C for 10 min and 1 ml of plasma taken for immediate analysis. The time from taking the sample to its injection into the HPLC was approximately 25 min.

Paracetamol N-acetyl-D-L-methionate undergoes rapid hydrolysis in plasma and whole blood (Chapter II, Section 3) and the extent to which this occurred during processing of samples was assessed by measuring plasma paracetamol and paracetamol N-acetyl-D-L-methionate concentrations in fresh whole blood spiked with paracetamol N-acetyl-D-L-methionate (20 $\mu g.ml^{-1}$). In addition fresh whole blood spiked with 0.04, 0.4 and 4.0 $\mu g.ml^{-1}$ of paracetamol N-acetyl-D-L-methionate was processed as described, to determine the threshold for detection of paracetamol N-acetyl-D-L-methionate.

Data Analysis

i) Plasma Concentration of Paracetamol and its Metabolites

Mean plasma concentrations of paracetamol and its sulphate and glucuronide conjugates were plotted on a semilogarithmic graph against time and Cmax, Tmax, AUC and $t_{\frac{1}{2}}$ values were determined as described in detail in Chapter III. The relative paracetamol bio-availability was calculated by dividing the 0-24 h area under the

plasma paracetamol concentration-time curve after administration of 2.146 g paracetamol N-acetyl-D-L-methionate by the corresponding plasma paracetamol area under the curve following administration of 1 g of paracetamol alone to the same healthy male subjects.

ii) Urinary Excretion of Paracetamol and its Metabolites

Each subject received 2.146 g of paracetamol N-acetyl-D-L-methionate which if completely hydrolysed would yield 1 g of paracetamol. As only paracetamol and its metabolites were measured in urine it was the percentage of the paracetamol component of the dose (1 g) recovered in urine in 24 h following administration of paracetamol N-acetyl-D-L-methionate which was determined.

In addition the cumulative urinary recovery, fractional urinary recovery in divided collections, urinary excretion rate, urinary elimination half life and renal clearance of paracetamol and its metabolites were calculated as described in detail in Chapter III.

iii) Statistical Analysis

Mean values $\pm$ standard deviation (SD) are presented. The relationship between quantitative observations on each subject (bivariate data) was determined by calculation of the correlation coefficient (r). The correlation coefficient was tested for statistical significance using tabulated values (Geigy Scientific Tables, page 61).

The Student t-test for paired data was used for statistical significance.

c) Results

i) Plasma Concentrations of Paracetamol N-acetyl-D-L-methionate

No measurable paracetamol N-acetyl-D-L-methionate was detected in any of the plasma samples taken from the 4 subjects. The limit of detection of plasma paracetamol N-acetyl-D-L-methionate was 0.1 $\mu g.ml^{-1}$.

Experiments showed that 42 $\pm$ 8% of the paracetamol N-acetyl-D-L-methionate was hydrolysed in the time taken to process a spiked whole blood sample (20 $\mu g.ml^{-1}$; n = 4) and that a whole blood concentration of 1 to 4 $\mu g.ml^{-1}$ would be required for the detection of any paracetamol N-acetyl-D-L-methionate under these experimental conditions.

ii) Plasma Concentrations of Paracetamol and its Sulphate and Glucuronide Conjugates

Mean plasma concentrations of paracetamol and its sulphate and glucuronide conjugates following oral administration of 2.146 g of paracetamol N-acetyl-D-L-methionate in 10 healthy male subjects are shown in Table 4.1 and Figure 4.1. Following administration of 2.146 g of paracetamol N-acetyl-D-L-methionate, plasma concentrations of the cysteine and mercapturic acid conjugates of paracetamol were below the limit of detection. Individual values for peak concentration (Cmax), time to reach peak concentration (Tmax) and apparent elimi-

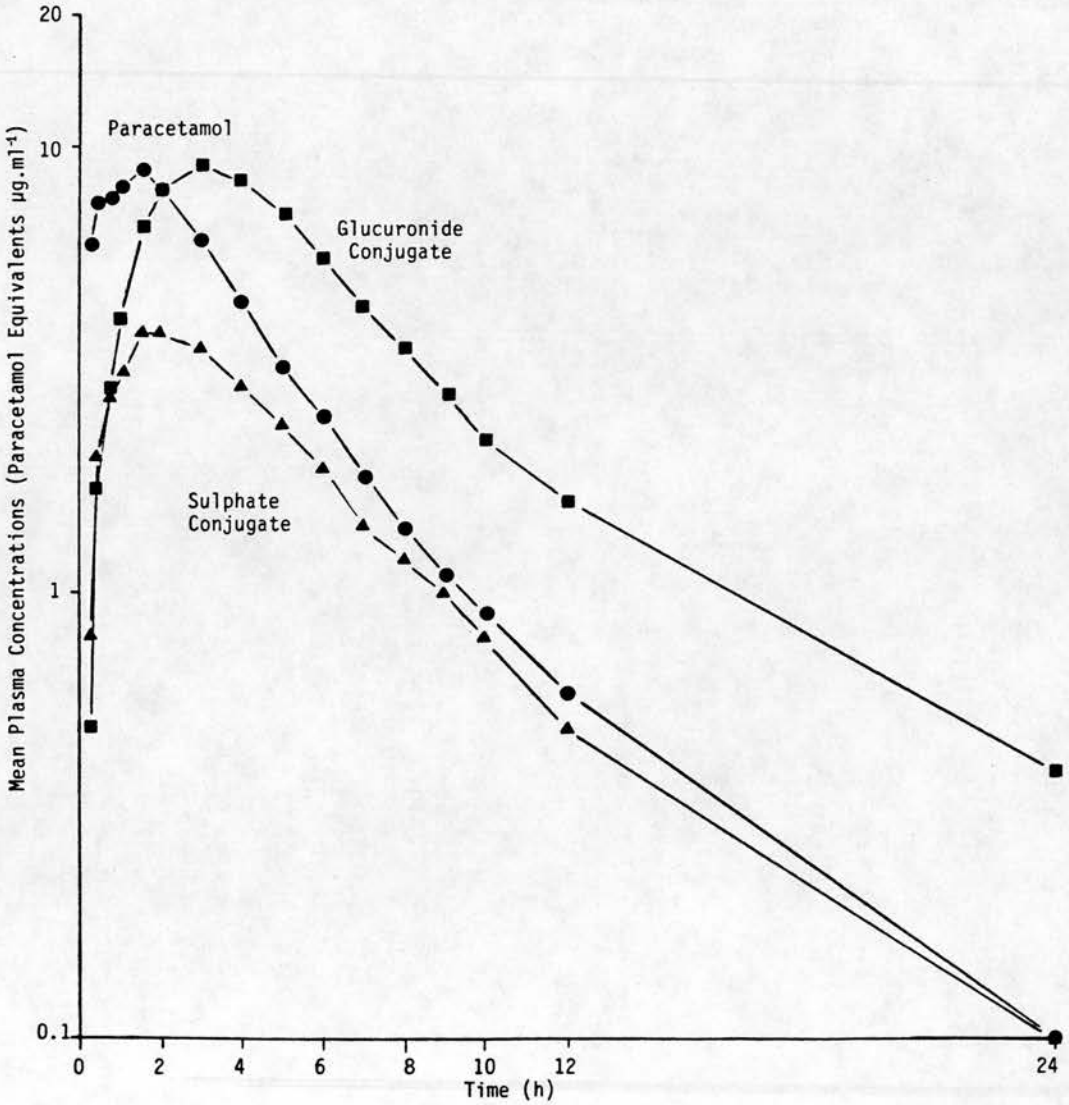
TABLE 4.1 Mean Plasma Concentrations of Paracetamol and its Sulphate and Glucuronide Conjugates in 10 Healthy Male Subjects Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate

Time After Ingestion (h)	Plasma Concentration ($\mu g.ml^{-1}$)		
	Paracetamol (Mean ± SD)	Paracetamol Sulphate (Mean ± SD)	Paracetamol Glucuronide (Mean ± SD)
0.25	6.0 ± 2.7	0.8 ± 0.7	0.5 ± 0.3
0.5	7.6 ± 3.0	2.0 ± 0.7	1.7 ± 0.7
0.75	7.7 ± 2.2	2.7 ± 0.9	2.8 ± 1.2
1.0	8.2 ± 2.0	3.1 ± 1.0	4.1 ± 1.5
1.5	8.9 ± 1.5+	3.8 ± 1.1	6.6 ± 2.3
2.0	8.0 ± 1.6+	3.8 ± 1.1	8.0 ± 2.5
3.0	6.2 ± 1.5+	3.5 ± 1.1	9.1 ± 2.5
4.0	4.5 ± 1.1+	2.9 ± 1.1	8.4 ± 2.2
5.0	3.2 ± 1.0+	2.4 ± 0.9	7.1 ± 1.5
6.0	2.5 ± 0.7+	1.9 ± 0.8	5.6 ± 1.5
7.0	1.8 ± 0.6+	1.4 ± 0.7	4.4 ± 1.1
8.0	1.4 ± 0.5+	1.2 ± 0.5	3.5 ± 0.8
9.0	1.1 ± 0.4+	1.0 ± 0.4	2.8 ± 0.6
10.0	0.9 ± 0.3+	0.8 ± 0.5	2.2 ± 0.5
12.0	0.6 ± 0.2	0.5 ± 0.3	1.6 ± 0.4*
24.0	0.1 ± 0.1	0.1 ± 0.2	0.4 ± 0.2

* = Mean of 9

+ = Significantly increased compared to administration of paracetamol alone; $P<0.05$

FIGURE 4.1 Mean Plasma Concentrations of Paracetamol and its Sulphate and Glucuronide Conjugates in 10 Healthy Male Subjects Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate



nation half life are shown in Tables 4.2 and 4.3 respectively. Plasma concentrations for each subject are shown in Appendices 4.1-4.3.

Paracetamol

Plasma paracetamol concentrations fluctuated in most subjects prior to reaching peak concentration. The mean individual peak plasma paracetamol concentration after administration of the methionate ester was significantly reduced (10.1 $\pm$ 1.8 $\mu g.ml^{-1}$ compared to 20.0 $\pm$ 8.4 $\mu g.ml^{-1}$ with paracetamol alone; $t = 4.183$, $P<0.01$) and delayed (1.08 $\pm$ 0.55 h compared to 0.35 $\pm$ 0.17 h with paracetamol alone; $t = 4.657$, $P<0.01$). From 2-8 h plasma paracetamol concentrations declined linearly when plotted on a semi-logarithmic graph. The mean elimination half life over this period was 2.3 $\pm$ 0.3 h (range 1.9-2.8) which was the same as that obtained after administration of paracetamol alone to the same subjects. From 1.5-10 h after dosing the mean plasma paracetamol concentrations with paracetamol N-acetyl-D-L-methionate were significantly higher than those obtained after administration of paracetamol. Too few data points were available to accurately determine the paracetamol half life from 10-24 h.

Paracetamol Sulphate

Paracetamol sulphate was detected in plasma after 15 min in most subjects. Compared with paracetamol alone, the mean individual time to reach peak plasma paracetamol sulphate concentration was significantly delayed (1.90 $\pm$ 0.61 h compared with 1.08 $\pm$ 0.53 h; $t = 3.587$,

TABLE 4.2 Peak Plasma Concentrations (Cmax) and Time to Reach Peak Plasma Concentrations (Tmax) of Paracetamol and its Sulphate and Glucuronide Conjugates in 10 Healthy Male Subjects Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate

	Paracetamol		Paracetamol Sulphate		Paracetamol Glucuronide	
Subject Number	Cmax ($\mu g.ml^{-1}$)	Tmax (h)	Cmax ($\mu g.ml^{-1}$)	Tmax (h)	Cmax ($\mu g.ml^{-1}$)	Tmax (h)
1	11.0	1.00	2.2	1.50	6.4	2.00
2	10.7	0.25	2.8	1.50	10.6	3.00
3	10.5	2.00	3.9	3.00	9.9	3.00
4	10.2	1.50	5.9	3.00	6.2	4.00
5	11.6	0.50	4.2	1.50	12.8	3.00
6	12.7	0.50	4.5	1.50	10.4	3.00
7	7.4	1.00	3.8	1.50	6.9	3.00
8	6.8	1.00	5.0	2.00	12.5	3.00
9	9.2	1.50	3.5	1.50	8.1	3.00
10	10.4	1.50	4.0	2.00	7.9	3.00
Mean $\pm$ SD	10.1 $\pm$ 1.8 +	1.08 $\pm$ 0.55 +	4.0 $\pm$ 1.0 +	1.90 $\pm$ 0.61 +	9.2 $\pm$ 2.4	3.00 $\pm$ 0.47 ++
* Mean $\pm$ SD	20.0 $\pm$ 8.4	0.35 $\pm$ 0.17	3.5 $\pm$ 0.7	1.08 $\pm$ 0.53	9.4 $\pm$ 2.6	2.00 $\pm$ 0.44

* = Mean data obtained following ingestion of 1 g of paracetamol

\+ = Significantly different from paracetamol alone; $P<0.02$

++ = Significantly different from paracetamol alone; $P<0.001$

TABLE 4.3 Apparent Plasma Elimination Half Life of Paracetamol and its Sulphate and Glucuronide Conjugates in 10 Healthy Male Subjects Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate

Subject Number	Apparent Plasma Elimination Half Life (h)		
	Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide
1	2.4	2.7	2.9
2	2.3	3.0	2.8
3	2.1	2.4	2.8
4	2.7	3.8	3.4
5	1.9	2.8	2.7
6	2.5	3.7	3.0
7	2.5	2.7	3.5
8	2.1	3.2	3.2
9	2.1	3.1	3.2
10	2.8	3.6	3.1
Mean ± SD	2.3 ± 0.3	3.1 ± 0.5	3.1 ± 0.3
* Mean ± SD	2.3 ± 0.3	3.2 ± 0.4	3.0 ± 0.3

* = Mean data obtained following ingestion of 1 g of paracetamol

Half life calculated over: 2-8 h for paracetamol
3-8 h for paracetamol sulphate
5-12 h for paracetamol glucuronide

$P<0.01$), and the mean individual peak plasma paracetamol sulphate concentration was significantly increased (3.5 ± 0.7 $\mu g.ml^{-1}$ compared with 4.0 ± 1.0 $\mu g.ml^{-1}$; $t = 2.313$, $P<0.02$).

The mean paracetamol sulphate half life from 3-8 h was 3.1 ± 0.5 h (range 2.4-3.7) which was similar to that obtained after paracetamol administration to the same subjects (3.2 ± 0.4 h). Mean plasma concentrations of paracetamol sulphate were lower than those of paracetamol at all times.

Paracetamol Glucuronide

Plasma paracetamol glucuronide was detected in plasma in all subjects from 15 min after administration of paracetamol N-acetyl-D-L-methionate and paracetamol glucuronide concentrations rose above those of paracetamol in most subjects after 2-3 h. The mean individual peak plasma paracetamol glucuronide concentration was 9.2 ± 2.4 $\mu g.ml^{-1}$ (range 6.4-12.8) which was similar to that obtained after paracetamol alone (9.4 ± 2.6 $\mu g.ml^{-1}$).

Compared to paracetamol, the mean individual time to reach the peak plasma paracetamol glucuronide concentration following paracetamol N-acetyl-D-L-methionate was significantly delayed (3.00 ± 0.47 h compared with 2.00 ± 0.44 h; $t = 6.034$, $P<0.001$). The mean paracetamol glucuronide half life from 5-12 h was 3.1 ± 0.3 h (range 2.7-3.5) which was similar to that obtained after paracetamol administration (3.0 ± 0.3 h).

iii) Areas Under the Plasma Concentration-time Curves of Paracetamol and its Sulphate and Glucuronide Conjugates

The individual areas under the plasma concentration time curves for paracetamol and its sulphate and glucuronide conjugates following administration of 1 g of paracetamol as (1) paracetamol and (2) paracetamol N-acetyl-D-L-methionate are shown in Table 4.4

The mean areas under the plasma concentration-time curves following administration of paracetamol N-acetyl-D-L-methionate for paracetamol, paracetamol sulphate and paracetamol glucuronide were 46.0 $\pm$ 9.2, 26.7 $\pm$ 10.2 and 68.4 $\pm$ 15.6 $\mu g.h.ml^{-1}$ respectively. These values did not differ significantly from those obtained following paracetamol administration to the same subjects (45.3 $\pm$ 10.6, 22.4 $\pm$ 5.1 and 65.0 $\pm$ 15.9 $\mu g.h.ml^{-1}$ respectively). The bioavailability of paracetamol after paracetamol N-acetyl-D-L-methionate administration was therefore the same as after administration of paracetamol alone. The mean relative paracetamol bioavailability was 102.7 $\pm$ 9.5%.

iv) Urine Recovery of Paracetamol and its Sulphate, Glucuronide, Cysteine and Mercapturic Acid Conjugates

The fraction of the paracetamol component of the dose (1 g) recovered in urine in 24 h as paracetamol and its metabolites after administration of 2.146 g paracetamol N-acetyl-D-L-methionate, and the mean cumulative urinary recovery of paracetamol and its metabolites following administration of 1 g of paracetamol as (1) paracetamol and

Table 4.4 Areas Under the Plasma Concentration-time Curves of Paracetamol and its Sulphate and Glucuronide Conjugates in 10 Healthy Male Subjects Following Ingestion of 1 g of Paracetamol as (1) Paracetamol (PARA) and (2) Paracetamol N-Acetyl-D-L-methionate (PNAM)

Subject Number	Area Under the Plasma Concentration-time Curve 0-24 h ($\mu g.h.ml^{-1}$)					
	Paracetamol		Paracetamol Sulphate		Paracetamol Glucuronide	
	PARA	PNAM	PARA	PNAM	PARA	PNAM
1	51.6	55.4	12.6	13.7	44.5	46.4
2	49.9	51.1	21.0	25.5	65.1	66.8
3	40.7	45.2	18.7	21.0	71.2	70.0
4	61.3	57.9	23.0	50.5	49.7	55.9
5	47.2	41.9	22.0	20.8	89.2	93.1
6	41.2	47.0	28.2	31.3	70.3	79.1
7	31.4	33.4	20.9	19.9	42.3	56.8
8	32.2	30.7	28.1	34.5	85.9	92.6
9	36.9	42.4	20.3	24.2	64.8	61.5
10	60.1	55.3	29.6	25.9	67.4	62.1
Mean $\pm$ SD	45.3 $\pm$ 10.6	46.0 $\pm$ 9.2	22.4 $\pm$ 5.1	26.7 $\pm$ 10.2	65.0 $\pm$ 15.9	68.4 $\pm$ 15.6

(2) paracetamol N-acetyl-D-L-methionate are shown in Tables 4.5 4.6(i), (ii) and Figure 4.2 respectively. Individual urinary excretion data are shown in Appendices 4.3-4.8.

The mean 24 h urinary recovery of paracetamol sulphate was significantly increased following paracetamol N-acetyl-D-L-methionate administration (258.9 $\pm$ 55.1 mg compared to 236.5 $\pm$ 45.2 mg with paracetamol; $t = 2.460$, $P<0.05$). From 0-4 h, however, the mean cumulative urinary recovery of paracetamol sulphate was not significantly different from that obtained after paracetamol administration. The mean urinary recoveries of paracetamol and its glucuronide, cysteine and mercapturic acid conjugates after 24 h were not significantly different from those obtained with paracetamol.

The mean urinary recovery of paracetamol glucuronide over 24 h after administration of paracetamol N-acetyl-D-L-methionate represented 497.3 $\pm$ 64.9 mg (range 407-572). The recovery of this conjugate was significantly reduced over the first 4 h (193.0 $\pm$ 34.0 mg compared to 223.1 $\pm$ 50.8 mg with paracetamol; $t = 2.534$, $P<0.05$).

Following paracetamol N-acetyl-D-L-methionate administration the mean 24 h urinary recovery of paracetamol represented 31.3 $\pm$ 10.2 mg (range 14-48). The mean 0-2 h urinary recovery was significantly reduced (11.7 $\pm$ 5.5 mg compared to 17.1 $\pm$ 8.0 mg with paracetamol; $t = 3.028$, $P<0.02$).

TABLE 4.5 Percentage of the Paracetamol Component of the Dose (1 g) Recovered in Urine as Paracetamol and its Sulphate, Glucuronide, Cysteine and Mercapturic Acid Conjugates in 24 h Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate in 10 Healthy Male Subjects

	Percentage of the Paracetamol Dose Recovered in Urine Over 24 h						
Subject Number	Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide	Paracetamol Cysteine	Paracetamol Mercapturic Acid	Cysteine + Mercapturic Acid	Total
1	3.6	22.0	42.9	7.0	7.3	14.3	82.8
2	2.5	21.5	56.6	1.9	3.3	5.2	85.8
3	3.7	20.1	45.3	2.4	2.2	4.6	73.7
4	4.8	35.0	40.7	5.1	5.8	10.9	91.4
5	2.0	21.6	57.1	3.4	4.4	7.8	88.5
6	2.6	29.9	57.2	1.6	2.0	3.6	93.3
7	4.0	34.8	43.5	3.9	5.7	9.6	91.9
8	1.4	25.1	50.7	1.0	1.9	2.9	80.1
9	3.7	23.1	55.4	1.1	2.7	3.8	86.0
10	3.0	25.7	47.9	2.1	3.2	5.3	81.9
Mean ± SD	3.1 ± 1.0	25.9 ± 5.5	49.7 ± 6.5	3.0 ± 1.9	3.9 ± 1.9	6.8 ± 3.7	85.5 ± 6.1
* Mean ± SD	3.6 ± 1.5	23.7 ± 4.5	49.8 ± 6.8	2.3 ± 1.2	3.4 ± 1.4	5.6 ± 2.5	82.6 ± 6.3

* = Mean data obtained following ingestion of 1 g of paracetamol

TABLE 4.6(i) Mean Cumulative Urinary Recovery of Paracetamol and its Sulphate and Glucuronide Metabolites in 10 Healthy Male Subjects Following Ingestion of 1 g of Paracetamol as (1) Paracetamol and (2) Paracetamol N-Acetyl-D-L-methionate

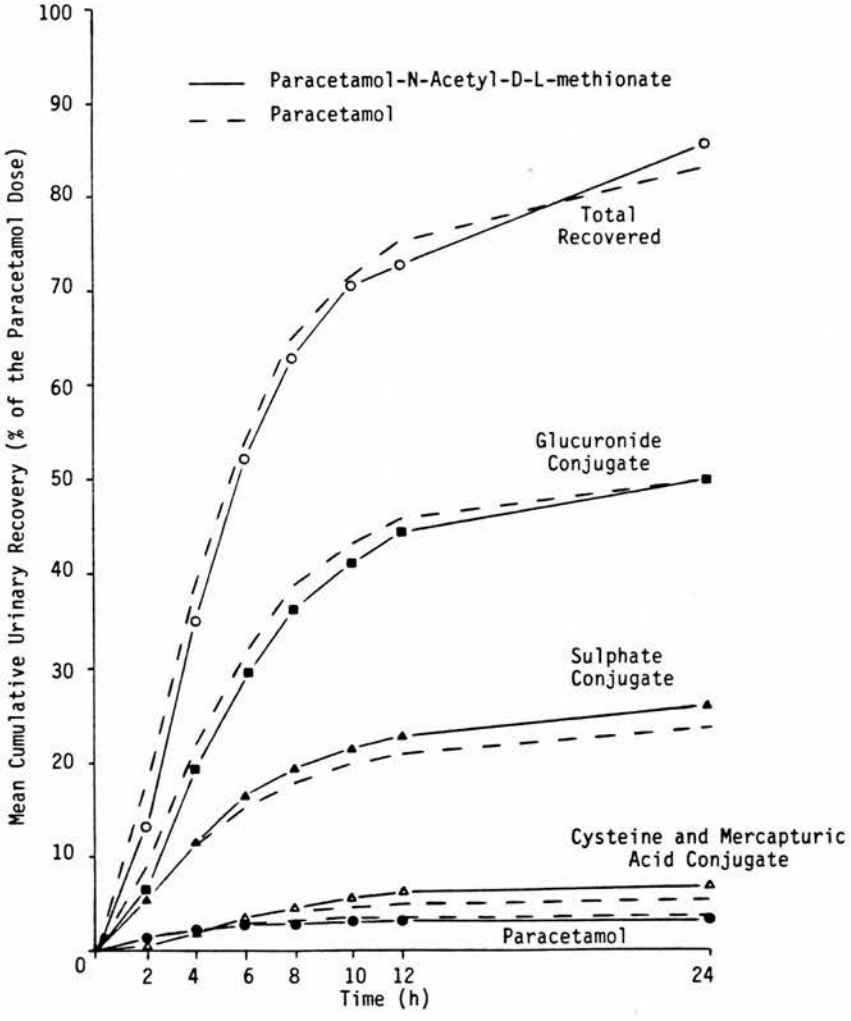
Collection Period (h)	Mean Cumulative Urinary Recovery (mg ± SD)	
	Paracetamol	Paracetamol N-Acetyl-D-L-methionate
Paracetamol		
0-2	17.1 ± 8.0	11.7 ± 5.5^{+}
0-4	26.3 ± 11.3	21.4 ± 7.4
0-6	31.2 ± 13.6	26.8 ± 8.4
0-8	34.3 ± 15.2	29.2 ± 9.1
0-10	35.4 ± 15.3	30.8 ± 10.0
0-12	35.4 ± 15.2	31.5 ± 10.2
0-24	35.4 ± 15.2	31.5 ± 10.2
Paracetamol Sulphate		
0-2	56.3 ± 20.8	54.2 ± 14.8
0-4	112.8 ± 25.1	118.8 ± 24.7
0-6	152.5 ± 33.6	163.4 ± 37.9^{+}
0-8	178.7 ± 33.9	193.0 ± 46.0^{+}
0-10	195.6 ± 34.9	214.4 ± 45.7^{+}
0-12	207.4 ± 37.8	228.5 ± 45.2^{+}
0-24	236.5 ± 45.2	258.9 ± 55.1^{+}
Paracetamol Glucuronide		
0-2	93.0 ± 37.7	64.8 ± 19.2^{+}
0-4	223.1 ± 50.8	193.0 ± 34.0^{+}
0-6	323.4 ± 60.9	294.7 ± 56.1
0-8	392.5 ± 52.6	361.4 ± 64.3
0-10	432.8 ± 52.7	409.6 ± 54.1
0-12	459.0 ± 53.2	442.3 ± 54.2
0-24	497.9 ± 67.6	497.3 ± 64.9

$^{+}$ = Significantly different from paracetamol alone; $P<0.05$

TABLE 4.6(ii) Mean Cumulative Urinary Recovery of Paracetamol Cysteine and Paracetamol Mercapturic Acid in 10 Healthy Male Subjects Following Ingestion of 1 g of Paracetamol as (1) Paracetamol and (2) Paracetamol N-Acetyl-D-L-methionate

Collection Period (h)	Mean Cumulative Urinary Recovery (mg + SD)	
	Paracetamol	Paracetamol N-Acetyl-D-L-methionate
Paracetamol Cysteine		
0-2	1.8 ± 1.4	1.2 ± 1.6
0-4	6.3 ± 3.1	7.4 ± 5.2
0-6	11.8 ± 6.2	14.4 ± 9.6
0-8	16.6 ± 8.0	19.6 ± 13.1
0-10	19.0 ± 9.3	24.3 ± 15.2
0-12	20.4 ± 10.1	26.9 ± 16.3
0-24	22.5 ± 11.8	29.5 ± 19.3
Paracetamol Mercapturate		
0-2	3.4 ± 2.0	2.4 ± 2.1
0-4	12.0 ± 4.9	12.4 ± 6.2
0-6	20.0 ± 8.6	21.9 ± 10.5
0-8	25.2 ± 9.7	28.1 ± 13.6
0-10	28.2 ± 10.6	32.7 ± 15.1
0-12	30.0 ± 11.1	35.4 ± 15.6
0-24	33.9 ± 13.9	38.5 ± 18.7
Cysteine + Mercapturate		
0-2	5.2 ± 3.2	3.6 ± 3.6
0-4	18.3 ± 7.9	19.8 ± 11.3
0-6	31.8 ± 14.1	36.4 ± 19.7
0-8	41.8 ± 17.0	47.7 ± 26.5
0-10	47.2 ± 19.1	57.0 ± 29.9
0-12	50.4 ± 20.4	62.2 ± 31.5
0-24	56.4 ± 24.9	68.1 ± 37.5

FIGURE 4.2 Mean Cumulative Urinary Recovery of Paracetamol and its Metabolites up to 24 h in 10 Healthy Subjects Following Ingestion of 1 g of Paracetamol as 1) Paracetamol and 2) Paracetamol N-Acetyl-D-L-methionate



Urinary excretion of paracetamol cysteine and paracetamol mercapturic acid following administration of paracetamol N-acetyl-D-L-methionate was similar to that obtained after administration of paracetamol alone to the same subjects. The mean combined 24 h urinary recovery of these metabolites was 68.1 ± 37.5 mg, with more mercapturic acid conjugate than cysteine conjugate recovered in most subjects over 24 h. The mean 24 h urinary recovery of paracetamol cysteine and paracetamol mercapturic acid was 29.5 ± 19.3 mg (range 11-70) and 38.5 ± 18.7 mg (range 19-73) respectively. Subject 1 had particularly high recoveries of paracetamol cysteine and mercapturic acid conjugate in urine, representing 7.0 and 7.3% of the paracetamol component of the dose respectively.

Following administration of paracetamol N-acetyl-D-L-methionate the mean percentage of the paracetamol component of the dose (1 g) recovered in urine after 6, 12 and 24 h represented 52.1, 76.5 and 85.5% respectively. These recoveries were similar to those obtained after administration of 1 g of paracetamol alone (53.9, 75.9 and 82.6% respectively).

v) Fractional Excretion of Paracetamol and its Sulphate Glucuronide, Cysteine and Mercapturic Acid Conjugates

The mean percentages of total paracetamol excreted in divided urine collections as paracetamol and its metabolites following ingestion of 2.146 g of paracetamol N-acetyl-D-L-methionate in 10 healthy subjects are shown in Table 4.7 and Figure 4.3. Individual data are shown in Appendices 4.4-4.8.

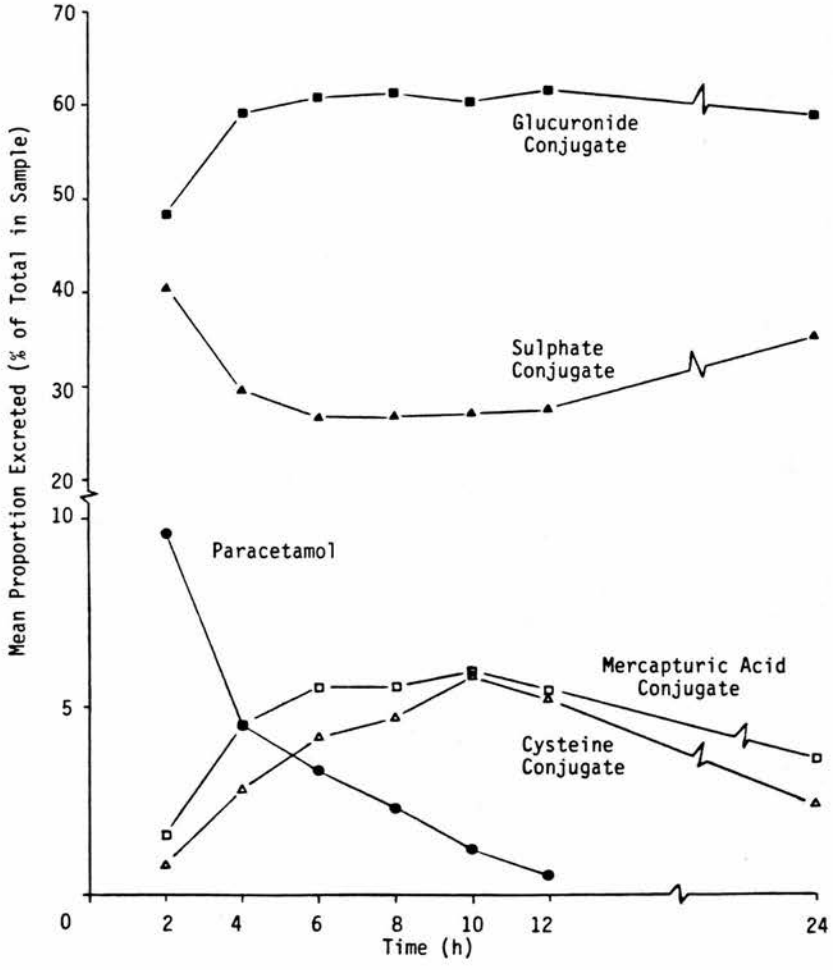
TABLE 4.7 Mean Percentage of Total Paracetamol Excreted in Divided Urine Collections as Paracetamol and its Metabolites Following Ingestion of 2.146 g Paracetamol N-Acetyl-D-L-methionate in 10 Healthy Male Subjects

Time After Ingestion (h)	Mean Proportion Excreted (As Percentage of Total in Sample) ± SD				
	Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide	Paracetamol Cysteine	Paracetamol Mercapturic Acid
0-2	9.2 ± 4.1	40.2 ± 4.7^{++}	48.2 ± 6.8^{++}	0.8 ± 1.1	1.6 ± 1.4
2-4	4.5 ± 1.5	29.4 ± 5.1^{+}	58.8 ± 7.2^{+}	2.8 ± 1.8	4.5 ± 2.3
4-6	3.3 ± 1.4	26.4 ± 4.9	60.6 ± 7.3^{+}	4.2 ± 2.5	5.5 ± 2.3
6-8	2.3 ± 1.9	26.4 ± 5.8	61.1 ± 8.8^{+}	4.7 ± 3.0	5.5 ± 2.5
8-10	1.2 ± 2.0	26.5 ± 3.6	60.1 ± 8.8^{+}	5.8 ± 3.5	5.9 ± 2.6
10-12	0.5 ± 1.7	27.5 ± 5.7	61.3 ± 8.0	5.2 ± 3.1	5.4 ± 2.2
12-24	-	35.4 ± 19.7	58.7 ± 22.0	2.4 ± 3.8	3.6 ± 4.2

$^{+}$ = Significantly different from paracetamol alone; $P<0.05$
$^{++}$ = Significantly different from paracetamol alone; $P<0.001$

Note: Paracetamol sulphate was significantly increased and paracetamol glucuronide significantly reduced

FIGURE 4.3 Changes in the Mean Proportional Urinary Excretion of Paracetamol and its Sulphate, Glucuronide, Cysteine and Mercapturic Acid Conjugates with Time Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate in 10 Healthy Male Subjects



The mean fractional excretion patterns of paracetamol and its metabolites following administration of paracetamol N-acetyl-D-L-methionate were similar to those obtained with paracetamol in the same subjects.

Paracetamol represented 9.2 ± 4.1% of the total excreted over the first 2 h and the fraction as the cysteine and mercapturic conjugates increased to 5.8 ± 3.5 and 5.9 ± 2.6% respectively by 10 h. Thereafter the fractional excretion of paracetamol and these metabolites declined.

The fraction excreted as paracetamol sulphate was significantly increased from 0-2 h (40.2 ± 4.7% compared to 32.9 ± 6.6% with paracetamol; $t = 7.167$, $P<0.001$) and 2-4 h (29.4 ± 5.1% compared to 27.1 ± 4.7% with paracetamol; $t = 4.518$, $P<0.01$). From 6-12 h the fraction excreted as paracetamol sulphate remained relatively constant representing approximately 26% of the total. This was similar to that obtained after paracetamol administration.

The fraction of the total excreted as paracetamol glucuronide in each divided urine collection was significantly reduced. From 0-2 h the fraction excreted was 48.2 ± 6.8% compared to 54.8 ± 8.4% with paracetamol ($t = 4.972$, $P<0.001$) and from 6-8 h, 61.1 ± 8.8% compared to 63.7 ± 7.8% ($t = 2.930$, $P<0.02$).

vi) Urinary Excretion Rate of Paracetamol and its Sulphate, Glucuronide, Cysteine and Mercapturic Acid Conjugates

The mean urinary excretion rates of paracetamol and its metabolites in 10 healthy male subjects following ingestion of 2.146 g of paracetamol N-acetyl-D-L-methionate are plotted semilogarithmically in Figure 4.4. Individual excretion rates fluctuated as was observed after paracetamol administration. Individual data are shown in Appendices 4.4-4.8.

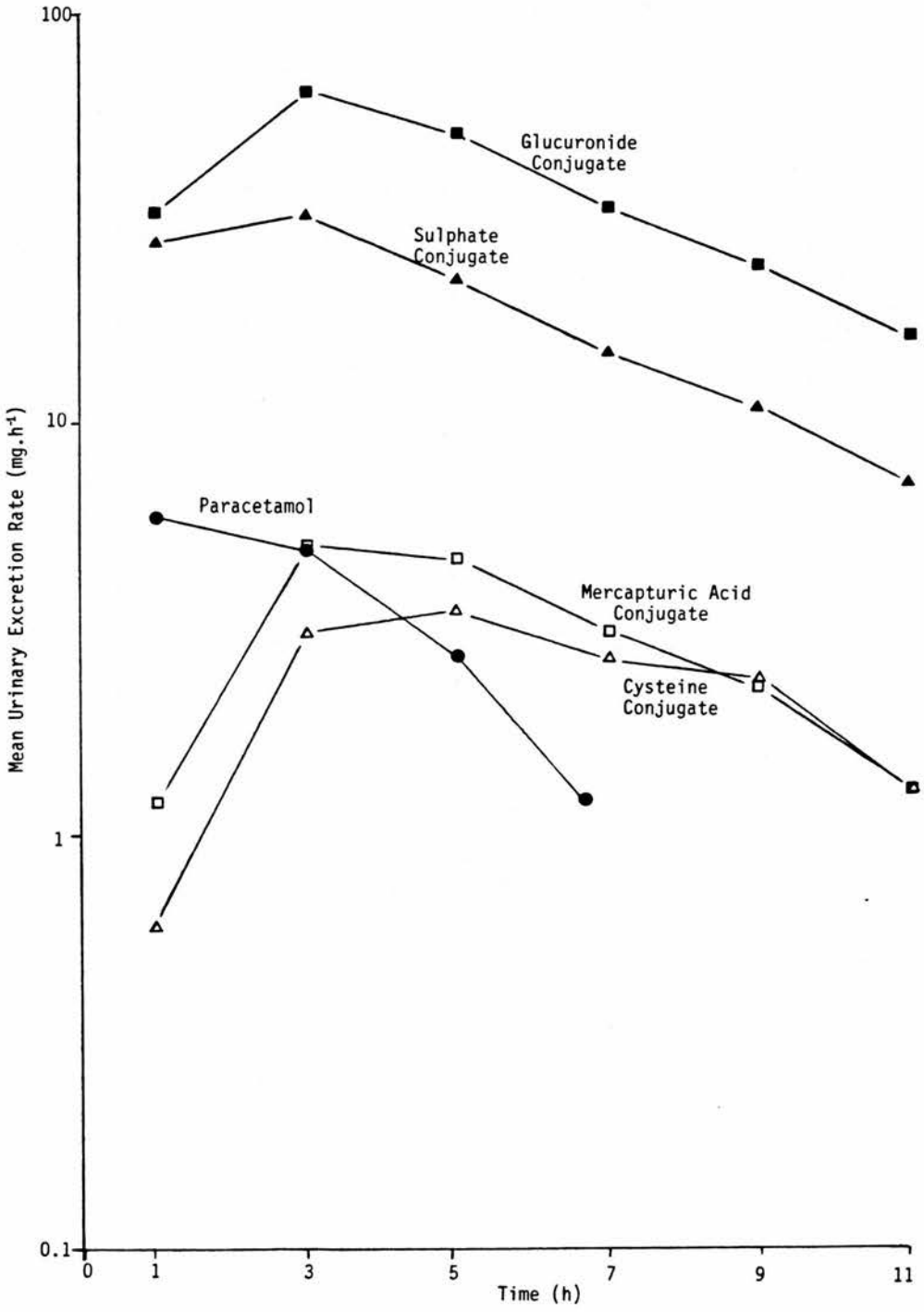
Paracetamol

The mean 0-2 h urinary excretion rate of paracetamol was significantly reduced after paracetamol N-acetyl-D-L-methionate administration (5.9 $\pm$ 2.8 $mg.h^{-1}$ compared to 8.6 $\pm$ 4.0 $mg.h^{-1}$ with paracetamol; $t = 3.028$, $P<0.02$). Thereafter the mean paracetamol excretion rates were similar to those obtained after paracetamol administration to the same healthy subjects.

Paracetamol Sulphate

The mean urinary excretion rate of paracetamol sulphate after administration of paracetamol N-acetyl-D-L-methionate rose to 32.3 $\pm$ 7.6 $mg.h^{-1}$ after 4 h and thereafter declined with a mean apparent urinary elimination half life ($n = 9$) from 2-12 h of 3.3 $\pm$ 0.5 h (range 2.5-4.3). The mean sulphate excretion rate from 2-4 h was 32.3 $\pm$ 7.6 $mg.h^{-1}$ compared to 28.3 $\pm$ 5.9 $mg.h^{-1}$ with paracetamol ($t =$

FIGURE 4.4 Mean Urinary Excretion Rates of Paracetamol and its Metabolites in 10 Healthy Male Subjects Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate



2.743, $P<0.05$) and from 4-6 h it was 22.3 $\pm$ 7.0 $mg.h^{-1}$ compared to 19.9 $\pm$ 5.3 $mg.h^{-1}$ with paracetamol ($t = 2.343$, $P<0.05$).

Paracetamol Glucuronide

The mean 0-2 h urinary excretion rate for paracetamol glucuronide was significantly reduced after paracetamol N-acetyl-D-L-methionate administration (32.4 $\pm$ 9.6 $mg.h^{-1}$ compared to 46.5 $\pm$ 18.8 $mg.h^{-1}$ with paracetamol; $t = 3.562$, $P<0.01$). Mean paracetamol glucuronide excretion rates thereafter were similar to those obtained with paracetamol.

The mean apparent urinary elimination half life ($n = 9$) from 2-12 h for paracetamol glucuronide was 3.4 $\pm$ 0.3 h (range 2.9-3.8) which was similar to that obtained after administration of paracetamol alone to the same subjects (3.1 $\pm$ 0.4 h).

Paracetamol Cysteine and Mercapturic Acid

The mean urinary excretion rates of the cysteine and marcapturic acid conjugates of paracetamol increased to 3.5 $\pm$ 2.2 $mg.h^{-1}$ from 4-6 h and 5.0 $\pm$ 2.5 $mg.h^{-1}$ from 2-4 h respectively. These rates were not significantly different from those obtained after paracetamol administration. The mean urinary excretion rate of paracetamol cysteine after administration of paracetamol N-acetyl-D-L-methionate was significantly increased from 8-10 h (2.4 $\pm$ 1.4 $mg.h^{-1}$ compared to 1.2 $\pm$ 0.7 $mg.h^{-1}$ with paracetamol; $t = 3.785$, $P<0.01$) and 10-12 h (1.3 $\pm$ 0.6 $mg.h^{-1}$ compared to 0.8 $\pm$ 0.6 $mg.h^{-1}$ with paracetamol; $t = 3.645$, $P<0.01$). Similar results were obtained for paracetamol mercapturate

from 8-10 h (2.3 ± 1.1 $mg.h^{-1}$ compared to 1.6 ± 0.6 $mg.h^{-1}$ with paracetamol; $t = 4.015$, $P<0.01$) and 10-12 h (1.3 ± 0.5 $mg.h^{-1}$ compared to 0.9 ± 0.4 $mg.h^{-1}$ with paracetamol; $t = 2.594$, $P<0.05$).

vii) Renal Clearances of Paracetamol and its Sulphate and Glucuronide Conjugates

After administration of paracetamol N-acetyl-D-L-methionate, the mean renal clearances of paracetamol, paracetamol sulphate and paracetamol glucuronide from 0-8 h were 13.0 ± 4.2 (range 8.8-18.1), 172.8 ± 57.9 (range 98.6-292.9) and 129.1 ± 29.1 $ml.min^{-1}$ (range 78.9 ± 175.7) respectively. These did not differ significantly from the renal clearances obtained after paracetamol administration to the same subjects (15.2 ± 7.0, 180.5 ± 31.6 and 136.9 ± 32.9 $ml.min^{-1}$ respectively). The 0-8 h renal clearance of paracetamol was positively correlated with urine flow rate, but the relationship was not as strong as after paracetamol administration. As with administration of paracetamol, no such correlation existed for the sulphate and glucuronide conjugates and there were no significant correlations between 0-8 h renal clearances and urinary pH for paracetamol or its sulphate and glucuronide conjugates.

d) Discussion

Paracetamol N-acetyl-D-L-methionate is a prodrug in which paracetamol is linked to N-acetyl-D-L-methionine by an ester bond. In stability experiments (Chapter II, Section 3) the hydrolysis rate of paracetamol N-acetyl-D-L-methionate increased with pH at 37°C. It is therefore likely that the further paracetamol N-acetyl-D-L-methionate passes down the gastro-intestinal tract prior to absorption the more susceptible it will be to hydrolysis. Esterases present in the intestine, liver and plasma contribute to the hydrolysis of orally administered ester prodrugs (Williams, 1985). Paracetamol N-acetyl-D-L-methionate was rapidly hydrolysed in plasma at 37°C (half life 0.4 h) and the rate of hydrolysis was even greater in whole blood at 37°C (Chapter II, Section 3). It is therefore probable that little, if any, administered paracetamol N-acetyl-D-L-methionate remains intact.

The inability to detect paracetamol N-acetyl-D-L-methionate in the plasma of any of the healthy subjects is consistent with previous reports. (SWRD Reports 304655, 1977; 305510, 1978). Paracetamol N-acetyl-D-L-methionate hydrolysis was reduced but not prevented by cooling of the blood samples. Whole blood concentrations of approximately 1 $\mu g.ml^{-1}$ would be required in order for any paracetamol N-acetyl-D-L-methionate to be detected in plasma under the present conditions.

Individual plasma paracetamol concentrations fluctuated in most subjects prior to reaching their peak values. In addition, following administration of paracetamol N-acetyl-D-L-methionate the mean peak plasma paracetamol concentration was only 50% of that obtained with paracetamol alone and the mean time to reach its peak was delayed by approximately 45 min. These results were consistent with other studies in animals and man (SWRD Reports 305212; 305213; 305510, 1978). The delayed absorption of paracetamol may be explained by the insolubility of paracetamol N-acetyl-D-L-methionate (see Appendix 1.2), however, it is not possible to ascertain whether the paracetamol N-acetyl-D-L-methionate is absorbed intact. Delay in paracetamol absorption may in itself provide some degree of protection against liver damage if paracetamol N-acetyl-D-L-methionate was taken in overdosage, however, there may also be a delayed and reduced pharmacological effect when taken in therapeutic doses. Because the absorption of paracetamol was slow there was no obvious distribution phase as observed after paracetamol administration.

The mean plasma paracetamol elimination half life from 2-8 h was 2.3 $\pm$ 0.3 h which was the same as that obtained with paracetamol in the same healthy subjects. Similarly, the mean area under the plasma paracetamol concentration-time curve from 0-24 h was not significantly different from that obtained after paracetamol. This indicates that although absorption was delayed and slow, the bioavailability of paracetamol after paracetamol N-acetyl-D-L-methionate administration was the same as after administration of paracetamol alone. The mean relative paracetamol bioavailability was 103%.

From 1.5 to 10 h mean plasma concentrations of paracetamol were significantly higher than those obtained with paracetamol. No pharmacodynamic studies following single oral administration of paracetamol N-acetyl-D-L-methionate have been performed in man, however, conflicting results have been obtained in animal models. Compared to paracetamol, paracetamol N-acetyl-D-L-methionate had a delayed onset of action but a longer dose dependent duration of analgesia in rats using the Randal-Selitto test, whereas similar analgesia and duration of effect for each compound was observed using a mouse writhing test (SWRD Report 105032, 1978).

Following paracetamol N-acetyl-D-L-methionate administration the mean 24 h urinary recoveries of paracetamol and its glucuronide, cysteine and mercapturic acid conjugates represented 3.1 $\pm$ 1.0, 49.7 $\pm$ 6.5, 3.0 $\pm$ 1.9 and 3.9 $\pm$ 1.9% of the paracetamol component of the dose respectively. These were not significantly different from those obtained following administration of paracetamol alone. The mean 24 h urinary recovery of paracetamol sulphate, was however, significantly increased, accounting for 259 $\pm$ 55 mg compared to 237 $\pm$ 45 mg after paracetamol administration ($P<0.05$). No significant differences in paracetamol metabolism have previously been reported following paracetamol N-acetyl-D-L-methionate administration.

As observed after paracetamol administration, paracetamol sulphate metabolism was initially a major metabolic route. The mean peak plasma paracetamol sulphate concentration was delayed by approximately 50 min due to the slower delivery rate of paracetamol, but the mean peak plasma paracetamol sulphate concentration was significantly

increased (4.0 ± 1.0 $\mu g.ml^{-1}$ compared to 3.5 ± 0.7 $\mu g.ml^{-1}$ with paracetamol, $P<0.02$). Although the mean area under the plasma paracetamol sulphate concentration-time curve was greater than that obtained after paracetamol administration to the same subjects, the difference was not significant.

The mean paracetamol sulphate urinary excretion rate from 0-2 h was similar to that obtained after paracetamol administration, but sulphate conjugation was increased relative to the other routes of metabolism over this period. Thus the mean paracetamol and paracetamol glucuronide urinary excretion rates from 0-2 h were significantly reduced over this period because of delayed paracetamol absorption, whereas the paracetamol sulphate excretion rate remained unaltered. The mean urinary excretion rate of paracetamol sulphate was significantly increased from 2-6 h. In addition, the mean fractional urinary excretion of paracetamol sulphate was significantly increased from 0-2 h following paracetamol N-acetyl-D-L-methionate administration (40.2 ± 4.7% compared to 32.9 ± 6.6% with paracetamol; $P<0.001$).

It was also observed that the mean paracetamol sulphate excretion rate over the first 4 h was not constant as was observed after administration of paracetamol to the same subjects. These results suggest that following paracetamol N-acetyl-D-L-methionate administration, saturation of the sulphate pathway did not occur. This may be due to methionine providing a source of inorganic sulphate and thus preventing its depletion, or alternatively the sulphate pathway may be less readily saturated since paracetamol was absorbed more slowly.

As with paracetamol sulphate, the mean peak plasma paracetamol glucuronide concentration was delayed, but it was not significantly different from that obtained after paracetamol administration. The mean apparent plasma and urinary elimination half life values for paracetamol sulphate and paracetamol glucuronide were also similar to those following paracetamol administration to the same subjects. The mean fractional urinary excretion of paracetamol glucuronide was significantly reduced and the increase in sulphate conjugation was at the expense of glucuronide conjugation. Previously it has been shown that decreased sulphate conjugation was accompanied by a corresponding increase in glucuronide conjugation (Clements et al, 1984).

Paracetamol N-acetyl-D-L-methionate administered to mice at a dose level equivalent to 400 $mg.kg^{-1}$ of paracetamol caused less depletion of hepatic glutathione than the equivalent paracetamol dose (Skoglund et al, 1986). Although paracetamol N-acetyl-D-L-methionate might be expected to increase the fraction of cysteine and mercapturic acid conjugates produced, the plasma concentrations of these metabolites were below the limit of detection and the mean 24 h urinary recoveries of these metabolites were not significantly different from those obtained following paracetamol administration. Again, the appearance of the cysteine and mercapturic acid conjugates was delayed relative to the other metabolites. The cysteine and mercapturic acid conjugation of paracetamol was therefore not influenced following administration of a therapeutic dose of paracetamol as the N-acetyl-D-L-methionate ester.

Following administration of paracetamol N-acetyl-D-L-methionate the mean percentage of the paracetamol component of the dose recovered in urine in 24 h was 85.5 + 6.1%, and absorption of paracetamol in this form was essentially complete. In a previous study the mean total 24 h urinary recovery of paracetamol was less after paracetamol N-acetyl-D-L-methionate administration (62%) than after paracetamol administration (74%) to healthy subjects (SWRD Report 305510, 1978).

There was a wide range in the 24 h urinary recoveries of paracetamol metabolites reflecting inter-individual differences in paracetamol metabolism as reported previously (Caldwell et al, 1980; Clements et al, 1984; Critchley et al, 1986). Subjects 4 and 7 who produced less paracetamol glucuronide and more paracetamol sulphate, cysteine and mercapturic acid conjugates than the other subjects after administration of 1 g of paracetamol produced a similar metabolic profile after paracetamol N-acetyl-D-L-methionate administration.

The mean renal clearances of paracetamol and its sulphate and glucuronide conjugates were not significantly different from those obtained after paracetamol administration to the same subjects. As before these results were consistent with glomerular filtration and passive reabsorption of paracetamol and active tubular secretion of the sulphate and glucuronide conjugates (Prescott and Wright, 1973; Duggin and Mudge, 1975).

The renal clearance of paracetamol sulphate is concentration dependent and the renal clearance decreases as the plasma concentration increases (Clements et al, 1984; Morris and Levy, 1984). This trend

was observed after paracetamol N-acetyl-D-L-methionate administration, but the result was not statistically significant.

e) Summary and Conclusion

Following administration of 1 g of paracetamol as paracetamol N-acetyl-D-L-methionate to 10 healthy male subjects the overall disposition of paracetamol was similar to that obtained after administration of 1 g of paracetamol alone to the same subjects.

Paracetamol N-acetyl-D-L-methionate was rapidly hydrolysed in the presence of ubiquitous esterases, and the peak plasma paracetamol concentration was reduced and the appearance of paracetamol and its metabolites delayed following administration of paracetamol N-acetyl-D-L-methionate. The area under the plasma paracetamol concentration-time curve and the total urinary recovery following administration of paracetamol N-acetyl-D-L-methionate were similar to those obtained after administration of paracetamol alone. The relative bioavailability of paracetamol was, therefore, the same and paracetamol absorption essentially complete following administration of paracetamol N-acetyl-D-L-methionate.

The urinary recovery of paracetamol and thus the extent of paracetamol metabolism following administration of the N-acetyl-D-L-methionate ester of paracetamol were similar to those obtained after administration of paracetamol alone. In particular the portion of the paracetamol dose metabolised via the toxic intermediate to form the cysteine and mercapturic acid conjugates of paracetamol was not increased even although methionine is a glutathione precursor. Sulphate conjugation was, however, significantly increased following administration of paracetamol N-acetyl-D-L-methionate. Saturation of

the sulphate pathway was observed after administration of paracetamol alone and may have been prevented following administration of paracetamol N-acetyl-D-L-methionate. This may be explained by methionine providing a source of inorganic sulphate thus preventing its depletion or alternatively that the paracetamol sulphate pathway was less readily saturated as paracetamol was released more slowly. The increase in sulphate conjugation was at the expense of glucuronide conjugation.

The elimination of paracetamol following administration of paracetamol N-acetyl-D-L-methionate was similar to that obtained after administration of paracetamol alone and consistent with glomerular filtration and passive reabsorption of paracetamol and active secretion of the sulphate and glucuronide conjugates.

Mean data obtained from healthy subjects following administration of 1 g of paracetamol as (1) paracetamol and (2) paracetamol N-acetyl-D-L-methionate are summarised in Table 4.8.

In conclusion, 2.146 g of paracetamol N-acetyl-D-L-methionate may provide a safer form of paracetamol, however, its pharmacological effectiveness may be reduced and delayed compared to paracetamol alone when administered as a single therapeutic dose to healthy subjects. Pameton (500 mg paracetamol + 250 mg D-L-methionine), which is already commercially available is, however, less likely to present this problem as the paracetamol component is probably rapidly absorbed. Paracetamol N-acetyl-D-L-methionate is, however, tasteless and, therefore, probably more palatable. In addition, paracetamol

N-acetyl-D-L-methionate contains more D-L-methionine than the equivalent paracetamol dose of Pameton. The efficacy of Pameton or paracetamol N-acetyl-D-L-methionate in preventing human paracetamol poisoning, however, remains to be established.

TABLE 4.8 Summary of Data Obtained Following Administration of 1 g of Paracetamol as (1) Paracetamol and (2) Paracetamol N-Acetyl-D-L-methionate to 10 Healthy Male Subjects

Results expressed as Mean ± (SD)

Plasma

Compound	Paracetamol				Paracetamol Sulphate				Paracetamol Glucuronide			
	Cmax ($\mu g.ml^{-1}$)	Tmax (h)	$t_{\frac{1}{2}}$ (2-8 h) (h)	$AUC_{(0-24\ h)}$ ($\mu g.h.ml^{-1}$)	Cmax ($\mu g.ml^{-1}$)	Tmax (h)	$t_{\frac{1}{2}}$ (3-8 h) (h)	$AUC_{(0-24\ h)}$ ($\mu g.h.ml^{-1}$)	Cmax ($\mu g.ml^{-1}$)	Tmax (h)	$t_{\frac{1}{2}}$ (5-12 h) (h)	$AUC_{(0-24\ h)}$ ($\mu g.h.ml^{-1}$)
Paracetamol	20.0 ± (8.4)	0.35 ± (0.17)	2.3 ± (0.3)	45.3 ± (10.6)	3.5 ± (0.7)	1.08 ± (0.53)	3.2 ± (0.4)	22.4 ± (5.1)	9.4 ± (2.6)	2.00 ± (0.44)	3.0 ± (0.3)	65.0 ± (15.9)
Paracetamol N-Acetyl-D-L-methionate	10.1* ± (1.8)	1.08* ± (0.55)	2.3 ± (0.3)	46.0 ± (9.2)	4.0* ± (1.0)	1.90* ± (0.61)	3.1 ± (0.5)	26.7 ± (10.2)	9.2 ± (2.4)	3.00* ± (0.47)	3.1 ± (0.3)	68.4 ± (15.6)

Urine

Compound	Paracetamol		Paracetamol Sulphate		Paracetamol Glucuronide		Paracetamol Cysteine	Paracetamol Mercapturic Acid	Total Paracetamol
	Urinary Recovery (%)	0-8 h Renal Clearance ($ml.min^{-1}$)	Urinary Recovery (%)	0-8 h Renal Clearance ($ml.min^{-1}$)	Urinary Recovery (%)	0-8 h Renal Clearance ($ml.min^{-1}$)	Urinary Recovery (%)	Urinary Recovery (%)	Recovered in 24 h (%)
Paracetamol	3.6 ± (1.5)	15.2 ± (7.0)	23.7 ± (4.5)	180.5 ± (31.6)	49.8 ± (6.8)	136.9 ± (32.0)	2.3 ± (1.2)	3.4 ± (1.4)	82.6 ± (6.3)
Paracetamol N-Acetyl-D-L-methionate	3.1 ± (1.0)	13.0 ± (4.2)	25.9* ± (5.5)	172.8 ± (57.9)	49.7 ± (6.5)	129.1 ± (29.1)	3.0 ± (1.9)	3.9 ± (1.9)	85.5 ± (6.1)

* = Significantly different from paracetamol; $P<0.05$

CHAPTER V

Paracetamol Disposition in Patients with Impaired Renal Function

PARACETAMOL DISPOSITION IN PATIENTS WITH IMPAIRED RENAL FUNCTION

a) Introduction

Most polar drugs and metabolites are dependent on renal excretion for elimination. Therefore, in patients with renal failure, drug disposition is abnormal. Protein binding is often decreased in patients with renal disease which affects drug distribution (Gibaldi, 1977; Reidenberg, 1977a) and drug metabolism may be normal, accelerated or slowed in patients with renal failure (Reidenberg, 1977b). To prevent cumulation and toxicity, drugs predominantly excreted unchanged in urine or metabolised to form active metabolites are usually administered at a reduced dose to patients with renal failure (Drayer, 1977; Mawer, 1982). In order to be effective, however, drugs that are metabolised by the liver to form inactive metabolites may require to be administered to renal patients at the full normal dose. This results in marked cumulation of metabolites which may account for the unpredictable toxicity and adverse drug reactions often observed in patients with renal impairment (Fabre and Balant, 1976; Stone and Walle, 1980; Lieberman et al, 1985).

Paracetamol is often administered to patients with renal impairment, and is predominantly metabolised by the liver to form inactive metabolites which are normally eliminated in the urine (see Chapter III). It is generally assumed that these metabolites of paracetamol are pharmacologically inactive but no information is available on this point. The excretion rate of paracetamol sulphate and paracetamol glucuronide is greatly reduced in patients with renal failure following paracetamol overdosage (Prescott and Wright, 1973), and the renal excretion of paracetamol and its metabolites was delayed in

proportion to the degree of renal impairment in patients with either analgesic nephropathy or nephropathy due to other causes, following administration of 650 mg of [^{14}C]-paracetamol (Thomas et al, 1980). In anephric patients the elimination of paracetamol metabolites was greatly impaired and haemodialysis was the major route for their elimination (Oie et al, 1975; Lowenthal et al, 1976). In this chapter the disposition of 1 g of paracetamol administered to non-dialysis and haemodialysis patients is investigated in more detail. Healthy male subjects who received 1 g of paracetamol (Chapter III) were used as the control group.

b) Methods

Patients

Eleven men and one woman with chronic renal impairment were studied. Their mean age was 52 ± 12 years and their body weight was 75 ± 8 kg. Seven had moderate to severe renal failure but were not being treated with dialysis and 5 with more advanced disease were receiving long term dialysis 2 or 3 times a week. Their clinical details are summarised in Table 5.1. Informed consent was given by each patient and the study was approved by the local Ethics Committee.

Drug Administration and Sampling

Patients attended the University Department of Clinical Pharmacology or the Medical Renal Unit after fasting overnight and an in-dwelling cannula was inserted into a forearm vein. Haemodialysis patients

TABLE 5.1 Clinical Details of Patients with Chronic Renal Failure

Patient Number	Weight (kg)	Age and Sex	Diagnosis	Hb ($g.dl^{-1}$)	Albumin ($g.litre^{-1}$)	ALT ($U.litre^{-1}$)	Bilirubin ($\mu mol.litre^{-1}$)	Alk Phosph ($U.litre^{-1}$)	Creatinine ($\mu mol.litre^{-1}$)	Creatinine Clearance ($ml.min^{-1}$)	Drugs
01	76.4	46♂	CF	16.3	44	17	14	40	334	17	AT
02	77.4	57♂	AG, RA	11.9	39	13	4	114	440	23	NF
03	85.3	43♂	PK	12.1	47	20	8	67	484	22	MT, BD, PZ
04	83.6	49♂	DB, HT	10.3	41	12	5	96	442	19	MT, NF, PZ, IN
05	83.1	65♂	PK, HT	11.3	43	14	11	59	378	17	NF, MT
06	61.0	48♀	PK	10.7	46	15	3	56	879	5	NF, QD, SB
07	74.5	70♂	CG, NE	10.3	23	16	3	119	727	9	NF, MZ, BU
08	68.4	46♂	CG	7.6	44	18	5	89	946	HD	AT, NF
09	79.5	33♂	CG, AN	7.2	45	24	4	128	1127	HD	
010	76.9	39♂	CF	6.9	45	22	5	51	760	HD	
011	64.9	65♂	GP	5.5	40	13	4	74	752	HD	
012	64.7	62♂	RA, AM	7.4	37	11	6	46	641	HD	MT, BP

HD = Haemodialysis, CF = Chronic renal failure of unknown aetiology, AG = Analgesic nephropathy, AN = Anephric, AM = Amyloidosis, NE = Nephrotic syndrome, CG = Chronic glomerulonephritis, DB = Diabetes, GP = Goodpasture's syndrome, HT = Hypertension, PK = Polycystic kidneys, RA = Rheumatoid arthritis, AT = Atenolol, BD = Bendrofluazide, BP = Buprenorphine, BU = Bumetanide, IN = Insulin, MT = Metoprolol, MZ = Metolazone, NF = Nifedipine, PZ = Prazosin, QD = Quinidine, SB = Salbutamol.

attended on an interdialysis day. Sachets containing 1 g of an effervescent formulation of paracetamol were supplied by Sterling Winthrop, Alnwick. Each patient received the contents of one sachet orally in 200 ml of water. The patients remained recumbent for 3 h and lunch and supper were allowed at 4 and 8 h respectively. Each patient received meals appropriate to their condition (eg low protein, controlled sodium or potassium intake). Fluid intake was restricted in haemodialysis patients and non-dialysis patients received 200 ml of water every 2 h for 12 h after administration of the paracetamol.

5 ml venous blood samples were taken into heparinised tubes at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 24 h. The blood was centrifuged at 1400 x g for 10 min and the plasma separated and stored at -20°C. Urine samples from non-dialysis patients were collected immediately before drug administration and then from 0-2, 2-4, 4-6, 6-8, 8-10, 10-12 and 12-24 h. The total urine volume and pH were recorded and 50 ml aliquots were stored at -20°C prior to analysis. Individual urine volumes and pH values are shown in Appendix 5.11.

Drug Analysis

Plasma concentrations of paracetamol, paracetamol sulphate and paracetamol glucuronide, and urinary concentrations of paracetamol, paracetamol sulphate, paracetamol glucuronide, paracetamol cysteine and paracetamol mercapturic acid were measured by HPLC as described

in Chapter II, Section 1. Metabolite concentrations are expressed as paracetamol equivalents. Electrochemical detection (Model LC - 4A, Bioanalytical Systems Incorporated) was also used to detect low concentrations and to increase selectivity of the assay for cysteine and mercapturic acid conjugates of paracetamol in plasma. Significant blank values were obtained in some patients and these were subtracted to give corrected concentrations. Patient No. 07 had a high urinary protein concentration and the paracetamol sulphate peak could not be properly resolved. The amount of sulphate excreted was, therefore, estimated from the relative areas under the plasma concentration-time curves of the sulphate and glucuronide conjugates and the urinary recovery of paracetamol glucuronide, assuming both metabolites had the same renal clearance and volume of distribution.

DATA ANALYSIS

i) Plasma Concentrations of Paracetamol and its Metabolites

Mean plasma concentrations of paracetamol and its sulphate, glucuronide, cysteine and mercapturic acid conjugates were plotted against time on a semilogarithmic graph and individual peak plasma concentrations (Cmax) and times to reach peak concentration (Tmax) were determined.

ii) Plasma Elimination Half Life

The plasma elimination half life for paracetamol and the apparent plasma elimination half life for paracetamol sulphate and paracetamol

glucuronide were determined: $t_{\frac{1}{2}}$ = 0.301/slope. The slope was determined by direct linear regression from semilogarithmic plots of plasma concentration against time over the period 2-8 and 8-24 h for paracetamol and 8-24 h for paracetamol sulphate and glucuronide. Formal model dependent curve fitting and compartmental analysis was not performed.

iii) Area Under the Curve

The 0-24 h area under the plasma concentration-time curve was calculated by the trapezoidal method for paracetamol and its sulphate and glucuronide conjugates.

iv) Non-dialysis Patients - Recovery of Paracetamol and its Metabolites in Urine

The percentage of the administered dose recovered in 24 h was determined. In addition, the fractional urinary recoveries of paracetamol and its metabolites in 24 h and in divided urine collections were calculated. The cumulative urinary recoveries of paracetamol and its metabolites were plotted against time.

v) Non-dialysis Patients - Renal Clearance

The 0-8 h renal clearances of paracetamol and its glucuronide and sulphate conjugates and the 0-24 h renal clearances of paracetamol cysteine and paracetamol mercapturic acid were calculated by dividing the amount recovered in the urine by the corresponding area under the plasma concentration-time curve. The area under the curve was calculated by the trapezoidal method.

vi) Statistical Analysis

Mean values ± standard deviation (SD) are presented. The relationship between quantitative observations on each subject (bivariate data) was determined by calculation of the correlation coefficient (r) and this was tested for statistical significance using tabulated values (Geigy Scientific Tables, page 61). Differences between means were compared by the Mann-Whitney two sample rank test for statistical significance.

c) Results

i) Plasma Concentrations of Paracetamol and its Sulphate, Glucuronide, Cysteine and Mercapturic Acid Conjugates

Mean plasma concentrations of paracetamol and its sulphate, glucuronide, cysteine and mercapturic acid conjugates following oral administration of 1 g of paracetamol to 7 non-dialysis and 5 haemodialysis patients are shown in Table 5.2, Figure 5.1 and Table 5.3, Figure 5.2 respectively. Individual values for peak concentration (Cmax) and time to reach peak concentration (Tmax), apparent elimination half life and area under the plasma concentration-time curves for paracetamol and its metabolites in non-dialysis patients are shown in Tables 5.4, 5.6 and 5.7 respectively. Individual values for Cmax and Tmax, and area under the plasma concentration-time curves for paracetamol and its metabolites in haemodialysis patients are shown in Tables 5.5 and 5.8 respectively. Plasma concentrations for each subject are shown in Appendices 5.1-5.5.

TABLE 5.2 Mean Plasma Concentrations of Paracetamol and its Sulphate, Glucuronide, Cysteine and Mercapturic Acid Conjugates in 7 Non-dialysis Patients Following Ingestion of 1 g of Paracetamol

	Plasma Concentration ($\mu g.ml^{-1}$)				
Time After Ingestion (h)	Paracetamol (Mean ± SD)	Paracetamol Sulphate (Mean ± SD)	Paracetamol Glucuronide (Mean ± SD)	Paracetamol Cysteine (Mean ± SD)	Paracetamol Mercapturic Acid (Mean ± SD)
0.25	13.8 ± 7.6	2.2 ± 1.3	1.5 ± 1.4	0.01 ± 0.01	0.00 ± 0.00
0.5	15.0 ± 8.4	5.3 ± 2.6	4.1 ± 2.7	0.02 ± 0.02	0.01 ± 0.01
0.75	13.1 ± 4.8	6.5 ± 2.5	6.8 ± 4.4	0.05 ± 0.02	0.01 ± 0.02
1.0	11.9 ± 4.2	7.4 ± 2.8	8.8 ± 4.9	0.08 ± 0.03	0.02 ± 0.03
1.5	10.6 ± 3.0	9.0 ± 3.3	11.4 ± 7.5	0.15 ± 0.05	0.05 ± 0.05
2.0	9.7 ± 2.6	10.2 ± 3.7	15.7 ± 6.8	0.20 ± 0.07	0.09 ± 0.06
3.0	7.1 ± 1.4	11.8 ± 4.7	16.6 ± 9.0	0.33 ± 0.11	0.16 ± 0.08
4.0	5.1 ± 1.0	12.3 ± 4.9	22.0 ± 7.2	0.43 ± 0.16	0.21 ± 0.08
5.0	3.9 ± 0.7	13.1 ± 5.0	23.5 ± 7.3	0.50 ± 0.18	0.25 ± 0.09
6.0	3.0 ± 0.6	12.9 ± 5.3	23.8 ± 7.3	0.51 ± 0.20	0.23 ± 0.07
7.0	2.3 ± 0.4	12.9 ± 6.4	24.0 ± 9.0	0.54 ± 0.22	0.23 ± 0.06
8.0	1.9 ± 0.3	12.3 ± 5.9	23.5 ± 8.4	0.53 ± 0.24	0.24 ± 0.05
9.0	1.5 ± 0.2	11.9 ± 6.1	22.8 ± 8.3	0.51 ± 0.25	0.23 ± 0.06
10.0	1.2 ± 0.2	11.2 ± 6.0	21.6 ± 8.7	0.49 ± 0.25	0.21 ± 0.06
12.0	0.9 ± 0.5[+]	9.9 ± 4.4	19.4 ± 6.4	0.45 ± 0.23	0.20 ± 0.06
24.0	0.5 ± 0.2	5.1 ± 2.0	13.2 ± 8.4	0.24 ± 0.17	0.11 ± 0.05

[+] = Mean of 6

FIGURE 5.1 Mean Plasma Concentrations of Paracetamol and its Sulphate, Glucuronide, Cysteine and Mercapturic Acid Conjugates in 7 Non-dialysis Patients Following Ingestion of 1 g of Paracetamol

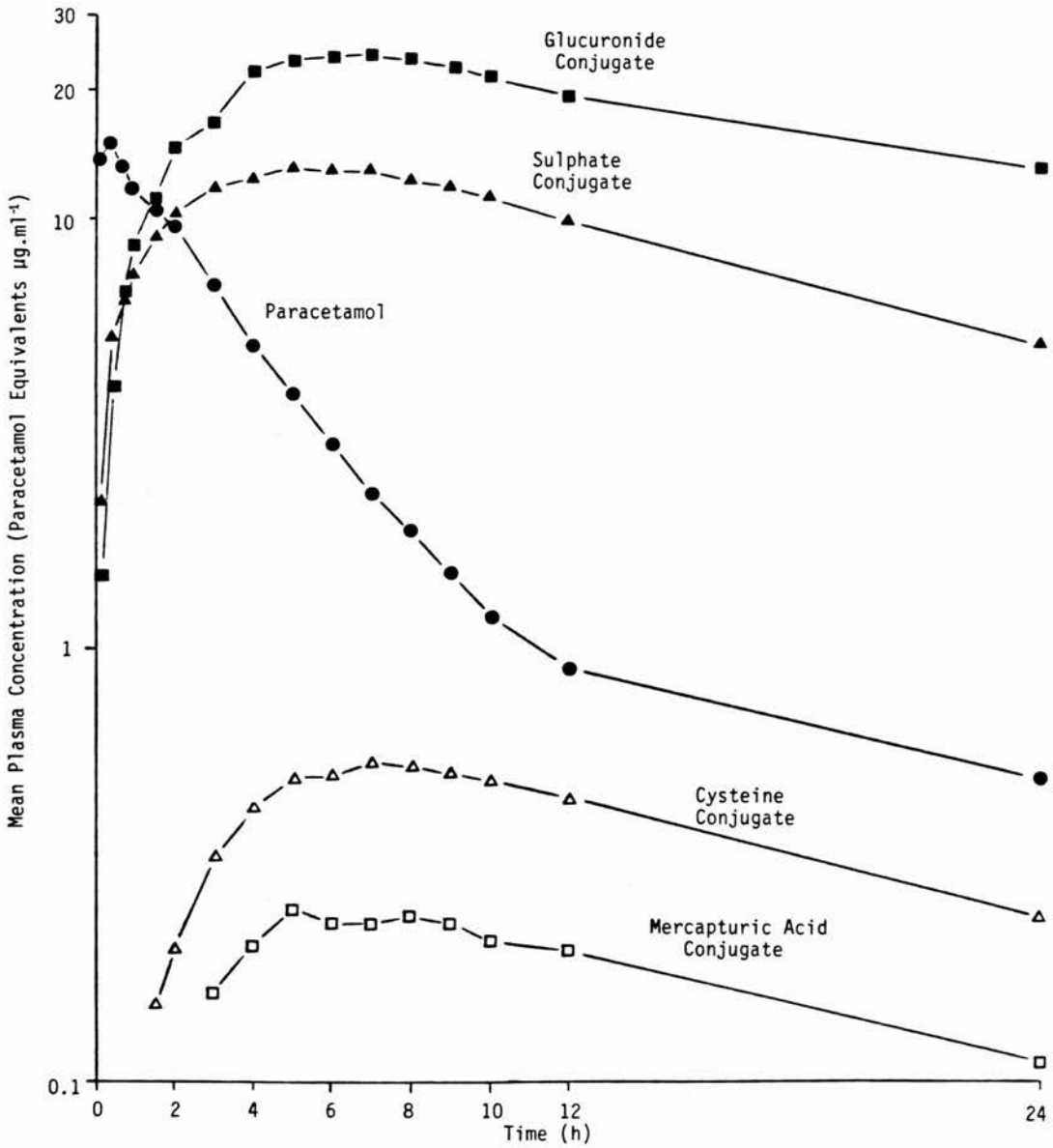


TABLE 5.3 Mean Plasma Concentrations of Paracetamol and its Sulphate, Glucuronide, Cysteine and Mercapturic Acid Conjugates in 5 Haemodialysis Patients Following Ingestion of 1 g of Paracetamol

	Plasma Concentration ($\mu g.ml^{-1}$)				
Time After Ingestion (h)	Paracetamol (Mean ± SD)	Paracetamol Sulphate (Mean ± SD)	Paracetamol Glucuronide (Mean ± SD)	Paracetamol Cysteine (Mean ± SD)	Paracetamol Mercapturic Acid (Mean ± SD)
0.25	15.6 ± 8.2	3.7 ± 2.7	0.7 ± 0.5	0.01 ± 0.01	0.00 ± 0.00
0.5	15.3 ± 2.5	7.7 ± 1.7	3.0 ± 1.7	0.04 ± 0.04	0.00 ± 0.00
0.75	14.2 ± 1.6	9.5 ± 1.7	5.8 ± 1.9	0.09 ± 0.06	0.01 ± 0.01
1.0	13.1 ± 2.1	10.7 ± 2.1	8.1 ± 2.8	0.14 ± 0.06	0.02 ± 0.03
1.5	10.9 ± 2.3	12.7 ± 1.6	11.7 ± 3.7	0.25 ± 0.06	0.07 ± 0.05
2.0	9.1 ± 2.6	14.6 ± 2.7	15.5 ± 4.8	0.42 ± 0.06	0.13 ± 0.06
3.0	6.3 ± 1.9	16.5 ± 3.4	20.4 ± 6.1	0.65 ± 0.12	0.25 ± 0.09
4.0	4.3 ± 1.5	17.7 ± 3.8	23.4 ± 6.8	0.92 ± 0.26	0.36 ± 0.12
5.0	3.1 ± 1.3	18.2 ± 3.6	25.3 ± 7.3	1.18 ± 0.34	0.49 ± 0.15
6.0	2.3 ± 1.0	19.6 ± 4.9	27.2 ± 7.5	1.19 ± 0.36	0.55 ± 0.17
7.0	1.6 ± 1.2	20.2 ± 5.7	28.0 ± 7.7	1.22 ± 0.34	0.58 ± 0.19
8.0	1.3 ± 0.9	19.9 ± 4.8	28.6 ± 8.1	1.24 ± 0.35	0.71 ± 0.20
9.0	1.2 ± 0.8[+]	20.5 ± 5.5	29.4 ± 8.2	1.17 ± 0.31	0.64 ± 0.22
10.0	1.0 ± 0.7[+]	20.0 ± 5.0	29.7 ± 8.6	1.19 ± 0.33	0.66 ± 0.22
12.0	0.7 ± 0.5[+]	19.5 ± 4.9	29.5 ± 8.4	1.05 ± 0.33	0.65 ± 0.26
24.0	0.4 ± 0.4	17.6 ± 5.7	29.1 ± 6.7	0.72 ± 0.25	0.66 ± 0.37

[+] = Mean of 4

FIGURE 5.2 Mean Plasma Concentrations of Paracetamol and its Sulphate, Glucuronide, Cysteine and Mercapturic Acid Conjugates in 5 Haemodialysis Patients Following Ingestion of 1 g of Paracetamol

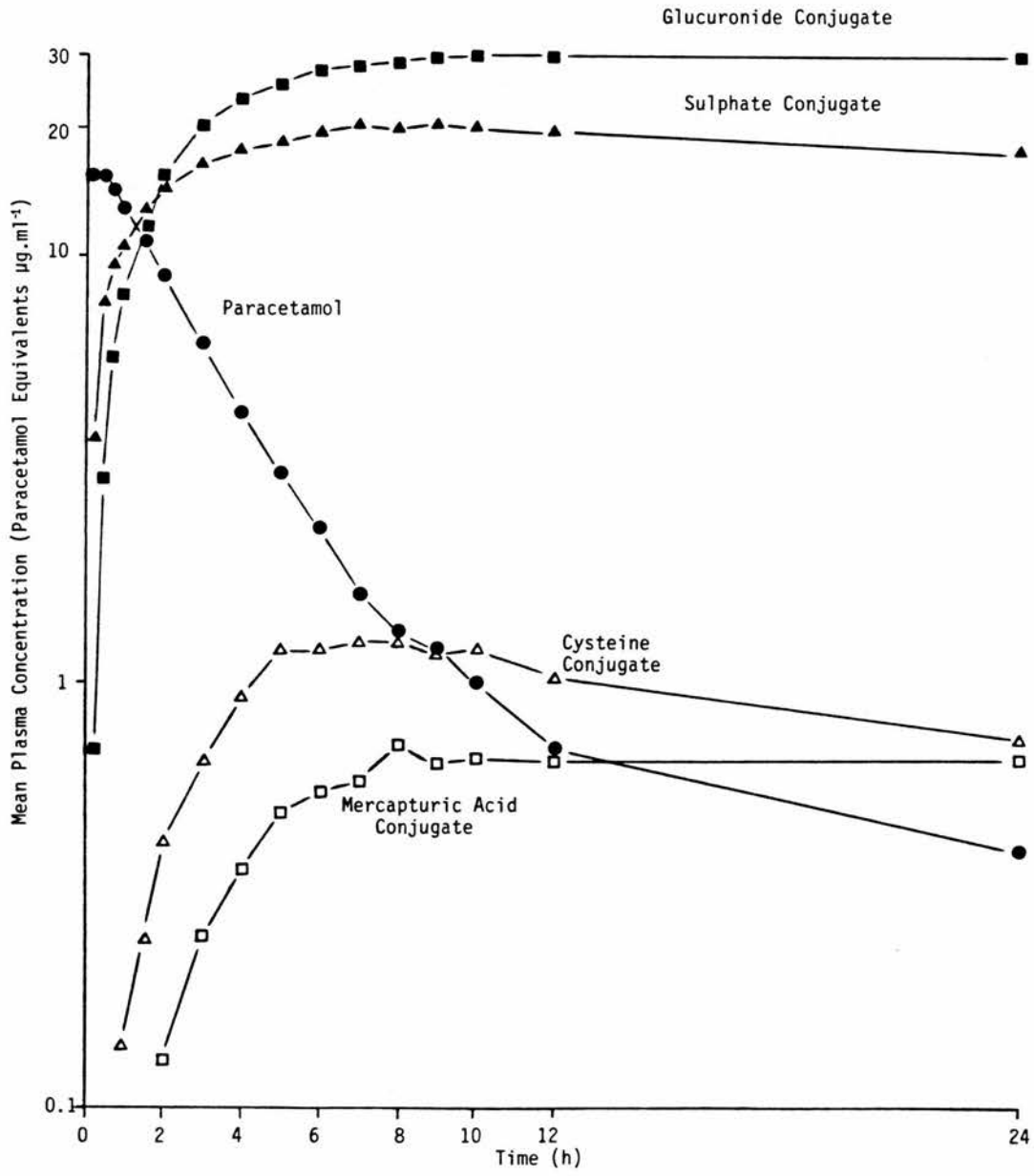


TABLE 5.4 Peak Plasma Concentrations (Cmax) and Time to Reach Peak Plasma Concentrations (Tmax) of Paracetamol and its Sulphate and Glucuronide Conjugates in 7 Non-dialysis Patients Following Ingestion of 1 g of Paracetamol

Patient Number	Paracetamol		Paracetamol Sulphate		Paracetamol Glucuronide	
	Cmax ($\mu g.ml^{-1}$)	Tmax (h)	Cmax ($\mu g.ml^{-1}$)	Tmax (h)	Cmax ($\mu g.ml^{-1}$)	Tmax (h)
01	23.6	0.25	9.4	3.00	29.9	3.00
02	19.5	0.25	11.8	5.00	18.8	8.00
03	8.9	0.25	11.3	5.00	20.0	6.00
04	7.8	1.50	13.5	5.00	19.3	6.00
05	11.9	0.50	12.5	7.00	18.8	7.00
06	30.3	0.50	26.9	7.00	42.6	7.00
07	19.2	0.50	10.8	7.00	26.9	7.00
Mean $\pm$ SD	17.3 $\pm$ 8.2	0.54 $\pm$ 0.44	13.7 $\pm$ 5.9 +	5.57 $\pm$ 1.51 +	25.2 $\pm$ 8.9 +	6.29 $\pm$ 1.60 +
* Mean $\pm$ SD	20.0 $\pm$ 8.4	0.35 $\pm$ 0.17	3.5 $\pm$ 0.7	1.08 $\pm$ 0.53	9.4 $\pm$ 2.6	2.00 $\pm$ 0.44

* = Mean data obtained following administration of 1 g of paracetamol to 10 healthy male subjects

+ = Significantly different from healthy subjects; $P<0.001$

TABLE 5.5 Peak Plasma Concentrations (Cmax) and Time to Reach Peak Plasma Concentrations (Tmax) of Paracetamol and its Sulphate and Glucuronide Conjugates in 5 Haemodialysis Patients Following Ingestion of 1 g of Paracetamol

	Paracetamol		Paracetamol Sulphate		Paracetamol Glucuronide	
Patient Number	Cmax ($\mu g.ml^{-1}$)	Tmax (h)	Cmax ($\mu g.ml^{-1}$)	Tmax (h)	Cmax ($\mu g.ml^{-1}$)	Tmax (h)
08	15.3	0.25	20.4	7.00	36.7	7.00
09	17.2	0.50	18.9	8.00	41.0	12.00
010	17.2	0.25	17.9	8.00	25.1	8.00
011	15.8	1.00	30.1	9.00	21.5	24.00
012	27.9	0.25	16.4	9.00	28.8	24.00
Mean $\pm$ SD	18.7 $\pm$ 5.2	0.45 $\pm$ 0.32	20.7 $\pm$ 5.4 +	8.20 $\pm$ 0.84 +	30.6 $\pm$ 8.1 +	15.00 $\pm$ 8.43 +
* Mean $\pm$ SD	20.0 $\pm$ 8.4	0.35 $\pm$ 0.17	3.5 $\pm$ 0.7	1.08 $\pm$ 0.53	9.4 $\pm$ 2.6	2.00 $\pm$ 0.44

* = Mean data obtained following administration of 1 g of paracetamol to 10 healthy male subjects

+ = Significantly different from healthy subjects; $P<0.005$

TABLE 5.6 Apparent Plasma Elimination Half Life of Paracetamol and its Sulphate and Glucuronide Conjugates in 7 Non-dialysis Patients Following Ingestion of 1 g of Paracetamol

Patient Number	Paracetamol $t_{\frac{1}{2}}$ (2-8 h) (h)	Paracetamol $t_{\frac{1}{2}}$ (8-24 h) (h)	Paracetamol Sulphate $t_{\frac{1}{2}}$ (8-24 h) (h)	Paracetamol Glucuronide $t_{\frac{1}{2}}$ (8-24 h) (h)
01	2.4	8.7	10.0	7.1
02	3.0	8.2	12.9	15.6
03	2.7	-	14.9	14.7
04	2.4	18.9	21.3	29.2
05	2.8	9.8	12.0	11.3
06	2.0	11.8	22.6	25.8
07	2.5	12.1	54.3	100.3
Mean ± SD	2.5 ± 0.3	11.6 ± 3.9 +	21.1 ± 15.4 ++	29.1 ± 32.3 ++
* Mean ± SD	2.3 ± 0.3	5.4 ± 2.4	3.2 ± 0.4	3.0 ± 0.3

* = Mean data obtained following administration of 1 g of paracetamol to healthy male subjects. The paracetamol sulphate and glucuronide elimination half life was calculated over 3-8 and 4-12 h respectively.

\+ = Significantly different from healthy subjects; $P<0.005$

++ = Significantly different from healthy subjects; $P<0.001$

TABLE 5.7 Area Under the Plasma Concentration-time Curves of Paracetamol and its Sulphate and Glucuronide Conjugates in 7 Non-dialysis Patients Following Ingestion of 1 g of Paracetamol

	Paracetamol		Paracetamol Sulphate	Paracetamol Glucuronide
Patient Number	AUC (0-24 h) ($\mu g.h.ml^{-1}$)	AUC (8-24 h) ($\mu g.h.ml^{-1}$)	AUC (0-24 h) ($\mu g.h.ml^{-1}$)	AUC (0-24 h) ($\mu g.h.ml^{-1}$)
01	63.5	13.4	125.0	389.5
02	68.4	20.1	190.9	343.4
03	42.1	5.9	187.6	333.4
04	47.1	16.5	232.4	358.3
05	63.7	18.3	204.8	306.0
06	80.6	9.7	465.0	738.3
07	72.1	18.8	217.3	551.1
Mean $\pm$ SD	62.5 $\pm$ 13.6 +	14.7 $\pm$ 5.3 +	231.9 $\pm$ 108.3 ++	431.4 $\pm$ 157.4 ++
* Mean $\pm$ SD	45.3 $\pm$ 10.6	8.0 $\pm$ 4.3	22.4 $\pm$ 5.1	65.0 $\pm$ 15.9

* = Mean data obtained following administration of 1 g of paracetamol to 10 healthy male subjects

\+ = Significantly different from healthy subjects; $P<0.05$

++ = Significantly different from healthy subjects; $P<0.001$

TABLE 5.8 Area Under the Plasma Concentration-time Curve of Paracetamol and its Sulphate and Glucuronide Conjugates in 5 Haemodialysis Patients Following Ingestion of 1 g of Paracetamol

Patient Number	Paracetamol AUC (0-24 h) ($\mu g.h.ml^{-1}$)	Paracetamol AUC (8-24 h) ($\mu g.h.ml^{-1}$)	Paracetamol Sulphate AUC (0-24 h) ($\mu g.h.ml^{-1}$)	Paracetamol Glucuronide AUC (0-24 h) ($\mu g.h.ml^{-1}$)
08	31.6	-	488.1	980.6
09	39.6	-	399.8	831.7
010	67.6	18.4	379.3	517.0
011	70.6	16.5	622.0	429.7
012	69.6	16.0	358.6	606.5
Mean $\pm$ SD	55.7 $\pm$ 18.8	17.0 $\pm$ 1.3 +	449.6 $\pm$ 108.3 ++	673.1 $\pm$ 227.9 ++
* Mean $\pm$ SD	45.3 $\pm$ 10.6	8.0 $\pm$ 4.3	22.4 $\pm$ 5.1	65.0 $\pm$ 15.9

* = Mean data obtained following administration of 1 g of paracetamol to 10 healthy male subjects

\+ = Significantly different from healthy subjects; $P<0.02$

++ = Significantly different from healthy subjects; $P<0.005$

Paracetamol

Paracetamol absorption was rapid in renal patients and the mean individual peak plasma paracetamol concentration in non-dialysis and dialysis patients was 17.3 ± 8.2 $\mu g.ml^{-1}$ (range 7.8-30.3) and 18.7 ± 5.2 $\mu g.ml^{-1}$ (range 15.3-27.9) respectively. The mean individual time to reach peak paracetamol concentration was 0.54 ± 0.44 h and 0.45 ± 0.32 h in non-dialysis and dialysis patients respectively. These values were not significantly different from those obtained in healthy male subjects which were 20.0 ± 8.4 $\mu g.ml^{-1}$ and 0.35 ± 0.17 h respectively. From 2-8 h mean plasma paracetamol concentrations declined linearly when plotted on a semilogarithmic graph. The mean plasma elimination half life over this period was similar in healthy subjects, non-dialysis and dialysis patients (2.3 ± 0.3, 2.5 ± 0.3 and 2.1 ± 0.4 h respectively). From 8-24 h the plasma paracetamol concentrations declined more slowly in both groups of renal patients. This was reflected in their combined mean plasma paracetamol elimination half life over this period which was 9.2 ± 4.8 h (range 5.3-19.7) compared to 5.4 ± 2.4 h in normal subjects; ($P<0.002$). The mean area under the plasma paracetamol concentration-time curve from 8-24 h for both renal groups combined was significantly greater than in normal subjects (13.4 ± 6.2 $\mu g.h.ml^{-1}$ compared to 8.0 ± 4.3 $\mu g.h.ml^{-1}$; $P<0.005$). The mean 0-24 h area under the plasma paracetamol concentration-time curve was significantly increased in renal patients compared to healthy subjects (59.7 ± 15.5 $\mu g.h.ml^{-1}$ compared to 45.3 ± 10.6 $\mu g.h.ml^{-1}$; $P<0.05$).

Paracetamol Sulphate

The plasma concentrations of paracetamol sulphate rose and exceeded those of the parent drug and were greatly elevated in patients with renal impairment. In Patient 011 sulphate conjugation was the major metabolic route. In non-dialysis and dialysis patients the mean individual peak plasma paracetamol sulphate concentration was significantly increased, at 13.7 $\pm$ 5.9 $\mu g.ml^{-1}$ (range 9.4-26.9) and 20.7 $\pm$ 5.4 $\mu g.ml^{-1}$ (range 16.4-30.1) respectively, compared to 3.5 $\mu g.ml^{-1}$ in normal subjects ($P<0.005$). The mean individual times to reach peak plasma paracetamol sulphate concentration in non-dialysis and dialysis patients were delayed correspondingly (5.6 $\pm$ 1.5 and 8.2 $\pm$ 0.8 h respectively, compared to 1.1 h in normal subjects; $P<0.005$). In the patients with impaired renal function the elimination of paracetamol sulphate was much slower and the mean elimination half life from 8-24 h in non-dialysis patients was significantly increased compared to the mean elimination half life from 3-8 h in normal subjects (21.1 $\pm$ 15.4 h and 3.2 $\pm$ 0.4 h respectively; $P<0.001$). The elimination of paracetamol sulphate in haemodialysis patients was negligible with no appreciable fall in plasma concentration up to 24 h. There was gross retention of paracetamol sulphate as reflected by the mean area under the 0-24 h plasma paracetamol sulphate concentration-time curves for non-dialysis and dialysis patients (231.9 $\pm$ 108.3 and 449.6 $\pm$ 108.3 $\mu g.h.ml^{-1}$ respectively compared to 22.4 $\pm$ 5.1 $\mu g.h.ml^{-1}$ in the healthy male subjects; $P<0.005$). These differences represented a 10 and 20-fold increase and in non-dialysis patients there was a significant

correlation between plasma creatinine concentration and area under the plasma paracetamol sulphate concentration-time curve ($r = 0.84$, $P<0.02$).

Paracetamol Glucuronide

As with paracetamol sulphate, plasma concentrations of the glucuronide conjugate were grossly elevated in the patients with impaired renal function. In the non-dialysis and dialysis patients the mean individual peak plasma paracetamol glucuronide concentrations were 25.2 $\pm$ 8.9 $\mu g.ml^{-1}$ (range 18.8-42.6) and 30.6 $\pm$ 8.1 $\mu g.ml^{-1}$ (range 21.5-36.7), compared to 9.4 $\mu g.ml^{-1}$ in normal subjects ($P<0.005$). The mean individual times to reach peak paracetamol glucuronide concentration in non-dialysis and dialysis patients were significantly delayed (6.3 $\pm$ 1.6 and 15.0 $\pm$ 8.4 h respectively, compared to 2.0 h in normal subjects; $P<0.005$). The mean paracetamol glucuronide elimination half life from 8-24 h in non-dialysis patients was 29.1 $\pm$ 32.3 h compared to 3.0 $\pm$ 0.3 h over the period 4-12 h in normal subjects ($P<0.001$). The elimination of paracetamol glucuronide in haemodialysis patients was negligible with no significant fall in plasma concentrations up to 24 h. In the non-dialysis and dialysis patients the mean area under the 0-24 h plasma paracetamol glucuronide concentration-time curves were 431.4 $\pm$ 157.4 and 673.1 $\pm$ 227.9 $\mu g.h.ml^{-1}$ respectively compared to 65.0 $\pm$ 15.9 $\mu g.h.ml^{-1}$ in the healthy male subjects ($P<0.005$). In the non-dialysis patients there was a significant correlation between the plasma creatinine concentration and

area under the plasma paracetamol glucuronide concentration-time curve ($r = 0.94$; $P<0.01$).

Paracetamol Cysteine and Mercapturic Acid Conjugates

Plasma concentrations of paracetamol cysteine and mercapturic acid could not be measured in healthy subjects and the concentrations in the patients with renal failure were low. The plasma concentrations of the cysteine conjugate were higher than those of the mercapturic acid conjugate and in the non-dialysis patients both conjugates disappeared at similar rates. In haemodialysis patients the rate of disappearance of each conjugate was slower than in the non-dialysis patients and there was no significant fall in the plasma mercapturic acid concentration up to 24 h.

ii) Renal Excretion of Paracetamol and its Metabolites in Non-Dialysis Patients

Individual total recoveries and 24 h fractional urinary recoveries of paracetamol and its metabolites following ingestion of 1 g of paracetamol in 7 non-dialysis patients are shown in Table 5.9 and the mean cumulative urinary recovery of paracetamol and its metabolites up to 24 h in Figure 5.3. The mean percentages of total drug excreted in divided urine collections as paracetamol and its metabolites are shown in Table 5.10 and Figure 5.4. Individual urinary excretion data are shown in Appendices 5.5-5.10.

TABLE 5.9 Twenty Four Hour Urinary Recovery and Fractional Urinary Recovery of Paracetamol and its Sulphate, Glucuronide, Cysteine and Mercapturic Acid Conjugates in 7 Non-dialysis Patients Following Ingestion of 1 g of Paracetamol

Patient Number	24 h Fractional Urinary Recovery (%)						
	Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide	Paracetamol Cysteine	Paracetamol Mercapturic Acid	Cysteine + Mercapturic Acid	Total (%)
01	1.6	23.6	67.3	3.1	4.4	7.5	88.5
02	2.8	29.5	60.5	3.4	3.6	6.9	56.2
03	2.1	29.9	60.1	3.2	3.2	6.4	55.5
04	-	41.9	50.6	4.3	3.2	7.5	62.3
05	4.2	36.0	54.5	2.2	2.8	5.0	49.9
06	3.1	26.9	64.1	3.5	2.4	5.9	45.4
07	1.6	27.5	69.5	1.3	-	1.3	30.5
Mean ± SD	2.6 ± 1.0	30.8 ± 6.2	60.9 ± 6.7	3.0 ± 1.0	2.8 ± 1.4	5.8 ± 2.2	+ 55.5 ± 17.8
* Mean ± SD	4.4 ± 1.5	28.7 ± 4.5	60.3 ± 6.8	2.8 ± 1.2	4.1 ± 1.4	6.8 ± 2.5	82.6 ± 6.3

* = Mean data obtained following ingestion of 1 g of paracetamol to 10 healthy male subjects

+ = Significantly different from healthy subjects; $P<0.02$

FIGURE 5.3 Mean Cumulative Urinary Recovery of Paracetamol and its Metabolites up to 24 h in 7 Non-dialysis Patients Following Ingestion of 1 g of Paracetamol

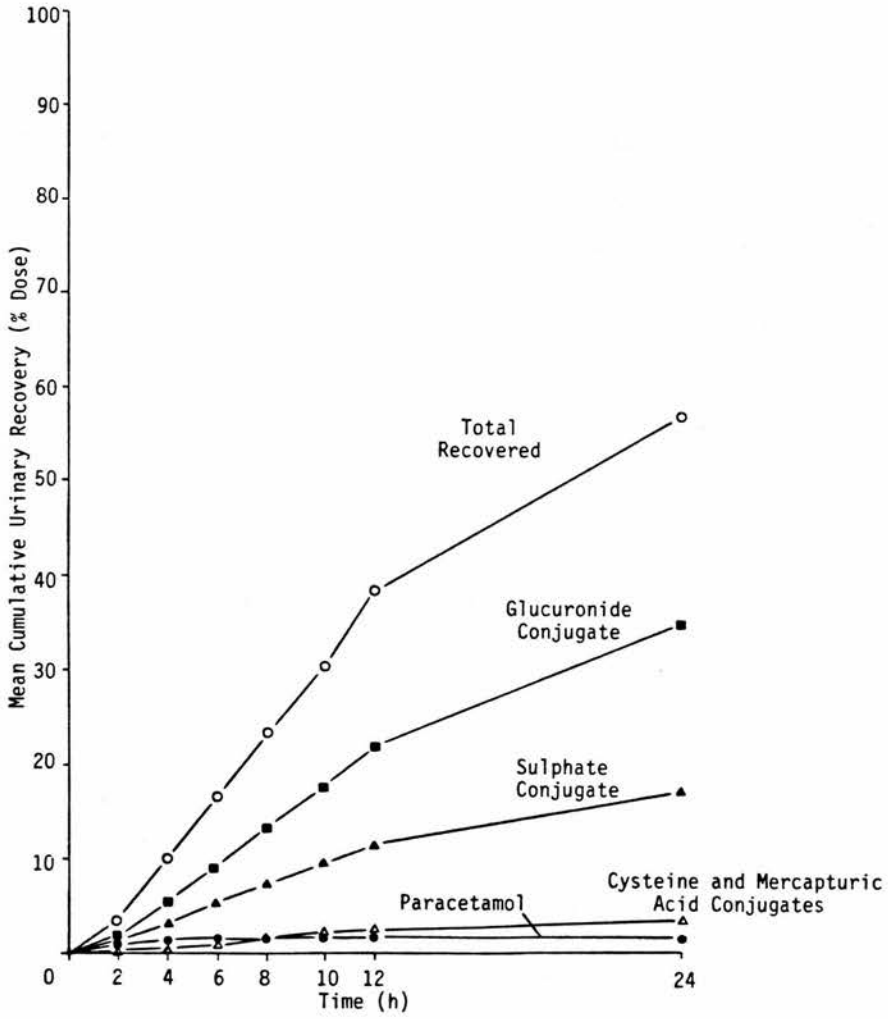
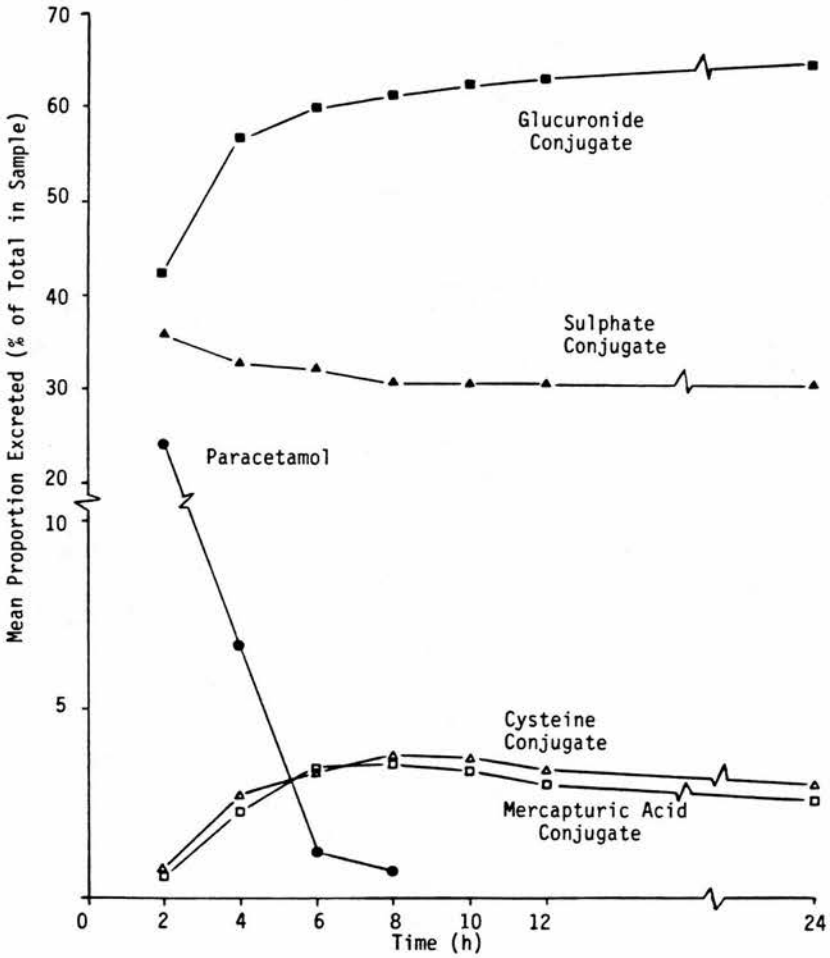


TABLE 5.10 Mean Percentage of Total Drug Excreted in Divided Urine Collections as Paracetamol and its Metabolites Following Ingestion of 1 g of Paracetamol in 7 Non-dialysis Patients

Time After Ingestion (h)	Mean Proportion Excreted (as Percentage of Total in Sample) + SD				
	Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide	Paracetamol Cysteine	Paracetamol Mercapturic Acid
0-2	+24.2 ± 9.4	35.7 ± 11.7	+42.2 ± 8.7	0.8 ± 0.8	0.6 ± 0.8
2-4	+6.7 ± 3.9	32.7 ± 8.2	56.6 ± 8.2	2.7 ± 1.1	2.3 ± 1.5
4-6	1.3 ± 2.1	+32.3 ± 5.8	59.7 ± 6.3	3.3 ± 1.3	3.4 ± 1.9
6-8	0.7 ± 1.7	+30.9 ± 5.8	61.1 ± 8.1	3.8 ± 1.6	3.6 ± 1.9
8-10	-	30.7 ± 6.2	62.1 ± 7.4	3.7 ± 1.0	3.4 ± 1.6
10-12	-	30.6 ± 5.4	63.0 ± 6.6	3.4 ± 1.1	3.0 ± 1.4
12-24	-	30.1 ± 7.5	64.4 ± 7.9	3.0 ± 0.7	2.6 ± 1.2

+ = Significantly different from healthy subjects; $P<0.05$

FIGURE 5.4 Changes in the Mean Proportional Urinary Excretion of Paracetamol and its Sulphate, Glucuronide, Cysteine and Mercapturic Acid Conjugates Following Ingestion of 1 g of Paracetamol in 7 Non-dialysis Patients



Urinary Recovery of Paracetamol and its Sulphate, Glucuronide, Cysteine and Mercapturic Acid Conjugates

The mean 24 h urinary recovery of the administered dose was significantly reduced in the non-dialysis patients 55.5 ± 17.8% of the dose (range 30.5-88.5) compared to 82.6% of the dose in healthy subjects ($P<0.02$). There was a significant negative correlation between the plasma creatinine concentration and the total urinary recovery ($r = -0.77$; $P<0.05$). The mean percentage of the dose recovered up to 24 h in non-dialysis patients as paracetamol and its sulphate, glucuronide, cysteine and mercapturic acid conjugates represented 1.4, 16.8, 34.9, 1.8 and 1.8% of the dose respectively.

Fractional Excretion of Paracetamol and Its Metabolites

The mean 24 h fractional urinary recovery of paracetamol and its sulphate, glucuronide, cysteine and mercapturic acid conjugates in the non-dialysis patients was 2.6 ± 1.0, 30.8 ± 6.2, 60.9 ± 6.7, 3.0 ± 1.0 and 2.8 ± 1.4% respectively which was similar to healthy subjects. The fractional urinary excretion pattern of paracetamol and its sulphate, glucuronide, cysteine and mercapturic acid conjugates in each divided urine collection in non-dialysis patients were generally similar to those in healthy subjects. The mean fraction recovered as paracetamol from 0-4 h and as paracetamol sulphate from 4-8 h however, were significantly increased and the mean fraction as paracetamol glucuronide from 0-2 h significantly reduced in non-dialysis patients. The mean fraction excreted as paracetamol cysteine was greater than that as paracetamol mercapturic acid from 6-24 h.

Renal Clearances of Paracetamol and its Metabolites

The renal clearances of paracetamol and its sulphate and glucuronide conjugates following ingestion of 1 g of paracetamol in non-dialysis patients are shown in Table 5.11.

In non-dialysis patients the mean renal clearances of the sulphate and glucuronide conjugates of paracetamol were greatly reduced compared to healthy subjects (15.6 $\pm$ 8.1 and 15.5 $\pm$ 7.1 $ml.min^{-1}$ versus 180.5 $\pm$ 31.6 and 136.9 $\pm$ 32.0 $ml.min^{-1}$ respectively; $P<0.001$) and there were significant negative correlations with the plasma creatinine concentrations ($r = -0.91$ and -0.81; $P<0.05$). Although the mean renal clearance of paracetamol was significantly reduced in non-dialysis patients (4.2 $\pm$ 2.0 $ml.min^{-1}$ compared to 15.2 $\pm$ 7.0 $ml.min^{-1}$ in healthy subjects; $P<0.002$) it was not reduced to the same extent as the sulphate and glucuronide conjugates and there were no significant correlations with the plasma creatinine or creatinine clearance. As in normal subjects, the renal clearance of paracetamol correlated with the urine flow rate in the non-dialysis patients ($r = 0.76$; $P<0.05$) but no such correlation existed for the sulphate or glucuronide conjugates. There was no significant correlation between the renal clearances of paracetamol and its sulphate and glucuronide conjugates and the urinary pH.

The mean renal clearance of paracetamol cysteine and mercapturic acid in the non-dialysis patients were 35 $\pm$ 25 and 80 $\pm$ 45 $ml.min^{-1}$ respectively.

TABLE 5.11 Renal Clearance of Paracetamol and its Sulphate and Glucuronide Conjugates in 7 Non-dialysis Patients Following Ingestion of 1 g of Paracetamol

Patient Number	0-8 h Renal Clearance ($ml.min^{-1}$)		
	Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide
01	4.5	27.8	23.3
02	5.4	16.2	17.1
03	5.5	19.2	21.4
04	-	13.6	10.3
05	7.2	20.9	21.8
06	3.4	4.8	7.4
07	1.5	6.7	6.9
Mean ± SD	4.6 ± 2.0 +	15.6 ± 8.1 ++	15.5 ± 7.1 ++
Mean ± SD *	15.2 ± 7.0	180.5 ± 31.6	136.9 ± 32.0

* = Mean 0-8 h renal clearance data obtained following administration of 1 g of paracetamol to 10 healthy male subjects

\+ = Significantly different from healthy subjects; $P<0.002$

++ = Significantly different from healthy subjects; $P<0.001$

d) Discussion

The absorption of paracetamol was normal in the patients with renal failure, but the elimination of paracetamol metabolites and paracetamol itself were impaired. The control group consisted of healthy subjects who were younger than the renal patients and was, therefore, not ideal. However, it is unlikely that the differences observed between the groups could be explained by factors other than renal disease. There was gross retention of the paracetamol sulphate and glucuronide conjugates in the patients with renal failure. The mean peak plasma concentrations, times to reach peak plasma concentrations, areas under the plasma concentration-time curves and apparent elimination half life values for the paracetamol sulphate and glucuronide conjugates were all increased compared to those in healthy subjects. The abnormalities in all cases were greatest in the haemodialysis patients. There was negligible elimination of the sulphate and glucuronide conjugates of paracetamol in the haemodialysis patients and this was consistent with previous studies (Oie et al, 1975; Lowenthal et al, 1976).

The plasma concentrations of the cysteine and mercapturic acid conjugates in patients with impaired renal function were low and the concentrations of the cysteine conjugate were higher than those of the mercapturic acid conjugate. In the non-dialysis patients these metabolites disappeared at similar rates. In the haemodialysis patients elimination of the paracetamol mercapturic acid conjugate was negligible but the paracetamol cysteine conjugate disappeared

slowly after approximately 8 h, indicating removal by a route other than urinary excretion.

Paracetamol was absorbed rapidly in the patients with renal failure and from 2-8 h it disappeared from plasma at the same rate in healthy subjects, non-dialysis and haemodialysis patients. From 8-24 h, however, paracetamol disappeared from plasma more slowly in the renal patients with a significant increase in the elimination half life and area under the plasma paracetamol concentration-time curve over this period compared to healthy subjects. This extended elimination phase in the patients with impaired renal function may be explained by slower release of residual parent compound from peripheral tissues back to the circulation, or more likely enterohepatic circulation of the paracetamol conjugates with regeneration of the parent drug. Enterohepatic circulation has previously been implicated in the unexpected impaired elimination of drugs such as oxazepam, diflunisal and clofibric acid in patients with renal failure (Odar-Cederlof et al, 1977; Verbeek et al, 1979; Faed and McQueen, 1979). Like paracetamol, these drugs are predominantly metabolised to glucuronide conjugates which in renal failure undergo enterohepatic circulation with subsequent hydrolysis which releases the parent compound in the gut. This is subsequently reabsorbed (Verbreek et al, 1981). Labile ester glucuronide conjugates have usually been implicated in this mechanism, and esterases rather than glucuronidase are responsible for hydrolysis (Meffin et al, 1983). However, paracetamol is metabolised to the more stable ether glucuronide conjugate. Paracetamol glucuronide is normally excreted into bile only to a limited extent in man

(Jayasinghe et al, 1986) but with the very high concentrations which occur in patients with renal failure hydrolysis of the glucuronide conjugate could explain the elevated plasma paracetamol concentrations from 8-24 h. Biliary excretion of the cysteine conjugate is only a minor route of elimination in man (Seigers et al, 1984; Jayasinghe et al, 1986). In the haemodialysis patients the mean plasma paracetamol cysteine concentrations declined from 8 h, which coincided with the decrease in disappearance of paracetamol. It may be, therefore, that the cysteine conjugate undergoes enterohepatic circulation in the haemodialysis patients. Enterohepatic circulation of paracetamol glutathione has been demonstrated in rats (Siegers et al, 1983) but there is no evidence for this in man.

The residual plasma paracetamol concentrations observed in the present study were low and clinically insignificant. However, paracetamol concentrations would probably be much higher with the gross cumulation of metabolites which must occur in renal patients on long term therapy. Multiple dose studies require to be performed (Prescott et al, 1989).

In the non-dialysis patients the mean 24 h urinary recovery of the administered dose was significantly reduced compared to healthy subjects but the fractional urinary recovery of paracetamol and its metabolites was similar. The ability to metabolise paracetamol by each pathway was, therefore, unaffected in the patients with chronic renal failure. Similar results have been reported in patients with either analgesic nephropathy or nephropathy due to other causes

(Thomas et al, 1980). In particular, the recovery of the cysteine and mercapturic acid conjugates was not increased in patients with renal disease and this indicates that conversion to the toxic intermediate was not enhanced. The mechanism by which paracetamol induces renal failure in overdosage is thought to be similar to the mechanism described for the liver. In the kidney a 50% reduction in renal gluthatione results in binding of the reactive intermediate to vital renal macromolecules (Mitchell et al, 1977).

In the non-dialysis patients the mean plasma concentrations and the mean total urinary recovery of paracetamol cysteine were greater than those of the mercapturic acid conjugate. Although the mean fractional urinary recovery of these metabolites was not significantly different from those obtained in healthy subjects, the urinary recovery of the mercapturic acid conjugate is normally greater than that of the cysteine conjugate in healthy subjects. It is possible that acetylation of the paracetamol cysteine conjugate to form paracetamol mercapturic acid is reduced in patients with renal failure. In this context acetylation in patients with renal disease may be normal or slowed (Reidenberg, 1977b).

In the non-dialysis patients the mean renal clearances of the cysteine and mercapturic acid conjugates were greater than the glomerular filtration rate. A possible explanation may be that the kidney is capable of forming these metabolites from precursors (Jones et al, 1979; Ross et al, 1980; Newton et al, 1982). However, one patient on

the present study was anephric and the plasma cysteine and mercapturic acid conjugate concentrations were comparable to those in the other patients.

In the non-dialysis patients the renal clearances of paracetamol sulphate and glucuronide were greatly reduced and were correlated with the plasma creatinine concentration but not with urine flow rate or pH. The renal clearance of paracetamol was also reduced in the non-dialysis patients but not to the extent observed with the sulphate and glucuronide conjugates. In addition, the renal clearance of paracetamol was unrelated to plasma creatinine and urinary pH, but was dependent on the urine flow rate. In healthy subjects paracetamol is freely filtered at the glomerulus and then passively reabsorbed, while the glucuronide and sulphate conjugates are actively secreted by the renal tubule (Prescott and Wright, 1973; Duggin and Mudge, 1975). The disproportionate decrease in the renal clearance of the conjugates suggests that the capacity for active tubular transport is reduced to a greater extent than passive reabsorption in patients with chronic renal failure. In addition to tubular defects caused by disease, the retention of endogenous organic anions may result in competition or saturation of the tubular transport system for acids.

e) Summary

The absorption, distribution, metabolism and elimination of paracetamol were studied in 7 non-dialysis and 5 haemodialysis patients with chronic renal failure after oral administration of an effervescent formulation of 1 g of paracetamol. Results were compared with those obtained following oral administration of an effervescent formulation of 1 g of paracetamol to 10 healthy male subjects.

The absorption of paracetamol was normal in the patients with impaired renal function but there was gross retention of paracetamol metabolites and the late elimination of the parent compound was also impaired. The retention of metabolites was greatest in haemodialysis patients and their elimination in this group was negligible.

From 2-8 h paracetamol disappeared from plasma at the same rate in healthy subjects and in both groups of patients with chronic renal failure. However, paracetamol elimination was delayed from 8-24 h in the renal failure patients. The cysteine and mercapturic acid conjugates of paracetamol were present at low concentrations in plasma in the patients with impaired renal function and both metabolites were eliminated at similar rates in the non-dialysis patients. In the haemodialysis patients elimination of paracetamol mercapturic acid was negligible but the plasma paracetamol cysteine concentrations gradually decreased over the period 8-24 h. The cysteine conjugate may undergo limited enterohepatic circulation with the subsequent reabsorption of the parent compound. Limited enterohepatic circu-

lation of this and the other metabolites of paracetamol which were present at elevated concentrations may therefore explain the delayed paracetamol elimination phase.

In the non-dialysis patients the mean 24 h urinary recovery of the administered dose was less than in healthy subjects. Paracetamol metabolism, however, was not abnormal as the fractional urinary recoveries of paracetamol and its metabolites in these patients and healthy subjects were similar.

One haemodialysis patient was anephric and the plasma concentrations of paracetamol and its metabolites were comparable to those of the other haemodialysis patients. The contribution of the kidney to the overall metabolism of paracetamol appears to be minimal.

The renal clearances of paracetamol and its sulphate and glucuronide conjugates were reduced in the non-dialysis patients. The decreases in the renal clearances of the conjugates were disproportionately greater than those of the parent compound. The capacity for active tubular transport appeared to be reduced to a greater extent than the capacity for passive reabsorption.

In the present study only the disposition of a single dose of paracetamol was investigated in patients with chronic renal failure. On long term therapy, however, paracetamol concentrations would probably be higher and there would be gross cummulation of metabolites. Multiple dose studies require to be performed.

CHAPTER VI

Paracetamol N-Acetyl-D-L-Methionate Disposition in Patients with Impaired Renal Function

PARACETAMOL N-ACETYL-D-L-METHIONATE DISPOSITION IN PATIENTS WITH IMPAIRED RENAL FUNCTION

a) Introduction

The idea to incorporate methionine into paracetamol tablets to make them safer if taken in overdosage was first suggested in 1975 (McLean and Day). Using this concept, Sterling Winthrop combined paracetamol with N-acetyl-D-L-methionine as the N-acetyl-D-L-methionine ester of paracetamol. This tasteless compound hydrolyses to yield equimolar quantities of paracetamol and methionine. Methionine is a glutathione precursor and is effective in preventing severe liver damage and death after paracetamol overdosage (Crome et al, 1976; Hamlyn et al, 1981; Vale et al, 1981).

After investigation of the disposition of the ester in healthy subjects (Chapter IV), it was administered to 7 non-dialysis and 5 haemodialysis patients with stable renal impairment.

The equivalent of 1 g of paracetamol was administered as 1) paracetamol and 2) paracetamol N-acetyl-D-L-methionate. At least one week was allowed between each administration. This chapter describes the disposition of paracetamol in patients with renal impairment following administration of paracetamol N-acetyl-D-L-methionate. Data obtained from

patients with renal failure who received 1 g of paracetamol alone (Chapter V) were used for comparison.

b) Methods

Patients

The same 11 men and one woman with chronic renal impairment previously described in Chapter V were entered onto the study. Their mean age was 52 ± 12 years and body weight 75 ± 8 kg. Seven had moderate to severe renal failure but were not being treated with dialysis and 5 with more advanced disease were receiving long term dialysis 2 or 3 times a week. Clinical details of these patients are summarised in Table 5.1. Informed consent was given by each patient and the study was approved by the local Ethics Committee.

Administration and Sampling

Patients attended the University Department of Clinical Pharmacology or the Medical Renal Unit after fasting overnight, and an in-dwelling cannula was inserted into a forearm vein. Haemodialysis patients attended on an interdialysis day. Sachets containing 2.146 g of an effervescent formulation of paracetamol N-acetyl-D-L-methionate (equivalent to 1 g of paracetamol) were supplied by Sterling Winthrop, Alnwick. Each patient received the contents of one sachet orally in 200 ml of water. The patients remained recumbent for 3 h and lunch and supper were allowed at 4 and 8 h respectively. Each

patient received meals appropriate to their condition (eg low protein, controlled sodium or potassium intake). Fluid intake was restricted as necessary in haemodialysis patients and non dialysis patients received 200 ml of water every 2 h up to 12 h.

5 ml venous blood samples were taken into heparinised tubes at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 24 h. The blood was centrifuged at 1400 x g for 10 min and the plasma separated and stored at -20°C. Urine samples from non-dialysis patients were collected immediately before drug administration and from 0-2, 2-4, 4-6, 6-8, 8-10, 10-12 and 12-24 h after dosing. The total urine volume and pH were recorded and 50 ml aliquots were stored at -20°C prior to analysis. Individual urine volumes and pH values are shown in Appendix 6.9.

Drug Analysis

Plasma concentrations of paracetamol, paracetamol sulphate and paracetamol glucuronide and urinary concentrations of paracetamol, paracetamol sulphate, paracetamol glucuronide, paracetamol cysteine and paracetamol mercapturic acid were measured by HPLC as described in Chapter II, Section 1. Significant blank values were obtained in some patients and these were subtracted to give corrected concentrations. Patient 07 had a high urinary protein concentration and the paracetamol sulphate peak was poorly resolved. The amount of sulphate excreted was therefore estimated from the relative areas under the plasma concentration-time curves of the sulphate and glucu-

ronide conjugates and the urinary recovery of paracetamol glucuronide, assuming both metabolites had the same renal clearance and volume of distribution.

Data Analysis

i) Plasma Concentrations of Paracetamol and its Metabolites

Mean plasma concentrations of paracetamol and its sulphate and glucuronide conjugates were plotted on a semilogarithmic graph against time and Cmax, Tmax, paracetamol $t_{\frac{1}{2}}$ (2-8 h), paracetamol sulphate and glucuronide $t_{\frac{1}{2}}$ (8-24 h) and AUC (0-24 h) values were determined as described in detail in Chapter III. The relative paracetamol bioavailability was calculated by dividing the 0-24 h area under the plasma paracetamol concentration-time curve after administration of 2.146 g paracetamol N-acetyl-D-L-methionate by the corresponding plasma paracetamol area under the curve following administration of 1 g of paracetamol alone to the same renal failure patient.

ii) Non Dialysis Patients - Urinary Excretion of Paracetamol and its Metabolites

Each subject received 2.146 g of paracetamol N-acetyl-D-L-methionate which, if completely hydrolysed would yield 1 g of paracetamol. As only paracetamol and its metabolites were measured in urine, the percentage of the paracetamol component of the dose (1 g) recovered in

urine in 24 h following administration of paracetamol N-acetyl-D-L-methionate was determined.

In addition, the fractional urinary recovery in 24 h and in each divided urine collection, and the 0-8 h renal clearances of paracetamol and its sulphate and glucuronide conjugates were calculated as described in Chapter III.

iii) Statistical Analysis

Mean values ± standard deviation (SD) are presented. The relationship between quantitative observations on each subject (bivariate data) was determined by calculation of the correlation coefficient (r). The correlation coefficient was tested for statistical significance using tabulated values (Geigy Scientific Tables, P61). Differences between means were compared by the Wilcoxon paired sample rank test for statistical significance.

c) Results

i) Plasma Concentrations of Paracetamol and its Sulphate and Glucuronide Conjugates

Mean plasma concentrations of paracetamol and its sulphate and glucuronide conjugates following oral administration of 2.146 g of paracetamol N-acetyl-D-L-methionate to 7 non dialysis and 5 haemodialysis patients are shown in Table 6.1, Figure 6.1 and Table 6.2,

TABLE 6.1 Mean Plasma Concentrations of Paracetamol and its Sulphate and Glucuronide Conjugates in 7 Non-dialysis Patients Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate

Time After Ingestion (h)	Plasma Concentration ($\mu g.ml^{-1}$)		
	Paracetamol (Mean ± SD)	Paracetamol Sulphate (Mean ± SD)	Paracetamol Glucuronide (Mean ± SD)
0.25	4.5 ± 3.6	0.6 ± 0.7	0.4 ± 0.4
0.5	8.9 ± 3.3	3.6 ± 1.2	1.8 ± 1.2
0.75	9.5 ± 2.5	5.0 ± 1.9	3.4 ± 1.9
1.0	10.4 ± 3.0	6.2 ± 2.2	4.9 ± 2.6
1.5	9.9 ± 2.6	8.2 ± 2.8	8.4 ± 4.5
2.0	8.7 ± 2.3	9.2 ± 3.2	11.2 ± 5.4
3.0	7.2 ± 2.1	11.0 ± 4.2	16.0 ± 6.2
4.0	5.9 ± 1.6	12.1 ± 4.1	19.0 ± 6.2
5.0	4.4 ± 1.3	12.7 ± 5.0	21.1 ± 7.5
6.0	3.3 ± 0.9	12.5 ± 5.1	21.2 ± 6.6
7.0	2.8 ± 0.9	12.5 ± 5.7	21.7 ± 7.6
8.0	2.3 ± 0.7	12.6 ± 6.5	21.7 ± 8.7
9.0	1.9 ± 0.5	11.9 ± 6.2	21.4 ± 8.0
10.0	1.5 ± 0.4	11.4 ± 5.3	20.1 ± 6.7
12.0	1.2 ± 0.3	10.2 ± 5.3	18.5 ± 7.2
24.0	0.6 ± 0.1	6.5 ± 4.2	12.4 ± 7.0

FIGURE 6.1 Mean Plasma Concentrations of Paracetamol and its Sulphate and Glucuronide Conjugates in 7 Non-dialysis Patients Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate

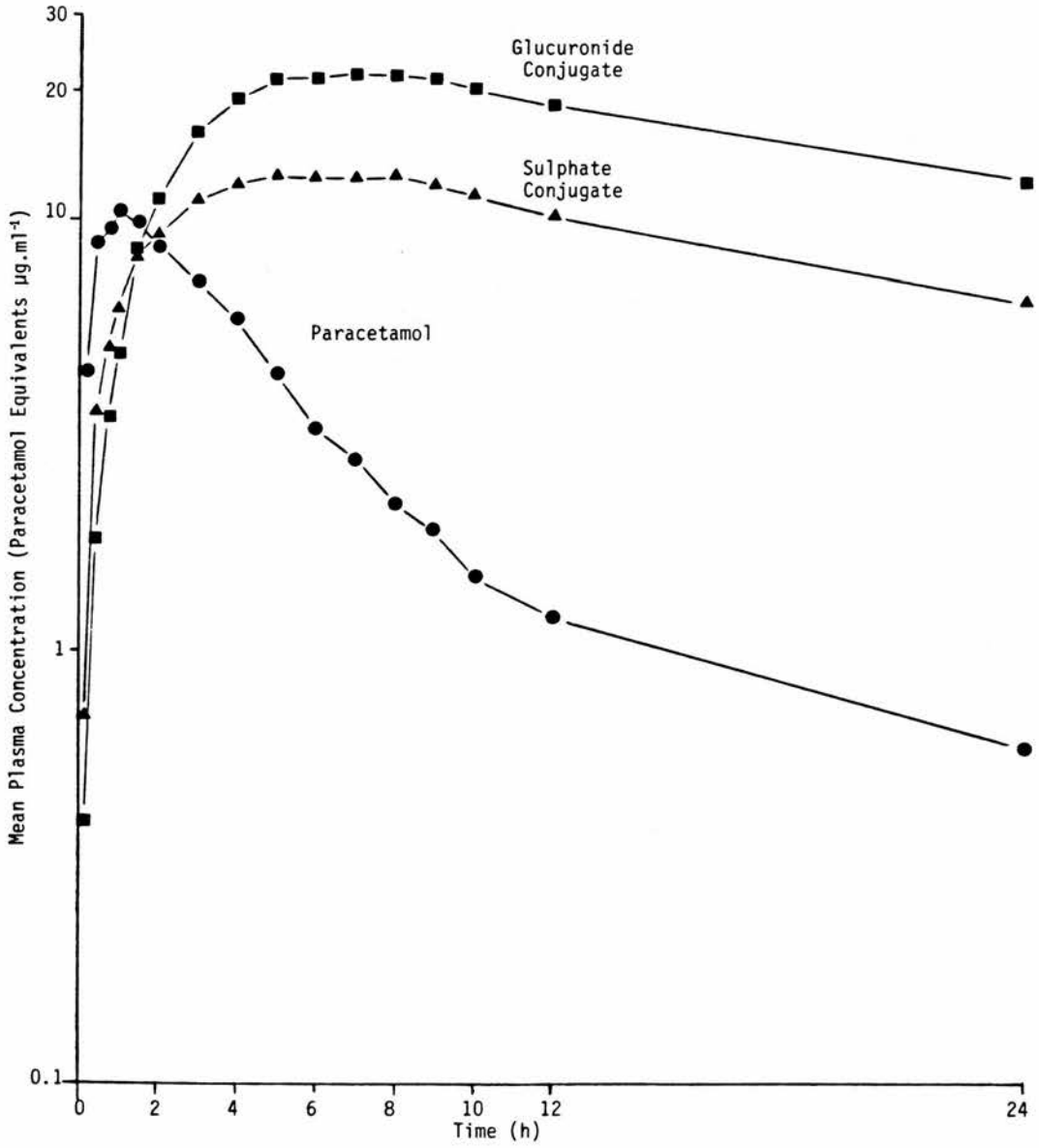


TABLE 6.2 Mean Plasma Concentrations of Paracetamol and its Sulphate and Glucuronide Conjugates in 5 Haemodialysis Patients Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate

Time After Ingestion (h)	Plasma Concentration ($\mu g.ml^{-1}$)		
	Paracetamol (Mean ± SD)	Paracetamol Sulphate (Mean ± SD)	Paracetamol Glucuronide (Mean ± SD)
0.25	4.2 ± 0.4	1.8 ± 2.0	0.6 ± 0.8
0.5	7.3 ± 0.4	4.4 ± 2.1	1.2 ± 1.1
0.75	8.1 ± 0.4	6.1 ± 2.0	2.4 ± 1.5
1.0	8.8 ± 1.1	7.3 ± 2.4	4.1 ± 1.1
1.5	9.0 ± 1.3	9.5 ± 2.3	6.9 ± 2.1
2.0	8.3 ± 1.4	11.0 ± 2.5	9.7 ± 3.0
3.0	7.0 ± 1.5	13.6 ± 3.1	14.8 ± 5.1
4.0	5.4 ± 1.4	16.2 ± 3.9	19.3 ± 5.8
5.0	3.1 ± 1.0	18.3 ± 5.8	21.7 ± 6.5
6.0	1.9 ± 1.2	18.5 ± 6.2	22.7 ± 6.3
7.0	1.4 ± 1.0	18.9 ± 6.6	23.9 ± 6.5
8.0	1.0 ± 1.0	19.0 ± 6.7	24.9 ± 6.9
9.0	0.7 ± 0.6	18.6 ± 7.0	24.7 ± 6.6
10.0	0.6 ± 0.6	19.1 ± 8.3	25.2 ± 6.1
12.0	0.5 ± 0.6[+]	18.3 ± 7.6	25.8 ± 7.4
24.0	0.3 ± 0.4	17.0 ± 8.4	26.3 ± 7.8

[+] = Mean of 4

Figure 6.2 respectively. Individual values for peak concentration (Cmax) and time to reach peak concentration (Tmax), apparent elimination half life and area under the plasma concentration-time curves for paracetamol and its metabolites in non-dialysis patients are shown in Table 6.3, 6.5 and 6.6 respectively. Individual values for Cmax and Tmax, and area under the plasma concentration-time curves for paracetamol and its metabolites in haemodialysis patients are shown in Tables 6.4 and 6.7 respectively. Plasma concentrations for each subject are shown in Appendices 6.1-6.3.

Paracetamol

Following administration of 2.146 g of paracetamol N-acetyl-D-L-methionate the mean plasma paracetamol profiles were similar in the non-dialysis and dialysis patients, and in most patients plasma paracetamol concentration fluctuated prior to reaching peak concentration. The mean individual peak plasma paracetamol concentration for the non-dialysis and dialysis patients combined following administration of paracetamol N-acetyl-D-L-methionate was significantly reduced (10.2 ± 2.1 $\mu g.ml^{-1}$ compared to 17.9 ± 6.9 $\mu g.ml^{-1}$; $P<0.005$) and delayed (1.31 ± 0.82 h compared to 0.50 ± 0.38 h; $P<0.01$) compared to that obtained following administration of 1 g of paracetamol alone to the same patients with impaired renal function. From 2-8 h plasma paracetamol concentrations declined linearly when plotted on a semilogarithmic graph and the mean plasma paracetamol elimination half life values over this period in the non-dialysis and dialysis

FIGURE 6.2 Mean Plasma Concentrations of Paracetamol and its Sulphate and Glucuronide Conjugates in 5 Haemodialysis Patients Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate

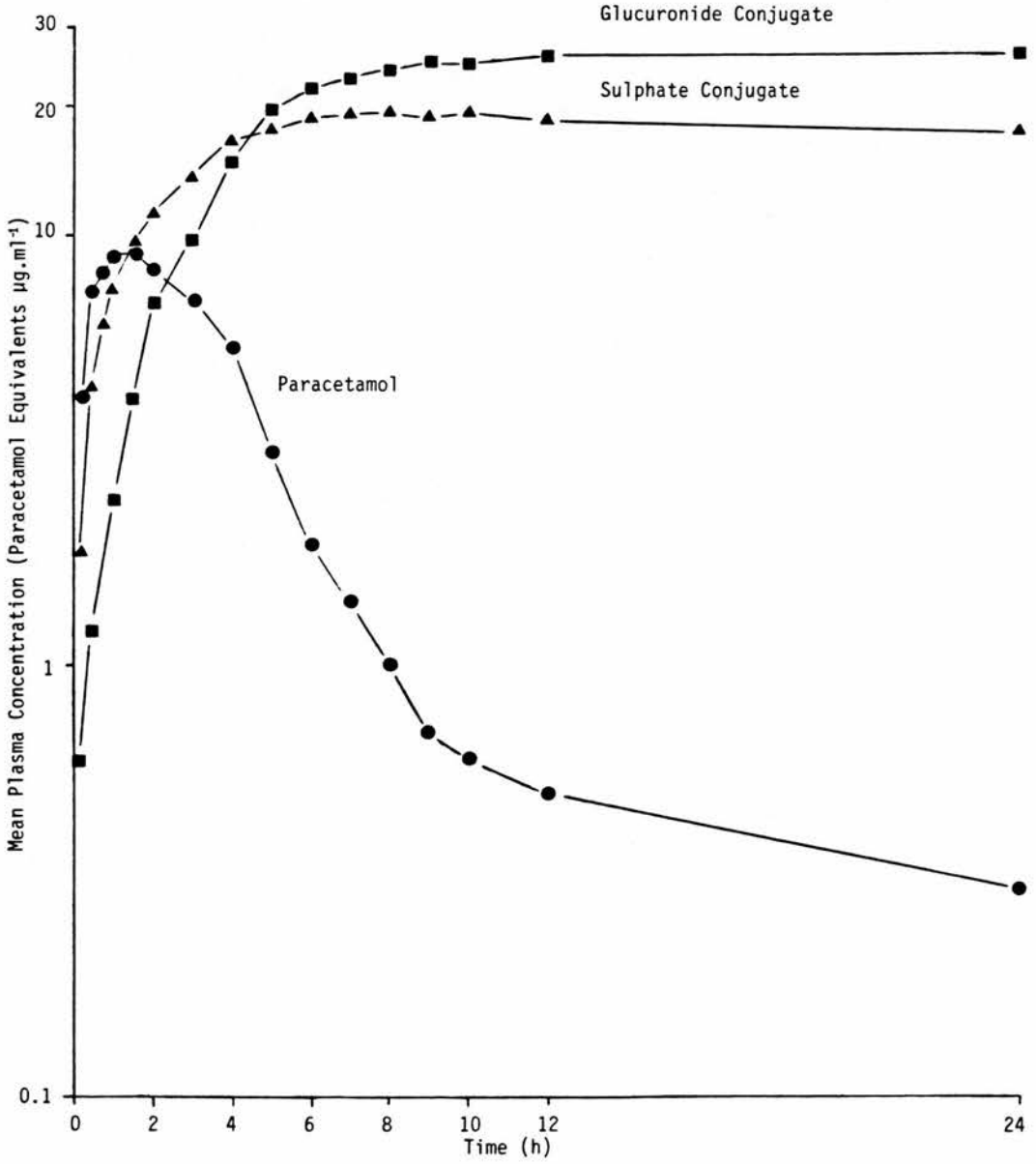


TABLE 6.3 Peak Plasma Concentrations (Cmax) and Time to Reach Peak Plasma Concentrations (Tmax) of Paracetamol and its Sulphate and Glucuronide Conjugates in 7 Non-dialysis Patients Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate

	Paracetamol		Paracetamol Sulphate		Paracetamol Glucuronide	
Subject Number	Cmax ($\mu g.ml^{-1}$)	Tmax (h)	Cmax ($\mu g.ml^{-1}$)	Tmax (h)	Cmax ($\mu g.ml^{-1}$)	Tmax (h)
01	12.1	0.50	9.7	5.00	28.1	5.00
02	12.5	1.00	10.8	5.00	19.7	7.00
03	7.7	1.00	10.3	5.00	18.2	9.00
04	9.1	0.75	12.0	4.00	19.1	9.00
05	11.5	1.00	14.4	6.00	14.7	6.00
06	14.3	1.00	26.5	8.00	40.5	8.00
07	8.7	3.00	10.6	10.00	23.1	7.00
Mean $\pm$ SD	10.8 $\pm$ 2.4 +	1.18 $\pm$ 0.83 +	13.5 $\pm$ 6.0	6.14 $\pm$ 2.12	23.3 $\pm$ 8.7	7.29 $\pm$ 1.50
* Mean $\pm$ SD	17.3 $\pm$ 8.2	0.54 $\pm$ 0.44	13.7 $\pm$ 5.9	5.57 $\pm$ 1.51	25.2 $\pm$ 8.9	6.29 $\pm$ 1.60

* = Mean data obtained following ingestion of 1 g of paracetamol to the same 7 non-dialysis patients

+ = Significantly different from renal failure patients administered 1 g of paracetamol; $P<0.005$

TABLE 6.4 Peak Plasma Concentrations (Cmax) and Time to Reach Peak Plasma Concentrations (Tmax) of Paracetamol and its Sulphate and Glucuronide Conjugates in 5 Haemodialysis Patients Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate

	Paracetamol		Paracetamol Sulphate		Paracetamol Glucuronide	
Subject Number	Cmax ($\mu g.ml^{-1}$)	Tmax (h)	Cmax ($\mu g.ml^{-1}$)	Tmax (h)	Cmax ($\mu g.ml^{-1}$)	Tmax (h)
08	7.8	1.00	18.4	4.00	27.5	24.00
09	8.2	1.00	19.1	8.00	37.9	24.00
010	10.6	1.00	14.4	6.00	23.9	6.00
011	9.5	3.00	33.2	10.00	17.5	10.00
012	10.6	1.50	16.3	12.00	27.3	24.00
Mean $\pm$ SD	+ 9.3 $\pm$ 5.2	+ 1.50 $\pm$ 0.87	20.3 $\pm$ 7.5	8.00 $\pm$ 3.16	26.8 $\pm$ 7.4	17.60 $\pm$ 8.88
* Mean $\pm$ SD	18.7 $\pm$ 5.2	0.45 $\pm$ 0.32	20.7 $\pm$ 5.4	8.20 $\pm$ 0.84	30.6 $\pm$ 8.1	15.00 $\pm$ 8.43

* = Mean data obtained following ingestion of 1 g of paracetamol to the same 5 haemodialysis patients

+ = Significantly different from renal failure patients administered 1 g of paracetamol; $P<0.005$

TABLE 6.5 Apparent Plasma Elimination Half Life of Paracetamol and its Sulphate and Glucuronide Conjugates in 7 Non-dialysis Patients Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate

Patient Number	Paracetamol $t_{\frac{1}{2}}$ (2-8 h) (h)	Paracetamol Sulphate $t_{\frac{1}{2}}$ (8-24 h) (h)	Paracetamol Glucuronide $t_{\frac{1}{2}}$ (8-24 h) (h)
01	2.3	11.0	10.0
02	3.4	13.4	16.0
03	3.5	11.0	12.0
04	2.5	21.2	26.6
05	3.4	11.3	14.8
06	2.6	20.1	23.3
07	2.8++	67.6	96.7
Mean ± SD	2.9 ± 0.5	22.2 ± 20.5	28.5 ± 30.7
* Mean ± SD	2.5 ± 0.3	21.1 ± 15.4	29.1 ± 32.3

* = Mean data obtained following administration of 1 g of paracetamol to the same 7 non-dialysis patients

++ = Calculated over 4-8 h

TABLE 6.6 Area Under the Plasma Concentration-time Curves for Paracetamol and its Sulphate and Glucuronide Conjugates in 7 Non-dialysis Patients Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate

Patient Number	Area Under the Plasma Concentration-time Curve 0-24 h ($\mu g.h.ml^{-1}$)		
	Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide
01	55.6	126.8	363.9
02	76.0	178.5	345.0
03	50.2	185.6	312.1
04	45.4	221.2	351.3
05	69.6	229.6	245.9
06	81.1	462.3	687.3
07	65.8	214.6	454.3
Mean $\pm$ SD	63.3 $\pm$ 13.4	231.2 $\pm$ 107.7	394.3 $\pm$ 143.4
* Mean $\pm$ SD	62.5 $\pm$ 13.6	231.9 $\pm$ 108.3	431.4 $\pm$ 157.4

* = Mean data obtained following administration of 1 g of paracetamol to the same 7 non-dialysis patients

TABLE 6.7 Area Under the Plasma Concentration-time Curves for Paracetamol and its Sulphate and Glucuronide Conjugates in 5 Haemodialysis Patients Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate

Patient Number	Area Under the Plasma Concentration-time Curve 0-24 h ($\mu g.h.ml^{-1}$)		
	Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide
08	30.8	438.4	727.5
09	36.4	406.2	763.5
010	37.6	233.4	472.5
011	59.5	658.9	342.4
012	59.7	355.1	566.9
Mean $\pm$ SD	44.8 $\pm$ 13.8	418.4 $\pm$ 155.4	574.6 $\pm$ 175.7
* Mean $\pm$ SD	55.7 $\pm$ 18.8	449.6 $\pm$ 108.3	673.1 $\pm$ 227.9

* = Mean data obtained following administration of 1 g of paracetamol to the same 5 haemodialysis patients

patients were 2.9 ± 0.5 h (range 2.3-3.5) and 2.0 ± 0.3 h (range 1.5-2.4) respectively. These values were not significantly different from those obtained following administration of 1 g of paracetamol alone (2.5 ± 0.3 and 2.1 ± 0.4 h respectively).

In both the non-dialysis patients and dialysis patients the plasma paracetamol concentrations declined more slowly from 10-24 h but too few data points were available to accurately determine the plasma elimination half life over this period. The mean 0-24 h areas under the plasma paracetamol concentration-time curve following administration of paracetamol N-acetyl-D-L-methionate were 63.3 ± 13.4 $\mu g.h.ml^{-1}$ (range 45.4-81.1) and 44.8 ± 13.8 $\mu g.h.ml^{-1}$ (range 30.8-59.7) in non-dialysis and dialysis patients respectively. These values were not significantly different from those obtained after administration of paracetamol alone to the same patients which were 62.5 ± 13.6 and 55.7 ± 18.8 $\mu g.h.ml^{-1}$ respectively.

The bioavailability of paracetamol after administration of paracetamol N-acetyl-D-L-methionate was therefore similar to that obtained after administration of 1 g of paracetamol alone to the same renal failure patients. The mean relative paracetamol bioavailability for both groups of renal patients combined following administration of paracetamol N-acetyl-D-L-methionate was 94.4 ± 16.3%.

Paracetamol Sulphate

Mean plasma concentrations of paracetamol sulphate following administration of 2.146 g paracetamol N-acetyl-D-L-methionate were similar to those obtained after administration of 1 g of paracetamol to the same patients with impaired renal function. None of the pharmacokinetic variables for plasma paracetamol sulphate following administration of paracetamol N-acetyl-D-L-methionate were significantly different from those obtained with paracetamol alone.

Following administration of paracetamol N-acetyl-D-L-methionate to renal failure patients, plasma concentrations of paracetamol sulphate were greatly elevated and exceeded those of the parent compound. In Patient 011 sulphate conjugation appeared to be the major metabolic route. In the non-dialysis and dialysis patients following administration of paracetamol N-acetyl-D-L-methionate, the mean individual peak plasma paracetamol sulphate concentrations were 13.5 $\pm$ 6.0 $\mu g.ml^{-1}$ (range 9.7-26.5) and 20.3 $\pm$ 7.5 $\mu g.ml^{-1}$ (range 14.4-33.2) respectively, and the mean individual times to reach peak plasma paracetamol sulphate concentration were 6.14 $\pm$ 2.12 h (range 4-10) and 8.00 $\pm$ 3.16 h (range 4-12) respectively. From 8-24 h the mean plasma paracetamol sulphate apparent elimination half life in non-daialysis patients following administraion of paracetamol N-acetyl-D-L-methionate was 22.2 $\pm$ 20.5 h (range 11.0-67.6) compared to 21.2 $\pm$ 15.4 h obtained after administration of 1 g of paracetamol to the same patients. The elimination of paracetamol sulphate in haemodialysis patients was negligible after administration of paracetamol

N-acetyl-D-L-methionate. The mean areas under the plasma paracetamol sulphate concentration-time curves in non-dialysis and dialysis patients following administration of paracetamol N-acetyl-D-L-methionate were 231.2 ± 107.7 $\mu g.h.ml^{-1}$ (range 127-462) and 418.4 ± 155.4 $\mu g.h.ml^{-1}$ (range 233-659) respectively. In the non-dialysis patients there was a significant correlation between plasma creatinine concentration and the area under the plasma paracetamol sulphate concentration-time curve ($r = 0.82$; $P<0.05$).

Paracetamol Glucuronide

As with paracetamol sulphate, none of the kinetic variables relating to paracetamol glucuronide following administration of 2.146 g paracetamol N-acetyl-D-L-methionate were significantly different from those obtained after administration of 1 g of paracetamol in the same patients.

In the non-dialysis and dialysis patients following administration of paracetamol N-acetyl-D-L-methionate, the mean individual peak plasma paracetamol glucuronide concentrations were 23.3 ± 8.7 $\mu g.ml^{-1}$ (range 14.7-40.5) and 26.8 ± 7.4 $\mu g.ml^{-1}$ (range 17.5-37.9) respectively, and the mean individual times to reach peak paracetamol glucuronide concentrations were 7.29 ± 1.50 h (range 5-9) and 17.60 ± 8.88 h (range 6-24) respectively. From 8-24 h the mean plasma paracetamol glucuronide apparent elimination half life in non-dialysis patients following administration of paracetamol N-acetyl-D-L-methionate was 28.5 ± 30.7 h (range 10.0-96.7) compared to 29.1 ± 32.3 h obtained after

administration of 1 g of paracetamol to the same patients. The elimination of paracetamol glucuronide in haemodialysis patients was negligible after administration of paracetamol N-acetyl-D-L-methionate. The mean areas under the plasma paracetamol glucuronide concentration-time curves in non-dialysis and dialysis patients following administration of paracetamol N-acetyl-D-L-methionate were 394.3 $\pm$ 143.4 $\mu g.h.ml^{-1}$ (range 246-687) and 574.6 $\pm$ 175.7 $\mu g.h.ml^{-1}$ (range 342-764) respectively. In the non-dialysis patients there was a significant correlation between plasma creatinine concentration and the area under the plasma paracetamol glucuronide concentration-time curve ($r = 0.90$; $P<0.01$).

ii) Renal Excretion of Paracetamol and its Metabolites in the Non-dialysis Patients

Individual total recoveries of the paracetamol component of the dose (1 g) and 24 h fractional urinary recoveries of paracetamol and its metabolites following ingestion of 2.146 g paracetamol N-acetyl-D-L-methionate in the 7 non-dialysis patients are shown in Table 6.8. The mean percentages of total paracetamol excreted in divided urine collections as paracetamol and its metabolites are shown in Table 6.9 and Figure 6.3. Individual urinary excretion data are shown in Appendices 6.4-6.8.

TABLE 6.8 Twenty Four Hour Urinary Recovery of the Paracetamol Component of the Dose (1 g) and Fractional Urinary Recovery of Paracetamol and its Sulphate, Glucuronide, Cysteine and Mercapturic Acid Conjugates in 7 Non-dialysis Patients Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate

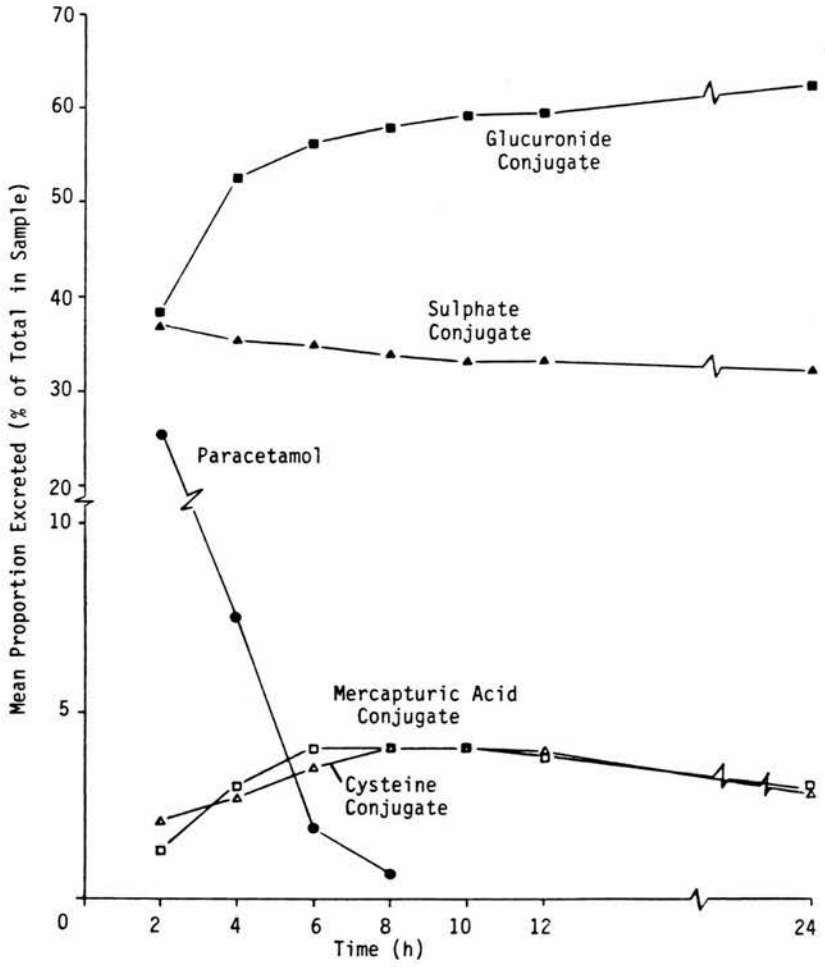
Patient Number	24 h Fractional Urinary Recovery (%)						
	Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide	Paracetamol Cysteine	Paracetamol Mercapturic Acid	Cysteine + Mercapturic Acid	Total (%)
01	1.7	24.8	63.0	3.5	7.0	10.5	63.2
02	2.6	30.8	59.9	3.7	3.1	6.8	62.1
03	1.8	31.4	59.8	3.4	3.7	7.1	70.4
04	-	39.2	50.8	5.5	4.4	9.9	52.8
05	2.1	43.9	48.1	2.6	3.4	6.0	69.9
06	3.7	30.9	59.5	3.4	2.5	5.9	43.7
07	1.4	31.3	65.3	1.7	-	1.7	35.2
Mean ± S.D.	2.2 ± 0.8	33.2 ± 6.3	58.1 ± 6.3	3.4 ± 1.2	3.4 ± 2.1	6.8 ± 2.9	56.8 ± 13.4
* Mean ± S.D.	2.6 ± 1.0	30.8 ± 6.2	60.9 ± 6.7	3.0 ± 1.0	2.8 ± 1.4	5.8 ± 2.2	55.5 ± 17.8

* = Mean data obtained following ingestion of 1 g of paracetamol to the same 7 non-dialysis patients

TABLE 6.9 Mean Percentage of Total Paracetamol Excreted in Divided Urine Collection as Paracetamol and its Metabolites Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate in 7 Non-dialysis Patients

Time After Ingestion (h)	Mean Proportion Excreted (as Percentage of Total in Sample) ± SD				
	Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide	Paracetamol Cysteine	Paracetamol Mercapturic Acid
0-2	25.2 ± 12.5	36.8 ± 10.0	38.3 ± 9.6	2.1 ± 1.3	1.3 ± 1.5
2-4	7.5 ± 5.0	35.4 ± 7.3	52.5 ± 6.5	2.7 ± 1.5	3.0 ± 2.5
4-6	1.9 ± 2.2	34.8 ± 6.9	56.1 ± 6.4	3.5 ± 1.6	4.0 ± 3.1
6-8	0.7 ± 1.6	33.8 ± 7.0	57.7 ± 7.9	4.0 ± 1.6	4.0 ± 2.6
8-10	-	33.0 ± 6.1	59.1 ± 6.7	4.0 ± 1.3	4.0 ± 2.4
10-12	-	33.1 ± 6.7	59.3 ± 7.1	3.9 ± 1.5	3.8 ± 2.1
12-24	-	32.0 ± 5.9	62.2 ± 6.9	2.8 ± 1.5	3.0 ± 1.4

FIGURE 6.3 Changes in the Mean Proportional Urinary Excretion of Paracetamol and its Sulphate, Glucuronide, Cysteine and Mercapturic Acid Conjugates Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate in 7 Non-dialysis Patients



Urinary Recovery of Paracetamol and Its Sulphate, Glucuronide, Cysteine and Mercapturic Acid Conjugates

The mean 24 h urinary recovery of the paracetamol component of the administered dose (1 g) after administration of 2.146 g paracetamol N-acetyl-D-L-methionate was 56.8 ± 13.4% (range 32.2-70.4) which was similar to that obtained after administration of 1 g of paracetamol alone (55.5 ± 17.8%) to the same non-dialysis patients. There was a significant negative correlation with plasma creatinine concentration and total urinary recovery ($r = -0.80$; $P<0.05$). The mean percentage of the paracetamol component of the dose recovered up to 24 h following administration of paracetamol N-acetyl-D-L-methionate in the non-dialysis patients as paracetamol and its sulphate, glucuronide, cysteine and mercapturic acid conjugates was 1.2, 18.9, 33.0, 1.9 and 1.9% respectively. These values were similar to those obtained after administration of paracetamol alone to the same non-dialysis patients (1.4, 16.8, 34.9, 1.8 and 1.8% respectively).

Fractional Excretion of Paracetamol and its Metabolites

The mean 24 h fractional urinary recovery of paracetamol and its sulphate, glucuronide, cysteine and mercapturic acid conjugates in non-dialysis patients following administration of paracetamol N-acetyl-D-L-methionate were 2.2 ± 0.8, 33.2 ± 6.3, 58.1 ± 6.3, 3.4 ± 1.2 and 3.4 ± 2.1% respectively. These values were not significantly different from those obtained after administration of 1 g of paracetamol to the same non-dialysis patients. The mean fractional

urinary excretion pattern of paracetamol and its metabolites in each divided urine collection in non-dialysis patients following administration of paracetamol N-acetyl-D-L-methionate was also not significantly different from that obtained in the same patients following administration of paracetamol alone.

Renal Clearances of Paracetamol and its Sulphate and Glucuronide Metabolites

The renal clearances of paracetamol and its sulphate and glucuronide conjugates following ingestion of 2.146 g paracetamol N-acetyl-D-L-methionate in non-dialysis patients are shown in Table 6.10.

Following administration of paracetamol N-acetyl-D-L-methionate the mean renal clearances of paracetamol and its sulphate and glucuronide conjugates were 4.4 $\pm$ 1.5, 16.6 $\pm$ 6.7 and 16.7 $\pm$ 6.5 $ml.min^{-1}$ respectively. These values were similar to those obtained after administration of 1 g of paracetamol to the same non-dialysis patients. There was a significant correlation with plasma creatinine concentration and paracetamol sulphate and glucuronide renal clearances ($r = -0.94$ and -0.81; $P<0.05$) but no such relationship existed for the paracetamol. Paracetamol renal clearance correlated with urine flow rate ($r = 0.84$; $P<0.05$) but not with urinary pH. The renal clearances of paracetamol sulphate and glucuronide did not correlate with either urine flow rate or urinary pH following administration of paracetamol N-acetyl-D-L-methionate to non-dialysis patients.

TABLE 6.10 Renal Clearance of Paracetamol and its Sulphate and Glucuronide Conjugates in 7 Non-dialysis Patients Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate

Patient Number	0-8 h Renal Clearance ($ml \cdot min^{-1}$)		
	Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide
01	4.2	21.1	18.0
02	4.9	17.4	18.6
03	6.3	22.7	25.2
04	-	18.6	15.8
05	5.0	21.8	22.5
06	4.1	5.3	7.4
07	1.8	9.4	9.4
Mean ± SD	4.4 ± 1.5	16.6 ± 6.7	16.7 ± 6.5
* Mean ± SD	4.6 ± 2.0	15.6 ± 8.1	15.5 ± 7.1

* = Mean 0-8 h renal clearance data obtained following administration of 1 g of paracetamol to the same non-dialysis patients

d) Discussion

The absorption of paracetamol appeared to be similar in the non-dialysis and dialysis patients following administration of 2.146 g paracetamol N-acetyl-D-L-methionate (equivalent to 1 g of paracetamol). However, the mean of the individual peak plasma paracetamol concentration was reduced and delayed by approximately 50 min compared to that obtained after administration of 1 g of paracetamol alone in the same renal failure patients. These results were consistent with the delayed absorption of paracetamol observed in healthy subjects following administration of paracetamol N-acetyl-D-L-methionate (SWRD Report 305510, 1978; Chapter IV of this thesis). Orally administered ester prodrugs are particularly susceptible to hydrolysis by esterases present in the intestine, liver and plasma (Williams, 1985). It is therefore likely that, as in healthy subjects, paracetamol N-acetyl-D-L-methionate is rapidly hydrolysed in patients with chronic renal failure. The delayed appearance of paracetamol may be explained by slow absorption because of the relative insolubility of paracetamol N-acetyl-D-L-methionate. It is not known whether the methionine ester of paracetamol is absorbed intact.

Following administration of paracetamol N-acetyl-D-L-methionate in patients with chronic renal failure, plasma paracetamol concentrations declined from 2-8 h at similar rates to those observed after administration of paracetamol alone in the same patients with renal failure. In addition, as was observed after administration of paracetamol alone, there was an extended phase of paracetamol elimination

from 10-24 h. The slower elimination of paracetamol during this period can probably be explained by enterohepatic circulation of paracetamol conjugates which are present at high concentrations in patients with chronic renal failure. These may be excreted to a limited extent into bile with subsequent hydrolysis releasing paracetamol back into the gut which is then reabsorbed. This mechanism has been described for drugs such as oxazepam, diflunisal and clofibric acid in patients with chronic renal failure (Odar-Cederlof et al, 1977; Faed and McQueen, 1979; Verbeek et al, 1979). Although the residual plasma paracetamol concentrations were relatively low, concentrations much higher are likely in patients with chronic renal failure receiving long term therapy.

Following administration of paracetamol N-acetyl-D-L-methionate the mean 0-24 h areas under the plasma paracetamol concentration-time curves in non-dialysis and dialysis patient were not significantly different from those obtained after administration of paracetamol alone to the same renal failure patients. This indicates that although absorption was delayed and slow, the bioavailability of paracetamol after administration of paracetamol N-acetyl-D-L-methionate was similar to that obtained after administration of paracetamol alone in patients with chronic renal failure. Similar findings were observed in healthy subjects (Chapter IV).

As observed after administration of paracetamol alone, there was gross retention of paracetamol sulphate and glucuronide conjugates following administration of paracetamol N-acetyl-D-L-methionate in patients with chronic renal failure. Plasma concentrations of these

conjugates in the non-dialysis and dialysis patients were similar to those obtained after administration of paracetamol alone in the same patients. The elimination of the sulphate and glucuronide conjugates in the haemodialysis patients was negligible following administration of paracetamol N-acetyl-D-L-methionate.

In the non-dialysis patients the mean 24 h urinary recovery of the paracetamol component of the dose was not significantly different from that obtained after administration of 1 g of paracetamol alone to the same patients. Similarly, the mean 24 h fractional urinary recoveries of paracetamol and its metabolites were not altered. The ability to metabolise paracetamol in this form was therefore unaffected in patients with chronic renal failure. In particular, even although methionine is a glutathione precursor, the cysteine and mercapturic acid conjugation of paracetamol was not influenced following administration of paracetamol N-acetyl-D-L-methionate in non-dialysis patients. As observed in the same patients following administration of paracetamol alone, the urinary recovery of the cysteine conjugate was higher than that of the mercapturic acid conjugate in 4 of the 7 non-dialysis patients following administration of paracetamol N-acetyl-D-L-methionate indicating that the ability to acetylate paracetamol cysteine may be reduced in these patients. Acetylation in patients with renal disease may be normal or slowed (Reidenber 1977b).

In healthy subjects there was a small but significant increase in the sulphation of paracetamol following administration of paracetamol

N-acetyl-D-L-methionate compared to that obtained after administration of paracetamol alone to the same subjects (Chater IV). The paracetamol sulphate pathway is susceptible to dose dependent saturation (Prescott, 1984) and is dependent on the availability of inorganic sulphate (Levy, 1986). In the non-dialysis patients, however there was no indication that sulphate conjugation of paracetamol was significantly increased following administration of paracetamol N-acetyl-D-L-methionate compared to paracetamol alone. It is possible that unlike the findings in healthy subjects, the sulphate conjugation pathway was not saturated following administration of paracetamol in the non-dialysis patients. Inorganic sulphate is predominantly eliminated by renal excretion (Cocchetto and Levy, 1981) and it is retained in patients with chronic renal failure (Freeman and Richards, 1979). Saturation of sulphate conjugation is probably less likely in patients with chronic renal failure.

Following administration of paracetamol N-acetyl-D-L-methionate in non-dialysis patients, the mean renal clearances of paracetamol and its sulphate and glucuronide conjugates were not significantly different from those obtained after administration of paracetamol alone to the same patients. As with paracetamol, the results obtained after administration of paracetamol N-acetyl-D-L-methionate to non-dialysis patients were consistent with glomerular filtration and passive reabsorption of paracetamol and active tubular secretion of the sulphate and glucuronide conjugates. Similarly, the capacity for active tubular transport seemed to be reduced to a greater extent than the capacity for passive reabsorption.

e) Summary and Conclusion

Following administration of 1 g of paracetamol as paracetamol N-acetyl-D-L-methionate (2.146 g) to 7 non-dialysis and 5 haemodialysis patients with chronic renal failure the overall disposition of paracetamol was similar to that obtained after administration of 1 g of paracetamol to the same patients.

Compared to paracetamol alone the appearance of paracetamol was delayed and the peak plasma paracetamol concentration was reduced following administration of paracetamol N-acetyl-D-L-methionate. These findings were consistent with those in healthy subjects. All other pharmacokinetic variables obtained following administration of paracetamol N-acetyl-D-L-methionate were not significantly different from those in the same renal failure patients following administration of paracetamol alone. Therefore the relative bioavailability, metabolism and elimination of paracetamol administered as the N-acetyl-D-L-methionine ester were not altered in patients with chronic renal failure. In particular, the production of the cysteine and mercapturic acid conjugates were not increased even although methionine is a glutathione precursor. In contrast to the findings in healthy subjects, the sulphation of paracetamol was not increased after administration of paracetamol N-acetyl-D-L-methionate in patients with chronic renal failure. Inorganic sulphate is retained in patients with chronic renal failure and this may account for the absence of saturation of sulphate conjugation following administration of paracetamol alone.

Mean data obtained in patients with chronic renal failure following administration of 1 g of paracetamol as 1) paracetamol and 2) paracetamol N-acetyl-D-L-methionate are summarised in Tables 6.11 and 6.12.

In conclusion 2.146 g paracetamol N-acetyl-D-L-methionate may provide a safer form of paracetamol, however when administered as a single therapeutic dose to patients with chronic renal failure the pharmacological response may be reduced and delayed compared to the equivalent paracetamol dose. This is less likely to occur with Pameton (500 mg paracetamol + 250 mg D-L-methionine) which is already available commercially. Paracetamol N-acetyl-D-L-methionate does, however, offer a tasteless formulation which yields paracetamol and methionine in equimolar quantities. The effectiveness of Pameton and paracetamol N-acetyl-D-L-methionate in preventing paracetamol toxicity remains to be established.

On long term administration of paracetamol N-acetyl-D-L-methionate to patients with chronic renal failure plasma paracetamol concentrations would probably be higher than those described in the present study and there would be gross cumulation of paracetamol metabolites. In addition the consequences of long term administration of N-acetyl-D-L-methionine to patients with chronic renal failure is not known and multiple dose studies should be performed.

TABLE 6.11 Summary of Plasma Data Obtained Following Administration of 1 g of Paracetamol as (1) Paracetamol and (2) Paracetamol N-Acetyl-D-L-methionate to Patients with Chronic Renal Failure

Results expressed as Mean ± (S.D.)

Compound	Paracetamol				Paracetamol Sulphate				Paracetamol Glucuronide			
	Cmax ($\mu g.ml^{-1}$)	Tmax (h)	$t_{\frac{1}{2}}$ (2-8 h) (h)	$AUC_{(0-24\ h)}$ ($\mu g.h.ml^{-1}$)	Cmax ($\mu g.ml^{-1}$)	Tmax (h)	$t_{\frac{1}{2}}$ (8-12 h) (h)	$AUC_{(0-24\ h)}$ ($\mu g.h.ml^{-1}$)	Cmax ($\mu g.ml^{-1}$)	Tmax (h)	$t_{\frac{1}{2}}$ (8-12 h) (h)	$AUC_{(0-24\ h)}$ ($\mu g.h.ml^{-1}$)
Paracetamol (Healthy Subjects)	20.0 ± (8.4)	0.35 ± (0.17)	2.3 ± (0.3)	45.3 ± (10.6)	3.5 ± (0.7)	1.08 ± (0.53)	3.2+ ± (0.4)	22.4 ± (5.1)	9.4 ± (2.6)	2.00 ± (0.44)	3.0++ ± (0.3)	65.0 ± (15.9)
Paracetamol (Non-dialysis)	17.3 ± (8.2)	0.54 ± (0.44)	2.5 ± (0.3)	62.5* ± (13.6)	13.7* ± (5.9)	5.57* ± (1.51)	21.1* ± (15.4)	231.9* ± (108.3)	25.2* ± (8.9)	6.29* ± (1.60)	29.1* ± (32.3)	431.4* ± (157.4)
Paracetamol (Dialysis)	18.7 ± (5.2)	0.45 ± (0.32)	2.1 ± (0.4)	55.7 ± (18.8)	20.7* ± (5.4)	8.20* ± (0.84)	-	449.6* ± (108.3)	30.6* ± (8.1)	15.00* ± (8.43)	-	673.1* ± (227.9)
Paracetamol N-Acetyl-D-L-methionate (Non-dialysis)	10.8** ± (2.4)	1.18** ± (0.83)	2.9 ± (0.5)	63.5 ± (13.4)	13.7 ± (6.0)	6.14 ± (2.12)	22.2 ± (20.5)	231.2 ± (107.7)	23.3 ± (8.7)	7.29 ± (1.50)	28.5 ± (30.7)	394.3 ± (143.4)
Paracetamol N-Acetyl-D-L-methionate (Dialysis)	9.3** ± (1.3)	1.50** ± (0.87)	2.0 ± (0.3)	44.8 ± (13.8)	20.3 ± (7.5)	8.00 ± (3.16)	-	418.4 ± (155.4))	26.8 ± (7.4)	17.60 ± (8.88)	-	574.6 ± (175.7)

+ = Half life calculated over 3-8 h

++ = Half life calculated over 5-12 h

* = Significantly different from paracetamol administered to healthy subjects; $P<0.05$

** = Significantly different from paracetamol administered to the same renal failure patients; $P<0.05$

TABLE 6.12 Summary of Urinary Excretion Data Obtained Following Administration of 1 g of Paracetamol as (1) Paracetamol and (2) Paracetamol N-Acetyl-D-L-methionate to Non-dialysis Patients with Chronic Renal Failure

Results expressed as Mean ± (S.D.)

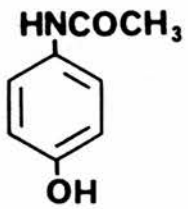
Compound	Paracetamol		Paracetamol Sulphate		Paracetamol Glucuronide		Paracetamol Cysteine	Paracetamol Mercapturic Acid	Total
	Fractional Urinary Recover (%)	0-8 h Renal Clearance ($ml.min^{-1}$)	Fractional Urinary Recovery (%)	0-8 h Renal Clearance ($ml.min^{-1}$)	Fractional Urinary Recovery (%)	0-8 h Renal Clearance ($ml.min^{-1}$)	Fractional Urinary Recovery (%)	Fractional Urinary Recovery (%)	Paracetamol Recovered in 24 h (%)
Paracetamol (Healthy Subjects)	4.4 ± (1.5)	15.2 ± (7.0)	28.7 ± (4.5)	180.5 ± (31.6)	60.3 ± (6.8)	136.9 ± (32.0)	2.8 ± (1.2)	4.1 ± (1.4)	82.6 ± (6.3)
Paracetamol (Non-dialysis)	2.6 ± (1.0)	4.6* ± (2.0)	30.8 ± (6.2)	15.6* ± (8.1)	60.9 ± (6.7)	15.5* ± (7.1)	3.0 ± (1.0)	2.8 ± (1.4)	55.5* ± 17.8
Paracetamol N-acetyl-D-L methionate (Non-dialysis)	2.2 ± (0.8)	4.4 ± (1.5)	33.2 ± (6.3)	16.6 ± (6.7)	58.1 ± (6.3)	16.7 ± (6.5)	3.4 ± (1.2)	3.4 ± (2.1)	56.8 ± (13.4)

* = Significantly different from paracetamol administered to healthy subjects; $P<0.05$

APPENDICES

APPENDIX 1.1

Chemical Data



Paracetamol [acetaminophen, N-acetyl-p-aminophenol, 4-hydroxyacetanilide, N-(4-hydroxyphenyl) acetamide] is a non-prescription analgesic and antipyretic agent.

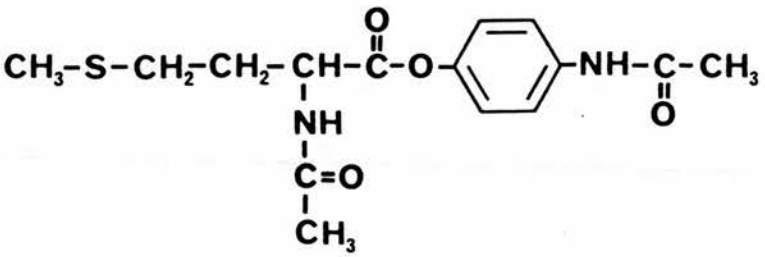
Description:	White odourless crystalline powder with bitter taste.
Molecular Weight:	151.2.
Melting Point:	168-172°C.
pKa:	9.5.
Soluble in:	70 parts water, 7 parts alcohol, 13 parts acetone, 40 parts glycerol, 9 parts propylene glycol, 50 parts chloroform and 10 parts methyl alcohol.
Insoluble in:	Ether, pentane and benzene.

APPENDIX 1.1 (continued)

Stability: At pH 6.0 at 25°C, an aqueous paracetamol solution has a half life of approximately 22 years. Degradation to acetic acid and p-aminophenol is accelerated by acidic or alkaline conditions (Koshy and Lach, 1961). Paracetamol metabolites in urine stored at -20°C were observed to be stable for 3 years (Adriaenssens, 1980).

APPENDIX 1.2

Chemical Data



Paracetamol N-acetyl-D-L-methionate (p-acetamidophenyl, 2-carbamoyl, 4-methylthiobutanoate; SUR 2647).

Description:	White tasteless powder with a characteristic odour derived from the N-acetyl-D-L-methionine.
Molecular Weight:	324.4.
Melting Point:	184-186°C.
Soluble in:	100 parts methanol, 350 parts ethanol, 300 parts acetone and 600 parts chloroform.
Insoluble in:	Water.
Stability:	See Chapter II, Section 3.

APPENDIX 2.1

Calculation of Factor (F) from Standards

From the standard regression line the concentration in test samples can be determined. From the regression equation: $y = mx + c$

ie ratio = slope x concentration + intercept

$$\text{Therefore: concentration} = \text{ratio} \times \frac{1}{\text{slope}}$$

The factor (F) represents the reciprocal of the slope of the regression line for the standards, ie concentration = ratio x F.

$$\text{Therefore: } F = \frac{\text{concentration}}{\text{ratio}}$$

(F) can therefore be calculated from the standards as follows. The example given is that for the plasma paracetamol assay.

APPENDIX 2.1 (continued)

Paracetamol Concentration ($\mu g.ml^{-1}$)	Peak Area Ratio	Peak Area Ratio for 25 $\mu g.ml^{-1}$	Mean Ratio + CV%
25	1.017	1.017	
25	1.015	1.015	1.016 ± 0.4%
5	0.204 (x 5)	1.020	
5	0.202 (x 5)	1.010	

$$F = \frac{\text{concentration}}{\text{ratio}} = \frac{25}{1.016} = \underline{\underline{24.62}}$$

These principles were applied to all established assays used during this work. The following mean values for F were obtained over the period of time the assay was in use.

Assay	Standards ($\mu g.ml^{-1}$)	Factor (F) ± SD	Period of Time (Months)
Plasma Paracetamol	5 and 25	24.61 ± 2.2	12
Urinary Paracetamol	1000	1.970 ± 0.071	12
Plasma Paracetamol N-acetyl-D-L-Methionate	5 and 25	9.733 ± 0.205	9

APPENDIX 3.1

Individual Plasma Paracetamol Concentrations in 10 Healthy Male Subjects Following Ingestion of 1 g of Paracetamol

Time (h)	Plasma Paracetamol Concentration ($\mu g.ml^{-1}$) Subject Number									
	1	2	3	4	5	6	7	8	9	10
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.25	14.9	22.8	20.3	6.7	40.7	24.7	15.2	15.1	12.4	14.5
0.5	13.2	16.4	13.8	10.8	15.1	14.3	9.5	11.6	12.9	20.7
0.75	11.6	11.9	9.8	12.9	11.3	10.8	8.3	9.0	9.5	14.7
1.0	11.0	9.9	8.7	11.4	9.4	9.4	7.8	7.8	8.7	13.2
1.5	9.0	8.2	7.0	10.3	8.1	7.5	6.4	6.5	7.0	10.6
2	7.5	6.7	5.9	9.5	5.8	6.0	5.3	4.9	5.7	8.1
3	6.3	4.7	4.2	7.2	4.4	4.2	4.3	3.5	5.0	6.4
4	4.3	3.8	3.2	5.5	2.9	2.9	2.8	2.3	3.2	4.9
5	3.3	2.7	2.1	3.9	2.1	2.3	1.7	1.6	2.2	3.7
6	2.6	2.0	1.7	3.3	1.5	1.6	1.3	1.1	1.5	2.0
7	2.1	1.8	1.2	2.4	1.1	1.1	1.0	0.8	1.0	1.9
8	1.5	1.3	0.9	2.0	0.9	0.9	0.7	0.6	0.7	1.8
9	1.4	0.9	0.8	1.8	0.7	0.7	0.6	0.6	0.6	1.2
10	0.9	0.7	0.7	1.2	0.6	0.6	0.5	0.3	0.5	1.2
12	0.7	0.6	0.5	1.0	0.4	0.3	-	0.4	0.3	0.9
24	-	0.3	-	0.4	0.2	-	-	-	-	0.3

APPENDIX 3.2

Individual Plasma Paracetamol Sulphate Concentrations in 10 Healthy Male Subjects Following Ingestion of 1 g of Paracetamol

Results expressed as paracetamol equivalents

Time (h)	Plasma Paracetamol Sulphate Concentration ($\mu g.ml^{-1}$) Subject Number									
	1	2	3	4	5	6	7	8	9	10
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.25	1.5	2.3	2.3	0.8	1.7	2.6	2.9	2.1	2.1	1.4
0.5	2.0	3.2	3.5	2.3	2.6	3.7	3.4	4.0	3.1	2.6
0.75	1.9	3.2	3.3	3.4	3.0	3.8	3.7	4.5	3.1	2.6
1.0	1.9	2.9	3.3	3.9	3.0	4.2	4.0	4.5	3.0	2.9
1.5	2.1	2.8	2.9	3.9	3.1	3.9	3.8	4.5	2.8	3.4
2	2.1	2.5	2.6	4.0	2.6	3.6	3.3	3.8	2.5	2.7
3	1.8	2.2	2.2	3.6	2.5	3.2	3.2	3.4	2.3	2.5
4	1.4	1.9	2.1	2.9	2.1	2.4	2.4	2.7	1.7	2.1
5	1.2	1.4	1.5	2.5	1.7	2.1	2.0	1.9	1.2	2.0
6	1.0	1.1	1.4	1.9	1.4	1.6	1.5	1.6	1.0	1.3
7	0.9	1.0	0.8	1.4	1.1	1.3	1.3	1.3	0.9	1.2
8	0.6	0.8	0.7	1.1	0.8	1.0	1.1	1.0	0.6	1.1
9	0.6	0.5	0.5	0.8	0.6	0.9	0.9	1.1	0.8	0.9
10	0.3	0.4	0.4	0.5	0.5	0.7	-	0.8	0.8	1.0
12	-	0.3	0.3	-	0.4	0.6	-	0.6	0.6	1.2
24	-	-	-	-	0.3	0.3	-	-	-	0.4

APPENDIX 3.3

Individual Plasma Paracetamol Glucuronide Concentrations in 10 Healthy Male Subjects Following Ingestion of 1 g of Paracetamol

Results expressed as paracetamol equivalents

Time (h)	Plasma Paracetamol Glucuronide Concentration ($\mu g.ml^{-1}$) Subject Number									
	1	2	3	4	5	6	7	8	9	10
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.25	1.7	2.5	2.7	0.3	1.6	1.7	1.2	1.8	1.6	1.7
0.5	3.5	5.2	6.4	1.3	5.3	4.3	2.6	4.9	4.9	3.8
0.75	4.6	7.5	8.6	2.6	7.8	6.3	4.0	8.4	7.2	5.2
1.0	5.5	8.1	10.5	3.5	9.4	8.4	5.0	9.8	8.4	7.1
1.5	7.3	9.8	11.0	4.5	11.8	9.5	5.5	13.0	8.9	8.5
2	6.9	9.7	11.1	5.8	11.7	9.9	5.8	12.3	9.3	9.0
3	7.0	9.5	9.8	5.7	13.3	9.8	5.7	12.2	9.1	8.5
4	5.1	8.5	8.2	5.3	12.0	8.0	4.5	10.0	7.0	7.4
5	3.9	6.5	6.4	4.6	9.4	6.5	3.7	7.6	5.2	6.0
6	3.5	5.4	5.0	3.8	7.6	5.1	3.1	5.9	4.7	5.0
7	2.2	4.8	3.8	2.9	5.5	3.9	2.4	5.5	3.5	3.9
8	1.8	3.5	3.0	2.3	3.7	3.0	2.2	3.7	2.6	3.0
9	1.5	2.7	2.4	1.8	2.6	2.5	2.0	3.0	2.4	2.5
10	1.1	2.0	1.8	1.5	2.2	1.9	1.7	2.8	1.7	2.1
12	0.7	-	1.1	1.0	1.3	1.2	0.7	1.5	0.8	1.5
24	-	0.4	-	0.8	0.2	0.3	-	-	0.6	0.3

APPENDIX 3.4

Urinary Recovery of Paracetamol In Each Sample Following Ingestion of 1 g of Paracetamol In 10 Healthy Male Subjects

Time (h)	Urinary Recovery of Paracetamol (mg) Subject Number									
	1	2	3	4	5	6	7	8	9	10
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0-2	23.4	15.5	1.0	21.3	15.8	17.0	19.9	8.2	30.0	18.5
2-4	17.4	-	11.5	13.6	7.7	5.9	10.6	2.8	13.4	9.4
4-6	11.4	-	3.3	5.2	1.9	4.5	5.1	4.7	6.3	6.5
6-8	7.6	-	7.5	4.2	1.7	-	3.7	-	4.0	2.6
8-10	-	-	6.5	2.0	-	-	-	-	2.1	-
10-12	-	-	1.7	-	-	-	-	-	-	-
12-24	-	-	-	-	-	-	-	-	-	-

APPENDIX 3.5

Urinary Recovery of Paracetamol Sulphate in Each Sample Following Ingestion of 1 g of Paracetamol in 10 Healthy Male Subjects

Results expressed as paracetamol equivalents

Time (h)	Urinary Recovery of Paracetamol Sulphate (mg) Subject Number									
	1	2	3	4	5	6	7	8	9	10
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0-2	54.6	53.9	5.0	66.5	47.7	51.1	75.1	75.8	73.4	60.0
2-4	55.9	43.3	58.0	73.2	45.1	55.8	76.5	43.2	51.0	63.1
4-6	38.3	37.7	21.8	58.2	29.8	41.6	42.6	52.0	31.0	43.8
6-8	18.0	15.4	46.3	36.4	20.1	28.3	29.9	32.3	17.8	17.0
8-10	14.2	13.6	30.1	25.8	10.9	17.7	17.5	10.8	11.3	17.6
10-12	16.2	4.2	12.3	17.0	7.4	10.9	16.5	10.2	9.9	13.2
12-24	63.6	18.6	11.7	35.2	19.6	31.0	38.7	14.3	29.7	28.7

APPENDIX 3.6

Urinary Recovery of Paracetamol Glucuronide In Each Sample Following Ingestion of 1 g of Paracetamol in 10 Healthy Male Subjects

Results expressed as paracetamol equivalents

Time (h)	Urinary Recovery of Paracetamol Glucuronide (mg) Subject Number									
	1	2	3	4	5	6	7	8	9	10
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0-2	110.7	101.8	11.4	61.5	106.5	104.4	70.0	103.6	153.2	106.6
2-4	137.7	130.7	134.4	101.0	121.0	159.3	111.4	98.5	159.5	147.7
4-6	99.4	109.7	73.1	83.8	122.2	94.4	83.7	127.6	105.8	103.7
6-8	64.3	48.1	142.3	52.0	80.3	63.8	59.2	84.9	59.6	36.4
8-10	29.7	46.6	81.5	32.0	40.9	38.4	33.3	29.5	33.9	37.3
10-12	36.1	14.1	37.2	21.5	26.5	21.6	29.6	26.1	23.5	25.0
12-24	-	61.1	19.8	40.1	42.7	52.6	39.1	32.6	101.6	-

APPENDIX 3.7

Urinary Recovery of Paracetamol Cysteine in Each Sample Following Ingestion of 1 g of Paracetamol in 10 Healthy Male Subjects

Results expressed as paracetamol equivalents

Time (h)	Urinary Recovery of Paracetamol Cysteine (mg) Subject Number									
	1	2	3	4	5	6	7	8	9	10
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0-2	4.0	2.1	-	2.4	2.0	-	3.0	-	1.6	2.6
2-4	5.4	3.8	2.6	6.7	4.5	3.1	7.7	2.1	3.2	6.0
4-6	7.5	4.5	2.0	9.8	6.0	3.6	11.7	2.2	2.5	5.2
6-8	6.0	1.5	10.6	7.8	4.9	3.5	8.4	1.8	1.5	1.8
8-10	4.9	1.4	2.6	5.0	2.4	1.6	2.8	0.9	0.8	2.1
10-12	3.0	0.5	0.9	3.8	1.3	1.5	1.3	0.8	-	1.3
12-24	-	7.5	-	8.8	-	-	2.3	-	-	2.1

APPENDIX 3.8

Urinary Recovery of Paracetamol Mercapturic Acid in Each Sample Following Ingestion of 1 g of Paracetamol in 10 Healthy Male Subjects

Results expressed as paracetamol equivalents

Time (h)	Urinary Recovery of Paracetamol Mercapturic Acid (mg) Subject Number									
	1	2	3	4	5	6	7	8	9	10
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0-2	5.3	4.9	-	3.5	3.6	2.6	5.4	-	4.9	3.5
2-4	9.8	9.7	3.9	10.8	8.6	7.0	14.3	3.2	9.8	9.1
4-6	7.9	7.6	2.8	12.1	8.4	5.7	16.3	4.7	7.5	7.2
6-8	5.1	2.6	8.6	7.2	4.8	4.0	9.9	3.6	3.6	2.2
8-10	3.7	2.5	3.5	4.9	3.1	1.9	4.9	1.5	2.0	2.3
10-12	2.3	1.0	1.2	3.6	1.9	1.4	2.2	1.5	1.4	1.8
12-24	13.8	5.0	-	9.3	-	-	3.2	-	4.6	2.1

APPENDIX 3.9

Individual Urine Volumes and pH in 10 Healthy Male Subjects Following Ingestion of 1 g of Paracetamol

Time After Ingestion (h)	Subject Number																			
	1		2		3		4		5		6		7		8		9		10	
	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH
0-2	92	6.5	31	5.7	13	6.2	295	6.7	295	7.3	274	7.2	272	7.3	67	6.4	430	6.9	110	7.6
2-4	345	7.2	45	6.1	260	6.2	215	7.5	155	7.5	478	7.2	315	7.5	43	6.6	570	7.2	274	6.7
4-6	92	6.3	70	7.0	79	6.0	83	7.0	53	6.4	108	6.9	86	7.5	258	6.9	350	7.3	168	6.5
6-8	200	6.0	76	7.2	235	6.3	98	6.5	94	6.9	66	6.2	93	6.5	165	6.9	295	7.2	120	6.5
8-10	195	5.9	188	6.4	240	6.3	97	5.9	214	6.9	122	5.6	130	6.4	50	6.0	180	6.4	144	6.3
10-12	495	6.5	192	6.1	155	6.8	165	7.3	212	7.1	88	5.4	220	6.4	54	5.9	335	6.4	180	6.5
12-24	1280	6.3	234	5.5	385	6.0	265	5.8	1059	6.3	500	5.7	730	6.6	970	5.9	1200	6.3	610	6.3

APPENDIX 4.1

Individual Plasma Paracetamol Concentrations in 10 Healthy Male Subjects Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate

Time (h)	Plasma Paracetamol Concentration ($\mu g.ml^{-1}$) Subject Number									
	1	2	3	4	5	6	7	8	9	10
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.25	6.0	10.7	2.2	4.0	10.1	5.9	5.3	7.1	3.4	5.3
0.5	9.8	8.4	3.5	6.5	11.6	12.7	4.6	5.7	6.1	7.1
0.75	9.2	9.4	4.3	7.3	10.4	11.3	6.8	6.3	5.5	6.8
1.0	11.0	7.6	5.7	8.0	11.6	10.2	7.4	6.8	6.0	7.9
1.5	11.0	7.9	7.5	10.2	9.3	9.4	7.4	6.6	9.2	10.4
2	9.0	7.6	10.5	9.5	6.9	7.5	5.8	5.8	7.8	9.1
3	7.1	7.5	7.3	8.0	4.6	5.9	4.3	3.9	5.8	7.1
4	5.3	4.8	5.1	6.2	3.4	4.1	3.2	2.9	4.5	5.4
5	4.2	3.6	3.6	4.8	2.1	3.0	1.9	2.1	2.9	4.1
6	2.9	2.7	2.8	3.5	1.5	2.5	1.7	1.5	2.9	3.2
7	2.2	1.8	2.1	2.7	1.1	1.8	1.4	1.1	1.6	2.6
8	1.6	1.4	1.3	2.1	0.8	1.4	1.1	0.8	1.0	2.1
9	1.4	1.3	1.1	1.8	0.6	1.0	0.9	0.6	0.9	1.8
10	1.1	1.0	0.8	1.4	0.5	0.8	0.7	0.5	0.8	1.4
12	0.7	0.9	0.4	1.0	0.5	0.5	0.4	0.3	0.7	0.9
24	0.2	0.1	0.2	-	0.2	0.2	-	-	0.1	0.2

APPENDIX 4.2

Individual Plasma Paracetamol Sulphate Concentrations in 10 Healthy Male Subjects Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate

Results expressed as paracetamol equivalents

Time (h)	Plasma Paracetamol Sulphate Concentration ($\mu g.ml^{-1}$) Subject Number									
	1	2	3	4	5	6	7	8	9	10
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.25	-	1.3	0.2	1.2	1.2	0.9	-	2.0	0.4	1.1
0.5	1.1	2.0	0.7	2.7	2.6	2.6	1.8	2.8	1.9	2.2
0.75	1.4	2.4	1.3	3.7	2.9	3.8	2.7	3.5	2.2	2.6
1.0	1.6	2.4	1.7	4.4	3.7	4.1	3.4	4.1	2.4	3.6
1.5	2.2	2.8	2.6	5.7	4.2	4.5	3.8	4.9	3.5	3.8
2	2.0	2.8	3.8	5.8	3.8	4.2	3.7	5.0	3.1	4.0
3	1.9	3.1	3.9	5.9	3.2	3.9	3.4	4.2	2.2	3.2
4	1.4	2.4	3.2	5.3	2.5	3.1	2.7	3.7	1.9	2.6
5	1.3	2.0	2.5	4.5	1.8	2.8	2.1	2.8	1.5	2.2
6	0.7	1.6	2.0	3.6	1.4	2.3	1.6	2.2	1.4	1.8
7	0.6	1.1	1.4	3.0	1.2	1.8	1.2	1.8	0.8	1.5
8	0.6	1.0	0.9	2.4	0.9	1.5	1.0	1.5	0.8	1.2
9	0.7	0.8	0.7	2.1	0.8	1.3	0.8	1.1	1.0	1.0
10	0.4	0.8	0.4	1.7	0.7	1.1	-	1.0	0.9	0.8
12	0.3	0.6	0.1	0.9	-	0.6	-	0.8	0.8	0.5
24	-	0.4	-	0.7	-	-	-	-	0.2	-

APPENDIX 4.3

Individual Plasma Paracetamol Glucuronide Concentrations in 10 Healthy Male Subjects Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate

Results expressed as paracetamol equivalents

Time (h)	Plasma Paracetamol Glucuronide Concentration ($\mu g.ml^{-1}$) Subject Number									
	1	2	3	4	5	6	7	8	9	10
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.25	0.2	0.7	0.2	0.2	0.4	0.3	1.0	0.9	0.3	0.5
0.5	1.2	2.2	0.8	0.7	2.5	1.7	2.1	2.5	1.4	1.6
0.75	2.3	4.2	1.4	1.3	3.4	4.2	1.9	4.4	2.7	2.5
1.0	3.5	4.9	2.3	1.9	5.4	5.7	3.1	6.5	3.9	4.2
1.5	5.4	7.6	4.1	3.6	9.5	8.7	4.4	10.0	6.5	5.7
2	6.4	8.4	6.7	4.4	11.8	9.9	5.3	11.9	7.9	7.3
3	6.4	10.6	9.9	5.9	12.8	10.4	6.9	12.5	8.1	7.9
4	5.9	9.9	9.8	6.2	11.5	9.3	5.4	10.8	8.0	7.3
5	4.8	8.6	7.4	5.7	8.9	8.2	5.3	9.0	6.3	6.8
6	3.4	6.6	6.4	4.6	7.7	6.6	3.8	7.2	4.6	5.1
7	2.6	5.0	5.1	3.8	6.0	4.9	3.3	6.0	3.7	4.0
8	2.0	3.8	3.8	2.9	4.4	4.1	2.7	4.7	2.9	3.2
9	1.7	3.2	3.0	2.5	3.2	3.2	2.3	3.5	2.4	2.5
10	1.3	2.5	2.2	2.1	2.6	2.6	1.9	2.9	2.0	2.2
12	0.9	-	1.4	1.9	2.1	1.6	1.7	2.1	1.3	1.4
24	0.3	0.3	0.5	0.3	0.5	0.3	0.7	0.2	0.2	0.2

APPENDIX 4.4

Urinary Recovery of Paracetamol in Each Sample Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate in 10 Healthy Male Subjects

Time (h)	Urinary Recovery of Paracetamol (mg) Subject Number									
	1	2	3	4	5	6	7	8	9	10
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0-2	16.4	10.8	6.0	19.5	3.8	12.0	17.8	5.3	15.3	10.1
2-4	8.7	7.7	11.5	14.1	10.2	12.0	9.3	3.3	12.1	8.2
4-6	10.8	4.2	2.7	5.6	6.4	2.4	6.1	4.1	6.2	5.3
6-8	-	2.0	4.2	5.1	-	-	3.2	1.5	3.8	4.4
8-10	-	-	6.5	4.0	-	-	3.6	-	-	2.4
10-12	-	-	6.0	-	-	-	-	-	-	-
12-24	-	-	-	-	-	-	-	-	-	-

APPENDIX 4.5

Urinary Recovery of Paracetamol Sulphate In Each Sample Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate In 10 Healthy Male Subjects

Results expressed as paracetamol equivalents

Time (h)	Urinary Recovery of Paracetamol Sulphate (mg) Subject Number									
	1	2	3	4	5	6	7	8	9	10
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0-2	56.2	48.0	17.5	59.1	60.8	68.5	69.1	61.6	47.4	54.1
2-4	57.1	47.9	56.8	84.9	55.7	50.8	96.4	67.4	63.2	65.7
4-6	36.7	40.1	17.2	63.4	35.0	45.1	62.3	55.6	40.8	49.1
6-8	26.2	23.1	19.3	50.1	18.8	32.6	38.5	26.8	23.8	36.8
8-10	17.0	15.8	41.7	29.1	12.6	21.9	24.9	17.5	15.8	18.3
10-12	9.3	13.0	27.1	11.6	9.2	14.3	17.4	13.6	10.7	14.9
12-24	17.2	27.2	21.9	51.7	24.2	66.0	39.9	8.1	29.5	17.7

APPENDIX 4.6

Urinary Recovery of Paracetamol Glucuronide in Each Sample Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate in 10 Healthy Male Subjects

Results expressed as paracetamol equivalents

Time (h)	Urinary Recovery of Paracetamol Glucuronide (mg) Subject Number									
	1	2	3	4	5	6	7	8	9	10
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0-2	67.7	74.0	24.2	41.2	86.5	85.3	59.8	72.7	70.1	66.1
2-4	104.0	119.2	117.3	96.4	157.9	126.2	136.1	133.1	152.7	139.7
4-6	81.5	132.8	43.7	85.6	123.1	103.5	109.5	125.9	101.8	109.2
6-8	62.5	78.9	47.0	59.5	70.0	71.5	60.5	78.2	67.1	71.8
8-10	33.9	51.3	102.8	41.0	38.3	49.2	43.5	42.2	44.9	35.1
10-12	24.9	45.3	71.8	15.2	26.6	27.4	26.1	29.4	32.5	28.3
12-24	54.0	64.6	46.6	68.0	68.4	108.7	-	25.4	84.4	29.3

APPENDIX 4.7

Urinary Recovery of Paracetamol Cysteine in Each Sample Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate in 10 Healthy Male Subjects

Results expressed as paracetamol equivalents

Time (h)	Urinary Recovery of Paracetamol Cysteine (mg) Subject Number									
	1	2	3	4	5	6	7	8	9	10
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0-2	5.1	2.0	-	1.8	2.2	-	-	1.0	-	-
2-4	15.1	3.2	5.0	7.8	7.4	2.2	9.5	3.1	3.5	5.1
4-6	16.6	6.4	3.0	9.8	7.5	4.5	11.3	2.4	3.5	4.7
6-8	13.5	3.6	3.7	8.4	5.1	3.4	7.7	1.4	2.0	4.0
8-10	8.7	2.0	8.3	6.4	3.9	3.7	7.4	1.1	1.2	4.2
10-12	4.8	2.2	3.7	3.0	2.5	2.4	2.9	0.8	0.6	2.5
12-24	6.6	-	-	13.8	5.8	-	-	-	-	0.4

APPENDIX 4.8

Urinary Recovery of Paracetamol Mercapturic Acid in Each Sample Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate in 10 Healthy Male Subjects

Results expressed as paracetamol equivalents

Time (h)	Urinary Recovery of Paracetamol Mercapturic Acid (mg) Subject Number									
	1	2	3	4	5	6	7	8	9	10
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0-2	6.7	4.0	-	2.6	3.1	2.6	-	1.8	2.9	-
2-4	20.5	7.8	5.2	11.7	10.2	4.8	16.0	5.3	8.1	10.4
4-6	16.5	9.3	2.7	13.0	10.4	5.0	16.3	5.3	6.8	8.8
6-8	11.9	4.8	3.0	10.0	6.9	3.0	9.6	3.3	3.7	5.8
8-10	7.0	2.8	7.1	7.3	4.8	2.4	6.7	2.1	2.2	3.9
10-12	3.8	2.0	3.7	2.5	3.0	2.4	3.5	1.4	1.3	2.8
12-24	6.7	1.7	-	10.9	5.5	-	5.2	-	1.9	-

APPENDIX 4.9

Individual Urine Volumes and pH in 10 Healthy Male Subjects Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate

Time After Ingestion (h)	Subject Number 1		2		3		4		5		6		7		8		9		10	
	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH
0-2	115	6.9	81	5.4	83	6.1	358	6.1	67	7.1	92	7.1	832	6.6	99	7.6	282	5.7	80	7.5
2-4	59	6.1	270	5.5	402	6.0	245	7.2	300	6.5	405	7.0	143	6.6	61	6.7	240	5.8	130	6.8
4-6	81	6.5	83	5.2	72	6.4	75	6.0	226	6.8	54	6.1	112	5.8	248	6.6	148	6.0	108	6.7
6-8	104	5.9	70	5.2	91	7.0	74	6.8	305	6.8	56	5.6	103	5.9	370	6.9	135	6.2	98	6.5
8-10	112	6.2	265	5.3	365	6.9	97	6.3	85	6.3	69	5.4	150	5.7	83	6.1	110	5.5	66	6.2
10-12	122	7.0	110	5.2	527	6.8	83	6.3	158	6.6	80	5.4	360	6.4	85	7.1	155	6.7	172	6.8
12-24	690	6.5	800	5.4	523	6.2	488	6.5	188	5.7	403	6.0	760	6.2	310	6.0	740	6.8	560	6.6

APPENDIX 5.1

Individual Plasma Paracetamol Concentrations in 7 Non-dialysis and 5 Dialysis Patients Following Ingestion of 1 g of Paracetamol

Time (h)	Plasma Paracetamol Concentration ($\mu g.ml^{-1}$)											
	Patient Number											
	Non-dialysis							Dialysis				
	01	02	03	04	05	06	07	08	09	010	011	012
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.25	23.6	19.5	8.9	1.3	9.7	18.7	14.8	15.3	12.5	17.2	5.2	27.9
0.5	17.3	13.4	8.3	4.4	11.9	30.3	19.2	13.3	17.2	14.8	12.5	18.5
0.75	15.3	12.9	8.4	6.8	11.8	21.2	15.2	12.3	14.1	12.9	15.5	16.0
1	12.3	11.4	8.6	6.4	11.2	19.7	13.9	10.3	12.8	12.4	15.8	14.3
1.5	10.5	9.6	8.5	7.8	10.1	16.9	11.0	8.3	9.2	10.6	14.1	12.3
2	9.1	8.8	7.7	7.8	9.4	15.3	10.0	5.8	7.4	9.8	12.4	10.1
3	6.9	6.7	5.8	5.7	7.0	9.6	8.1	3.8	4.9	7.2	8.5	6.9
4	4.9	5.2	4.4	3.6	5.3	6.9	5.7	2.3	3.2	5.4	5.9	4.7
5	3.8	4.4	3.1	2.9	4.1	4.8	4.0	1.8	1.9	3.7	4.7	3.6
6	2.7	3.5	2.3	2.2	3.1	3.6	3.6	1.1	1.2	3.1	3.2	2.7
7	2.0	2.8	2.0	1.6	2.5	2.4	2.7	-	0.9	2.5	2.7	2.1
8	1.7	2.1	1.7	1.4	2.2	2.0	1.9	-	0.8	2.2	1.6	1.7
9	1.3	1.7	1.5	1.2	1.6	1.3	1.6	-	-	1.7	1.4	1.5
10	1.0	1.5	1.2	1.0	1.4	1.0	1.5	-	-	1.5	1.1	1.2
12	0.8	1.3	-	1.0	1.1	-	1.1	-	-	1.0	0.8	0.9
24	0.4	0.5	-	0.7	0.6	0.6	0.7	-	0.1	0.7	0.8	0.6

APPENDIX 5.2

Individual Plasma Paracetamol Sulphate Concentrations in 7 Non-dialysis and 5 Dialysis Patients Following Ingestion of 1 g of Paracetamol

Results expressed as paracetamol equivalents

Time (h)	Plasma Paracetamol Sulphate Concentration ($\mu g.ml^{-1}$)											
	Patient Number											
	Non-dialysis							Dialysis				
	01	02	03	04	05	06	07	08	09	010	011	012
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.25	3.6	2.7	2.4	0.9	2.7	-	3.3	4.0	2.2	6.4	-	6.0
0.5	5.8	4.2	3.5	2.4	5.0	10.5	6.0	9.0	5.2	9.1	6.8	8.4
0.75	7.2	5.4	4.6	4.0	6.9	11.7	6.0	11.2	6.8	9.9	10.4	9.0
1	7.1	5.8	5.2	5.3	7.9	13.3	7.4	12.8	7.8	10.6	12.5	9.7
1.5	8.4	7.1	7.1	7.3	9.1	16.3	7.8	13.1	11.8	12.3	15.2	10.9
2	8.8	8.1	8.2	9.1	10.8	18.2	8.2	16.2	13.1	13.5	18.4	11.8
3	9.4	9.3	9.4	11.8	11.8	22.1	8.9	17.5	15.0	14.9	21.9	13.0
4	8.7	9.9	10.0	13.0	11.6	22.9	9.7	18.4	16.2	15.9	23.9	13.9
5	8.9	11.8	11.3	13.5	12.4	23.9	10.0	16.9	16.6	17.4	24.4	15.5
6	8.2	10.6	10.9	13.1	12.4	24.4	10.4	19.5	17.8	17.5	27.9	15.3
7	7.4	10.3	10.7	11.4	12.5	26.9	10.8	20.4	17.9	17.4	29.8	15.3
8	7.4	9.5	10.4	11.5	12.0	25.2	10.4	19.5	18.9	17.9	28.0	15.3
9	5.9	9.3	9.9	12.0	10.9	25.0	10.4	19.6	18.9	17.3	30.1	16.4
10	5.3	9.5	9.2	10.7	9.8	24.3	9.7	19.6	18.9	16.9	28.6	16.4
12	4.3	9.4	8.3	10.5	8.5	18.9	9.3	17.6	18.9	16.9	28.1	15.9
24	2.2	4.2	4.9	7.0	4.6	15.5	8.4	13.5	16.0	15.1	27.6	15.7

APPENDIX 5.3

Individual Plasma Paracetamol Glucuronide Concentrations in 7 Non-dialysis and 5 Dialysis Patients Following Ingestion of 1 g of Paracetamol. Predose Values were Subtracted to Give Corrected Concentrations

Results expressed as paracetamol equivalents

Time (h)	Plasma Paracetamol Glucuronide Concentration ($\mu g.ml^{-1}$)											
	Patient Number											
	Non-dialysis							Dialysis				
	01	02	03	04	05	06	07	08	09	010	011	012
Predose	2.2	0.0	0.0	0.0	0.0	0.0	0.0	3.8	2.1	0.0	2.2	1.9
0.25	3.0	1.0	0.9	-	-	0.8	0.9	0.7	0.6	1.1	-	1.3
0.5	9.4	2.7	1.9	1.2	4.2	4.9	4.6	3.6	3.5	3.5	-	4.4
0.75	15.5	5.0	3.2	2.1	6.2	8.3	7.2	6.8	7.1	5.2	2.8	7.3
1	17.6	6.2	4.3	3.3	8.1	11.6	10.3	9.6	10.6	6.6	3.9	9.6
1.5	24.4	9.1	7.2	6.0	10.9	18.8	13.6	14.0	14.9	10.2	5.9	13.3
2	26.2	11.2	9.8	8.8	14.1	23.5	16.0	20.7	19.0	12.7	8.9	16.2
3	29.9	14.2	13.9	14.4	16.6	30.8	20.3	25.7	26.9	16.9	12.3	20.1
4	29.0	16.5	16.7	17.6	17.2	34.6	22.5	29.3	31.0	19.9	14.6	22.4
5	29.5	17.2	19.7	19.2	18.8	37.1	23.2	31.8	32.7	21.8	15.2	25.1
6	26.8	18.3	20.0	19.3	18.4	38.4	25.6	33.8	35.6	23.5	17.6	25.6
7	24.9	18.7	19.1	17.0	18.8	42.6	26.9	36.7	35.4	23.1	19.4	25.6
8	24.3	18.8	18.8	17.7	18.5	41.3	25.1	35.3	38.3	25.1	18.4	25.8
9	20.2	18.5	18.2	19.2	17.1	40.3	26.1	36.2	39.5	23.5	20.3	27.7
10	17.8	17.4	17.9	17.1	15.1	39.4	26.8	36.7	40.1	24.1	19.5	28.3
12	14.9	17.4	16.0	17.3	13.7	31.3	25.3	34.8	41.0	23.9	20.2	27.6
24	4.8	9.4	9.0	12.6	6.8	26.7	23.1	35.5	36.1	23.4	21.5	28.8

APPENDIX 5.4

Individual Plasma Paracetamol Cysteine Concentrations in 7 Non-dialysis and 5 Dialysis Patients Following Ingestion of 1 g of Paracetamol. Predose Values were Subtracted to Give Corrected Concentrations

Results expressed as paracetamol equivalents

Time (h)	Plasma Paracetamol Cysteine Concentration ($\mu g.ml^{-1}$)											
	Patient Number											
	Non-dialysis							Dialysis				
	01	02	03	04	05	06	07	08	09	010	011	012
Predose	0.00	0.02	0.00	0.00	0.01	0.02	0.00	0.00	0.09	0.01	0.04	0.00
0.25	0.01	0.01	0.01	0.04	-	-	-	-	-	0.02	-	0.01
0.5	0.05	0.02	0.03	0.06	-	0.01	-	0.09	-	0.06	-	0.05
0.75	0.08	0.04	0.06	0.04	0.03	0.06	0.03	0.18	0.06	0.07	0.04	0.11
1	0.13	0.06	0.09	0.08	0.04	0.11	0.05	0.23	0.08	0.15	0.09	0.17
1.5	0.20	0.11	0.15	0.17	0.12	0.23	0.09	0.35	0.21	0.29	0.18	0.31
2	0.25	0.16	0.22	0.23	0.11	0.30	0.14	0.49	0.34	0.41	0.39	0.46
3	0.33	0.25	0.32	0.43	0.20	0.52	0.28	0.60	0.52	0.59	0.81	0.72
4	0.39	0.32	0.42	0.61	0.21	0.67	0.40	0.69	0.77	0.78	1.31	1.04
5	0.45	0.40	0.51	0.64	0.26	0.83	0.44	0.88	0.95	0.97	1.62	1.47
6	0.45	0.40	0.43	0.68	0.28	0.87	0.48	0.87	1.02	0.92	1.70	1.44
7	0.38	0.44	0.44	0.75	0.31	0.94	0.54	0.89	1.06	0.97	1.60	1.57
8	0.37	0.42	0.45	0.71	0.24	0.97	0.54	0.97	0.98	1.07	1.80	1.37
9	0.34	0.41	0.45	0.62	0.22	0.99	0.51	0.93	0.89	1.04	1.57	1.43
10	0.27	0.41	0.41	0.56	0.21	0.97	0.60	0.87	0.93	1.08	1.59	1.49
12	0.21	0.34	0.38	0.53	0.23	0.85	0.58	0.74	0.77	0.93	1.37	1.42
24	0.06	0.20	0.13	0.41	0.07	0.41	0.43	0.48	0.54	0.62	1.01	0.97

APPENDIX 5.5

Individual Plasma Paracetamol Mercapturic Acid Concentrations in 7 Non-dialysis and 5 Dialysis Patients Following Ingestion of 1 g of Paracetamol. Predose Values were Subtracted to Give Corrected Concentrations

Results expressed as paracetamol equivalents

Time (h)	Plasma Paracetamol Mercapturic Acid Concentration ($\mu g.ml^{-1}$)											
	Patient Number											
	Non-dialysis							Dialysis				
	01	02	03	04	05	06	07	08	09	010	011	012
Predose	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.12	0.00
0.25	-	-	-	-	-	-	0.02	-	-	-	-	-
0.5	0.03	-	-	-	-	-	0.02	0.04	-	-	-	0.01
0.75	0.05	0.01	-	-	-	-	0.02	0.03	-	-	-	0.01
1	0.08	0.01	0.03	-	-	-	0.02	0.06	-	-	-	0.04
1.5	0.16	0.03	0.05	0.04	0.01	0.04	0.04	0.14	0.05	0.04	0.01	0.09
2	0.22	0.06	0.10	0.05	0.10	0.06	0.05	0.22	0.08	0.09	0.12	0.14
3	0.32	0.10	0.19	0.11	0.20	0.15	0.07	0.32	0.14	0.21	0.35	0.23
4	0.36	0.14	0.27	0.20	0.17	0.22	0.11	0.44	0.25	0.25	0.51	0.36
5	0.39	0.18	0.32	0.23	0.19	0.27	0.14	0.51	0.35	0.35	0.69	0.56
6	0.35	0.19	0.27	0.25	0.16	0.27	0.15	0.56	0.45	0.33	0.78	0.62
7	0.27	0.21	0.25	0.26	0.13	0.31	0.20	0.61	0.41	0.37	0.79	0.72
8	0.24	0.23	0.24	0.26	0.18	0.35	0.20	0.69	0.47	0.71	1.03	0.64
9	0.23	0.24	0.24	0.22	0.13	0.34	0.19	0.68	0.45	0.43	0.96	0.69
10	0.16	0.21	0.21	0.20	0.17	0.33	0.19	0.60	0.51	0.44	0.99	0.76
12	0.13	0.19	0.21	0.19	0.18	0.32	0.17	0.57	0.47	0.39	1.01	0.81
24	0.07	0.08	0.09	0.10	0.10	0.20	0.16	0.46	0.51	0.28	1.24	0.79

APPENDIX 5.6

Urinary Recovery of Paracetamol in Each Sample Following Ingestion of 1 g of Paracetamol in 7 Non-dialysis Patients

Time (h)	Urinary Recovery of Paracetamol (mg) Patient Number						
	01	02	03	04	05	06	07
Predose	0.0	0.0	0.0	-	0.0	0.0	0.0
0-2	6.9	5.4	7.0	-	11.6	9.5	5.1
2-4	4.5	4.7	5.5	-	9.0	4.8	-
4-6	2.6	3.0	-	-	-	-+	-
6-8	-	3.4	-	-	-	-	-
8-10	-	-	-	-	-	-	-
10-12	-	-	-	-	-	-	-
12-24	-	-	-	-	-	-+	-

+ = Incomplete collection

APPENDIX 5.7

Urinary Recovery of Paracetamol Sulphate in Each Sample Following Ingestion of 1 g of Paracetamol in 7 Non-dialysis Patients

Results expressed as paracetamol equivalents

Time (h)	Urinary Recovery of Paracetamol Sulphate (mg) Patient Number						
	01	02	03	04	05	06	07
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0-2	21.5	8.7	11.6	11.0	18.1	7.6	4.0
2-4	28.6	16.9	22.4	20.9	33.2	12.2	8.0
4-6	24.7	19.3	21.7	26.8	29.7+	12.6	7.7
6-8	31.8	24.4	26.9	9.1	26.5	15.1	9.4
8-10	24.8	21.2	20.5	44.2	30.0	5.9	8.4
10-12	22.1	17.5	22.2	35.8	27.0	18.9	9.0
12-24	55.7	58.3	43.0	113.6	15.7+	50.0	37.3

+ = Incomplete collection

APPENDIX 5.8

Urinary Recovery of Paracetamol Glucuronide in Each Sample Following Ingestion of 1 g of Paracetamol in 7 Non-dialysis Patients

Results expressed as paracetamol equivalents

Time (h)	Urinary Recovery of Paracetamol Glucuronide (mg) Patient Number						
	01	02	03	04	05	06	07
Predose	Trace	0.0	0.0	0.0	0.0	0.0	0.0
0-2	47.9	12.1	10.8	7.0	18.1	11.0	5.7
2-4	75.4	28.1	37.4	18.5	49.8	28.9	17.7
4-6	63.6	34.2	39.7	31.3	45.4+	28.7	18.0
6-8	89.2	46.6	56.7	10.1	44.3	37.0	23.1
8-10	73.7	43.7	41.6	50.2	51.2	14.2	21.4
10-12	55.1	37.6	49.0	46.2	43.2	47.2	24.4
12-24	191.3	137.8	103.2	151.5	20.5+	124.1	101.9

+ = Incomplete collection

APPENDIX 5.9

Urinary Recovery of Paracetamol Cysteine in Each Sample Following Ingestion of 1 g of Paracetamol in 7 Non-dialysis Patients

Results expressed as paracetamol equivalents

Time (h)	Urinary Recovery of Paracetamol Cysteine (mg) Patient Number						
	01	02	03	04	05	06	07
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0-2	0.7	-	-	0.4	0.4	0.4	-
2-4	3.1	1.8	2.0	1.9	1.6	1.2	0.3
4-6	3.2	2.2	2.7	3.4	1.7+	1.6	0.4
6-8	4.5	3.0	3.5	1.5	2.1	2.1	0.5
8-10	4.0	2.7	2.7	5.5	2.8	0.9	0.6
10-12	3.2	2.1	2.7	4.5	1.7	2.7	0.5
12-24	8.1	7.2	4.8	9.6	1.1+	6.7	2.0

+ = incomplete collection

APPENDIX 5.10

Urinary Recovery of Paracetamol Mercapturic Acid in Each Sample Following Ingestion of 1 g of Paracetamol in 7 Non-dialysis Patients

Results expressed as paracetamol equivalents

Time (h)	Urinary Recovery of Paracetamol Mercapturic Acid (mg)						
	Patient Number						
	01	02	03	04	05	06	07
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0-2	2.2	-	-	0.1	0.3	0.1	-
2-4	5.7	1.3	1.8	1.0	2.1	0.7	-
4-6	6.5	2.2	2.9	2.4	2.7+	1.1	-
6-8	7.3	3.3	3.6	1.2	2.8	1.4	-
8-10	5.1	3.4	2.7	4.3	3.1	0.6	-
10-12	3.3	2.5	2.7	3.5	2.1	1.9	-
12-24	8.6	7.6	4.2	7.8	1.1+	5.0	-

+ = Incomplete collection

APPENDIX 5.11

Individual Urine Volumes and pH in 7 Non-dialysis Patients Following Ingestion of 1 g of Paracetamol

Time (h)	Patient Number													
	01		02		03		04		05		06		07	
	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH
0-2	200	5.4	150	6.0	270	6.4	160	4.7	318	5.4	190	6.3	65	6.2
2-4	175	5.3	152	6.2	325	6.6	105	4.7	380	5.8	122	6.2	106	6.9
4-6	90	5.5	93	6.1	250	6.7	215	5.0	-	-	96	6.1	90	6.7
6-8	265	5.8	178	6.4	340	7.0	45	5.0	145	5.5	195	6.5	86	6.6
8-10	285	5.6	126	6.1	260	6.8	320	5.0	200	5.3	41	6.1	93	6.6
10-12	270	5.8	140	6.3	335	6.9	350	5.0	225	5.4	200	6.3	85	6.6
12-24	1350	5.5	840	6.2	830	6.4	1780	5.5	-	-	830	6.6	495	6.5

APPENDIX 6.1

Individual Plasma Paracetamol Concentrations in 7 Non-dialysis and 5 Dialysis Patients Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-Methionate. Predose Values were Subtracted to Give Corrected Concentrations

Time (h)	Plasma Paracetamol Concentration ($\mu g.ml^{-1}$)											
	Patient Number											
	Non-dialysis							Dialysis				
	01	02	03	04	05	06	07	08	09	010	011	012
Predose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.0	2.0	0.0	0.0
0.25	9.1	11.9	2.8	1.7	3.1	10.3	2.5	4.2	4.6	3.9	3.8	4.6
0.5	12.1	12.3	7.3	6.4	6.9	12.7	4.7	7.5	7.1	7.2	7.8	6.7
0.75	10.8	11.7	7.0	9.1	9.5	12.8	5.9	7.7	8.1	8.6	8.2	7.8
1	11.8	12.5	7.7	9.0	11.5	14.3	5.9	7.8	8.2	10.6	8.2	9.1
1.5	11.2	10.3	7.3	8.3	11.1	14.2	6.7	7.4	8.0	10.0	8.8	10.6
2	8.7	9.6	6.3	6.5	9.8	12.8	7.2	7.2	6.8	8.8	8.6	10.2
3	6.2	7.4	5.0	4.7	7.8	10.6	8.7	6.0	6.3	6.0	9.5	7.1
4	4.7	6.4	4.7	3.4	6.4	7.5	7.9	5.6	3.9	4.8	7.6	5.0
5	3.4	5.0	3.6	2.4	4.9	5.8	5.5	2.3	2.5	2.6	4.6	3.6
6	2.4	3.9	2.6	2.0	4.3	4.0	3.9	-	1.7	1.9	3.1	2.8
7	1.9	3.6	2.3	1.5	3.4	3.4	3.8	-	1.2	1.2	2.3	2.3
8	1.5	2.7	2.0	1.3	2.9	2.7	2.7	-	0.9	0.5	1.7	1.8
9	1.3	2.6	1.8	1.1	2.4	2.0	2.1	-	0.7	0.1	1.2	1.4
10	1.0	2.1	1.4	1.1	2.0	1.4	1.8	-	0.6	-	1.0	1.3
12	0.7	1.6	1.0	1.0	1.4	1.2	1.4	-	-	-	0.7	1.2
24	0.6	0.7	0.6	0.6	0.5	0.6	0.7	-	0.2	-	0.7	0.7

APPENDIX 6.2

Individual Plasma Paracetamol Sulphate Concentrations in 7 Non-dialysis and 5 Dialysis Patients Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-Methionate. Predose Values were Subtracted to Give Corrected Concentrations

Results expressed as paracetamol equivalents

Time (h)	Plasma Paracetamol Sulphate Concentration ($\mu g.ml^{-1}$)											
	Patient Number											
	Non-dialysis							Dialysis				
	01	02	03	04	05	06	07	08	09	010	011	012
Predose	0.0	0.0	2.0	0.0	0.0	0.0	0.0	5.2	0.0	18.2	0.0	0.0
0.25	1.4	1.7	0.8	-	-	-	-	1.7	2.9	-	-	4.6
0.5	3.5	3.4	3.1	3.2	2.6	6.1	3.0	4.3	4.3	1.1	6.8	5.6
0.75	4.5	4.3	4.1	5.0	4.5	9.1	3.2	7.3	5.7	2.9	8.2	6.5
1	5.6	5.4	5.3	5.9	6.3	10.9	3.7	7.6	6.6	4.3	10.9	7.1
1.5	8.0	7.0	6.8	8.3	8.7	13.8	4.6	9.6	10.9	5.8	12.0	9.2
2	8.6	7.6	7.6	9.7	10.1	15.6	5.5	11.5	11.5	7.0	14.0	10.9
3	9.1	9.2	8.5	11.1	11.9	20.0	7.5	14.3	15.0	9.3	17.5	11.8
4	9.4	10.4	10.2	12.0	13.1	20.7	8.9	18.4	15.8	11.5	21.6	13.9
5	9.7	10.6	10.3	11.7	13.1	23.6	9.7	17.3	18.3	12.5	27.9	15.5
6	8.3	10.1	9.8	11.5	14.4	23.3	10.4	16.3	17.4	14.4	29.4	14.8
7	7.7	10.7	9.5	11.4	13.4	24.9	9.9	16.5	18.3	13.5	30.4	15.9
8	6.9	10.1	9.6	11.2	13.5	26.5	10.1	16.5	19.1	12.7	30.3	16.6
9	6.2	9.2	9.5	10.4	12.9	25.2	9.9	16.2	18.2	12.4	30.5	15.7
10	5.5	9.7	8.9	10.8	12.1	22.5	10.6	16.8	18.2	11.6	33.2	15.7
12	4.6	7.8	7.4	10.0	10.4	21.3	10.1	15.6	18.3	10.5	30.8	16.3
24	2.4	4.4	3.6	6.6	5.1	14.8	8.7	13.5	18.2	7.6	30.3	15.6

APPENDIX 6.3

Individual Plasma Paracetamol Glucuronide Concentrations in 7 Non-dialysis and 5 Dialysis Patients Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-Methionate. Predose Values were Subtracted to Give Corrected Concentrations

Results expressed as paracetamol equivalents

Time (h)	Plasma Paracetamol Glucuronide Concentration ($\mu g.ml^{-1}$)											
	Patient Number											
	Non-dialysis							Dialysis				
	01	02	03	04	05	06	07	08	09	010	011	012
Predose	0.0	0.0	3.3	0.0	0.0	0.0	2.5	12.9	3.1	21.3	0.0	0.0
0.25	0.9	0.4	0.1	-	-	0.5	1.0	0.3	2.0	-	-	0.7
0.5	4.3	1.7	1.0	1.5	0.8	1.9	1.3	1.3	2.8	0.5	-	1.5
0.75	7.1	3.0	1.6	3.2	1.9	4.5	2.3	2.6	4.0	2.6	-	2.6
1	10.0	4.6	3.2	4.1	3.0	6.7	2.7	4.0	5.6	4.3	2.5	4.3
1.5	16.8	8.0	5.9	7.9	5.3	11.5	3.5	6.9	9.2	7.4	3.5	7.5
2	20.8	9.8	8.1	11.0	7.3	15.9	5.4	9.5	12.1	10.3	4.7	11.9
3	24.7	14.1	11.4	14.7	10.2	24.7	11.9	15.6	21.3	13.3	7.2	16.5
4	26.3	17.6	15.2	16.7	12.3	29.0	15.6	23.2	25.0	18.0	10.2	20.1
5	28.1	18.6	17.2	17.0	13.3	34.6	19.2	22.2	30.5	20.2	12.3	23.3
6	25.2	18.6	17.1	17.2	14.7	34.1	21.4	22.1	31.0	23.9	13.4	23.2
7	22.7	19.7	17.3	18.0	13.6	37.2	23.1	24.2	32.8	22.9	14.6	24.9
8	20.5	19.4	17.8	17.7	14.0	40.5	21.9	24.7	34.9	23.1	15.5	26.1
9	19.3	18.4	18.2	19.1	14.0	38.6	22.1	24.9	34.1	23.3	15.6	25.5
10	17.0	19.5	17.1	17.6	13.5	34.2	22.1	26.3	34.2	22.3	17.5	25.5
12	14.4	16.5	14.5	16.6	11.8	32.8	22.7	26.8	36.7	21.6	16.9	27.1
24	6.7	9.9	7.4	12.2	6.8	24.3	19.8	27.5	37.9	21.5	17.1	27.3

APPENDIX 6.4

Urinary Recovery of Paracetamol in Each Sample Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate in 7 Non-dialysis Patients

Time (h)	Urinary Recovery of Paracetamol (mg) Patient Number						
	01	02	03	04	05	06	07
Predose	0.0	0.0	Trace	-	0.0	0.0	0.0
0-2	4.6	4.7	4.4	-	7.6	9.7	4.8
2-4	4.7	4.7	5.6	-	7.5	6.1	-
4-6	1.7	3.5	3.1	-	-	-	-
6-8	-	2.8	-	-	-	-	-
8-10	-	-	-	-	-	-	-
10-12	-	-	-	-	-	-	-
12-24	-	-	-	-	-	-	-

APPENDIX 6.5

Urinary Recovery of Paracetamol Sulphate in Each Sample Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate in 7 Non-dialysis Patients

Results expressed as paracetamol equivalents

Time (h)	Urinary Recovery of Paracetamol Sulphate (mg) Patient Number						
	01	02	03	04	05	06	07
Predose	0.0	0.0	Trace	0.0	0.0	0.0	0.0
0-2	12.1	7.6	11.7	10.0	13.4	5.9	4.2
2-4	26.9	19.3	25.3	16.1	32.1	12.1	7.4
4-6	17.7	23.9	24.9	25.8	32.9	14.4	11.9
6-8	22.6	21.8	28.2	37.4	37.8	16.4	10.9
8-10	22.3	23.3	21.9	21.8	34.8	17.4	12.3
10-12	17.8	22.0	28.5	24.6	31.5	15.5	13.1
12-24	38.3	73.0	80.5	72.1	124.2	52.9	50.6

APPENDIX 6.6

Urinary Recovery of Paracetamol Glucuronide In Each Sample Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate In 7 Non-dialysis Patients

Results expressed as paracetamol equivalents

Time (h)	Urinary Recovery of Paracetamol Glucuronide (mg) Patient Number						
	01	02	03	04	05	06	07
Predose	0.0	0.0	Trace	0.0	0.0	0.0	0.0
0-2	20.7	8.6	11.7	7.4	8.2	7.9	3.3
2-4	61.4	34.5	25.3	17.8	29.9	21.6	11.2
4-6	42.3	42.0	24.9	30.4	34.2	27.3	23.1
6-8	56.8	40.5	28.2	45.9	37.1	32.2	24.2
8-10	56.5	44.6	21.9	30.6	37.8	34.9	26.5
10-12	48.4	43.1	28.5	30.3	35.8	32.1	28.5
12-24	111.4	159.0	80.5	105.5	153.0	104.4	114.4

APPENDIX 6.7

Urinary Recovery of Paracetamol Cysteine in Each Sample Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate in 7 Non-dialysis Patients

Results expressed as paracetamol equivalents

Time (h)	Urinary Recovery of Paracetamol Cysteine (mg) Patient Number						
	01	02	03	04	05	06	07
Predose	0.0	0.0	Trace	0.0	0.0	0.0	0.0
0-2	1.5	0.7	0.7	0.4	0.3	0.4	-
2-4	4.1	1.6	2.1	1.7	1.3	1.2	-
4-6	3.5	2.6	2.5	3.8	1.7	1.5	0.4
6-8	4.3	2.7	3.3	6.4	3.1	1.9	0.5
8-10	4.4	3.0	2.6	3.4	3.0	2.2	0.6
10-12	3.8	2.8	3.7	3.8	1.8	1.8	0.7
12-24	-	9.9	9.1	9.1	6.4	5.7	3.8

APPENDIX 6.8

Urinary Recovery of Paracetamol Mercapturic Acid in Each Sample Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate in 7 Non-dialysis Patients

Results expressed as paracetamol equivalents

Time (h)	Urinary Recovery of Paracetamol Mercapturic Acid (mg) Patient Number						
	01	02	03	04	05	06	07
Predose	0.0	0.0	Trace	0.0	0.0	0.0	0.0
0-2	1.8	0.2	0.5	0.2	0.2	0.1	-
2-4	8.4	1.0	2.2	1.4	1.9	0.8	-
4-6	7.4	2.1	3.0	2.8	2.8	1.1	-
6-8	8.1	2.4	3.6	4.6	3.6	1.4	-
8-10	7.1	2.7	3.0	2.8	3.5	1.7	-
10-12	5.2	2.5	3.9	3.2	2.6	1.4	-
12-24	6.3	8.0	9.3	8.5	9.0	4.6	-

APPENDIX 6.9

Individual Urine Volumes and pH in 7 Non-dialysis Patients Following Ingestion of 2.146 g of Paracetamol N-Acetyl-D-L-methionate

Time (h)	Patient Number													
	01		02		03		04		05		06		07	
	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH	Vol (ml)	pH
0-2	142	5.4	164	6.1	256	5.6	120	4.7	255	5.5	242	6.5	103	6.2
2-4	175	5.4	106	6.1	315	5.6	82	4.9	250	5.6	142	6.3	77	5.8
4-6	88	5.5	104	6.2	205	5.8	174	5.1	140	5.4	115	6.1	108	6.7
6-8	214	5.6	142	6.5	280	6.2	270	4.9	235	5.6	140	6.4	104	6.6
8-10	258	5.2	120	6.1	193	5.7	275	4.7	160	5.3	130	6.1	105	6.6
10-12	310	5.0	156	6.4	275	5.6	240	4.9	225	5.6	148	6.3	115	6.6
12-24	1325	5.1	950	6.5	1110	5.7	1060	5.5	1114	5.6	690	6.7	630	6.6

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Paracetamol Disposition and Metabolite Kinetics in Patients with Chronic Renal Failure

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Summary. The disposition of paracetamol following an oral dose of 1.0 g was compared in 10 healthy volunteers, 7 patients with moderate chronic renal failure and 6 patients with end stage renal failure on maintenance haemodialysis.

Paracetamol absorption was normal in the patients with renal failure. The mean plasma half-life of paracetamol from 2 to 8 h was similar in the 3 groups (2.1 to 2.3 h) but from 8 to 24 h it disappeared much more slowly in the renal failure patients (half-life 11.7 compared with 4.9 h in the healthy volunteers). Plasma concentrations of paracetamol glucuronide and sulphate conjugates were greatly increased in the patients with moderate renal failure and the mean plasma half-lives were 30.5 and 21.8 h respectively compared with about 3 h in the healthy volunteers. Plasma concentrations of these metabolites were even higher in the dialysis patients and there was no significant fall over 24 h. The cysteine and mercapturic acid conjugates of paracetamol could only be measured in plasma in the patients with renal failure and concentrations were very low.

The fractional urinary recovery of paracetamol and its glucuronide, sulphate, cysteine and mercapturic acid conjugates was similar in healthy volunteers and patients with moderate renal failure. The mean renal clearances of paracetamol and its glucuronide and sulphate conjugates in the healthy volunteers and patients with moderate renal failure were 15.7, 137 and 172, and 5.9, 14.5 and 14.8 ml/min respectively. In the latter patients the mean renal clearances of the cysteine and mercapturic acid conjugates were much greater at 35.4 and 80.2 ml/min. In the patients with moderate renal failure the AUC's of the glucuronide and sulphate conjugates were related to the plasma creatinine and there were significant negative correlations with the renal clearances of these metabolites and total urinary recovery. Marked cumulation of the polar glucuronide and sulphate conjugates of paracetamol would seem inevitable in patients with renal failure and the parent drug is apparently regenerated to a limited extent from retained metabolites.

Key words: paracetamol, renal failure; drug disposition, polar metabolites, cumulation, pharmacokinetics

The disposition of drugs is often abnormal in patients with chronic renal failure [1-3], and those which are excreted largely unchanged by the kidney or converted to active metabolites are usually given to patients with renal failure in reduced dosage to avoid cumulation and toxicity [4, 5]. However, drugs which are primarily inactivated by metabolism in the liver are often continued in full dosage and in such circumstances marked cumulation of their polar metabolites is inevitable unless there are alternative routes of elimination. Under normal conditions these metabolites may be inactive but at the remarkably high concentrations which must be achieved during chronic therapy in patients with renal failure their biological effects are unknown. Such patients are particularly susceptible to adverse drug reactions and in some cases unsuspected toxicity may be caused by cumulation of drug metabolites [6, 7].

Paracetamol (acetaminophen) is one of the most commonly used drugs and about 90% of a therapeutic dose is normally excreted in the urine in 24 h as glucuronide, sulphate and glutathione-derived conjugates [8]. The excretion rate of the glucuronide and sulphate conjugates is markedly reduced in patients with renal failure following paracetamol overdosage [9] while in patients with end-stage renal

Table 1. Clinical details of patients with renal failure

Patient	Weight (kg)	Age (years)/ Sex	Diagnosis	Hb ($g \cdot dl^{-1}$)	Albumin ($g \cdot l^{-1}$)	ALT ($u \cdot l^{-1}$)	Bili-rubin ($\mu mol \cdot l^{-1}$)	Alk Phosph ($u \cdot l^{-1}$)	Creatinine ($\mu mol \cdot l^{-1}$)	Creatinine clearance ($ml \cdot min^{-1}$)	Drugs
1	76.4	46/M	CF	16.3	44	17	14	40	334	17	AT
2	77.4	57/M	AG, RA	11.9	39	13	4	114	440	23	NF
3	85.3	43/M	PK	12.1	47	20	8	67	484	22	MT, BD, PZ
4	83.6	49/M	DB, HT	10.3	41	12	5	96	442	19	MT, NF, PZ, IN
5	68.4	46/M	CG	7.6	44	18	5	89	946	HD	AT, NF
6	79.5	33/M	CG, AN	7.2	45	24	4	128	1127	HD	
7	74.5	70/M	CG, NE	10.3	23	16	3	119	727	9	NF, MZ, BU
8	64.9	65/M	GP	5.5	40	13	4	74	752	HD	
9	61	48/F	PK	10.7	46	15	3	56	879	5	NF, QD, SB
10	83.1	65/M	PK, HT	11.3	43	14	11	59	378	17	NF, MT
11	76.9	39/M	CF	6.9	45	22	5	51	760	HD	
12	64.7	62/M	RA, AM	7.4	37	11	6	46	641	HD	MT, BP
13	65.7	60/F	CF	4.8	39	12	4	60	1175	HD	

HD = Haemodialysis, CF = Chronic renal failure of unknown aetiology, AG = Analgesic nephropathy, AN = Anephric, AM = Amyloidosis, NE = Nephrotic syndrome, CG = Chronic glomerulonephritis, DB = Diabetes, GP = Goodpasture's syndrome, HT = Hypertension, PK = Polycystic kidneys, RA = Rheumatoid arthritis. AT = Atenolol, BD = Bendrofluazide, BP = Buprenorphine, BU = Bumetanide, IN = Insulin, MT = Metoprolol, MZ = Metolazone, NF = Nifedipine, PZ = Prazosin, QD = Quinidine, SB = Salbutamol

failure the elimination of these metabolites is greatly impaired and haemodialysis appears to be the major route for their removal from the body [10, 11]. We report detailed studies of the disposition and kinetics of paracetamol and its polar metabolites in patients with moderate to severe impairment of renal function.

Methods, Patients and Subjects

Eleven men and 2 women with chronic renal impairment were studied. Their mean age was 53 (11) years and their body weight was 74 (8) kg. Seven had moderate to severe renal failure but were not being treated with dialysis, and 6 with more advanced disease were receiving long-term haemodialysis 2 or 3 times a week. Their clinical details are summarized in Table 1. Ten healthy male volunteers with a mean age of 29 (7) years and body weight 72 (11) kg served as controls. They did not take drugs regularly or smoke and they denied excessive regular consumption of ethanol. Physical examination was normal as were liver function tests, haemoglobin and plasma urea and creatinine concentrations estimated by standard automated methods. The study was approved by the local Ethics Committee and all patients and volunteers gave informed consent.

Drug Administration and Sampling

After an overnight fast, the patients and volunteers took 1.0 g of soluble paracetamol in 200 ml of water at approximately 08.30 h. They remained recumbent for the next 3 h and received 200 ml of water every 2 h up to 12 h. The usual diet was resumed 4 h after dosing. Blood was sampled at frequent intervals for 24 h and except in the haemodialysis patients (who produced little or no urine), divided urine collections were made over the same period. In the latter patients the study was carried out on a day preceding dialysis.

Drug Analysis

Paracetamol and its glucuronide, sulphate, cysteine and mercapturic acid conjugates in plasma and urine were assayed by high performance liquid chromatography using UV detection. Electrochemical detection was also used for low concentrations of paracetamol and cysteine and mercapturic acid conjugates in the presence of potentially interfering peaks [12]. Variable blank values were obtained in some patients with renal failure and these were subtracted to give corrected concentrations. In Patient 7 paracetamol sulphate could not be measured in urine because of interference. The amount excreted was therefore estimated from the relative areas under the plasma concentration time curves of the glucuronide and sulphate conjugates and the urinary recovery of glucuronide assuming that both metabolites had the same renal clearance and volume of distribution.

Data Analysis

The plasma half-life ($t_{1/2}$) of paracetamol and its metabolites was determined from the log-linear phases of the concentration-time curves by weighted linear re-

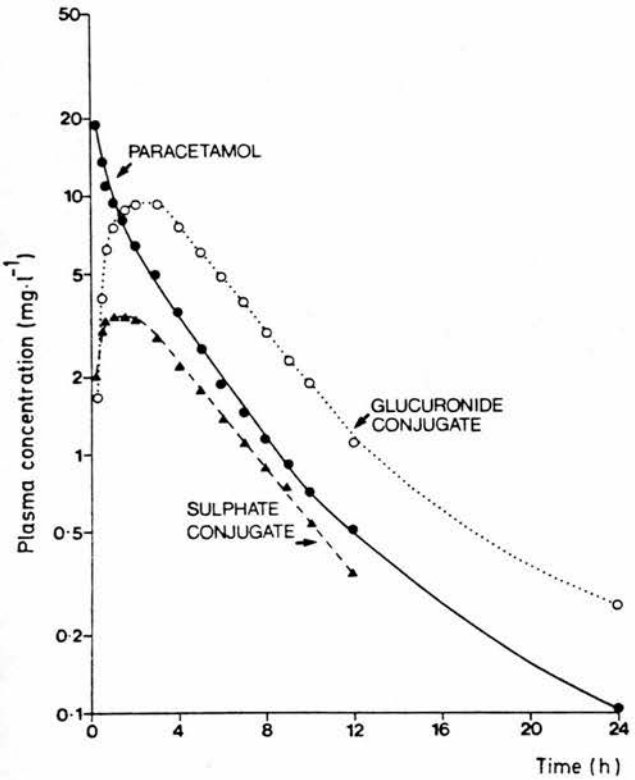

Fig. 1. Mean plasma concentrations of paracetamol and its glucuronide and sulphate conjugates in 10 healthy volunteers following an oral dose of 1.0 g

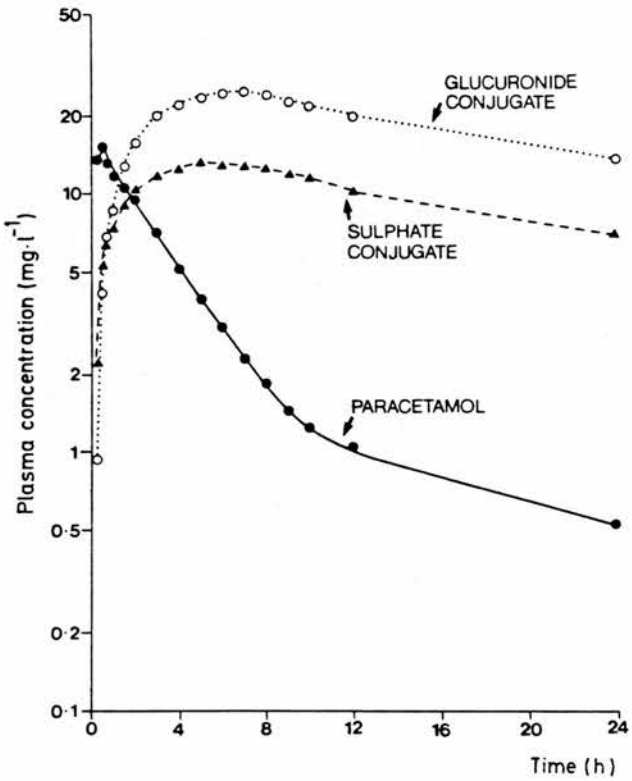

Fig. 2. Mean plasma concentrations of paracetamol and its glucuronide and sulphate conjugates in 7 patients with moderate renal failure following an oral dose of 1.0 g

gression and the area under the curve (AUC) was estimated by the trapezoidal method. Renal clearance in the healthy volunteers and patients with moderate renal failure was calculated by dividing the amount recovered in the urine by the corresponding AUC. The "SIPHAR"[1] modeling and parameter estimation programme was used for compartmental and model-independent analysis of the plasma paracetamol and metabolite concentration data. To obtain the volumes of distribution (V) of the glucuronide and sulphate conjugates in the patients with moderate renal failure, the "dose" of conjugate was taken as the amount formed from the administered dose of paracetamol (1.0 g × the fractional 24-h urinary recovery of the metabolite). V_z was calculated as total clearance/β and V_{ss} as the product of the clearance and mean residence time. It was assumed that the absorption of paracetamol was complete and that there was no extra-renal loss of the conjugates.

Results are given as means (SD) and metabolite concentrations are expressed as paracetamol equivalents. Differences between means were compared by the Mann-Whitney test or non-parametric analysis of variance with $p = < 0.05$ as the level of significance.

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Results

Plasma Paracetamol

Paracetamol was rapidly absorbed in the healthy volunteers and renal failure patients with mean peak plasma concentrations of 20.0 and 17.9 $mg \cdot l^{-1}$ occuring on average at 0.35 and 0.5 h respectively after administration. The mean plasma half-life from 2 to 8 h was similar in the volunteers, patients with moderate renal failure and dialysis patients (2.2 (0.3), 2.3 (0.5) and 2.1 (0.4) h). However, after 8 h there were significant differences between the groups (Figs. 1-3). Paracetamol continued to disappear rapidly from 8 to 24 h in the healthy volunteers with a mean half-life of 4.9 (2.1) h while low levels persisted in the renal patients and the corresponding half-life in both renal groups combined was 11.7 (5.2) h ($p = < 0.001$). As a result, the total AUC for paracetamol was greater in the renal patients than in the healthy volunteers (Table 2) and there was a highly significant difference in the area from 8 to 24 h (12.4 compared with 6.7 $mg \cdot l^{-1} \cdot h$, $p = < 0.005$).

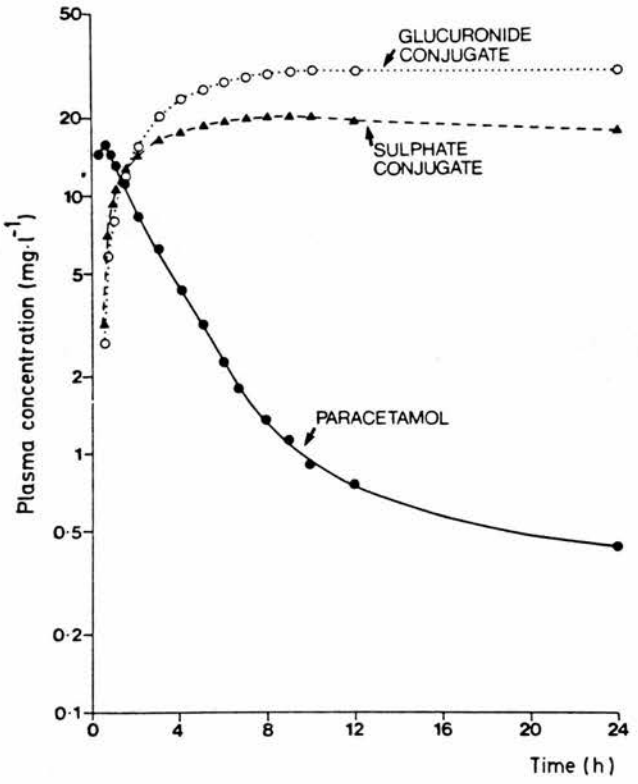

Fig. 3. Mean plasma concentrations of paracetamol and its glucuronide and sulphate conjugates in 6 patients with end-stage renal failure on maintenance haemodialysis following an oral dose of 1.0 g

Table 2. Mean plasma half-life (h) from 8 to 24 h and area under the plasma concentration-time curve (AUC, $mg \cdot l^{-1} \cdot h$) from 0 to 24 h for paracetamol and its glucuronide and sulphate conjugates in healthy volunteers and patients with renal failure

	Paracetamol		Glucuronide conjugate		Sulphate conjugate	
	$t_{1/2}$	AUC	$t_{1/2}$	AUC	$t_{1/2}$	AUC
Healthy volunteers	4.9 (2.1)	45 (11)	2.9 (0.3)[a]	66 (16)	3.5 (1)[a]	21.4 (4.5)
Moderate renal failure patients	11.0 (5.3)	63 (12)	30.5 (40.7)	431 (157)	21.8 (15.2)	232 (108)
Dialysis patients	12.7 (5.5)	57 (15)	-	671 (204)	-	438 (101)

[a] 4 to 12 h

Table 3. Mean maximum plasma concentration (C_{max}, $mg \cdot l^{-1}$) of paracetamol and its glucuronide and sulphate conjugates, and the time to peak concentrations (t_{max}, h) in healthy volunteers and patients with chronic renal failure following an oral dose of 1.0 g of paracetamol

	Paracetamol		Glucuronide		Sulphate	
	C_{max}	t_{max}	C_{max}	t_{max}	C_{max}	t_{max}
Healthy volunteers	20.0 (8.4)	0.35 (0.17)	9.4 (2.6)	2.0 (4.4)	3.7 (0.9)	1.1 (0.5)
Moderate renal failure patients	17.3 (8.2)	0.54 (0.44)	25.2 (8.9)	6.0 (1.6)	13.7 (5.9)	5.6 (1.5)
Dialysis patients	18.6 (4.7)	0.46 (0.29)	31.4 (7.4)	13.8 (8.5)	20.8 (6.5)	8.3 (1.2)

Plasma Concentrations of Paracetamol Conjugates

The plasma concentrations of the glucuronide and sulphate conjugates of paracetamol were greatly elevated in the patients with moderate renal failure, and were even greater in the dialysis patients (Figs. 1-3). There were corresponding highly significant increases in the mean AUC with more than 10- and 20-fold increases for glucuronide and sulphate respectively in the dialysis patients (Table 2). The mean peak plasma concentrations of glucuronide and sulphate (C_{max}) and the time to reach peak concentrations (t_{max}) were significantly increased in the renal patients with the greatest increase in those on dialysis (Table 3).

The elimination of the glucuronide and sulphate conjugates was very slow in the patients with moderate renal failure and the mean plasma half-lives were 30.5 and 21.8 h compared with 2.9 and 3.5 h respectively in the healthy volunteers ($p = < 0.001$, Table 2). There was gross retention of these metabolites in the dialysis patients with no significant fall in concentrations up to 24 h (Fig. 3). In the patients with moderate renal failure there were significant correlations between the plasma creatinine concentration and the AUCs of the glucuronide and sulphate conjugates ($r = 0.91$ and 0.84, $p = < 0.02$).

The cysteine and mercapturic acid conjugates could not be measured in plasma in the healthy volunteers and concentrations were low in the patients with renal failure. Cysteine conjugate concentrations were consistently higher than those of the mercapturic acid conjugate and it disappeared slowly in both groups of renal patients. In the dialysis patients there was no significant fall in plasma mercapturic acid conjugate concentrations up to 24 h (Fig. 4).

Renal Excretion of Paracetamol and Metabolites

In the healthy volunteers a mean of 82.5% of the administered dose of paracetamol was recovered in the urine in 24 h. The mean recovery in the patients with moderate renal failure was 56.9% (range 30-86%) and there was a significant negative correlation with the plasma creatinine concentration ($r = -0.77$, $p = < 0.05$). There were no important differences between the groups in respect of the fractional urinary recovery of paracetamol and its metabolites (Table 4).

In the patients with moderate renal failure the renal clearances of the glucuronide and sulphate conjugates were greatly reduced (mean 14.5 and 14.8 ml·min^{-1} versus 137 and 172 ml·min^{-1} respectively in the healthy volunteers), and were inversely related to the plasma creatinine concentration ($r = -0.87$ and -0.84, $p = < 0.02$). In contrast, the decrease in renal clearance of unchanged paracetamol in these patients ws proportionately much smaller (Table 5) and there was no significant correlation with plasma creatinine or creatinine clearance. As expected, the renal clearance of paracetamol was correlated with the urine flow rate ($r = 0.96$, $p = < 0.01$) and there was no such relationship with the glucuronide and sulphate conjugates.

The renal clearances of the cysteine and mercapturic acid conjugates in the patients with moderate renal failure were surprisingly high (Table 5) and greatly exceeded the creatinine clearance in every case.

Distribution Volumes of the Glucuronide and Sulphate Conjugates

The volumes of distribution of the glucuronide and sulphate conjugates were similar. The respective mean values for V_z and V_{ss} were 0.24 (0.05), 0.28 (0.04), 0.27 (0.06) and 0.29 (0.06) l·kg^{-1}.

Discussion

The absorption of paracetamol appeared to be normal in the patients with renal failure but there were major abnormalities in other aspects of its disposition. Not only were the glucuronide and sulphate conjugates retained as expected, but the elimination of paracetamol itself was also impaired. The rate at which it disappeared from the plasma during the first 8 h was virtually identical in the healthy volunteers and patients with renal failure but in the latter low concentrations persisted after this time and the half-life from 8 to 24 h was greatly prolonged. The healthy volunteers were younger than the renal patients and thus not ideal controls, but it is most unlikely that these differences could be related to factors other than renal disease.

After rapid oral absorption or bolus intravenous administration, paracetamol normally disappears from the plasma in 3 distinct phases. Distribution is largely complete in 1 to 1.5 h, and the log-linear decline from 2 to about 8 h is normally used to calculate the elimination half-life of the drug. However, after 8 h there is a third phase of slower disappear-

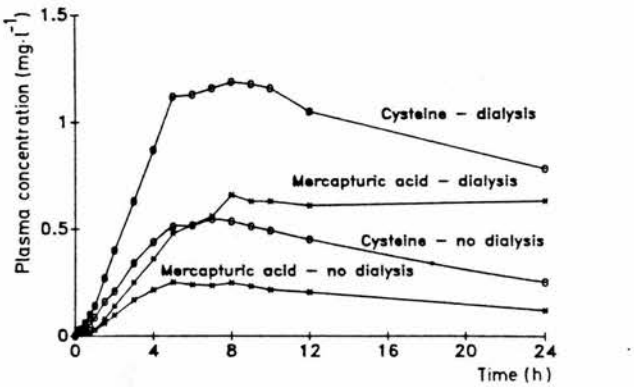

Fig. 4. Mean plasma concentrations of cysteine and mercapturic acid conjugates of paracetamol following an oral dose of 1.0 g in 7 patients with moderate renal failure not on dialysis and 6 patients on regular haemodialysis

Table 4. Mean 24-h urinary recovery of paracetamol and its major metabolites in healthy volunteers and patients with moderate renal failure

	Fractional urinary recovery %					
	Paracetamol	Glucuronide conjugate	Sulphate conjugate	Cysteine conjugate	Mercapturate conjugate	% of dose recovered in 24 h
Healthy volunteers	4.1 (1.4)	60.7 (7.5)	28.1 (5.1)	3.0 (1.7)	4.1 (1.7)	82.5 (6.1)
Moderate renal failure patients	2.4 (1.1)	61.0 (6.8)	30.8 (6.1)	3.0 (0.9)	2.8 (1.4)	56.9 (17.1)

Table 5. Renal clearance (ml·min^{-1}) of paracetamol and its conjugates in healthy volunteers and patients with moderate renal failure

	Paracetamol	Glucuronide conjugate	Sulphate conjugate	Cysteine conjugate	Mercapturic acid conjugate	24-h urine volume (l)
Healthy volunteers	15.7 (6.0)	137 (31)	172 (39)	-	-	1.81 (0.69)
Moderate renal failure patients	5.9 (2.5)	14.5 (6.6)	14.8 (7.6)	35.4 (45)	80.2 (45)	2.08 (0.64)

ance in which the half-life is increased to 4 to 5 h [13]. This late elimination phase was greatly extended in the patients with renal disease and possible rate-limiting mechanisms include slow transfer of residual drug from peripheral tissues back to the circulation and augmented enterohepatic circulation of paracetamol conjugates with regeneration of the parent drug. The latter is more likely since similar unexpected impairment of elimination of drugs such as oxazepam, diflunisal and clofibric acid has been described in patients with chronic renal failure [14-16]. Like paracetamol, these drugs are extensively metabolized by glucuronide conjugation. In patients with renal failure there may be enterohepatic circulation of the retained glucuronide conjugates with regeneration of the parent drug by hydrolysis and subsequent reabsorption [3]. This effect is probably greatest with drugs which form labile ester glucuronides [17]. Paracetamol is converted to a more stable ether glucuronide which is normally excreted into bile to only a limited extent [18]. Although the residual plasma concentrations of paracetamol observed in the present single dose study were low and clinically insignificant, higher concentrations might be expected with the extensive cumulation of conjugates which would occur during long-term treatment in patients with severe renal failure.

The overall metabolic fate of paracetamol in the patients with moderate renal failure and the healthy volunteers was similar as judged by the fractional urinary recovery of the drug and its major metabolites. In particular, the recovery of glutathione-derived conjugates was not increased and there was no evidence of enhanced conversion to potentially toxic metabolites. In the patients with moderate renal failure the renal clearances of paracetamol glucuronide and sulphate were greatly reduced and correlated with the plasma creatinine concentration. In contrast, the renal clearance of unchanged paracetamol was unrelated to the plasma creatinine and in proportion it was reduced much less than the renal clearances of the conjugates. Indeed, the mean renal clearance of paracetamol was within the normal range of 5 to 20 $ml \cdot min^{-1}$, and as in healthy subjects its clearance (but not those of the glucuronide and sulphate conjugates) was dependent on the urine flow rate [8]. The polar glucuronide and sulphate conjugates are excreted primarily by active tubular secretion but the renal clearance of paracetamol depends on glomerular filtration with extensive passive tubular reabsorption [9, 19]. The strikingly disproportionate decrease in the clearance of the conjugates implies much greater reduction in the capacity for active tubular transport than for passive reabsorption. It is also possible that competition or saturation of tubular transport by retained endogenous anions contributes to the very low clearance of the conjugates in patients with renal failure.

Plasma concentrations of the cysteine and mercapturic acid conjugates were very low and the disappearance of the former in the haemodialysis patients indicates a route of elimination other than renal excretion. In the patients with moderate renal failure the renal clearances of both metabolites were considerably greater than the glomerular filtration rate, raising the possibility of their formation from precursors in the kidney. The findings are consistent with renal acetylation of cysteine to form the mercapturic acid conjugate. The volumes of distribution of the individual glucuronide and sulphate conjugates of paracetamol have not been reported previously. Similar values of about 0.27 $ml \cdot kg^{-1}$ were obtained for both conjugates in the patients with moderate renal failure, and these are of the same order as reported previously for the two metabolites combined [9, 11].

The glucuronide and sulphate conjugates of paracetamol appear to be eliminated almost entirely by renal excretion, and marked cumulation would occur with continued use of the drug in patients with renal failure. Predictions based on the clearance of these metabolites by haemodialysis [10] indicate that the average maximum plasma concentrations of glucuronide and sulphate conjugates would exceed 500 and 400 $mg \cdot l^{-1}$ respectively during regular therapy with 1 g of paracetamol 4 times daily in patients with end stage renal disease on twice weekly maintenance haemodialysis. Further studies with multiple doses should be carried out in patients with renal disease and similar cumulation may be expected with other drugs that are extensively converted to polar metabolites which depend on renal excretion for their removal from the body.

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