PARACETAMOL METABOLISM IN MAN

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Ph.D.
UNIVERSITY OF EDINBURGH
1980

UNIV. EDINBURGEN EX BIBL

Abstract

The absorption, metabolism and elimination of paracetamol was investigated in healthy subjects after therapeutic doses and also in patients with paracetamol overdosage, some of whom developed liver damage. Paracetamol disposition was also studied in relation to treatment with N-acetylcysteine (NAC), cysteamine and methionine, which were used to prevent paracetamol hepatotoxicity.

Sensitive, specific and reproducible analytical methods were developed for the estimation of paracetamol and its sulphate, glucuronide, mercapturic acid and cysteine conjugates in plasma and urine. These methods, which employed high-performance liquid chromatography, were significant improvements on existing procedures, especially in terms of simplicity and total assay times.

Following a therapeutic dose, absorption and metabolism of paracetamol was rapid and essentially complete. Paracetamol metabolism showed wide but reproducible individual variation. NAC had little appreciable effect on paracetamol elimination after a therapeutic dose but may have delayed absorption.

The renal clearance of paracetamol was very low, indicating extensive renal tubular reabsorption whereas that of the sulphate and glucuronide conjugates was high, suggesting active tubular secretion. The mercapturic acid and cysteine conjugates were probably also actively secreted since they were not measureable (< 1 μg/ml) in plasma. Renal clearance of paracetamol and its conjugates was not appreciably altered at high plasma concentrations following overdosage.

Following overdosage, paracetamol elimination was rapid but prolonged relative to therapeutic dosage, particularly in patients who developed severe liver damage. The proportions of overall drug recovery excreted

as the sulphate and glucuronide conjugates were lower and higher respectively, indicating saturation of sulphate conjugation. Also, the proportion excreted as the mercapturic acid and cysteine conjugates was increased in patients who developed severe liver damage.

Early treatment of paracetamol overdosage with NAC was associated with reduced hepatotoxicity, enhanced formation of paracetamol sulphate, increased excretion of the mercapturic acid and cysteine conjugates and decreased plasma paracetamol half-life. Cysteamine treatment also resulted in reduced toxicity and enhanced formation of paracetamol sulphate but decreased excretion of the mercapturic acid and cysteine conjugates. Methionine had little appreciable effect on the metabolism of paracetamol and was the least effective treatment. NAC probably protects the liver by "unsaturating" sulphate conjugation and assisting removal of the toxic intermediate metabolite of paracetamol by direct conjugation and/or repleting hepatic glutathione. Cysteamine apparently inhibits the microsomal oxidation of paracetamol to its toxic intermediate metabolite.

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SECTION I

INTRODUCTION AND HISTORICAL REVIEW

Introduction

I) Brief History

The analgesic and antipyretic activity of acetanilide, phenacetin and paracetamol (Fig. I) was first recognised towards the end of the last century. Paracetamol was little used as an analgesic until after it was discovered to be the major metabolite of both acetanilide and phenacetin (Brodie and Axelrod I948a, I949).This suggested that the analgesic effect of these two drugs may have been due to the paracetamol (Greenberg and Lester I946; Brodie and Axelrod, I949). The haematotoxic effects of acetanilide and phenacetin are not shown by paracetamol and this has led to its increasing use over the last 20 years as an alternative to salicylates, which have considerable toxicity (Prescott and Thompson, 1974; Prescott, 1975, 1977, 1979).

The "over-the-counter" availability of paracetamol and growing public awareness of the gastro-intestinal side-effects of aspirin have resulted in declining consumption of aspirin while paracetamol takes an increasing share of the market for mild analgesics (Prescott, I979). Paracetamol either alone or in combination with other drugs accounted for about half the sales of "over-the-counter" mild analgesics and more than double the quantity of aspirin prescribed in

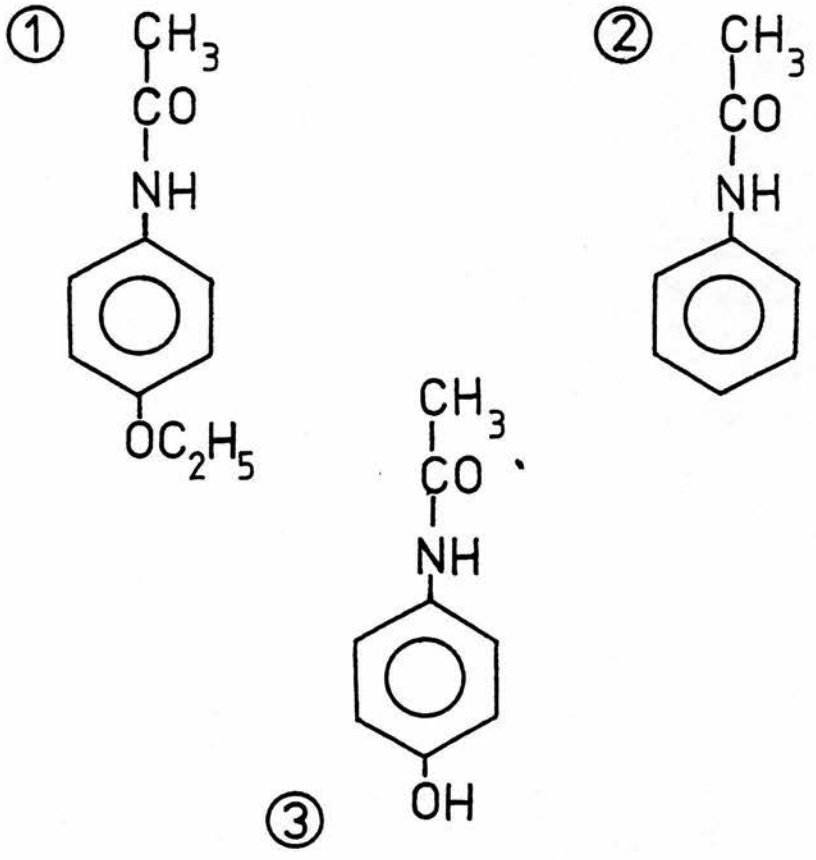


Fig. 1 Structural similarities between Phenacetin (1), Acetanilide (2) and Paracetamol (3).

England and Wales during I973/74 (Spooner and Harvey, I976).

With the increased use of paracetamol there have been increasing reports of overdosage, from which the mortality rate is about 2 - 3% (Volans, I976). Death usually results from fulminant hepatic failure following massive hepatic necrosis (Davidson and Eastham I966). Thus paracetamol, a remarkably safe drug at low doses, is potentially lethal after a large overdose. With large doses paracetamol is converted to a reactive metabolite which binds covalently to liver cell macromolecules (Mitchell et al I973a; Jollow et al I973).

The treatment of paracetamol overdosage has included attempts to remove the drug by dialysis, enhance excretion by diuresis and reduce covalent binding with agents which bind the reactive metabolite of paracetamol. (Farid et al, I972; MacLean et al, I968; Prescott et al, I974, I979 ; Crome et al, I976).

(2) Pharmacology

Paracetamol exhibits antipyretic, analgesic, and to a much lesser extent, anti-inflammatory actions (Beaver, I965, I966). It is ineffective against severe pain but useful against headache, musculo-skeletal pains and, to a lesser extent, rheumatic complaints.

The mechanism of action of some antipyretic analgesics appears to be inhibition of prostaglandin synthesis (Ferreira and Vane, I974). This inhibitory action varies greatly for different drugs at different sites (Flower, I974) and probably explains the variability in pharmacological action of these drugs. The relative lack of anti-inflammatory action of paracetamol was attributed by Flower and Vane (I972) to the fact that paracetamol inhibits prostaglandin synthesis strongly in central nervous tissues, eg the hypothalamus, where whole body temperature is regulated, but weakly in peripheral tissues where local inflammation may be generated (Ferreira and Vane, I974). Salicylates, which are effective anti-inflammatory agents, strongly inhibit prostaglandin synthesis at both sites. Prostaglandins are widely accepted as being associated with inflammation, pyrexia and pain (Ferreira and Vane, I974).

The dose-response of paracetamol as an analgesic

antipyretic in humans is well established. Flinn and Brodie (I948) investigated the effect of paracetamol on the threshold of pain produced by heated needles. They demonstrated that 325 mg taken orally increased the threshold significantly for about 3 hours and that a dose of Ig was not much more effective. Wallenstein and Houde (I954) used a subjective estimation of "relief scores" in patients with cancer pain and showed 600mg paracetamol orally to be as effective as aspirin and significantly more effective than placebo and salicylamide. A similar study by Moertel et al (I972) showed aspirin to be more effective than paracetamol but the differences were not statistically significant. Batterman and Grossman (I955), studying the long term use of paracetamol in subjects with musculo-skeletal pain, demonstrated the analgesic effect of 300 - 600 mg doses four times a day for periods of up to 25 weeks. Thus tolerance does not appear to occur with paracetamol. They also noted the lack of effect in rheumatic patients and this was confirmed by Hajnal et al (I959).

The antipyretic action of paracetamol was investigated by Colgan and Mintz (I957) in children with temperatures above I02^{o}F due to various conditions. A comparison using age-related doses was made with aspirin showing that both drugs were effective anti-

pyretics with maximum effect after about three hours. Anti-diuretic action has been demonstrated in patients with diabetes insipidus using normal doses of paracetamol (Nusynowitz and Forsham, I966). The mechanism of action is unclear but paracetamol was found to increase the rate of water transport across toad bladder (Nusynowitz et al, I966).

(3) Contra-Indications and Adverse Reactions

Paracetamol is generally well tolerated in therapeutic doses. Doses of up to 3.6g daily for periods of up to II6 weeks were better tolerated than aspirin and produced no evidence of blood, kidney or liver disturbances in patients with musculo-skeletal complaints (Batterman and Grossman, I955). In febrile children receiving "therapeutic" doses of paracetamol four times daily for two weeks a similar lack of side-effects was noted (Colgan and Mintz, I957). Methaemoglobinaemia occurs with acetanilide and phenacetin but has not been described with paracetamol (Prescott and Thomson, I974; Prescott, I975). Agranulocytosis, a much more serious haemotoxic effect, has been described in a single patient taking paracetamol regularly for some months (Lloyd, I96I).

The term "Analgesic Nephropathy" has been ascribed to toxic effects on the kidney after chronic use

of excessive doses of mild analgesics, usually with particular reference to phenacetin (Murray and Goldberg, I975; Mitchell et al, I977). Prescott (I965) compared the effects of paracetamol, phenacetin, aspirin and caffeine on the urinary excretion of renal tubular and blood cells. There was some evidence of mild nephrotoxicity with all the drugs but particularly aspirin. Paracetamol appeared to be the least toxic. No adequately documented case of analgesic nephropathy has yet been attributed to paracetamol alone (Prescott, I979). In a study of the chronic use of paracetamol in middle-aged subjects, Edwards et al (I97I) discovered no correlation between renal impairment and ingestion of amounts up to 30 kg over a period of years.

Paracetamol rarely causes allergic reactions but a case of fixed drug eruption attributed to paracetamol has been described (Wilson, I975). In contrast to aspirin, paracetamol does not cause gastric irritation, erosions and bleeding or effects on platelet aggregation (Mielke and Britten, I970).

Paracetamol is not extensively bound to plasma protein at therapeutic concentrations (Gazzard et al, I973) and will therefore be unlikely to cause interactions by displacing other extensively bound drugs.

Eade and Lasagna (I967) studied subjective side-effects of paracetamol and phenacetin in relation to effects on mood, energy and mentation in healthy volunteers. Paracetamol produced the least number of unwanted secondary actions and was almost equivalent to placebo.

4) Absorption, Distribution, Metabolism and Excretion.

Absorption: Paracetamol is taken orally as tablets or elixir and is normally rapidly absorbed from the gastro-intestinal tract. In 8 fasted healthy subjects, mean plasma paracetamol concentrations of I7.8 - 57.7 µg/ml were obtained 30 minutes after ingesting 2g of 6 commercial preparations on different occasions (Richter and Smith, I974). Nimmo et al (I975) found peak concentrations of 20.0 ± I.8 µg/ml I5 - 30 minutes after administering 20 mg/kg of paracetamol in aqueous solution to healthy subjects.

Most drugs are absorbed by passive diffusion in the unionised state (Levine, I970). Paracetamol is a very weak acid with a pKa of 9.5, and therefore exists almost totally unionised in the gastro-intestinal tract, where the pH varies from I - 2 in the stomach to a maximum of about 6 - 7 in the duodenum and 8 towards the large intestine. Weikel and Lish (I959) found that the rate of paracetamol absorption in rats

was dependent on the gastric emptying rate since the major absorption site was the small intestine. This pattern was verified in man by Heading et al (I973).

McGilveray and Mattok (I972) found a similar delay in reaching peak levels (which were also lower) when comparing oral doses in fasting and non-fasting subjects. No difference was found in the total amount of drug absorbed. Paracetamol absorption can also be influenced by the co-administration of other drugs. Nimmo et al (I973a) showed that propantheline (which inhibits gastric motility) and metoclopramide (which stimulates gastric peristalsis) slow and enhance respectively the rate of absorption. The implications may be important in relation to paracetamol toxicity after overdosage.

Distribution: In dogs given 2.7g of phenacetin orally, paracetamol, its major metabolite, was found to be fairly evenly distributed throughout the tissues with the highest concentrations to be found in the kidneys, blood and liver (Brodie and Axelrod, I949). Gwilt et al (I963) confirmed these results in dogs given 300 mg/kg paracetamol orally and noted that fat contained much lower concentrations than other tissues. In man, paracetamol was distributed between red blood cells and plasma in a ratio of I.06 for concentrations between 4 and 25 µg/ml. Glynn and Bastain (I973) found a

good correlation between levels of paracetamol in plasma and saliva and Gazzard et al (I973) observed no binding to human plasma proteins at concentrations below 80 µg/ml and only I5% binding at 280 µg/ml.

Intracellular distribution has been studied in animals with regard to toxic effects on the kidney (Duggin and Mudge, I976; McMurtry et al, I976) and the liver (Jollow et al, I973).

Metabolism: For most drugs, the primary sites of elimination are the liver (metabolism and biliary excretion) and kidney (excretion). Drugs absorbed from the gastro-intestinal tract pass directly to the liver and thence to other organs and sites of action. Thus many drugs taken orally will undergo considerable hepatic metabolism before reaching the systemic circulation. This "first-pass" metabolism of paracetamol in rats removes up to half the paracetamol delivered to the liver, depending on the rate of its delivery (Cohen et al, I974). The fraction of paracetamol normally removed by first-pass metabolism in man was estimated by Chiou (I975) and Forrest et al (I977) to be 0.I02 and 0.I5 respectively. Rawlins et al (I977) observed incomplete bioavailability of paracetamol after oral administration to volunteers and concluded that "first-pass" metabolism was likely to account for

this.

Large amounts of glucuronyl transferases and sulphotransferases together with their cofactors UDP-glucuronic acid and active sulphate (PAPS) are present in the gut and liver. These enzymes convert paracetamol to its phenolic glucuronide and sulphate conjugates. The mixed function oxidase system is also principally located in the liver and performs a variety of oxidative actions, including the addition of oxygen to aromatic molecules to form epoxides and the hydroxylation of aromatic amino groups. Both these actions are potentially damaging to the cell since the products can be highly reactive and bind covalently to macromolecules leading to cell necrosis (Judah et al, 1970). This occurs with paracetamol (Mitchell et al, 1973a) and will be discussed further in the following chapter on toxicity.

However, other enzyme systems such as the glutathione-S-transferases may deactivate these toxic metabolites (Boyland and Chasseaud, 1969). Addition of glutathione to the substrate results in a non-reactive conjugate from which glycine and glutamic acid are often lost to form the cysteine conjugate. Acetylation of the amino group of cysteine results in the formation of mercapturic acid conjugates.

The above reactions account for the metabolism of most of a dose of paracetamol in animals and man (Fig. 2). In humans, the amounts of paracetamol sulphate and glucuronide conjugates recovered in urine normally account for 20 - 30% and 45 - 60% respectively of a therapeutic dose. Only 5% or less is recovered as free drug (Cummings et al, I967; Jagenburg et al, I968; Levy and Yamada, I97I). The cysteine and mercapturic acid conjugates, normally together account for up to IO% of the dose (Jagenburg et al, I968; Mitchell et al, I974). Other minor metabolites include 3-hydroxy and 3-methoxy paracetamol sulphates and glucuronides (Mrochek et al, I974; Andrews et al, I976) and 3-methylthioparacetamol (Klutch et al, I978).

The relative amounts of each metabolite excreted in urine differ considerably after overdosage and normal dosage. In overdosage, Prescott and Wright (I973) found that about I5% was excreted as unchanged drug. Howie et al (I977) observed that in patients with severe liver damage after overdosage, proportionally less was excreted as the sulphate and more as the cysteine and mercapturate conjugates. Knox and Jurand (I977, I978) reported the presence of considerable amounts of 3-methoxyparacetamol in human urine after paracetamol overdosage.

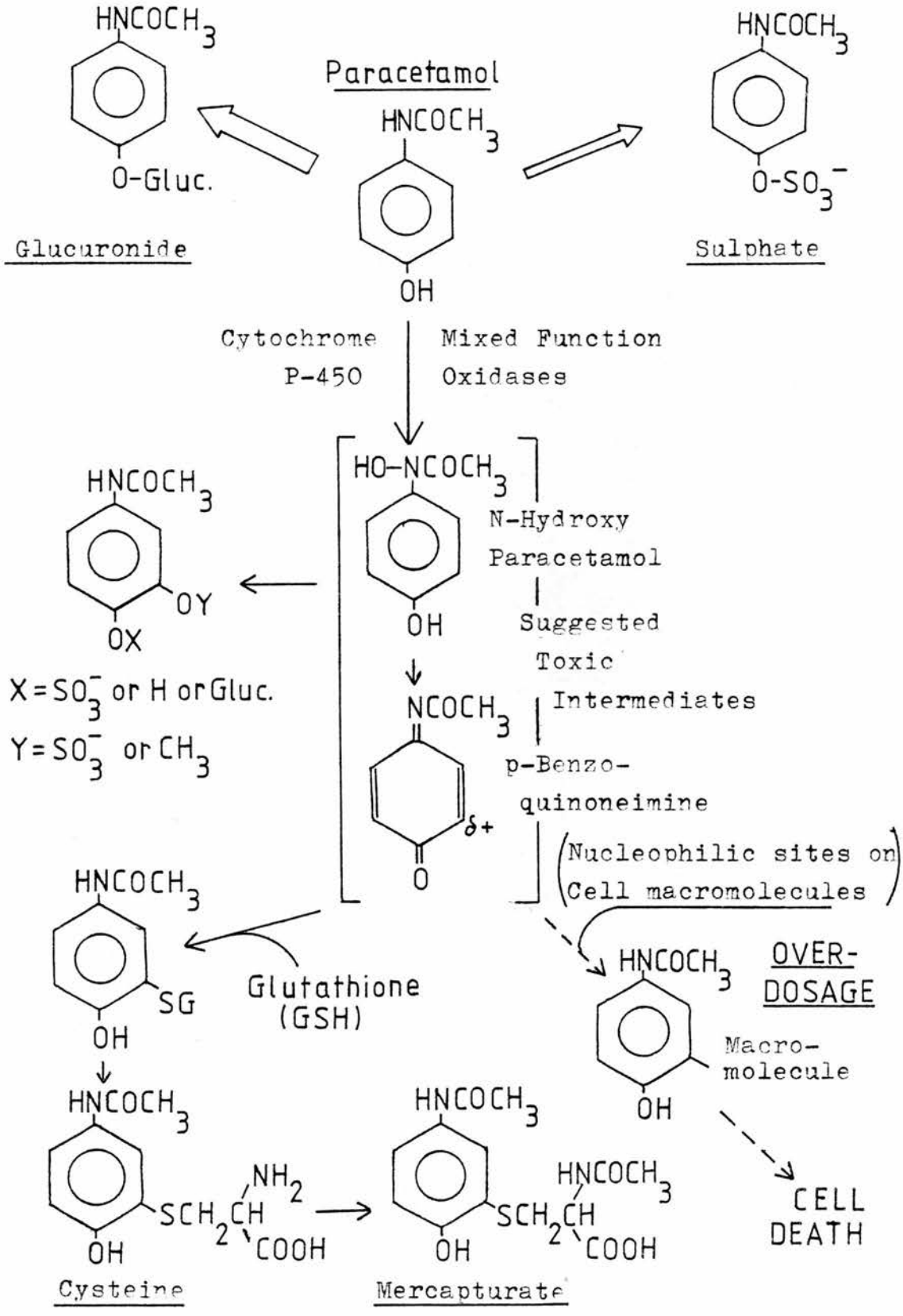


Fig. 2 Metabolism of Paracetamol in Man. (Modified from Mitchell et al, 1974; Andrews et al, 1976).

Much attention has been focussed on N-oxidation of paracetamol and similar aromatic amino- and acetylamino-compounds. Such oxidation by cytochrome P-450 - dependent mixed function oxidases is thought to be the first step in the cytotoxicity often associated with aromatic amino compounds, including paracetamol (Potter et al, I973; Thorgeirsson et al, I973, I975; Hinson et al, I974).

Urinary Excretion:

The elimination half-life of paracetamol after therapeutic doses in normal subjects is I½ - 3 hours with a mean value of about 2 hours (Nelson and Morioka, I963; Cummings et al, I967; Prescott et al, I968, I97I). Cummings et al (I967) found a parallel relationship between semilogarithmic plots of the urinary excretion rate versus time after dose for paracetamol and its sulphate and glucuronide conjugates. Thus paracetamol elimination appears to be limited by the rate of formation of its conjugates. The rate constants of excretion of the sulphate and glucuronide conjugates were considerably greater than for their formation and the excretion rate constant for unchanged drug was very small (Cummings et al, I967; Prescott and Wright, I973).

Prescott and Wright (I973) showed in man that the

renal excretion of unchanged paracetamol was dependent on urine flow rate whereas the sulphate and glucuronide conjugates were actively secreted and renal clearance was therefore independent of flow. This was confirmed by Duggin and Mudge (I975) in dogs. The unchanged drug appeared to be filtered and reabsorbed by passive diffusion so that the excretion rate was low and dependent on urine flow rate. Changes in urine pH had no effect on excretion. These findings are explained by the lipid solubility of paracetamol and the fact that the molecule is practically unionised over the physiological range of urine pH. The conjugates are actively secreted and, due to their high water solubility, are not extensively reabsorbed. Thus their renal clearance was greater than the glomerular filtration rate (Prescott and Wright, I973).

5) Toxicity of Paracetamol:

Introduction

Paracetamol hepato-toxicity was first described in cats (Eder, I964) and later in rats (Boyd and Bereckzy, I966) and at about this time the earliest reports of severe liver damage after overdosage in man appeared (Davidson and Eastham, I966; Thomson and Prescott, I966).

Subsequent reports confirmed these findings (Rose, I969; Proudfoot and Wright, I970; Prescott et al, I97I;

Clark et al, I973). Histological examination of the liver showed acute centrilobular hepatic necrosis. In severe cases necrotic areas coalesce, sometimes resulting in total liver failure and death. In less severe cases regeneration begins soon after overdosage and normal structure and function is usually restored within 7 - 28 days. Acute renal tubular necrosis may occur but is less common (Prescott, I978).

Individual susceptibility to paracetamol toxicity varies widely (Mitchell, I977). Complete recovery has been claimed after a dose of 75g but there was no biochemical evidence that paracetamol had been taken at all (Chakrabarti and LLoyd, I973). On the other hand Wilson et al (I978) reported the death of an adolescent after an estimated dose of only 6 - 9 gm (confirmed by plasma paracetamol concentrations) taken together with phenobarbital.

Mechanisms of Toxicity:

a) Covalent Binding and Cell Necrosis:

The mechanism of paracetamol toxicity was elucidated by Mitchell and his co-workers (Mitchell et al, I973a, I973b; Jollow et al, I973; Potter et al, I973). They demonstrated the role of paracetamol metabolism by cytochrome P-450-dependent mixed function oxidases and implicated an intermediate metabolite as the

indirect cause of centrilobular hepatic necrosis. The extent of damage was dose-dependent and could be altered by modifying the activity of the cytochrome P-450-dependent oxidation system. Animals pre-treated with phenobarbital, (which induces this enzyme system) showed significantly greater liver damage with paracetamol compared with non-pretreated animals. The reverse was found with a mixed-function oxidase inhibitor (piperonyl butoxide) and an inhibitor of cytochrome P-450 synthesis (cobaltous chloride).

Jollow et al (I973) showed that administration of ^{3}H-labelled paracetamol to mice resulted in the covalent binding of a small proportion of radio_activity to cell macromolecules. The metabolite was bound almost entirely in liver cells although later work showed similar affinity for liver and kidney cells (Mitchell et al, I977). Within the cells binding is localised particularly to the endoplasmic reticulum. Mudge and Duggin (I976) and McMurtry et al (I976) have shown that dose-dependent covalent binding of a paracetamol metabolite can occur in the kidney in response to cytochrome P-450-dependent enzyme induction.

The relationship between reactive metabolite toxicity in the liver and other tissues has been discussed from a pharmacokinetic viewpoint by Gillette (I974).

Covalent binding which causes loss of metabolic activity in the liver may switch activity to other sites, such as the kidney, thus setting up a new site for covalent binding and cell necrosis. A second possibility is the migration of reactive metabolite to other sensitive sites. This was thought to be unlikely in the case of paracetamol (Jollow et al, I973; Mudge and Duggin, I976; Mitchell et al, I977) although recent studies suggest otherwise (Healey et al, I978; Gemborys et al, I978).

b) Role of Glutathione

Hepatotoxic doses of paracetamol deplete hepatocellular glutathione and, when stores are reduced by about 70%, covalent binding and cell damage increase dramatically (Mitchell et al, I973b). A similar relationship was found in kidney cells (McMurtry et al, I976). Mitchell et al (I973b) also showed that mice pretreated with diethyl maleate (which depletes glutathione) are more sensitive to the hepatotoxicity of paracetamol. Conversely pretreatment with cysteine, a glutathione precursor, protects against covalent binding and liver damage (Mitchell, I973b; Strubelt et al, I974).

Davis et al (I974) investigated species differences in susceptibility to paracetamol toxicity. They found a direct correlation between the rate of hepatic glutathione

depletion and the rate of covalent binding and severity of necrosis produced by similar dose levels in mice and hamsters (susceptible) and rats, guinea pigs and rabbits (relatively resistant). Jollow et al (I974) compared the metabolism of paracetamol in mice, hamsters and rats and found larger proportions excreted as the mercapturic acid conjugate in species highly susceptible to paracetamol toxicity, such as hamsters. Increasing the dose resulted in increasing excretion of paracetamol mercapturic acid conjugate until a plateau was reached, indicating maximum depletion of glutathione stores. A similar plateau in paracetamol sulphate excretion was also found, suggesting the dependence of both pathways on the same precursors.

The relevance of glutathione depletion to the hepatotoxicity of paracetamol in humans was demonstrated by Mitchell et al (I974) who showed that with increasing doses of paracetamol the proportions of glutathione-derived metabolites excreted in urine increased correspondingly. The toxic dose of paracetamol in mice was extrapolated to man and it was estimated that a dose of I5g was likely to produce hepatotoxicity in humans. Thus the remarkable safety of paracetamol in therapeutic doses is attributable to the ability of the glutathione-conjugating system to inactivate the toxic arylating metabolite.

c) Time Course of Toxicity:

Maximal covalent binding of paracetamol to macromolecules occurs within two hours in mice, well after peak plasma paracetamol concentrations and after glutathione stores have been depleted (Jollow et al, I973; Mitchell et al, I973b). However, biochemical and histological evidence of cell damage is not apparent in mice for at least 3 hours (Mitchell et al, I973a) and in man for I2 - 72 hours after a hepatotoxic dose (Prescott, I975; Davis et al, I976).

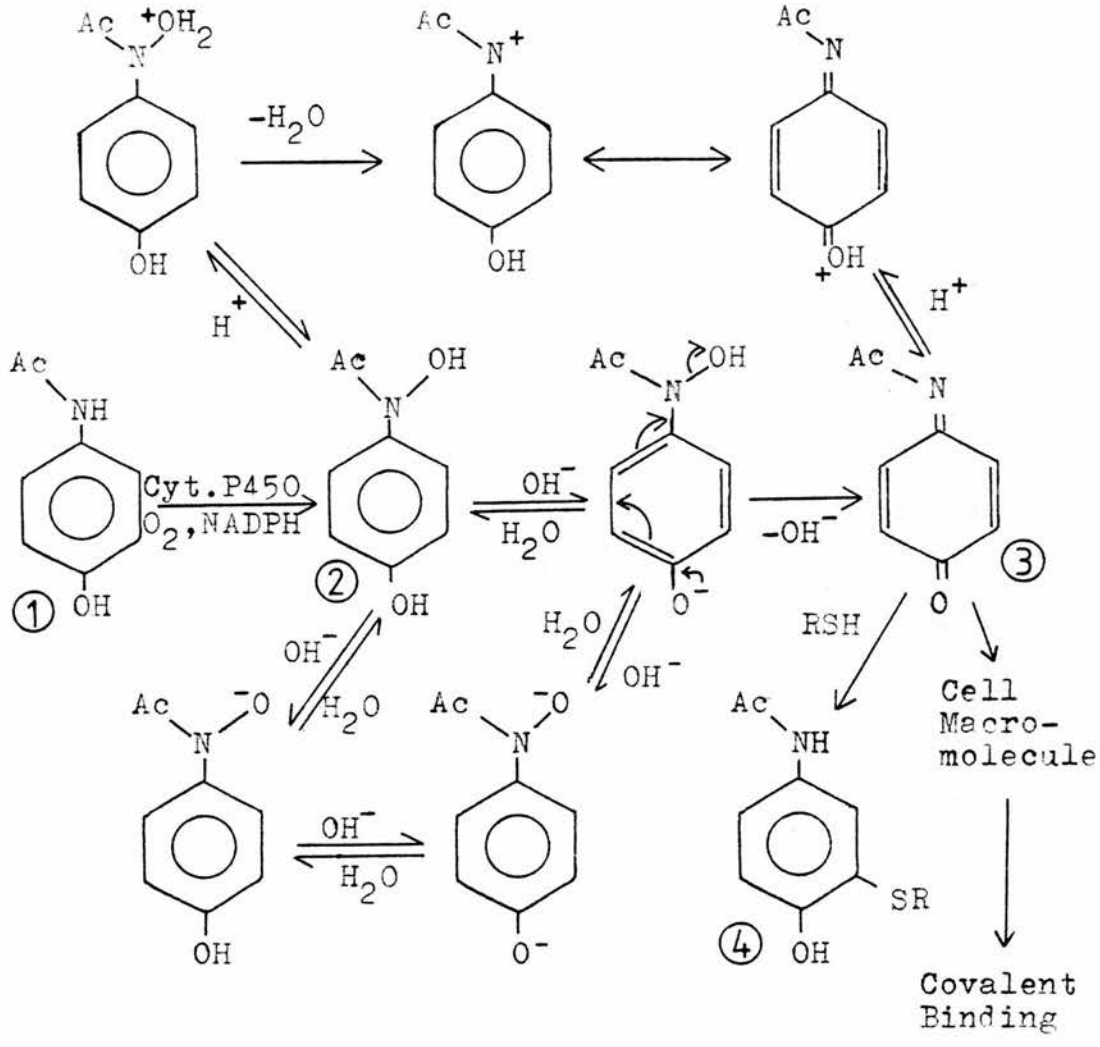
d) N-hydroxylation:

Of the variety of oxidative reactions involving cytochrome P-450-dependent enzymes, N-hydroxylation is the most likely to be responsible for paracetamol toxicity. Thorgeirsson et al (I973) demonstrated that N-hydroxylation of 2-acetylaminofluorene, a carcinogen, is dependent on this enzyme system and Davis et al (I974) noted that hamsters, which are very susceptible to 2-acetylaminofluorene toxicity, produce large amounts of the N-hydroxy derivative. However, guinea pigs are resistant to 2-acetylaminofluorene toxicity and produce little N-hydroxy compound. Thorgeirsson et al (I975) found that mice with genetic loss of the ability to increase cytochrome P-450-dependent enzymic oxidative capacity were less susceptible to paracetamol

and 2-acetylaminofluorene toxicity than normal mice. Also, pretreatment with enzyme inducers produced no effect on N-hydroxy-2-acetylaminofluorene excretion. More indirect evidence in favour of N-hydroxyparacetamol as a precursor to cytotoxicity is the low toxicity of the N-methyl analogue of paracetamol (Jollow et al, 1974; Nelson et al, 1978) which must be demethylated before N-oxidation can occur.

A complex scheme implicating N-hydroxylation as the first step in paracetamol toxicity was suggested by Gemborys et al (1978). N-hydroxyparacetamol probably rearranges to the highly reactive N-acetyl-p-benzoquinoneimine with loss of water (see Figs 2 and 3) and this may explain why glutathione conjugates of paracetamol are substituted in the 3-position (Calder et al, 1974). The 3-position carbon will exhibit electrophilic properties and attract nucleophilic divalent sulphur, such as in sulphydryl compounds and nucleophilic centres of macromolecules (Fig 4).

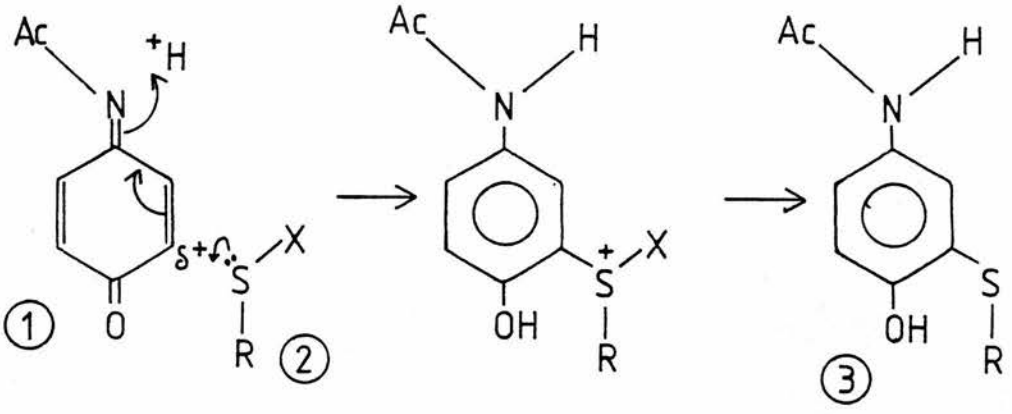
Mulder et al (1977, 1978) suggested that N-hydroxylation may be only the first step towards toxicity. Other enzymic reactions such as sulphation or deacetylation may be involved before covalent binding to macromolecules occurs. Recently, Hinson et al (1979) found that N-hydroxyparacetamol can be formed from phenacetin by microsomal oxidation but evidence for its formation from paracetamol was not found.



Key :- Ac = CH_3CO

(1) Paracetamol
(2) N-Hydroxyparacetamol
(3) N-Acetyl-p-benzoquinoneimine
(4) Detoxification of (3) with sulphydryl compounds (RSH) eg Glutathione

Fig. 3 : Suggested fate of paracetamol following N-Hydroxylation (after Gemborys et al, 1978).



Key :- Ac = CH_3CO

(1) N-Acetyl-p-benzoquinoneimine
(2) Glutathione or Macromolecule (X = CH_3 or H)
(3) 3 - Substituted Paracetamol

Fig. 4: 3-Substitution of Paracetamol in glutathione conjugation and covalent binding to macromolecules.

e) Toxic Dose and Liver Function:

Paracetamol hepatotoxicity results in changes in the ability of the liver to carry out its normal functions. This can be assessed by standard clinical tests of liver function such as for hyperbilirubinaemia and prolongation of the prothrombin time. Also, marked increases in the plasma concentrations of enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) occur as a result of leakage from damaged liver cells.

The toxic dose-response relationship in animals is less easily identifiable in humans. The observation of Proudfoot and Wright (1970) that about 15g appeared to be the threshold toxic dose was confirmed with the similar biochemical estimate of Mitchell et al (1974). However, a single dose of 5.85g, not much above the therapeutic dose, was said to produce severe liver damage in one subject (Fernandez & Fernandez-Brito, 1977) whereas 15.3g apparently produced no signs of damage in another (Prescott and Wright, 1973) and 6 - 9g has been fatal (Wilson et al, 1978).

There is an apparent wide variation in the individual susceptibility to paracetamol toxicity. Unreliability in reporting the actual dose ingested (Matthew, 1971)

and the common occurrence of vomiting after ingestion both contribute to the uncertainty in correlating dose with toxicity. The only way to check the amount said to have been taken is by measurement of paracetamol or its metabolites in body fluids. The recovery of paracetamol and its conjugates in urine has been used as a measure of the dose ingested (Prescott et al, I974. I976; Davis et al, I976). A positive relationship between the severity of liver damage and urinary recovery of drug was found by Davis et al (I976). Prescott et al (I97I) found that the plasma paracetamol concentration at 4 and I2 hours after ingestion could provide a useful indication of the likelihood of liver damage and concluded that levels above 200 µg/ml at 4 hours and 50 µg/ml at I2 hours were usually associated with hepatotoxicity. Concentrations of less than I20 µg/ml at 4 hours and 25 µg/ml at I2 hours were never associated with serious toxicity. Paracetamol concentrations at these times correlated with abnormalities of liver function tests carried out subsequently. However, the dependability of this correlation rests on accurate knowledge of the time of paracetamol ingestion. A more reliable guide was the plasma paracetamol half-life, which indicated probable liver damage if greater than 4 hours (Prescott et al, I97I). An increase in the half-life reflected the general impairment of liver function caused by hepatic necrosis. These findings were confirmed by Stewart and Simpson (I973).

f) Factors Affecting Toxicity:

The effect of enzyme induction on toxicity in animals has already been mentioned (p. 18). Self poisoning often involves more than one drug, frequently including known enzyme inducers such as ethanol and barbiturates. Wright and Prescott (1973) studied the effect of possible enzyme induction in paracetamol overdosage and found increased severity of liver damage in potentially-induced patients. Evidence for increased production of the toxic metabolite in such patients was provided by Mitchell et al (1974) who found that volunteers pre-treated with phenobarbital produced more paracetamol mercapturic acid conjugate than non-pretreated subjects.

Nutrition influences paracetamol toxicity in rats (McLean and Day, 1975). Low protein or yeast diets produced lower hepatic levels of cytochrome P-450 but an even greater reduction in glutathione levels. Enzyme induction and negative nitrogen balance have been implicated as potentiators of hepatotoxicity with the chronic use of paracetamol (Barker et al, 1977).

Competition for glucuronide and sulphate conjugation with paracetamol may also potentiate hepatotoxicity. Oxazepam and ascorbic acid compete with paracetamol for glucuronide (Dybing, 1976) and sulphate conjugation

respectively (Houston and Levy, I976). In subjects ingesting I - 4g of paracetamol, the amount of drug excreted as the sulphate conjugate tended to plateau with increasing dosage , indicating saturation of the sulphate conjugation pathway (Davis et al, I976).

Young children are more resistant to the hepatotoxicity of paracetamol than adults , which could reflect their relatively greater production of paracetamol sulphate (Miller et al, I976). The elimination half-life of paracetamol may be longer in young children and neonates (Levy et al, I975; Miller et al., I976) suggesting that the overall metabolism of paracetamol is slower than in adults.

Other factors such as the ability to repair damaged tissue may also be important in determining the extent of liver damage after paracetamol overdosage.

g) Effect of Toxicity on Paracetamol Elimination.

Hepatotoxicity after paracetamol overdosage affects the metabolism of paracetamol (Prescott and Wright, I973). The elimination half-life of paracetamol was prolonged within a few hours of the ingestion of toxic doses indicating early onset of liver damage and impairment of paracetamol metabolism. This was confirmed by the low ratio of conjugated to free drug

in the plasma of patients with liver damage compared to those without. Patients with liver damage who had previously taken enzyme inducers such as phenobarbital and ethanol showed similar reductions in the rate of paracetamol elimination (Wright and Prescott, 1973).

Comparison of the kinetics of elimination of therapeutic and toxic doses revealed that elimination was rapid in the absence of liver damage, but was progressively impaired with increasing severity of liver damage, and significantly more paracetamol was excreted unchanged in patients with severe liver damage. Urinary excretion of paracetamol and its metabolites was rapid whether liver damage was present or not, provided that kidney function was unimpaired.

6) Treatment of Paracetamol Overdosage:

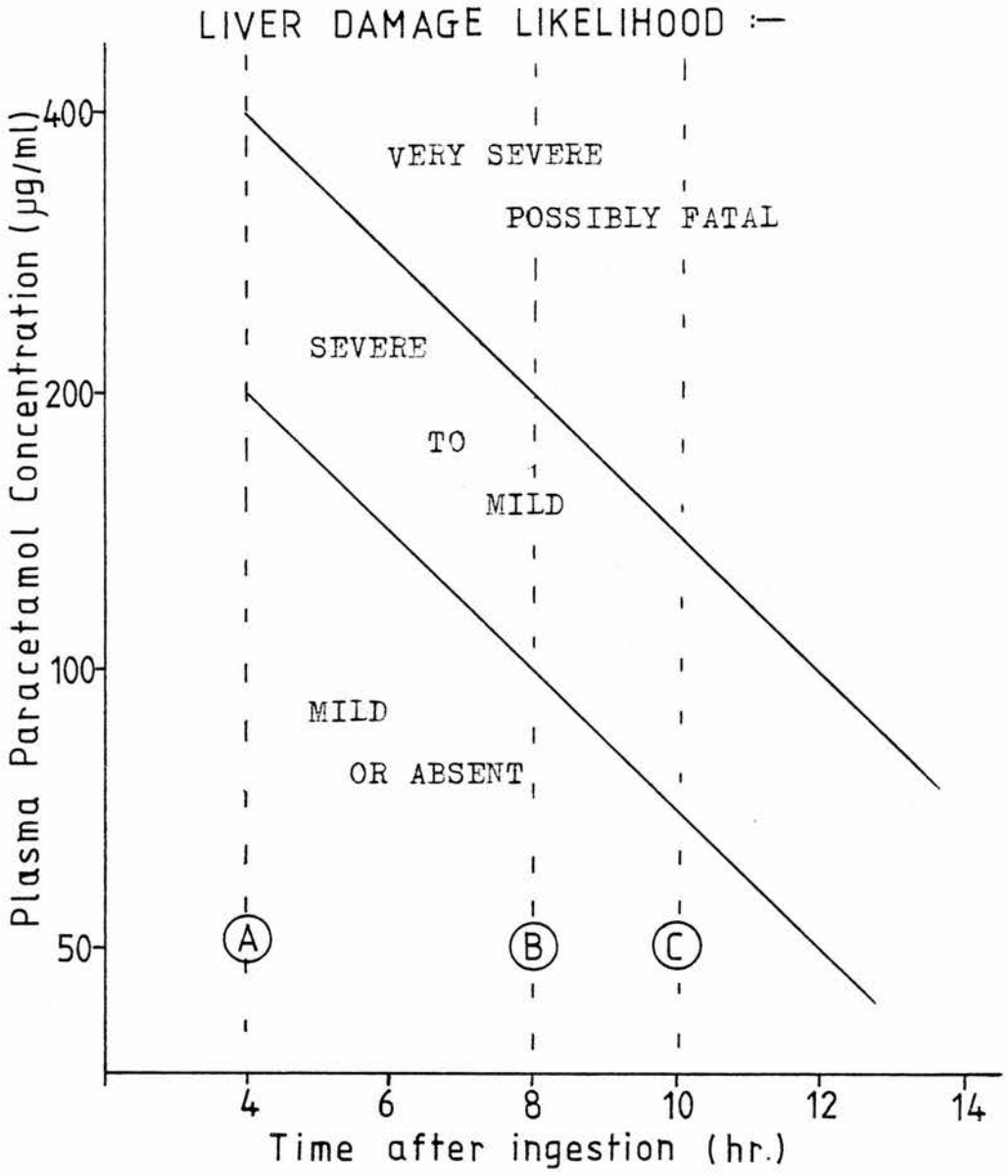
The hepatotoxicity of paracetamol depends on its absorption, distribution, metabolism and excretion. Attempts have been made to modify each of these processes in the hope of preventing toxicity. It may be possible to remove the drug before absorption is complete, haemodialysis has been used to enhance its removal from the blood and its metabolism may be modified to prevent formation of the toxic metabolite and its covalent binding (Prescott and Matthew, 1974;

Farid et al, I972; Strubelt et al, I974; Harvey and Levitt, I976).

Time is an important factor since treatment must be instituted before glutathione depletion and irreversible covalent binding occurs. Some "treatment" is potentially harmful, especially if given too late (see below). To aid the early recognition of potential toxicity and the need for treatment, Prescott et al (I974) constructed a nomogram of plasma paracetamol concentration against time after ingestion (Fig 5). Patients with plasma paracetamol concentrations above the line joining 200 µg/ml at 4 hours and 50 µg/ml at I2 hours were considered to be at considerable risk of liver damage and therefore likely to benefit from treatment. With values below the line, treatment would be unnecessary.

a) Prevention of Absorption:

Gastric aspiration and lavage are normally carried out in patients admitted not later than 4 hours after ingestion (Matthew, I97I; Prescott and Matthew, I974) since absorption is usually complete within this time. Dordoni et al (I973) recommended the administration of activated charcoal or cholestyramine to bind unabsorbed paracetamol and therefore prevent its absorption. Although paracetamol absorption was considerably



Ingestion to treatment interval:-

Ⓐ Continuing absorption likely up to 4 hrs - paracetamol concentration not diagnostic.
Ⓑ Time limit for maximum efficacy of treatment.
Ⓒ Limited efficacy up to 10 hrs; no protection beyond 15 hrs after ingestion.

Treatment is indicated if the paracetamol concentration is above the lower line.

Fig. 5 : Paracetamol "Treatment Lines" (modified from Prescott et al, 1974).

reduced when drug and adsorbent were taken together, reduction was insignificant when adsorbent was given one hour or more after the paracetamol. Very few self-poisoning cases are admitted to hospital within such a short time of ingestion.

b) Removal from Blood:

Haemodialysis was used by Farid et al (I972) who stated that such treatment might be valuable up to I2 hours after ingestion. Subsequent analysis of their data by Prescott (I972) revealed that potentially toxic quantities of paracetamol had been taken by only 3 out of I5 patients dialysed and that liver damage had occurred in all three. Most patients were dialysed unneccessarily and only very small amounts of paracetamol were removed in this way, even in the more severely poisoned patients.

Haemoperfusion through coated charcoal columns has been proposed as a more rapid means of removing paracetamol from the circulation (Willson et al, I973). However, in a controlled trial by the same workers (Gazzard et al, I974b) haemoperfusion was of no clinical benefit. The treatment was probably given too late and only small quantities of paracetamol were removed. The authors stated that possible harm may have been done by the treatment.

Forced diuresis does not enhance elimination significantly. Although urinary excretion of paracetamol is dependent on urine flow rate, elimination by conjugation is far more important, even when liver damage is present (Prescott and Wright, I973). Furthermore, paracetamol has antidiuretic properties (Nusynowitz and Forsham, I966) and renal failure sometimes occurs after overdosage. Attempts at forced diuresis in such circumstances could be harmful.

c) Sulphydryl Compounds:

The major role of glutathione conjugation in protecting against paracetamol toxicity was elucidated by Mitchell et al (I973b, I974). Intravenous administration of glutathione in high doses was shown to protect mice against paracetamol toxicity (Strolin-Benedetti et al, I975) but Gazzard et al (I974a) obtained no protection in rats. Glutathione does not enter cells easily. Other compounds such as cysteamine, methionine, cysteine and dimercaprol have been shown to be much more effective protective agents in animals (Mitchell et al, I973b, I974; McLean and Nuttall, I978; Strubelt et al, I974).

Cysteamine may act by inhibition of microsomal oxidation, since it reduces the amounts of divalent sulphur conjugates produced in isolated perfused rat

liver (Harvey and Levitt, I976). Cysteamine and the other compounds are very effective in restoring depleted hepatic glutathione (Strubelt et al, I974) and may act as glutathione precursors or substitutes.

Mitchell et al (I974) proposed that cysteamine and similar nucleophiles should prevent liver damage after paracetamol overdosage in man, even when given many hours later. Of several sulphydryl compounds tested, D-penicillamine appeared to aggravate the nephrotoxicity of paracetamol (Prescott et al, I976) and dimercaprol was not particularly effective (Hughes et al, I976). Intravenous cysteamine has been widely used in man since Prescott et al (I974) reported its first successful use in preventing severe liver damage in patients with severe paracetamol poisoning.

However, cysteamine produces unpleasant side effects and therefore alternatives were sought. Oral L-methionine has since been shown to afford some protection in man without unpleasant side effects (Crome et al, I976) although Prescott et al (I976) considered cysteamine to be more consistently effective. It is now recognised that all treatment should be given within 8 -10 hours of ingestion since effectiveness decreases dramatically beyond this time (Prescott et al, I979). In addition, large doses of sulphydryl compounds

may be dangerous if severe liver damage has already occurred. Methionine in particular aggravates pre-existing liver disease (Phear et al, I956).

Prescott and Matthew (I974) suggested the widely-available mucolytic N-acetylcysteine as a possible alternative to cysteamine. Subsequently Piperno and Berssenbruegge (I976) demonstrated its effectiveness in mice up to 4½ hours after paracetamol ingestion, ie after the first signs of liver damage had occurred. Since then Prescott et al (I977, I978, I979) have reported its effectiveness and lack of side-effects in man and further experience has shown it to be more effective than cysteamine and methionine. The time limit for maximum effectiveness with all three compounds is 8 hours after paracetamol ingestion (Prescott et al, I978, I979).

d) Other Methods:

Mepyramine and hydrocortisone were recommended by MacLean et al (I968). However subsequent studies showed that this treatment greatly increased mortality from paracetamol in rats (Nimmo et al, I973b). Walker et al (I974) showed that vitamin E protects rats against paracetamol hepatotoxicity, possibly by an antioxidant effect on cell membrane lipids which would otherwise be damaged by the toxic metabolite of

paracetamol. It has been suggested that incorporation of vitamin E, methionine or N-acetylcysteine, into paracetamol tablets would provide "built in" protection against liver damage after overdosage (McLean and Day, 1975; Jondorf, 1978).

SECTION II

OUTLINE AND PURPOSE OF THE PRESENT STUDY

Section II

Outline and Purpose of the Present Study.

Previous investigations into the metabolism and pharmacokinetics of paracetamol in man were limited owing to the difficulty in measuring metabolites and the small numbers of subjects generally used. Usually only therapeutic dosage has been studied and often drug and metabolite estimations were based on non-specific analytical methods. The results of these studies do not always agree and more precise information, particularly following overdosage, is necessary to gain a better understanding of the fate of paracetamol in man and its effects on the liver.

1) Estimation of Paracetamol and its Major Metabolites

Studies by many workers have demonstrated widely varying metabolite patterns and overall recovery of paracetamol (Mrochek et al, 1974; Davis et al, 1976; Andrews et al, 1976). With the advantages of High Performance Liquid Chromatography (HPLC) and careful quantitation procedures for estimating paracetamol and its metabolites, it is possible to obtain more reliable data. Three such procedures were developed and evaluated -

a) Estimation of paracetamol and its major metabolites, the sulphate, glucuronide, cysteine and mercapturic

acid conjugates in urine.

b) Estimation of paracetamol alone in plasma.

c) Estimation of paracetamol and its sulphate and glucuronide conjugates in plasma.

2) Studies in Healthy Subjects

Control data were obtained from healthy subjects taking a therapeutic dose of paracetamol. A similar study was carried out with the simultaneous administration of N-acetyl-L-cysteine (NAC) in an attempt to elucidate the mechanism whereby NAC protects the liver from damage after paracetamol overdosage.

3) Hepatic Damage after Paracetamol Overdosage

There have been many clinical reports concerning the damaging effects of paracetamol on the liver (Davidson and Eastham, 1966; 'Paracetamol Hepatoxicity,' Lancet, 1975). In this study paracetamol metabolism and elimination was investigated in overdosage patients, comparing those who subsequently developed liver damage with those who did not.

4) Effects of Treatment on Paracetamol Metabolism and the Prevention of Liver Damage

In the last 6 years L-methionine, cysteamine and N-acetyl-L-cysteine (NAC) have been used in the Edinburgh Regional Poisoning Treatment Centre to prevent liver damage after paracetamol overdosage. The protective

actions of these compounds were investigated in relation to their effect on the metabolism and elimination of paracetamol in an attempt to throw light on the mechanisms involved.

SECTION III

ESTIMATION OF PARACETAMOL AND ITS METABOLITES IN URINE AND PLASMA

ESTIMATION OF PARACETAMOL AND ITS MAJOR METABOLITES IN URINE AND PLASMA

Chapter 1 Review of Analytical Methods

Various methods have been developed to rapidly and reliably estimate plasma concentrations of paracetamol with minimum interference from other materials. This is important since the prognosis after overdosage may be greatly improved if an early estimation of the plasma paracetamol concentration can be made, thus allowing specific therapy to be given when such treatment will be most effective (Prescott et al, 1976). Specific procedures are necessary because about 25% of self-poisoning attempts are made with more than one drug (Chambers, 1976) and some methods are liable to interference by other drugs.

The earliest methods were colorimetric (Greenberg and Lester, 1946; Brodie and Axelrod, 1948), employing an acid hydrolysis of paracetamol to p-aminophenol. This was followed by a coupling reaction with α-naphthol to produce a blue colour which was measured at 620nm. Other procedures broadly based on these methods are numerous (reviewed by Wiener, 1978), the advantage being that spectrophotometers are in wide use and are simple to operate. They must be used with care when specificity for free paracetamol is important since p-aminophenol is also produced by hydrolysis of the sulphate and gluc-

uronide conjugates of paracetamol. A rapid method not susceptible to metabolite interference is that of Glynn and Kendal (1975). Paracetamol is nitrated and the resulting orange-yellow colour in alkaline solution is measured. Free paracetamol may be assayed directly by its ultra-violet absorbance after solvent extraction (Routh et al, 1968).

With the colorimetric and UV absorbance assays, interference from unrelated compounds can occur (Wiener, 1978) and none of the methods are particularly suitable for measuring therapeutic concentrations of paracetamol. Several direct gas chromatographic methods have been reported which overcome some of these problems (Grove, 1971; Windorfer and Röttger, 1974; Stewart and Willis, 1975). However, paracetamol is chromatographed as the free drug and considerable adsorption losses are liable to occur using this approach due to the polar nature of the drug. Most workers favoured derivatisation to improve chromatography, particularly at very low concentrations (Prescott, 1971 a and b; Thomas and Coldwell, 1972; Street, 1975; Dechtiaruk et al, 1976; Garland et al, 1977; Evans and Harbison, 1977). The major problem with gas chromatography is the need to concentrate and derivatise the extracts, which adds to the total analysis time. Also, some methods are still liable to interference (Street, 1975; Dechtiaruk et al, 1976; Kalra et al, 1977).

All the above procedures can measure either free paracetamol or total paracetamol after enzymic or acid hydrolysis. Estimation of the individual conjugates was laborious and unsatisfactory. Jagenburg et al (1968) employed column chromatography with Sephadex G10 to separate paracetamol and its sulphate, glucuronide, cysteine and mercapturic acid conjugates. Separation and quantitation was a very lengthy process requiring large quantities of urine. Simpler, more rapid separations were achieved using Thin Layer Chromatography (TLC) with either one or two dimensional systems (Cummings et al, 1967; Davis et al, 1976; Andrews et al, 1976). Quantitation was attempted by UV absorbance after elution from the silica gel or by scanning densitometry. None of these techniques was found to be satisfactory for routine use in this laboratory (Howie, 1977). The ion exchange system of Mrochek et al (1974) provided excellent separations but took between one and two days per analysis.

Analysis of polar compounds was greatly facilitated with the advent of High Performance Liquid Chromatography (HPLC), allowing fast separations of many compounds on short columns. It is now possible to separate many components of complex mixtures in a single analysis on columns as short as 100 mm or less. Another major advantage of HPLC is the ease of sample preparation. Analysis can be performed in an aqueous, organic or mixed mobile

phase and the sample needs only to be miscible with this eluting phase before chromatography is performed. However, many drugs and metabolites are not easily detectable at the low concentrations often encountered in untreated biological fluids. Detection is no problem with paracetamol since the free drug and its conjugates all have adequate UV extinction coefficients and can be quantified in plasma and urine without concentration after therapeutic doses. Paracetamol alone has been analysed using HPLC by many workers, mostly after an extraction stage (Wiener, 1978).

The divalent sulphur-containing conjugates of paracetamol were measured by Buckpitt et al (1977) in liver microsomal preparations. Quantitation was achieved using ^{14}C - labelled paracetamol and three mobile-phase systems were required to separate the compounds of interest. Knox and Jurand (1977, 1978) have described the separation of paracetamol and its metabolites in urine using HPLC from a theoretical viewpoint and they identified at least one previously unknown metabolite. Quantitation was not attempted but useful guidance was given in the formulation of suitable systems for separating compounds of widely varying physico-chemical characteristics.

Howie, Adriaenssens and Prescott (1977) first described the quantitation of paracetamol and its major conjugates in urine. The only sample preparation

required was the addition of aqueous internal standard solution. Free paracetamol in plasma was measured after the addition of internal standard in aqueous trichloracetic acid, which served to remove plasma proteins prior to analysis. Columns dry-packed with octadecyl silica (Spherisorb-ODS 10 μm) were used in each case. Modifications of these procedures were used in subsequent studies on paracetamol and its major conjugates in both urine and plasma.

Chapter 2

b) Development of HPLC Methods for the Estimation of Paracetamol and its Metabolites in Plasma and Urine

a) Reference Compounds

Paracetamol was obtained from BDH, Poole, Dorset and was assumed to be 100% pure. Paracetamol sulphate potassium salt monohydrate, paracetamol glucuronide, paracetamol cysteine and paracetamol mercapturate were kindly donated by Dr R S Andrews, Sterling-Winthrop, Research and Development Division, Newcastle-upon-Tyne, England and Dr W Madison, McNeil Laboratories Inc., Fort Washington, USA. The conjugates were obtained either synthetically or from natural sources.

Purity was determined by UV spectrophotometry of aqueous solutions and HPLC. The figures obtained were:
Paracetamol sulphate - 91%
Paracetamol Glucuronide - 96%
Paracetamol Cysteine - 93.7%
Paracetamol Mercapturate - Not determined at source.
The purity of paracetamol mercapturate as determined by Raney Nickel reduction followed by GLC was 41.7% (Howie, 1977).

The internal standard, N-propionyl-4-aminophenol was synthesised and purified as described below. Analytical grade reagents were used for HPLC solvents without further purification. 4-fluorophenol was obtained from Aldrich Chemical Co, Gillingham, Dorset. 60% W/V

Perchloric acid and Trichloracetic acid were analytical grade and obtained from BDH, Poole, Dorset.

b) Internal Standard

The original internal standard used in the method of Howie, Adriaenssens and Prescott (1977) was 4-fluorophenol. This compound is hygroscopic and its UV absorption is relatively poor at the optimum wavelength for detecting paracetamol and its conjugates (242 nm). A new internal standard, N-propionyl-4-aminophenol (NPA) was synthesised and evaluated.

Synthesis of N-propionyl-4-aminophenol (NPA)

Reagents: 4-aminophenol (BDH, reagent grade); propionic anhydride (BDH, 97% min. containing about 3% propionic acid); methanol (James Burrough, reagent grade); diethyl ether (May and Baker analytical grade).

Method: 2g of 4-aminophenol was vigorously shaken with 10 ml of methanol to remove dark brown impurities present in the reagent grade material. The mixture was then filtered and the colourless crystals remaining were washed with a further 10 ml of methanol and dried.

1.09g (10 millimole) of 4-aminophenol was dissolved in 30 ml of methanol in a 100 ml round bottomed flask with gentle warming and a reflux condenser fitted. 1.43g (1.42 ml, 11 millimole) of propionic anhydride, diluted in 20 ml of diethyl ether was added slowly over

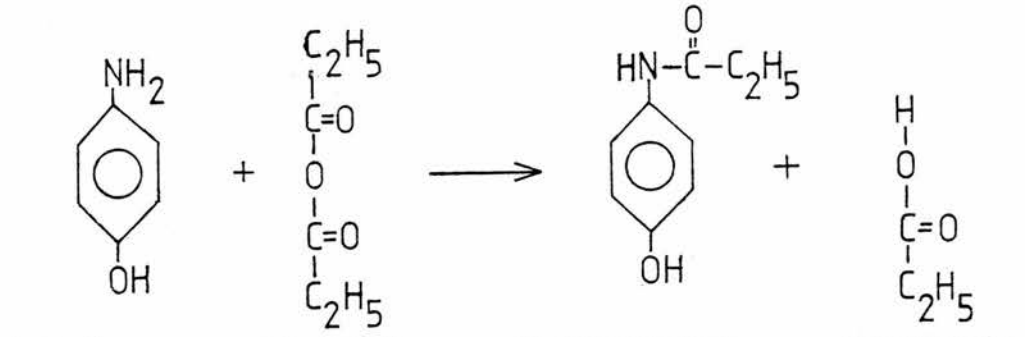
about 2 minutes to the methanolic solution of 4-amino-phenol. The mixture was refluxed for 15 minutes, cooled and then evaporated under vacuum to remove the diethyl ether at room termperature and then the methanol on a water bath at 60°c. When about 3 ml remained, 30 ml of distilled water was added and the product was allowed to crystallise at 4°c for about 30 minutes.

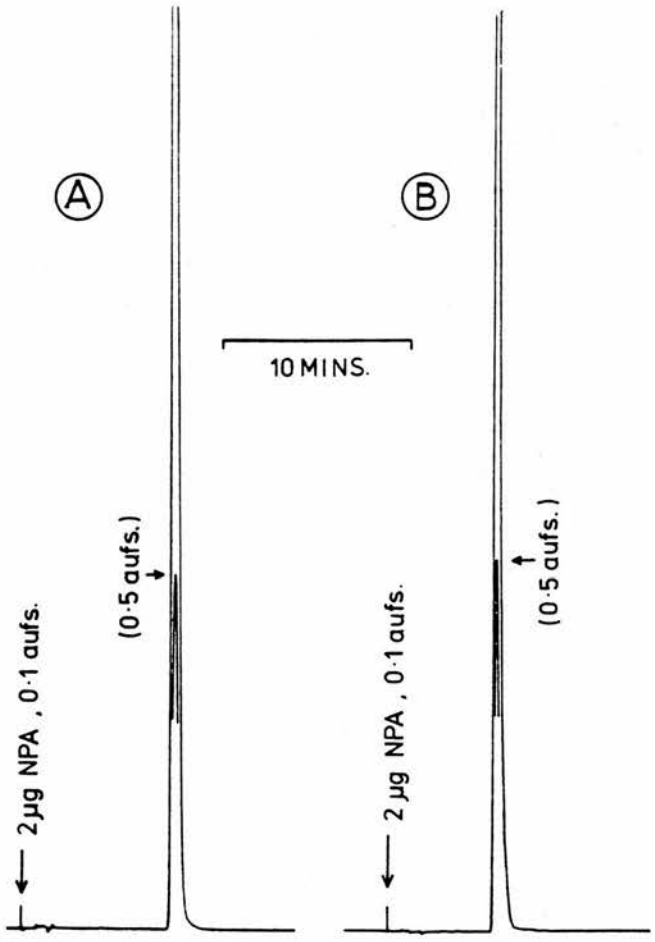
The faintly-pink crystals were filtered and washed successively with 20 ml of distilled water, 2 x 10 ml of 3% aqueous sodium bicarbonate and 20 ml of distilled water. The washed product was redissolved in the minimum volume of hot methanol, 30 ml of hot water was added and the solution allowed to recrystallise at 4°c for 1 hour.

After filtration and drying, 0.86g of a colourless, odourless, non-hygroscopic crystalline product was obtained, equivalent to a 52.1% yield. The reaction scheme is shown in Fig. 6. Chromatographic purity was determined in the HPLC systems used for urine and plasma analysis. A single peak was obtained at 242 and 254 nm (Fig.6). The UV spectrum of an aqueous solution was practically identical to that of paracetamol (Fig 7 ; molar extinction coefficient 0.99 relative to paracetamol at 242 nm).

c) Apparatus

The HPLC system consisted of an Orlita model AE10 - 4 high pressure pump which delivered solvent via a simple





(A) COLUMN: 90 x 4.9 mm ODS-Hypersil. MOBILE PHASE: 0.1 M potassium dihydrogen phosphate: 98% formic acid : isopropanol (100: 0.1: 1.7 v/v/v); 1.3 ml/min. WAVELENGTH: 254 nm.

(B) COLUMN: 170 x 4.6 mm Spherisorb 5-ODS. MOBILE PHASE: water: glacial acetic acid: isopropanol (98: 1: 1 v/v/v); 1.5 ml/min. WAVELENGTH: 242 nm.

Fig. 6 : Above - Reaction scheme for the preparation of N-propionyl-4-aminophenol (NPA) from 4-aminophenol and propionic anhydride.

Below - Chromatographic purity of NPA.

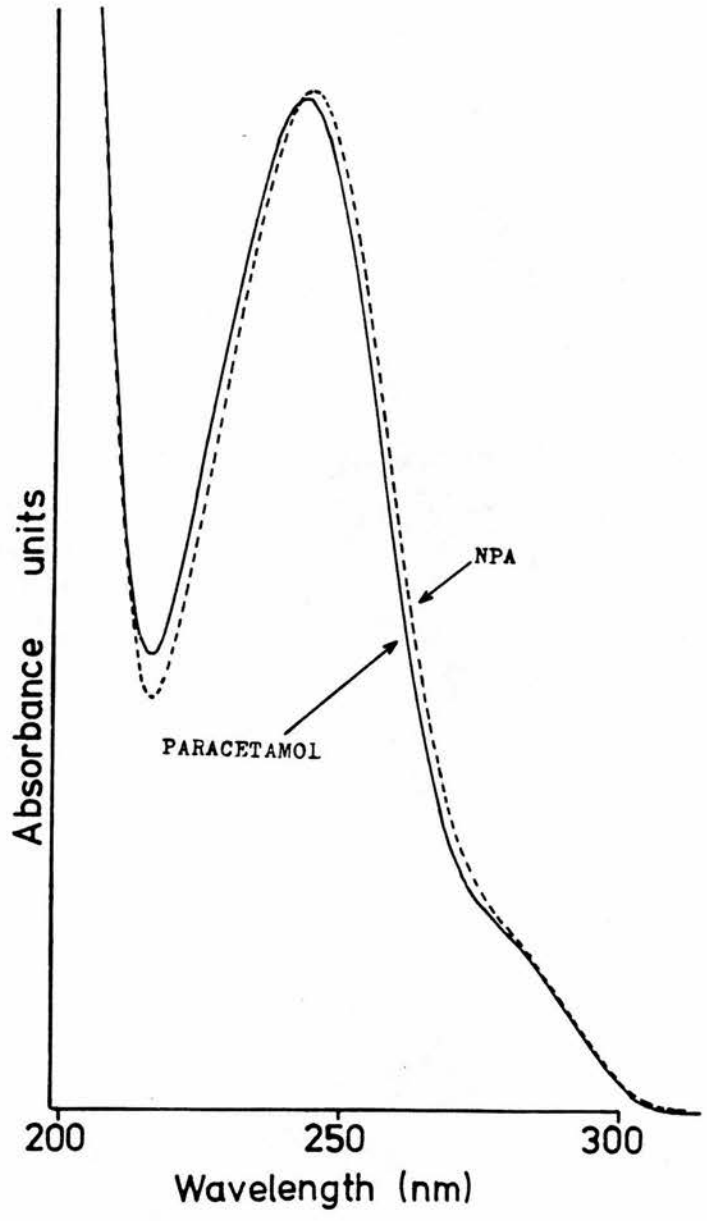


Fig. 7 UV absorbance spectra of paracetamol and N-propionyl-4-aminophenol (NPA) in water.

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septum injector to the stainless steel, internally-polished analytical column (90 - 180 mm long and 4.5 or 4.9 mm internal diameter). A pressure gauge was included to monitor solvent delivery. The eluate from the column passed through a short length of PTFE tubing (.005" int. diameter) into an 8 or 10 µl flow cell mounted in a UV absorbance detector (Cecil 212 variable wavelength or Waters model 440 fixed wavelength). Peak areas were integrated using either a Hewlett-Packard model 3370 - A integrator or a Spectra-Physics Minigrator. A permanent trace of each chromatogram was obtained using a suitable strip chart recorder connected to the integrator (Fig. 8). The Orlita pump is of the diaphragm type and delivers the solvent in pulses which were damped to avoid excess noise in the signal output to the integrator. In practice, the Bourdon tube of the pressure gauge acted as an expansion chamber which, coupled with the flow resistance of the column itself, provided adequate pulse dampening.

Two types of column and injector were used. In both designs solvent is introduced into the injector and passes concentrically around its central shaft and onto the column. The two designs are shown diagrammatically in Fig. 9 .

Sample injection was performed using a high-pressure micro-syringe (SGE types 5 and 25 BL-RD-50NG), the needle tip being placed within the glass beads, just touching

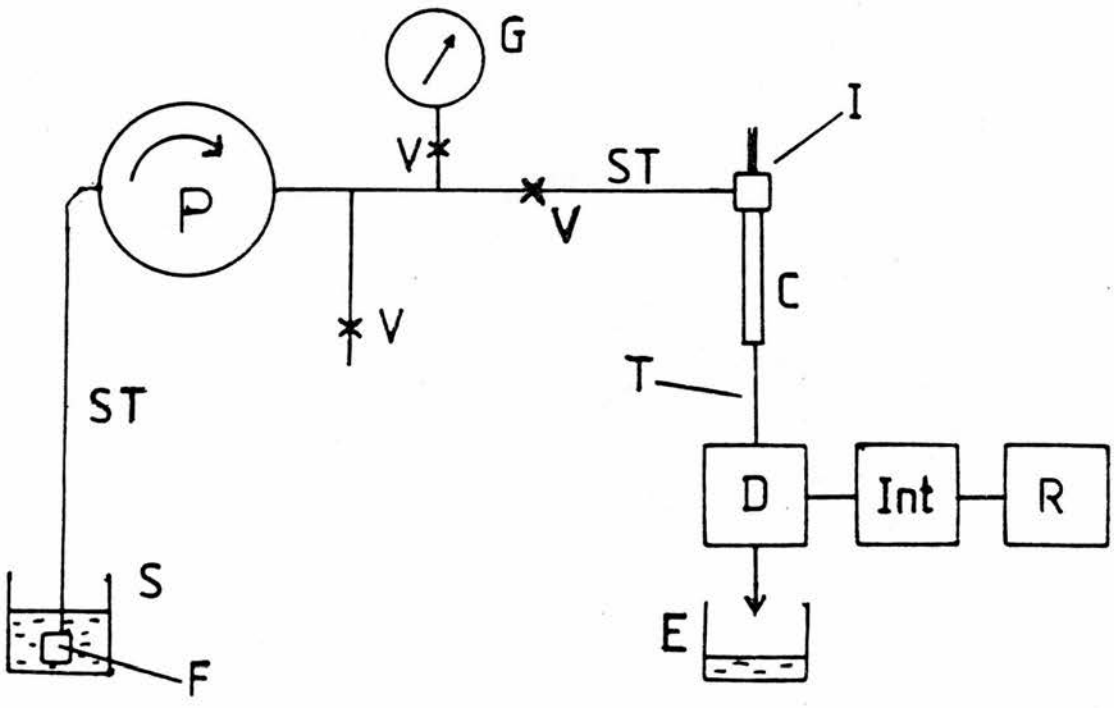


Fig 8 HPLC Assembly

Key:-
S - Solvent reservoir and solvent;
F - Solvent filter;
ST - Type 316 stainless steel tubing (1/16" or 1/8" O.D);
P - Solvent delivery pump;
V - On-off valves;
G - Pressure Gauge;
I - Septum injector assembly;
C - Analytical column;
T - Microbore tubing;
D - UV detector;
E - Eluate reservoir;
Int - Peak area integrator;
R - Strip chart recorder.

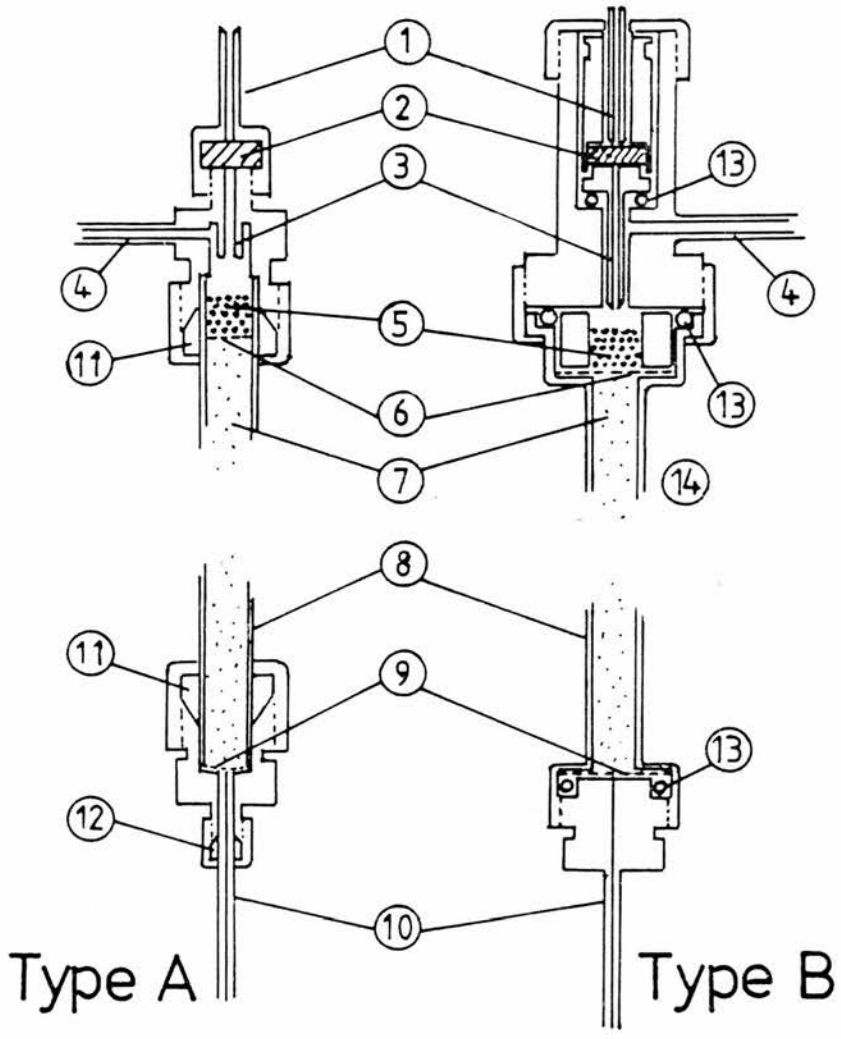


Fig 9 : Analytical Column and Septum Injector Assemblies.*

key:- 1 - Upper syringe needle guide; 2 - Septum; 3 - Lower syringe needle guide; 4 - Solvent inlet; 5 - Glass beads (80-100 mesh); 6 - Woven stainless steel gauze; 7 - Packing material; 8 - Column tube (internally polished); 9 - Packing retaining gauze (woven stainless steel); 10 - Microbore outlet tubing; 11 - Steel ferrule; 12 - PTFE ferrule; 13 - PTFE 'O'-ring; 14 - PTFE insert.

* Type A according to Bristow, HETP Ltd, Macclesfield, Cheshire. Type B according to Knox and Wall, Wolfson Liquid Cromatography Unit, University of Edinburgh.

the stainless steel gauze above the packing (Fig. 9). In this way the sample is placed directly onto the packing with the minimum of spreading. This makes possible the "infinite diameter effect" (Knox and Parcher, 1969) where sample components never touch the column walls during separation, ensuring maximum chromatographic efficiency. In practice, some band spreading and wall effects are probably introduced by too-large sample volumes and the disturbance caused by the syringe needle during sample injection (Majors, 1973; Bristow et al, 1977).

d) Column Packing Material

Octadecyl silica has been shown to be the most appropriate stationary phase material for the separation of weakly acidic small molecules (Twitchett and Moffat, 1975) and this was proved to be so for paracetamol and its metabolites also (Howie, 1977; Knox and Jurand, 1977, 1978). Separation efficiency is inversely related to stationary phase particle size (Martin and Synge, 1941; Majors, 1972,1973; Knox, 1973) and improved chromatography was obtained by substituting the 10 μm material used earlier (Howie, Adriaenssens and Prescott, 1977) with 5 μm material. Spherisorb-ODS (5 μm) was used in urine analyses and for paracetamol alone in plasma, and ODS-Hypersil (5 - 6 μm) was used for analysis of paracetamol and its conjugates in plasma.

e) Column Packing Procedure

Early columns were packed by the 'tap-fill' method of Snyder (1967). Packing material was poured into the column in small amounts and the column was then tapped at the sides and bottom between each addition until no further settling occurred. When the column was adequately filled, the high-pressure pump was connected to the top of the column and about 100 column volumes of the appropriate mobile phase were passed through at high pressure. The column was then disconnected, a stainless-steel gauze was fitted, and this was covered with a 5 mm layer of glass beads (80 - 100 mesh, Fig. 9). The column was then ready for use.

The more efficient columns using 5 μm particles were slurry packed for optimum performance, using two procedures shown diagramatically in Fig. 10. The balanced density technique (Kirkland, 1971; Majors, 1972; Cassidy et al, 1974) was employed using an 8 - 12% w/v suspension of the packing material in a mixture of iodomethane/ethyl acetate (4:Iv/v). This slurry was first briefly ultrasonicated to disperse particle agglomerates and the column tube, with the packing-retaining gauze and the outlet fitting attached, was connected to the slurry reservoir tube. Both tubes were filled with slurry (total volume about 15 ml), topping up with more solvent if necessary. The reservoir tube was then connected to the high pressure pump (see

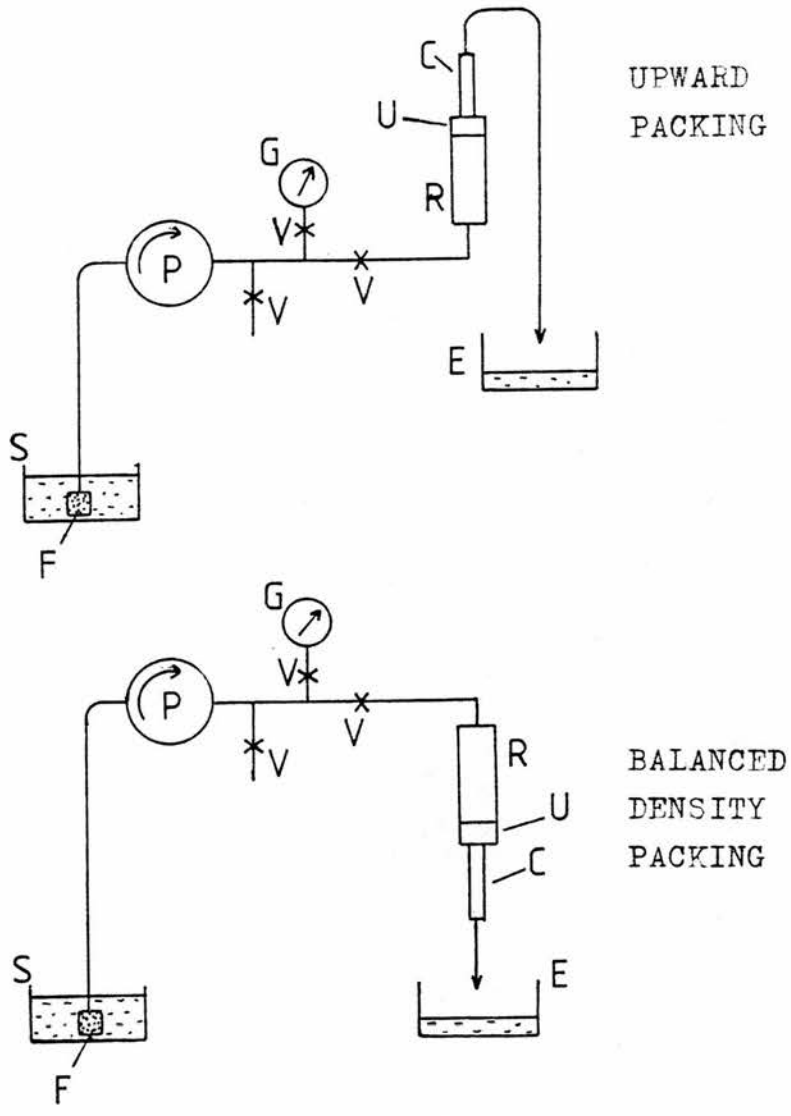


Fig 10: Column packing apparatus and methods

key:- S - Column packing solvent and reservoir
F - Filter (7 or 30 μM)
P - Solvent delivery pump
V - on/off valve
G - Pressure gauge
R - Reservoir for packing slurry (25 cm long x 3/8" OD.)
U - Reducing union (3/8" to 1/4")
C - Column
E - Eluate reservoir.

Apparatus) via a length of 1/16" o.d. stainless steel tubing and packing commenced with a flow rate of 8 - 10 ml/min, using ethyl acetate and the driving solvent. About 50 column volumes were passed through the packing tube and column. The solvent was then changed to a 1:1 v/v mixture of methanol: ethyl acetate for about 20 column volumes, followed by methanol alone for another 20 column volumes. The column was then detached from the slurry reservoir and inspected to check that the packing had bedded down evenly. The weight of packing used was sufficient to fill the whole length of the column (about 1.5g/100 mm). The reservoir tube was then reconnected and solvent flow re-started. The methanol content of the solvent was then progressivley reduced with water over about 50 column volumes, ending up with water alone for another 20 column volumes. The flow rate was adjusted during this sequence so that solvent viscosity changes did not overburden the pump and pressure gauge.

The packing apparatus was dismantled and enough packing removed from the top of the column to allow for insertion of a stainless steel gauze directly on top of the remaining packing, followed by 80 - 100 mesh glass beads (about 5mm depth). The column was now ready for connection to the injector assembly. Before use each column was tested with standard mixtures of paracetamol metabolites to ensure that performance and

solute retention were adequate and stable.

In the second procedure, a more polar, unbalanced slurry was used (Kirkland, 1971; Bristow et al, 1977; Shandon Southern Products technical note). An 8 - 12% w/v suspension of packing material was prepared in a solvent consisting of 85:15 v/v methanol/water. Particle agglomeration was clearly visible in this medium and was minimized by brief ultrasonication and vigorous shaking before packing. The reservoir tube and column were connected and filled with slurry as in the first method. The solvent delivery tube was attached and the reservoir and column vigorously shaken before commencing solvent flow.

A flow rate of 8 - 10 ml/min of 85:15 v/v methanol: water was used and the column and reservoir were inverted so that packing proceeded upwards. Eluate was collected via a flexible outlet tube (Fig. 10). Upward packing ensured a uniform column bed with a minimum of radial particle sizing, since sedimentation of larger particles and agglomerates occurs in the opposite direction to the laying down of the column bed. Longitudinal particle sizing (where larger particles pack last) does occur but is unlikely to affect column performance (Bristow et al, 1977). After about 80 column volumes had passed, the water content of the solvent was progressively increased to 100% over 50 column volumes. The rest of the procedure was

as for the first method.

The two methods appeared to produce equivalent chromatographic results with similar column efficiencies, as determined by the number of theoretical plates per column (Martin and Synge, 1941) with a mixture of standards (paracetamol, its metabolites and the internal standard). The number of theoretical plates (N) was determined from the equation $N = 16 \frac{R^2}{W^2}$ where R = peak retention time and W = baseline peak width (in the same units as R) for tangents extrapolated to the baseline from the inflection at each side of the peak. The most efficient column produced about 6000 theoretical plates for N-propionyl-4-aminophenol in a 90 mm long column after using the balanced density method.

f) HPLC Systems for the Analysis of Urine Samples

With the use of a new internal standard, different mobile phases relative to that of the earlier method (Howie, Adriaenssens and Prescott, 1977) were required for optimum separations. It was found that changing the mobile phase from 1% aqueous acetic acid with 15% methanol and 0.1% ethyl acetate(V/V) to 1% acetic acid containing 1 - 2%each(V/V) of methanol and/or isopropanol,with the more efficient 5 µm Spherisorb-ODS provided better separations. The columns used were 120 to 180 mm long and 4.5 to 4.9 mm internal diameter.

Resolution of poorly-retained peaks close to paracetamol sulphate and glucuronide conjugates was improved.

Chromatograms obtained from drug-free urine, urine collected 3 - 4 hours after a therapeutic dose of paracetamol (20 mg/kg) and urine collected 9 -23 hours after an overdose of paracetamol are shown in Fig. 11 . A chromatogram of standards is shown for comparison (Fig. 12a). Components of interest are well resolved from each other and endogenous compounds. Other drugs commonly ingested with paracetamol in overdosage (eg barbiturates, salicylates, benzodiazepines) did not interfere although the possibility of interference from metabolites of these drugs cannot be ruled out.

Paracetamol and its major conjugates could still be measured 24 hours after a therapeutic dose and sometimes up to 5 days after an overdose using the system described.

g) HPLC Systems for the Analysis of Plasma Samples

i) Free Paracetamol Only: The system used was as described by Howie, Adriaenssens and Prescott (1977) using a Spherisorb 10-ODS column (90 mm long x 4.5 mm id) and a mobile phase composed of 1% aqueous acetic acid and ethyl acetate (99:1 v/v), UV detection was at 253 nm. The system described below for paracetamol and its conjugates could also be used . Fig. 13 shows chromatograms obtained from plasma after a therapeutic dose of para-

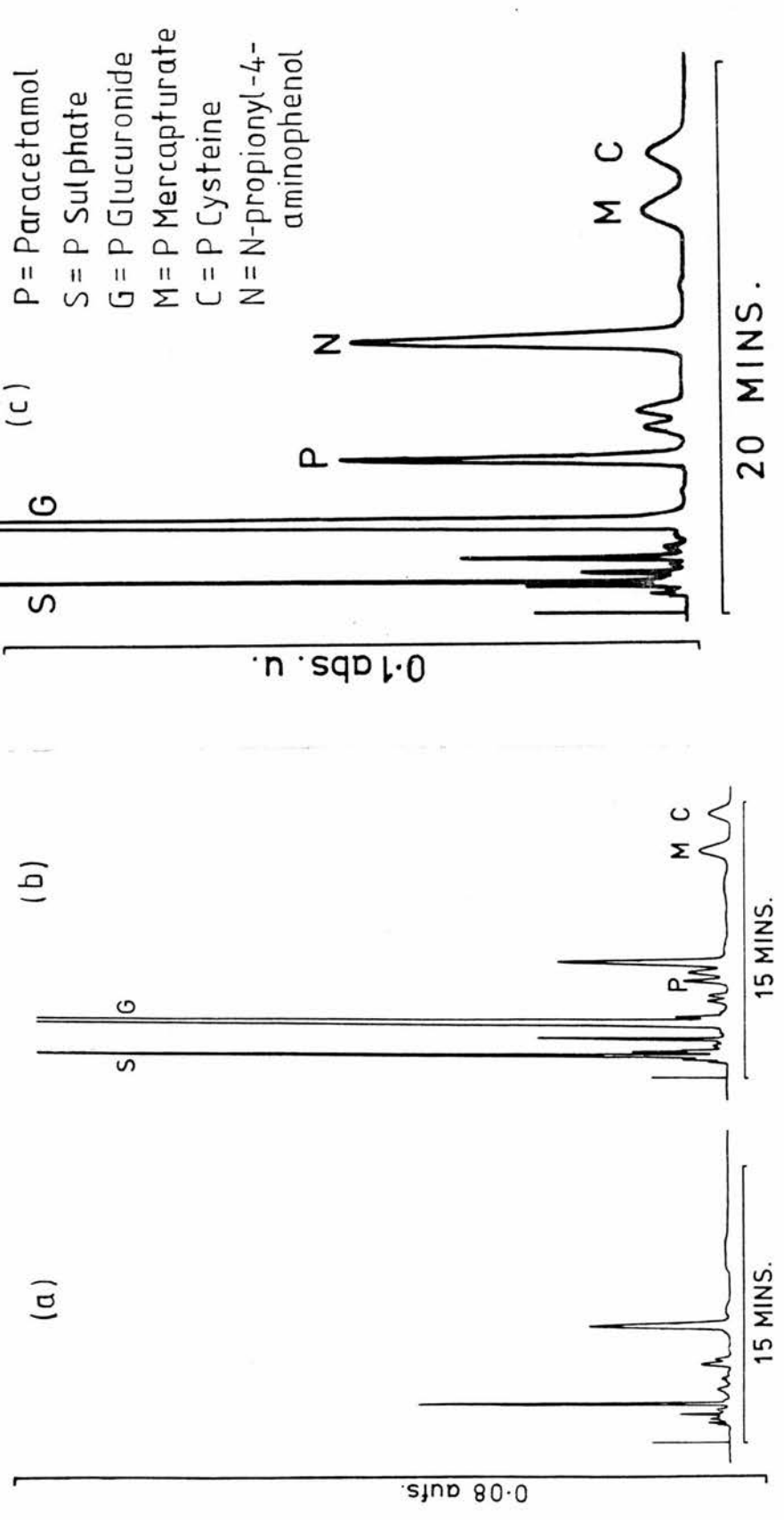


Fig. 11: HPLC of paracetamol and its conjugates in (a) drug-free urine, (b) urine collected 3 -4 hours after 20 mg/kg paracetamol orally, (c) urine collected 9 - 23 hours after an overdose of paracetamol. (Column : Spherisorb-5-ODS. Mobile Phase: 1% aqueous acetic acid with (a, b) isopropanol (1.0% v/v), methanol (1.5% v/v), (c) methanol (1.6% v/v). Flow Rate: 1.8 ml/min. Wavelength: 242 nm.)

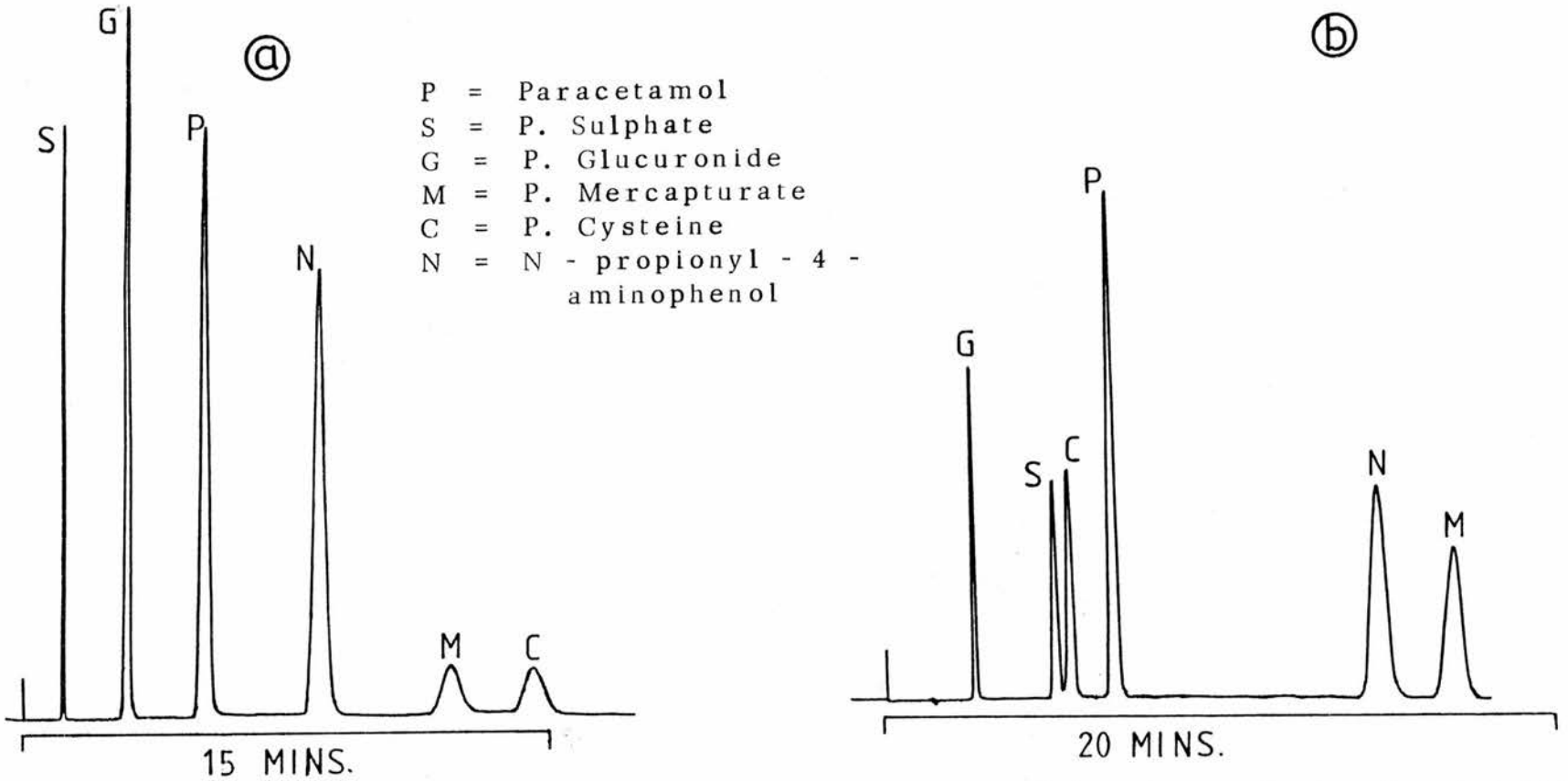


Fig. 12: HPLC of reference compounds on the systems used for the analysis of urine (a) and plasma (b).

(a) COLUMN :- Spherisorb 5-ODS (170 x 4.9 mm); MOBILE PHASE :- water : glacial acetic acid: methanol : isopropanol (96.5 : 1.0 : 1.5 : 1.0 v/v/v/v) at 1.8 ml/min. WAVELENGTH :- 242 nm. (b) COLUMN :- ODS - Hypersil (90 x 4.9 mm); MOBILE PHASE :- 0.1 M potassium dihydrogen phosphate : 98% formic acid : isopropanol (100: 0.1 : 1.7 v/v/v) at 0.9 ml/min. WAVELENGTH :- 254nm.

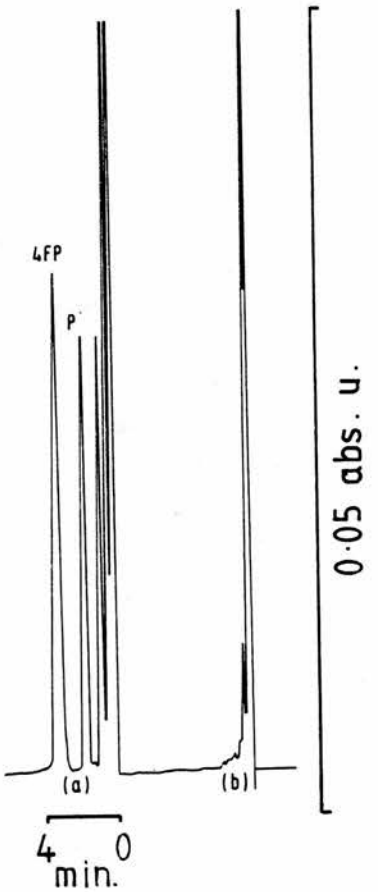


Fig. 13: HPLC of paracetamol alone in plasma

(a) Plasma collected 2 hours after 20 mg/kg paracetamol orally.
P = paracetamol
4FP = 4-fluorophenol

(b) Drug-free plasma.

Column - 90 x 4.5 mm Spherisorb-10-ODS.
Mobile Phase - 1% aqueous acetic acid:ethyl acetate (99:1 V/V).
Flow rate - 3ml/min.
Wavelength - 253 nm.

cetamol and also from drug-free plasma.

ii) Paracetamol and its Conjugates: The columns were either 90 or 120 mm, 4.9 or 4.5 mm internal diameter, packed with Hypersil-ODS. The mobile phase was 0.1M potassium dihydrogen phosphate, 98% formic acid and isopropanol (100: 0.1: 1.7 V/V/V). Chromatograms of drug-free plasma, plasma taken 2 hours after a therapeutic dose of paracetamol and plasma taken 60 hours after a severe overdose are shown in Fig. 14. Standards alone are shown for comparison in Fig. 12b. Most endogenous compounds eluted early in this system. No commonly-used drugs interfered.

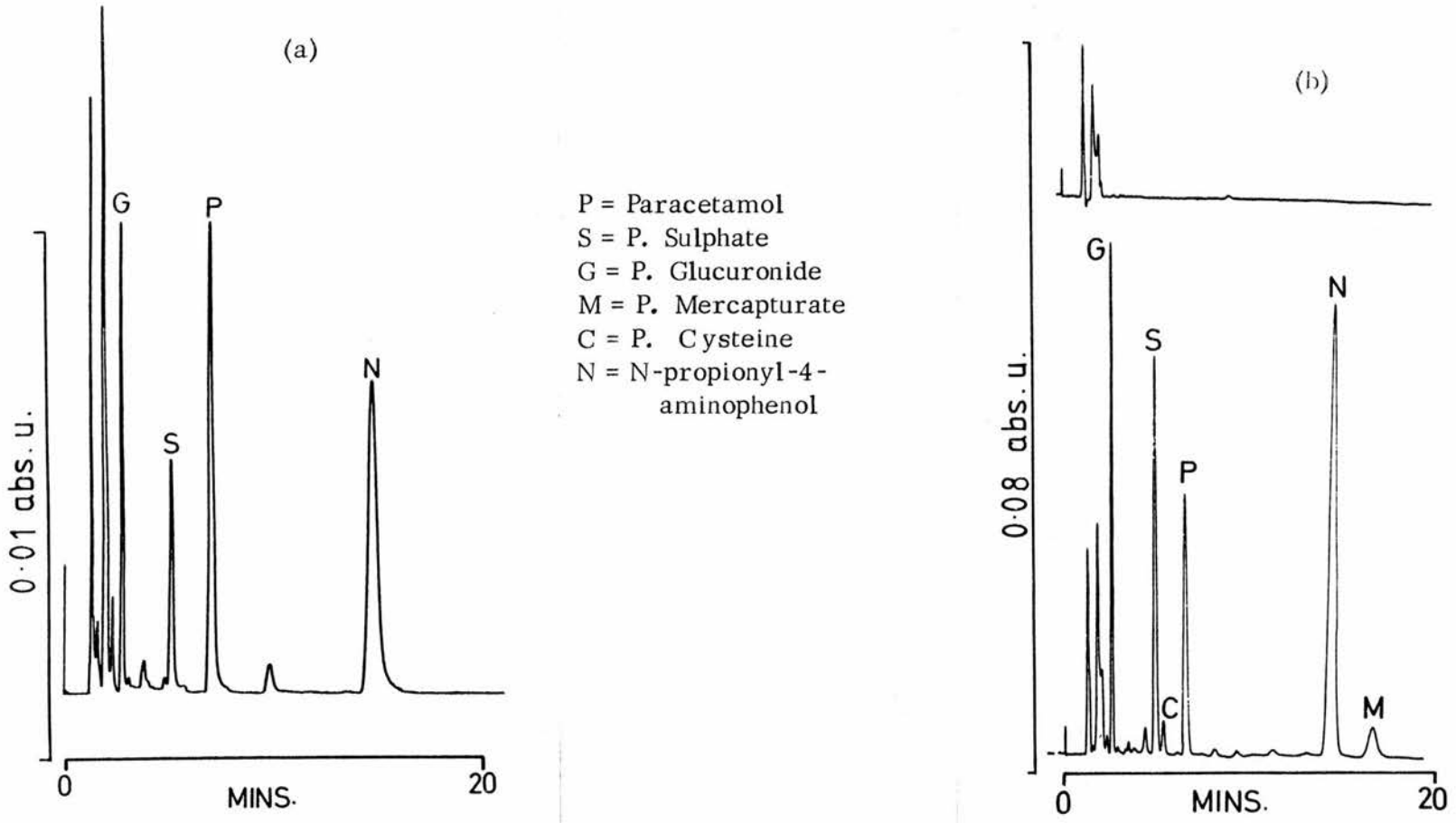


Fig. 14: HPLC of paracetamol and its conjugates in plasma.
(a) Plasma collected 2 hours after 20 mg/kg paracetamol orally. (b) Drug-free plasma (above) and plasma collected 60 hours after paracetamol overdosage with severe liver damage and renal failure (below).
(Column: 90 x 4.9 mm Hypersil-ODS. Mobile Phase : potassium dihydrogen phosphate: 98% formic acid: isopropanol, 100 : 0.1 : 1.7 v/v/v, flow rate 0.9 ml/min. Wavelength : 254 nm).

Chapter 3

UV Absorbance of Paracetamol Metabolites Relative to Paracetamol.

It has been assumed in the past that paracetamol and its major conjugates have essentially equal molar UV extinction coefficients and that their UV spectra are superimposable (Mrochek et al, 1974). Howie (1977) calculated the extinction coefficients at " λ max 242 nm" and found that the values for the sulphate and glucuronide conjugates were about 8% higher than for free paracetamol, this difference was ignored in subsequent quantitation procedures. Both Mrochek et al (1974) and Howie (1977) made measurements at wavelengths below the absorbance maximum and presumably assumed that the spectra were superimposable. However, these assumptions are not warranted.

a) UV Absorbance Spectra

Dilute solutions (15 - 20 µg/ml) of paracetamol, its sulphate and glucuronide conjugates and NPA were prepared in the solvents used for HPLC. The UV spectra were determined against reference cells containing solvent alone, using a Pye-Unicam model SP 1750 spectrophotometer. The spectra appeared almost identical but there were slight differences around the absorbance maxima. The maximum absorbance of paracetamol in each solvent was at 243 nm. The sulphate and glucuronide conjugates both peaked within 1 nm below the paracetamol peak and NPA peaked at about 244 nm.

The relative changes in the absorbance spectra and hence in the extinction coefficients at other wavelengths likely to be used for the measurement of paracetamol were also studied.

Method

Five wavelengths in the region 240 - 254 nm were chosen and six absorbance measurements were taken at each wavelength using the solutions described above in aqueous acetic acid plus a solvent blank. The SP1750 spectrophotometer wavelength setting dial was placed manually at the desired wavelength then moved away and reset for each of the six readings. This was done to overcome any variation in wavelength setting when measuring each solution.

Results

The results are shown in Table 1. The absorbance of the conjugates did not change at the same rate as that of paracetamol. This was obvious when the absorbance of the solution of each compound at each wavelength was compared to its maximum absorbance ($A_\lambda \div A$ max, Table 2). Each compound exhibits maximum absorbance at around 242 nm and the absorbance is maintained above 99% of maximum for at least one nanometer either side of λmax. The absorbance of each compound was considerably reduced at 254 nm, the wavelength most commonly used by other workers in UV measurements of paracetamol and other aromatic compounds. The absorbance of paracetamol and its sulphate and glucuronide conjugates dropped by 23, 37 and 29.5 per cent respectively relative to their absorbance at 242 nm. The decrease in absorbance for N-propionyl-4-aminophenol was 19.3%.

The data was further analysed to determine the change in absorbance for each compound relative to paracetamol from the relationship $F_{x,p} = \frac{A_x - A_p}{Ap}$ where $F_{x,p}$ is the fractional change in absorbance of the compound x relative to paracetamol. A_x is the absorbance of this compound at the wavelength in question relative to its absorbance at 242 nm ($A_\lambda \div A$ max in Table 2). Ap is the absorbance of paracetamol at this wavelength relative to its absorbance at 242 nm. The results (Table 3) show that

TABLE 1

Change in Absorbance with wavelength for Paracetamol, its sulphate and glucuronide and N-propionyl-4-aminophenol (NPA)

(Absorbance ±S.D, n = 6 readings; Solutions were 15-20 µg/ml in 1% aqueous acetic acid)

Wavelength (nm)	Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide	NPA	Blank
240	.795 (±.008)	.968 (±.005)	.643 (±.003)	.920 (±.004)	0 (±.003)
242	.805 (±.008)	.971 (±.005)	.647 (±.003)	.939 (±.004)	0 (±.003)
243	.805 (±.008)	.965 (±.005)	.645 (±.004)	.943 (±.004)	0 (±.002)
249	.746 (±.007)	.825 (±.005)	.576 (±.004)	.895 (±.005)	0 (±.003)
254	.620 (±.006)	.612 (±.005)	.456 (±.003)	.763 (±.004)	0 (±.003)

TABLE 2

Change in Absorbance with Wavelength

(Absorbance relative to maximum value, A_{λ}/A_{max})

A_{λ}/A_{max} / λ (nm)	Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide	NPA*
240	.988	.997	.994	.976
242	1.000	1.000	1.000	.996
243	1.000	.994	.997	1.000
249	.927	.850	.890	.949
254	.770	.630	.705	.807

* N-propionyl-4-aminophenol

TABLE 3

Change in Absorbance with Wavelength relative to Paracetamol for paracetamol sulphate and paracetamol glucuronide and N-propionyl-4-aminophenol (NPA)*

$F^{*}_{x,p}$ / λ(nm)	Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide	NPA
240	0	+0.009	+0.006	-0.012
242	0	0	0	-0.004
243	0	-0.006	-0.003	0
249	0	-0.083	-0.040	+0.024
254	0	-0.182	-0.084	+0.048

* For calculation see text, p 69

the absorbance of the sulphate and glucuronide conjugates decreased more rapidly than that of free paracetamol with increased wavelength (18.2% and 8.4% relative to paracetamol on changing from 242 to 254 nm). The absorbance spectrum of N-propionyl-4-aminophenol followed that of paracetamol very closely, the discrepancy being less than 5% at 254 nm. Accurate measurement of extinction coefficients relative to paracetamol are therefore required for each compound before results obtained at different wavelengths can be compared.

b) Absorbance of the sulphate and glucuronide conjugates relative to paracetamol determined by enzymic hydrolysis

The sulphate and glucuronide conjugates can be easily hydrolysed to paracetamol enzymically, and the process can be monitored by direct HPLC of the reaction mixture. Neither the purity nor the actual amount of conjugate in the sample taken for hydrolysis needs to be accurately known before incubation with the enzymes.

Method

Aqueous solutions of paracetamol and its sulphate and glucuronide conjugates were prepared containing 1.0, 1.0, and 1.2 mg/ml respectively. An aqueous solution containing 2 mg/ml N-propionyl-4-aminophenol (NPA) was also prepared. 200 µl of the paracetamol sulphate solution and 100 µl of the NPA solution were mixed with

200 µl of 0.2M sodium acetate buffer (pH 5.0). Three 150 µl aliquots of this mixture were pipetted into 3 ml polypropylene tubes. To the first was added 2 µl Glusulase (Endo Laboratories, Garden City, USA), to the second was added 2 µl S. Helix Pomatia extract (Industrie Biologique Francaise, Gennevilliers, France), and to the third was added 20 µl of reconstituted dried S. Helix Pomatia extract (Sigma Chemical Co, Poole, England). These three enzyme preparations contained arylsulphatase 47,800, 800,000 and unspecified units per ml and β-glucuronidase 145,500, 100,000 and 129,000 units per ml respectively. The samples were mixed and incubated at 37°c for three hours together with the remaining control mixture without enzyme. This procedure was repeated for the paracetamol glucuronide and paracetamol solutions.

After incubation, 2.5 µl aliquots of each sample were injected directly into the HPLC column, the paracetamol samples served as controls. The samples were analysed at 242 nm and 254 nm with the HPLC system used for analysis of paracetamol and its conjugates in plasma. The 3 mixtures without added enzyme were analysed similarly.

Results

Table 4 shows the peak area ratios obtained for each solution without adding enzyme divided by the ratio obtained after enzyme addition (using NPA as internal

TABLE 4

Relative absorbance of solutions of paracetamol sulphate and paracetamol glucuronide before and after enzymic hydrolysis to paracetamol*

Enzyme Preparation	Wavelength (nm)	Paracetamol Glucuronide (Gl)	Paracetamol Sulphate (Su)	Free Paracetamol (pa)
Glusulase	242	1.199	1.291	1.000
S. Helix Pomatia	242	1.204	1.293	0.999
Reconst. dried S. Helix Pom.	242	1.199	1.299	0.997
Mean	242	1.201	1.294	0.999
Glusulase	254	1.116	1.049	1.000

* Equivalent to extinction Coefficients Relative to paracetamol,

i.e. E_{Gl}/E_{pa} , E_{Su}/E_{pa} - see text for explanation, pp 74, 76.

standard). These values are equivalent to the extinction coefficients of each compound relative to paracetamol. The three enzyme preparations produced almost equal results which showed that the absorbances of the paracetamol glucuronide and sulphate solutions at 242 nm are higher by 20.1 and 29.4% (mean values) respectively relative to the equivalent amount of paracetamol released after hydrolysis (ie relative extinction coefficients of 1.201 and 1.294). The absorbance of the control paracetamol samples did not change under the same conditions. A single series of measurements at 254 nm showed that the glucuronide and the sulphate absorbed respectively 11.6% and 4.9% more light than the equivalent amount of free paracetamol at this wavelength.

The extinction coefficients of the glucuronide and sulphate conjugates relative to paracetamol at 254 nm may also be calculated by multiplying the value at 242 nm (see Table 4) by the relative absorbance change from 242 to 254 nm (Fx,p, Table 3). The appropriate value for paracetamol glucuronide is 1.201 x (1 - 0.084) = 1.100 and for paracetamol sulphate, 1.294 x (1 - 0.182)= 1.058. These two values agree well with those obtained directly at 254 nm, ie 1.116 and 1.049 respectively (Table 4).

c) Absorbance of the Cysteine and Mercapturic Acid Conjugates Relative to Paracetamol

Since the sulphate and glucuronide conjugates showed

considerable differences in their extinction coefficients relative to paracetamol, it was possible that the cysteine and mercapturic acid conjugates did likewise. These two metabolites cannot be simply hydrolysed to release paracetamol and they generally contribute only a few per cent each to the total recovery of paracetamol in urine. Their extinction coefficients relative to paracetamol were estimated on the basis of the peak area ratios obtained for weighed concentrations of each conjugate and paracetamol relative to the internal standard (NPA) after HPLC analysis. This procedure has the advantage over direct spectral analysis of independence from interference produced by impurities known to be present in the reference materials. However, only very small quantities of each compound were available and reliable values for relative extinction coefficients depend on accurate knowledge of the percentage purity of each compound.

Method

Stock aqueous solutions of paracetamol and its cysteine and mercapturic acid conjugates were prepared containing 0.42, 1.18 and 1.07 mg/ml respectively. 500 µl of the paracetamol solution was pipetted into each of four 3 ml tubes followed by 100 µl of the paracetamol cysteine solution into two of the tubes and 100 µl of the paracetamol mercapturate solution into the other two. After thorough mixing, the solutions were analysed by

HPLC using the system described for plasma . Relative extinction coefficients at each wavelength were calculated from the equation

$$\frac{Ecy}{Epa} = \frac{Acy}{Apa} \times \frac{Cpa}{Ccy} \times \frac{Mcy}{Mpa} \times \frac{Ppa}{Pcy}$$

where $\frac{Ecy}{Epa}$ is the extinction coefficient of the cysteine conjugate relative to paracetamol at the appropriate wavelength. A is the peak area, C the concentration, M the molecular weight and P the purity of each compound (Pa = paracetamol, Cy = paracetamol cysteine conjugate). This equation also holds for paracetamol mercapturate but since its purity was known to be low, the corresponding $\frac{E_{me}}{E_{pa}}$ could not be found. However, the fractional change in absorbance (^{F}me) of paracetamol mercapturate relative to paracetamol between 242 and 254 could be found since this depends only on the peak area ratio $\frac{A_{me}}{A_{pa}}$ at each wavelength.

$$\text{Thus } \underset{(242 \rightarrow 254nm)}{Fme} = \left[\frac{Ame}{Apa}\right]_{(242nm)} \Big/ \left[\frac{Ame}{Apa}\right]_{(254nm)}$$

The corresponding values for paracetamol cysteine were calculated for comparison.

Results

$\frac{E_{cy}}{E_{pa}}$ at 242 nm, 249 nm and 254 nm are shown in Table 5, together with the fractional absorbance changes

TABLE 5

Extinction Coefficients and Fractional Absorbance Changes for the Cysteine and mercapturate Conjugates Relative to Paracetamol

Wavelength (nm)	Relative Extinction Coefficient E_{cy}/E_{pa}	Fractional Absorbance Change vs 242 nm	
		F_{cy}	F_{me}
242	1.32	-	-
249	1.31	0.995	1.002
254	1.35	1.025	1.037

pa = paracetamol

cy = paracetamol cysteine

me = paracetamol mercapturate

for the cysteine and mercapturic acid conjugates relative to paracetamol from 242 nm to 249 nm and 242 nm to 254 nm (F_{me} and F_{cy}). The mean value of the duplicate samples was taken in each case. The results suggest that the extinction coefficient of the cysteine conjugate is about 30% higher than that of paracetamol and that it changes little relative to paracetamol at the wavelengths studied. The fractional absorbance changes for the mercapturic acid conjugate relative to paracetamol are very similar to those of the cysteine conjugate (Table 5). However, since the mercapturic acid and cysteine conjugates account for only a small proportion of the total quantity of paracetamol and its metabolites excreted, the extinction differences relative to paracetamol were ignored.

Summary

The UV extinction coefficients of paracetamol and its major conjugates differ significantly according to wavelength. Thus in order to estimate the conjugates, absorbance calibration factors are required to correct for the extinction differences relative to paracetamol.

At 242 nm the extinction coefficients of the sulphate and glucuronide conjugates relative to paracetamol were 1.294 and 1.201 respectively. The corresponding calibration factors were therefore $\frac{1}{1.294}$ and $\frac{1}{1.201}$ respectively. At 254 nm, the corresponding factors

were $\frac{1}{1.058}$ and $\frac{1}{1.100}$ for the sulphate and glucuronide conjugates respectively.

Correcting factors for the cysteine and mercapturic acid conjugates were not used.

Chapter 4

Stability of Paracetamol Conjugates

Howie (1977) showed that the sulphate, glucuronide, cysteine and mercapturic acid conjugates of paracetamol were stable in urine for at least seven months stored at -20°c. No losses were observed with repeated thawing and freezing. This stability study was continued and the same batch of samples was assayed three years later. The original procedure of Howie (1977) was used.

Method

Four sets of urine samples (designated A, B, E and F), each sample containing the sulphate, glucuronide, cysteine and mercapturic acid conjugates of paracetamol, were stored at -20°c. The pH of A and B was adjusted to 5.3 and E and F to 7.2 before freezing. Samples A and E were used once only and discarded, whereas B and F were refrozen after thawing and re-analysed later, together with "fresh" once-frozen samples of A and E. Thus the effect of pH, as well as thawing and refreezing could be determined.

Duplicate samples of A, B, E and F were thawed and 0.2 ml of each sample was pipetted into 3 ml polypropylene tubes. 50 µl of internal standard solution (4 mg/ml aqueous 4-fluorophenol) was added to each tube and the samples were mixed. A set of standards was prepared containing 25, 50, 100, 150 and 200 µg/ml aqueous paracetamol and these were treated in the same way as the urine samples. 1 - 3 µl aliquots of all the solutions were injected into the HPLC column (170 x 4.9 mm Spherisorb ODS-5µm). The solvent was 1% aqueous acetic acid, methanol, ethyl acetate (90:15:0.2 V/V/V), flow rate 1.6 ml/min. UV detection was at 250 nm and peak areas were measured using a Spectra-Physics Minigrator. Peak area ratios of the conjugates to internal standard were related to those of the paracetamol standards. The results (expressed as µg/ml paracetamol equivalent) are shown in Table 6 together with the original data obtained by Howie (1977).

Results: In all but two cases the concentrations obtained in the present study were within two standard deviations of the values obtained during the first 7 months (maximum deviation 7.9%). The two exceptions were the concentrations of cysteine conjugate in samples A and E which were 15.1% and 7.7% high respectively. The results suggest that decomposition of paracetamol conjugates in urine stored at -20°c occurs slowly, if at all.

TABLE 6

Stability of Paracetamol Conjugates in Urine After Three Years at -20°C

Conjugate / Sample / Conc^{n} (μg/ml)		pH 5.3		pH 7.2	
		A	B	E	F
Sulphate	DH†	118 ± 3.9	119 ± 7.4	119 ± 3.3	119 ± 6.1
	PA*	120	111	122	123
	% difference	< S.D.	<2S.D.(6.7%)	<S.D.	<S.D.
Glucuronide	DH	161 ± 7.2	160 ± 8.6	169 ± 11.5	164 ± 7.4
	PA	161	153	164	151
	% difference	< S.D.	< S.D.	<S.D.	<2S.D.(7.9%)
Mercapturate	DH	72 ± 1.3	71 ± 1.9	74 ± 2.9	70 ± 2.6
	PA	73.8	67.7	73.7	66.3
	% difference	<2S.D.(2.5%)	<2S.D.(4.6%)	< S.D.	<2S.D.(5.3%)
Cysteine	DH	67 ± 1.5	66 ± 2.4	69 ± 2.3	66 ± 2.1
	PA	77.1	70.5	74.3	67.4
	% difference	> 2S.D.(15.1%)	<2S.D.(6.8%)	>2S.D.(7.7%)	< S.D.

Samples A and E were analysed and discarded after being frozen once only.

Samples B and F were thawed, analysed and refrozen a number of times.

* analysed 3 years after initiating study, mean of 2 samples.

† mean and standard deviation of 5 analyses over 7 months.

Chapter 5 Analytical Procedures

Paracetamol and its conjugates in urine and plasma were estimated after the addition of a measured quantity of aqueous internal standard to a measured volume of urine or plasma. Estimation in plasma was only different in that the internal standard solution contained a protein precipitant, thus plasma proteins were removed prior to chromatography.

a) Collection of Samples

The volume and pH of urine samples was recorded and duplicate aliquots stored at -20°c. Blood samples were separated immediately and the plasma stored at -20°c.

b) Apparatus and Materials

Reference compounds, internal standard, apparatus and analytical columns were as described previously (Chap. 2) . Solvents were analytical grade and water was glass distilled; aqueous components were vacuum degassed before use.

c) Sample Preparation and Analysis

i) Urine

Aliquots of 100 - 500 µl of urine were pipetted into 3 ml polypropylene tubes and 100 - 500 µl of internal standard solution (2 mg/ml NPA) was added. The amount of internal standard depended on the anticipated drug and metabolite concentrations in the

sample. A set of three standards was prepared with each batch of unknowns by mixing aliquots of 1.0 mg/ml aqueous paracetamol (P) and the internal standard solution (NPA) in the ratios 200 µl P: 100 µl NPA, 100 µl P: 100 µl NPA, and 100 µl P: 200µl NPA.

The chromatographic system was as described on pp 60-61, 0.5-4 µl aliquots of each sample were injected onto the HPLC column. The concentration of unknown samples was calculated from the relationship :-

$$C_x(\text{mg/ml}) = Rp \times \frac{A_x}{A_{NPA}} \times \frac{1}{E_{x,p}} \times \frac{V_{NPA}}{V_u}$$

C_x is the concentration of the compound of interest (paracetamol or its conjugates expressed as paracetamol equivalent), A_x is the peak area of this compound and A_{NPA} is the area of the internal standard peak. $\frac{1}{E_{x,p}}$ is the absorbance calibration factor (reciprocal of the relative extinction coefficient) of the compound relative to paracetamol at the appropriate wavelength (see pp 80 - 81). V_{NPA} and V_u are the volumes of internal standard solution and urine sample respectively. R_p is the standardisation factor calculated as the reciprocal of the slope of the line obtained from the three paracetamol standards run with each batch of the unknowns.

It was considered unnecessary to prepare more than three paracetamol standards with each batch of unknowns

since the peak area ratios (and hence Rp values) were very reproducible over many months of use (Rp = 1.813 ± 0.043 over 19 months). The only sources of variation were the preparation of standard solutions of paracetamol and NPA. There is no extraction step and any variation in solvent and column characteristics is minimised by the use of peak areas rather than heights for quantitation.

ii) Plasma - Paracetamol Alone

Two stock solutions (A and B) were prepared containing the internal standard 4-Fluorophenol (4FP) in aqueous trichloracetic acid (TCA). Solution A contained 5 mg/ml 4FP in 25% W/W aqueous TCA and solution B contained 7 mg/ml 4FP in 75% W/W aqueous TCA.

1.0 ml aliquots of plasma containing paracetamol after overdosage were pipetted into 10 ml glass tubes and 1.0 ml of solution A was added slowly while the tubes were held on a vortex mixer. The tubes were centrifuged for 5 minutes at about 1200 g to separate the precipitated protein from the clear supernatant. Up to 5 µl of this supernatant was injected into the HPLC column. For plasma samples containing low concentrations, such as after therapeutic dosage, 100 µl of solution B was added slowly to 1.0 ml aliquots of plasma and up to 25 µl of the clear supernatant was chromatographed. The chromatographic system was as described on p 61.

Quantitation was by peak height ratio, overdose samples were related to plasma standards containing 50 and 200 μg/ml paracetamol and therapeutic samples were related to plasma standards containing 5 and 20 μg/ml paracetamol. The standards were treated in the same way as unknown samples. The limit of measurement was about 0.1 μg/ml.

iii) Plasma - Paracetamol and Metabolites

Sample preparation was identical to (ii) above except that solution A (for overdose concentrations) contained 360 μg/ml NPA in 6% W/V aqueous perchloric acid and solution B (for low concentrations) contained 300 μg/ml NPA in 30% W/V aqueous perchloric acid. Up to 5 μl aliquots of the clear supernatant were chromatographed using the HPLC system described on p 65. Peak areas of paracetamol and its sulphate and glucuronide conjugates were related to standards containing 5 and 20 μg/ml paracetamol (low concentration range) and 25 and 250 μg/ml paracetamol (overdose range). The limit of measurement of each compound was less than 1 μg/ml. Appropriate correction factors relating concentrations of paracetamol sulphate and glucuronide to paracetamol in plasma were determined as described below. The mercapturate and cysteine conjugates were seldom seen in plasma except in severe overdosage, usually accompanied by renal failure. Therefore it was considered unnecessary to determine similar factors for

these compounds and calibration considerations (ie linearity and absorbance factors) were assumed to be equivalent to those already described for the urine assay.

d) Correction Factors for the Sulphate and Glucuronide Conjugates

Estimations of paracetamol and its sulphate and glucuronide conjugates in plasma produce different peak area ratios relative to internal standard when compared to estimations of the same concentrations in water (or urine). Paracetamol alone was used for standardisation therefore appropriate correction factors were required to account for these differences and the differences in molar absorptivity relative to paracetamol. The correction factors were calculated by comparing the peak area ratios of measured concentrations of paracetamol and its sulphate and glucuronide conjugates in plasma and water taken through the procedures described above. The values obtained are shown in Table 7.

e) Linearity of the Assay Procedures.

The Linearity of each assay for concentrations found in therapeutic and overdose situations was assessed as follows :-

Urine: Urine from a patient who had taken paracetamol in overdosage was diluted serially with water.

This provided concentration ranges

TABLE 7

Correction Factors for the quantitation of Paracetamol sulphate and glucuronide conjugates in plasma using Paracetamol only for standardisation.

Conjugate	Wavelength (nm)	Correction Factor	
		Therap. Assay	Overdose Assay
Sulphate	242	0.84	0.76
	254	1.03	0.93
Glucuronide	242	0.77	0.77
	254	0.84	0.84

of 1.6 - 1600, 28 - 28000, 2.0 - 1980, 13 - 1300 and 9.8 - 980 μg/ml of paracetamol and its glucuronide, sulphate, mercapturic acid and cysteine conjugates respectively. The dilutions were assayed according to c(i) above.

Plasma - Paracetamol Alone: Serial dilutions of plasma containing 500 μg/ml paracetamol were prepared using drug-free plasma to cover the range 0.5 - 500 μg/ml. The dilutions were assayed in two ranges (0.5 - 50 and 25 - 500 μg/ml) according to c(ii) above.

Plasma - Paracetamol and Metabolites: Plasma containing 420, 239 and 51.5 μg/ml of paracetamol and its glucuronide and sulphate conjugates respectively was serially diluted with drug-free plasma down to one-tenth of the stock concentrations. These dilutions covered typical concentrations of the three compounds after overdosage. Dilutions of plasma containing 25, 28 and 10 μg/ml of paracetamol and its glucuronide and sulphate conjugates respectively, covering typical therapeutic concentrations, were prepared similarly. The two concentration ranges were assayed according to c(iii) above.

Peak height or peak area ratios were plotted against concentration for the plasma assays and the measured concentrations of each compound were plotted against dilution for the urine assay.

Results: The plots are shown in Fig. 15 (urine), Fig. 16 a and b (paracetamol alone in plasma), and Fig. 17 a and b (paracetamol and metabolites in plasma). Linearity was maintained throughout each concentration range for each assay and the plots obtained for the plasma assays passed through the origin in each case.

f) Precision and Accuracy

Batches of six replicate samples of urine and plasma containing typical concentrations of paracetamol and its metabolites at therapeutic and overdose levels were assayed by the appropriate procedures already described to assess the precision and accuracy of each method. Concentrations are all paracetamol equivalent, corrected for purity.

Urine: Paracetamol and its glucuronide, sulphate, cysteine and mercapturic acid conjugates were dissolved in drug-free urine at concentrations of 0.63, 2.30, 1.22, 0.40 and approximately 0.35 mg/ml respectively. Portions of this solution were diluted 1 in 4 and 1 in 30 with drug-free urine to provide three concentration levels which were then assayed according to c(i) above.

Plasma - Paracetamol Alone: Plasma samples containing 2, 10 and 30 µg/ml were assayed on six separate occasions according to the therapeutic level procedure in c(ii) above. This was repeated for the overdose procedure

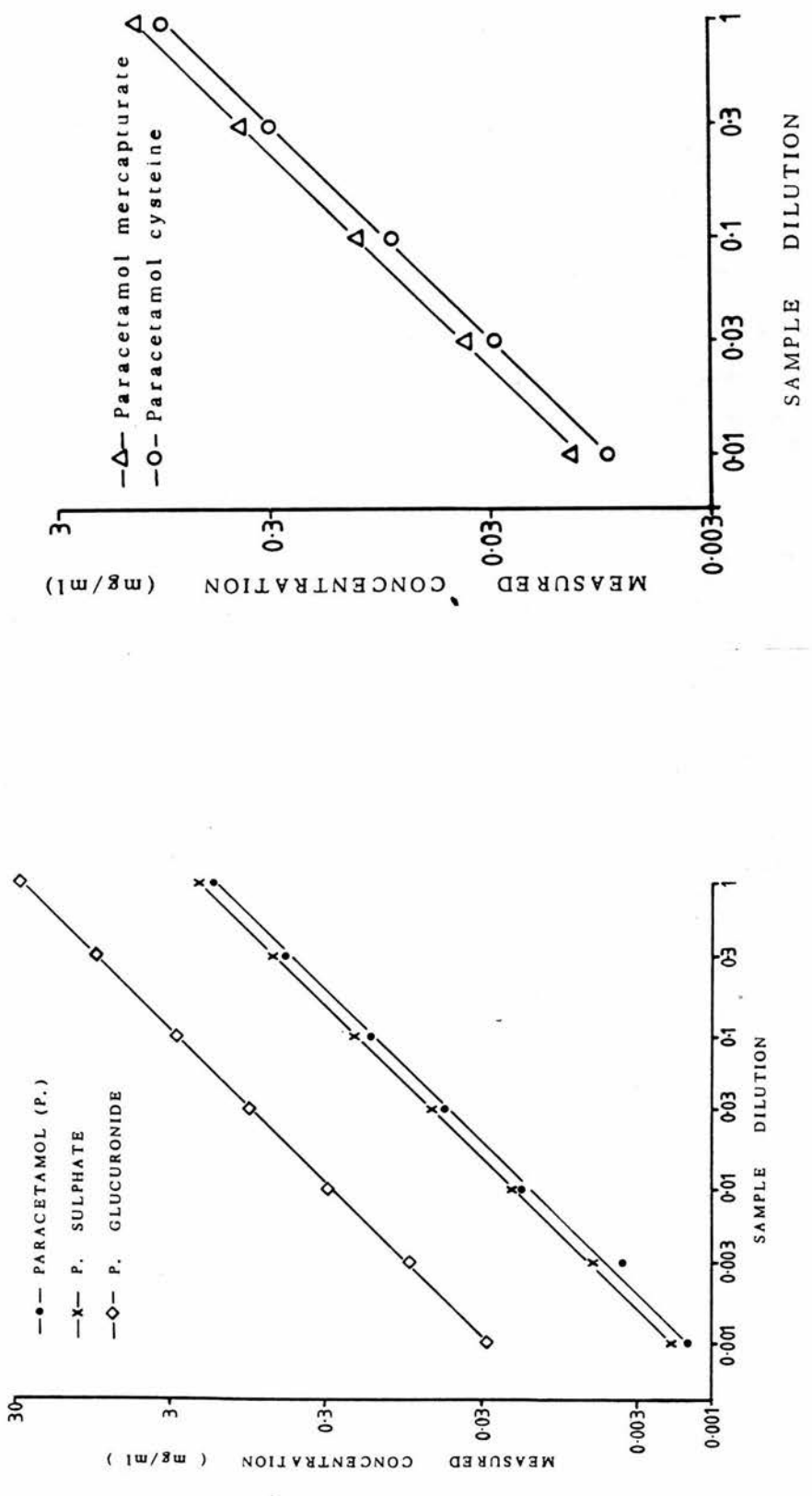


Fig. 15: Linearity of the HPLC assay for paracetamol and its conjugates in urine using NPA as internal standard.

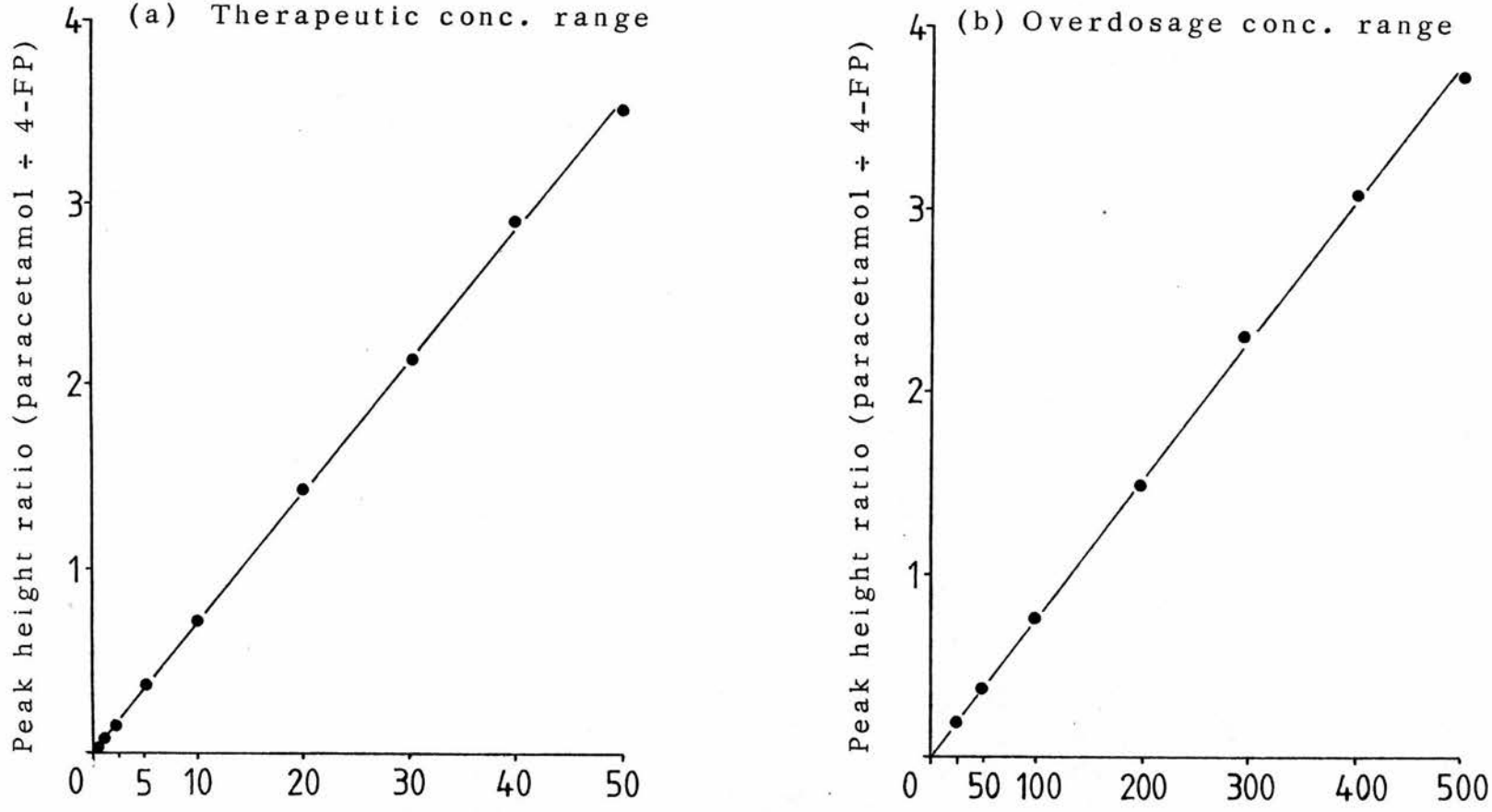


Fig.16: Linearity of calibration graphs for the estimation of paracetamol alone in plasma using HPLC with 4-fluorophenol (4-FP) as internal standard.

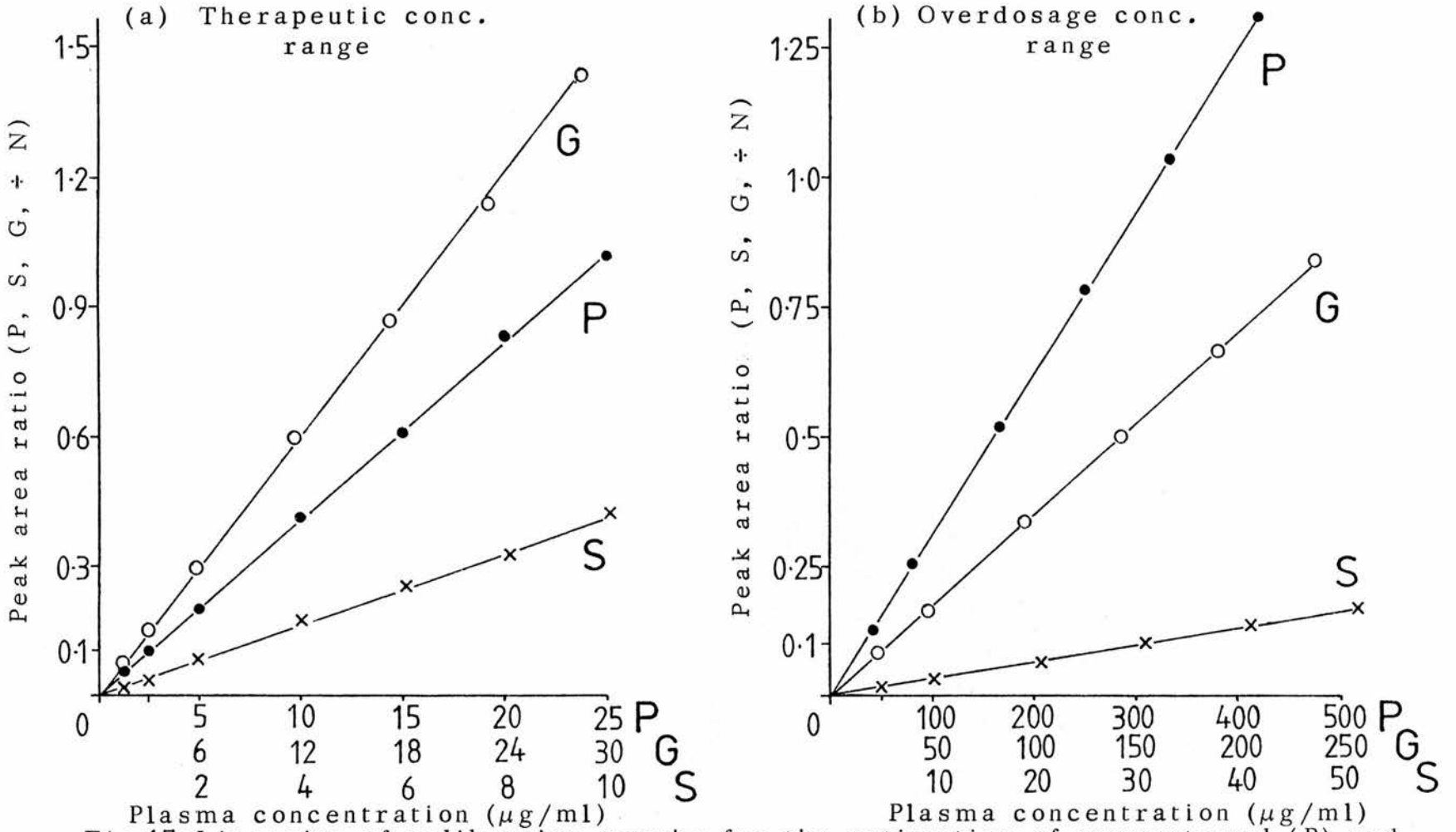


Fig.17: Linearity of calibration graphs for the estimation of paracetamol (P) and its sulphate (S) and glucuronide (G) conjugates in plasma using HPLC with N-propionyl-4-aminophenol (N) as internal standard.

using samples containing 25, 100 and 300 μg/ml.

Plasma - Paracetamol and Metabolites: Concentrations assayed using the therapeutic range procedure in c(iii) above were 2.5, 2.8 and 1.0 μg/ml and 20.0, 22.8 and 8.1 μg/ml of paracetamol and its glucuronide and sulphate conjugates respectively.

For the overdose range assay, the concentrations were 42, 24 and 5.2 μg/ml and 252, 143 and 31 μg/ml of paracetamol and its glucuronide and sulphate conjugates respectively.

Results: Means, standard deviations, coefficients of variation and percentage accuracy for each assay are shown in Tables 8 - 10.

Urine: For the urine assay (Table 8), coefficients of variation were less than 2% at all concentrations for paracetamol and its sulphate and glucuronide conjugates whereas the mercapturate and cysteine conjugates, which chromatographed as broader peaks, showed somewhat greater variability (maximum 7.2%). Accuracy was within 6 percent at all levels for paracetamol and its sulphate and glucuronide conjugates. The high values obtained for the cysteine conjugate reflect the difference in its UV extinction coefficient relative to paracetamol.

Plasma-Paracetamol Alone: For free paracetamol alone in

TABLE 8

Precision and accuracy of the measurement of Paracetamol and its conjugates in urine

Compound	High level conc. (μg/mℓ)			Medium level conc. (μg/mℓ)			Low level conc. (μg/mℓ)		
	Weighed -in	measured **	% Accur.	Weighed -in	measured **	% Accur.	Weighed -in	measured **	% Accur.
Paracetamol	630	649 ± 3 (0.5%)	103	158	162 ± 2 (1.2%)	103	21.0	21.9 ± 0.3 (1.2%)	104
Paracetamol Sulphate	1220	1258 ± 9 (0.7%)	103	305	312 ± 3 (0.9%)	102	40.7	43.2 ± 0.2 (0.5%)	106
Paracetamol Glucuronide	2300	2205 ± 20 (0.9%)	96	575	549 ± 5 (0.9%)	96	76.7	73.7 ± 1.4 (2.0%)	96
Paracetamol * Mercapturate	350 *	340 ± 5 (1.6%)	97	88 *	85 ± 5 (5.8%)	97	11.7 *	12.0 ± 0.8 (6.3%)	103
Paracetamol Cysteine	400	482 ± 12 (2.6%)	120	100	116 ± 5 (4.3%)	116	13.3	15.2 ± 1.1 (7.2%)	114

* Based on purity estimate of 41.7% (Howie, 1977)

** Values are mean ± S.D.; coefficient of variation in brackets.

TABLE 9

Accuracy and Precision for the Rapid Estimation of Paracetamol Alone in Plasma.

Conc. (µg/ml)	Therapeutic Level			Overdose Level		
Weighed -in	2	10	30	25	100	300
Estimated	2.02	9.91	30.2	23.2	98.3	300.3
± S.D	0.10	0.30	0.3	1.3	2.6	8.1
Accuracy %	101.0	99.1	100.7	92.8	98.3	100.1
C.V % *	4.9	3.0	1.1	5.7	2.6	2.7

* Coefficient of variation

TABLE 10

Precision and accuracy of the measurement of Paracetamol and its conjugates in plasma

Compound	Overdose Range Assay						Therapeutic Range Assay					
	High conc. (μg/mℓ)			Low conc. (μg/mℓ)			High conc. (μg/mℓ)			Low conc. (μg/mℓ)		
	Weighed-in	Meas*-ured	% Accur.	Weighed-in	Meas*-ured	% Accur.	Weighed-in	Meas*-ured	% Accur.	Weighed-in	Meas*-ured	% Accur.
Paracetamol	252	254 ±.9 (0.3%)	101	42	42±.1 (0.3%)	100	20.0	19.7±.4 (1.9%)	98	2.5	2.5±.03 (1.4%)	100
Paracetamol Sulphate	31	31 ±.1 (0.4%)	100	5.2	5.3±.1 (1.5%)	103	8.1	8.4±.2 (2.1%)	104	1.0	1.1±.03 (2.4%)	113
Paracetamol Glucuronide	143	137±.5 (0.4%)	96	24	23±.06 (0.3%)	96	22.8	23.3±.5 (2.0%)	102	2.8	2.8±.03 (1.1%)	100

* values are mean ± S.D.; coefficient of variation in brackets

plasma (Table 9), coefficients of variation ranged from 1.1 to 5.7%. Measured concentrations were within 2% of weighed-in values for all but the 25 µg/ml samples in the overdose range (within 7%).

Plasma-Paracetamol and Metabolites: The ranges of values obtained for precision were 0.3 - 1.9% (paracetamol), 0.3 - 2.0% (paracetamol glucuronide) and 0.4 - 2.4% (paracetamol sulphate, Table 10). The higher values were all at the low therapeutic level. Accuracy was within 4% for all compounds at each level except for paracetamol sulphate at the 1.0 µg/ml concentration (accuracy 113%).

Chapter 6

Comparisons with other Methods

The above procedures were compared with published methods for estimating "total" paracetamol in urine and plasma and free paracetamol in plasma.

(i) Urine

Aliquots of urine samples collected from 10 patients after paracetamol overdosage were pooled and assayed by HPLC as in chapter 5. The same pooled samples were assayed following enzymic hydrolysis of the sulphate and glucuronide conjugates to free paracetamol which was then estimated by gas-liquid chromatography (Prescott, 1971a,b). The total free paracetamol obtained after hydrolysis and gas-liquid chromatography (T_G) was plotted against the corresponding total of paracetamol and its

sulphate and glucuronide conjugates obtained by HPLC (T_L). There was a highly significant correlation between the values obtained by the two methods ($r = 0.995$; $p < .001$) and the slope of the regression line was close to unity ($T_G = 0.95T_L - 0.56$; Fig. 18).

(ii) Plasma - Paracetamol Alone:

125 plasma samples collected from poisoned patients and healthy subjects, with concentrations ranging from 1.3 to 440 μg/ml were assayed as in Chapter 5 and by the gas-liquid chromatographic (GLC) method of Prescott (1971 b). Values obtained by HPLC were plotted against those measured by GLC(Fig 19). A highly significant correlation was obtained ($r = 0.997$; $p < .001$). The equation of the regression line was $C_l = 1.02\ C_G - 0.21$, where C_l and C_G are concentrations measured by HPLC and GLC respectively.

(iii) Plasma - Paracetamol and Metabolites:

Twenty-four samples collected from patients after paracetamol overdosage and containing a wide range of paracetamol concentrations were assayed by the following methods:-

a. Glynn and Kendal (1975), which measured free paracetamol only. After protein removal with trichloracetic acid, paracetamol was nitrated in acid. This was

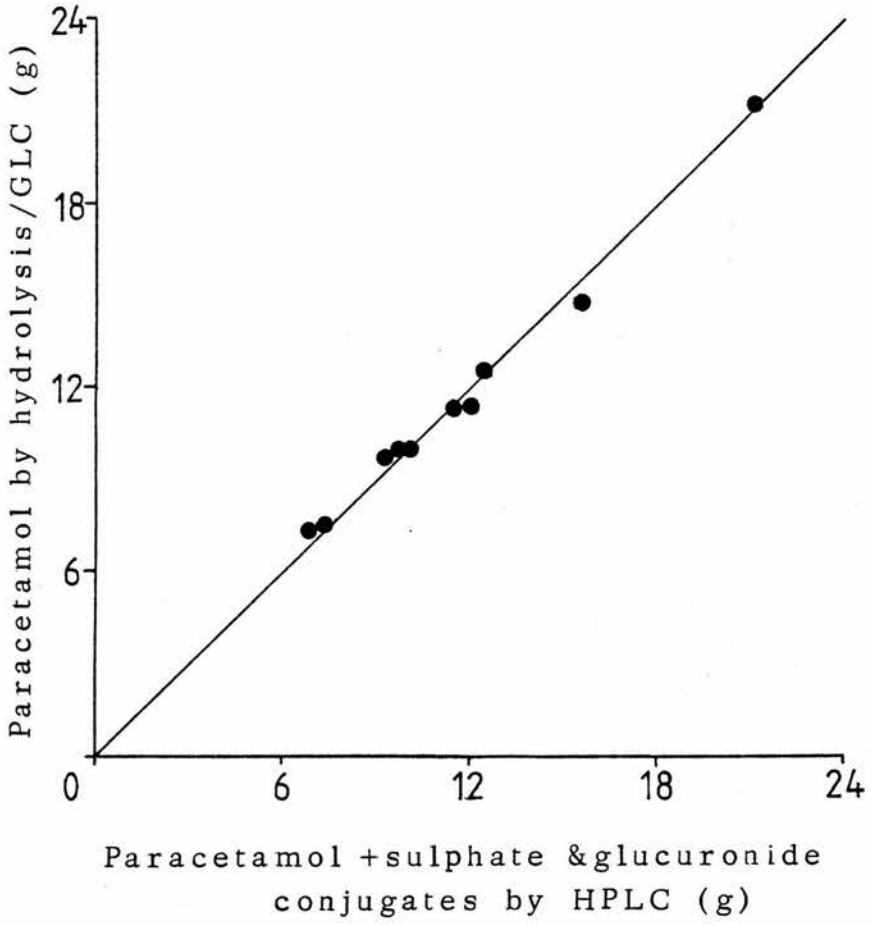


Fig. 18 Comparison of methods for the estimation of paracetamol and its sulphate and glucuronide conjugates in urine using gas-liquid chromatography (GLC; Prescott 1971a, b) and high performance liquid chromatography (HPLC; Chapter 5).

The line denotes 1:1 relationship. n = 10 samples.

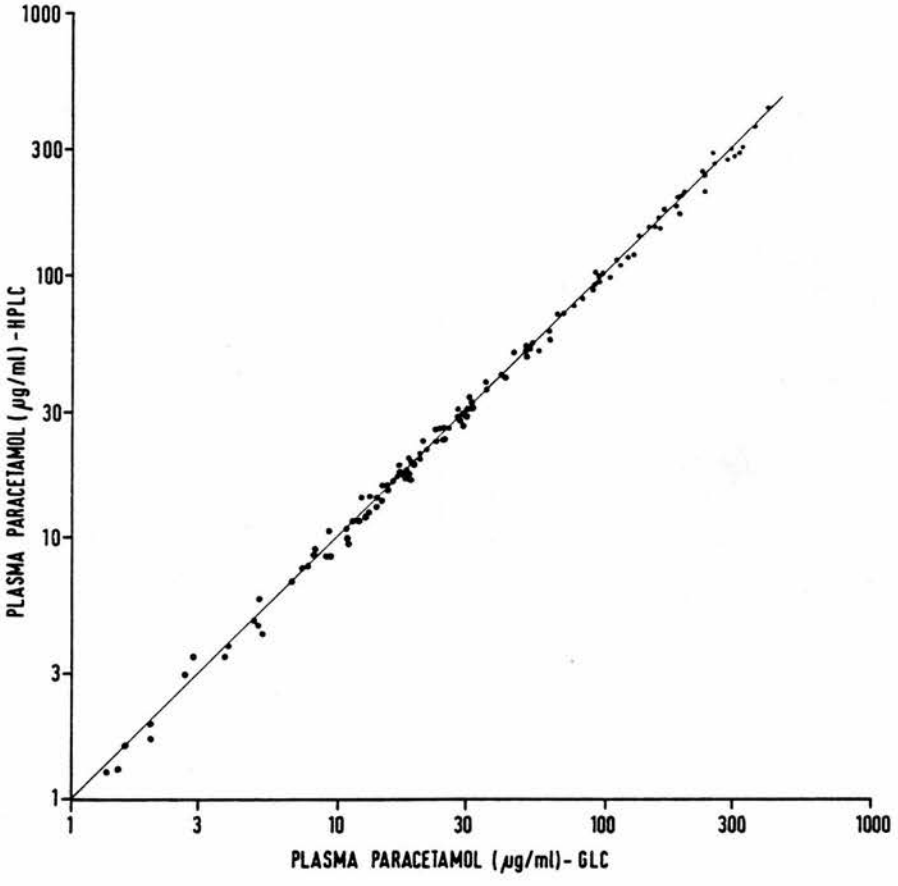


Fig. 19: Comparison of methods for the estimation of paracetamol alone in plasma by high performance liquid chromatography (HPLC; Chapter 5) and gas-liquid chromatography (GLC; Prescott, 1971b).

The line denotes 1:1 relationship
n = 125 samples.

followed by addition of base to produce and orange-yellow ion which was read at 450 nm (Mace and Walker, 1976).

b. Wilkinson (1976), which involved protein removal and hydrolysis using perchloric acid. Hydrolysis was carried out at 100°c for 40 minutes. The p-aminophenol released was coupled with o-cresol in the presence of aqueous ammonia and the resulting blue colour was read at 615 nm.

c. Smith et al (1978), in which protein was first removed with trichloracetic acid followed by hydrolysis in 4 N hydrochloric acid at 100°c for one hour. The p-aminophenol released was coupled with phenol in alkaline bromine water and the blue colour obtained was read at 620 nm (Welch and Conney, 1965).

d. By HPLC, as already described in Chapter 5.

Each method was standardised with the same set of weighed-in paracetamol standards in horse serum. The "paracetamol" concentrations measured using methods (a), (b) and (c) were plotted against those obtained by HPLC.

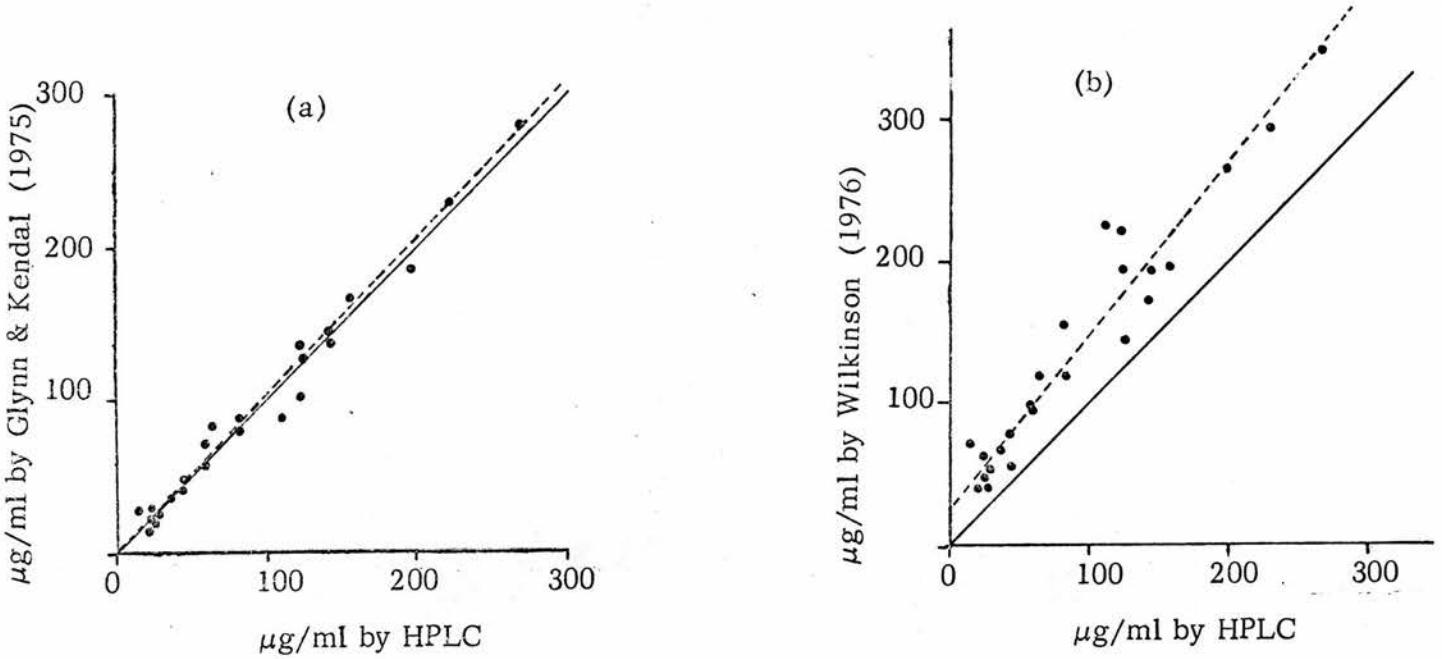
The extents of hydrolysis using method (b) and the modification advocated by Love (1977) were also assessed. Plasma (0.5 ml) containing added paracetamol and its sulphate and glucuronide conjugates was treated with 0.33M perchloric acid (4.5 ml) to precipitate the proteins and the supernatant was removed for hydrolysis.

An aliquot (0.5 ml) was removed before commencing hydrolysis. Further aliquots (0.5 ml) were removed after hydrolysis for 10 minutes at 100°c (according to Love 1977) and after 40 minutes at 100°c (according to Wilkinson 1976). Equal amounts of internal standard (50 µl of aqueous NPA, 2.0 mg/ml) were mixed with each aliquot prior to chromatography as in method (d).

Results

Good correlation coefficients were obtained in each case (Figs. 20 and 21) but only with the method of Glynn and Kendal (1975) was the slope of the regression line close to unity (m = 1.01; Fig. 20 a), indicating that values were essentially equivalent using this method and the HPLC procedure. With the methods of Wilkinson(1976) and Smith et al (1978) the slope values were 1.20 and 1.87 respectively (Figs. 20b and 21 a), suggesting that more than just free paracetamol was being measured by these two methods. When the paracetamol concentration measured according to Smith et al (1978) was plotted against the total paracetamol concentration by HPLC (ie equivalent to the free drug plus its sulphate and glucuronide conjugates), the relationship obtained suggested that the former method was measuring total paracetamol in plasma (Fig. 21 b).

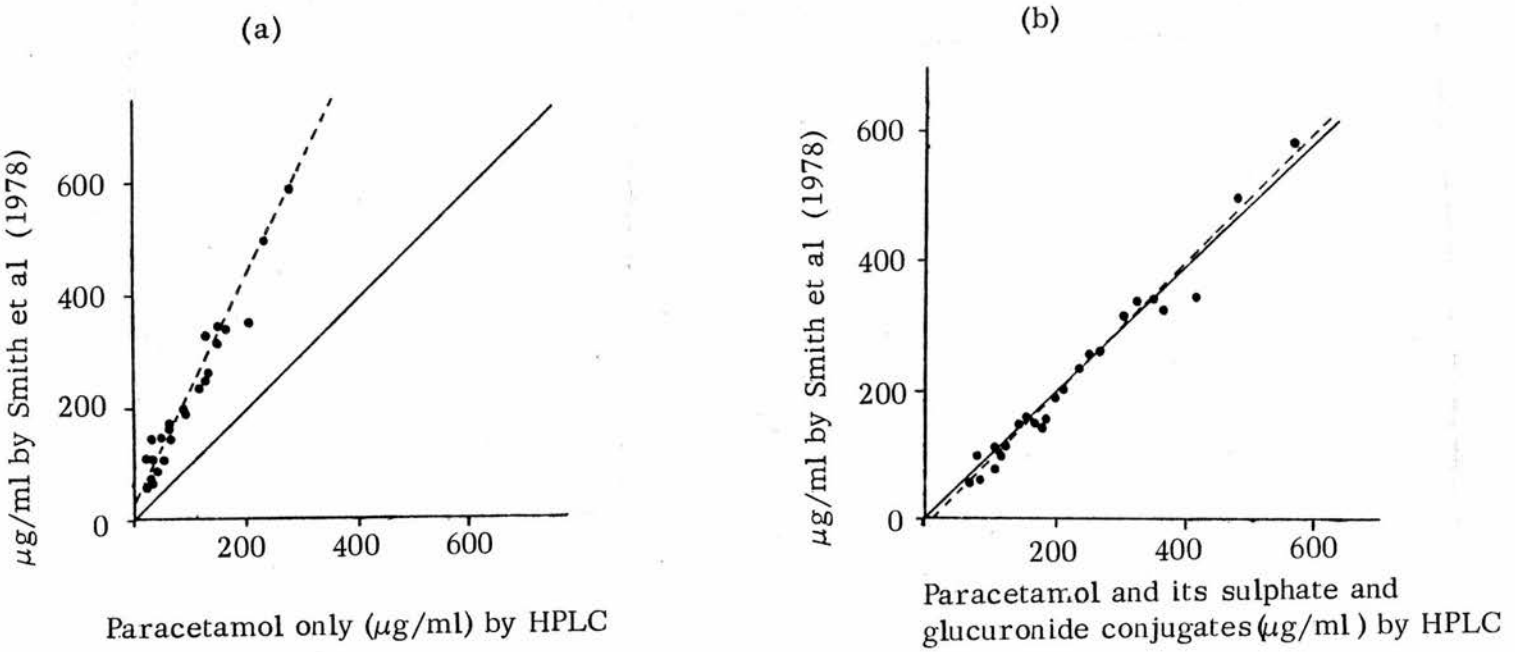
Further investigation of the methods of Wilkinson (1976) and Love (1977) by HPLC showed that paracetamol sulphate completely disappeared after both hydrolyses



(a) y = 1.01x - 1.9; r = 0.99

(b) y = 1.20x + 26; r = 0.96

Fig: 20: Comparison of plasma paracetamol concentrations obtained using HPLC (see chap. 5) and
(a) the nitration method of Glynn and Kendal (1975)
(b) the perchloric acid hydrolysis method of Wilkinson (1976)
(n = 24; ---- regression line; ——— line describing 1:1 relationship).



(a) $y = 1.87x + 37$; $r = 0.98$

(b) $y = 1.02x - 17$; $r = 0.99$

Fig 21: Comparison of plasma paracetamol concentrations obtained using HPLC (see chapter 5) and the method of Smith et al (1978).

(a) concentration of paracetamol only by HPLC compared with " paracetamol concentration " obtained using the method of Smith et al (1978).

(b) concentration of paracetamol together with its sulphate and glucuronide conjugates obtained by HPLC compared with " paracetamol concentration " obtained using the method of Smith et al (1978).

(n = 24; ------ regression line; ——— line describing 1:1 relationship).

(Fig. 22) but even after 40 minutes at 100°c considerable amounts of free paracetamol and its glucuronide conjugate were present. Thus the "paracetamol" concentration measured by these methods did not reflect either free or total paracetamol in plasma. Clinical interpretation would be more difficult using these methods than if complete hydrolysis had been achieved.

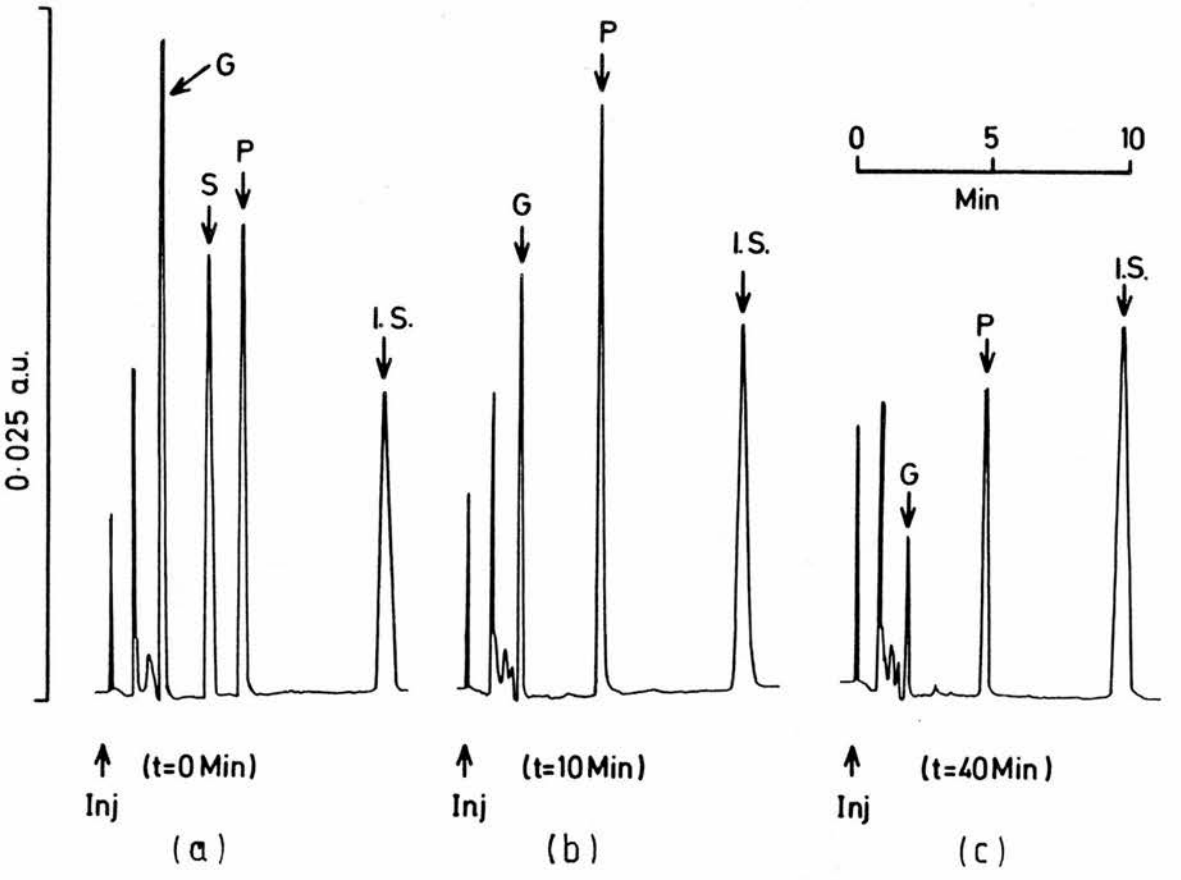


Fig. 22: HPLC of paracetamol (P) and its glucuronide (G) and sulphate (S) conjugates in plasma before and after hydrolysis in perchloric acid.

(a) before hydrolysis; (b) after hydrolysis for 10 mins (Love, 1977); (c) after hydrolysis for 40 mins (Wilkinson, 1976).

IS = internal standard (N-propionyl-4-aminophenol).

Chapter 7 Summary and Discussion

HPLC methods were developed and evaluated for the estimation of paracetamol and its four major conjugates in urine and plasma. These methods were shown to be significant improvements on existing procedures, especially in terms of simplicity and total assay times. The 5 μm particle-size spherical reverse-phase column stationary phase materials now available were particularly suitable for the analysis of paracetamol and its conjugates. Fast, efficient separations were achieved with simple column/injector assemblies at relatively low pressures and solvent flow rates. Mobile phase composition could be easily modified to improve peak detection and separation from endogenous materials.

An internal standard (N-propionyl-4-aminophenol, NPA) was prepared and shown to be suitable for quantitation at different detector wavelengths, owing to its chemical similarity to paracetamol. The assay procedures each required only simple one-step sample preparation (basically addition of internal standard) followed by chromatography. Peak area or height ratios of each compound to the internal standard could be related to standards containing paracetamol only. Linearity, reproducibility and accuracy were demonstrated for each method over all concentrations likely to be encountered in practice.

Each HPLC method was shown to correlate well with appropriate alternative methods for measuring either free paracetamol or paracetamol plus its sulphate and glucuronide conjugates. Also, more information concerning the pattern of metabolism could be obtained more quickly by HPLC due to the specificity and speed of these methods.

Urine samples containing paracetamol conjugates were shown to be stable at -20°c for at least 3 years, an important finding since samples were collected over a period of years.

SECTION IV

PARACETAMOL DISPOSITION IN HEALTHY SUBJECTS

PARACETAMOL DISPOSITION IN HEALTHY SUBJECTS

Introduction

The metabolism of paracetamol was studied after a single oral dose in 8 healthy subjects. In addition, the effect of oral N-acetylcysteine, the current treatment of choice for paracetamol overdosage (Prescott et al, 1979) on paracetamol metabolism was investigated.

Chapter 1: Subjects and methods .

a) Paracetamol Alone: Eight healthy male laboratory and clinical staff volunteered for the study. None had received any other medication for at least one week beforehand and each subject fasted overnight before taking 20 mg/kg paracetamol B.P dissolved in 400 ml "Coca-cola" over 10 minutes. The ages and weights of the subjects and doses administered are shown in Table 11. The bladder was emptied and an in-dwelling cannula was inserted into a suitable forearm vein prior to paracetamol administration. The subjects refrained from smoking and fluids during the first 2 hours and lunch was allowed 4 hours after dosing. 10 ml blood samples were collected into heparinised tubes at 0, 15, 30, 45, 60 and 90 minutes and 2, 3, 4, 6 and 8 hours after dosing. The plasma was separated immediately and stored at -20°c prior to analysis. Urine samples were collected hourly for the first 12 hours and from 12 - 24 hours. The volume and pH of each sample was measured and duplicate

TABLE 11

Paracetamol and healthy subjects - ages weights and doses administered*

Subject	Age (yr)	Weight (kg)	Dose (mg)
PA	28	67	1340
AB	28	70	1400
JF	34	75	1500
IK	21	62	1240
AP	29	68	1360
JP	32	67	1340
LP	42	75	1500
HR	32	63	1260

* All subjects were male; dose 20mg/kg Paracetamol BP

aliquots were stored at -20°c prior to analysis.

b) Paracetamol with N-acetylcysteine: The protocol was identical to (I) above except that

(a) 75 mg/kg N-acetylcysteine* was added to the paracetamol solution in "Coca-cola". This was followed by two further doses of 37.5 mg/kg each in 100 ml "Coca-cola" at 2 and 4 hours after the first dose.

b) In five subjects no blood samples were taken and urine was collected at 0 -2, 2 - 4, 4 - 8, and 8 - 24 hours.

* Supplied as a 20% W/V solution buffered to pH7 ("Airbron", Duncan-Flockhart Ltd.)

c) Analytical Methods

Plasma concentrations of paracetamol and its sulphate and glucuronide conjugates and urine concentrations of paracetamol and its sulphate, glucuronide, cysteine and mercapturic acid conjugates were measured as described in Section III.

Chapter 2

Paracetamol Disposition

The absorption, distribution and elimination of most drugs are usually first-order processes (Gibaldi, 1971). Thus the rate of delivery of drug to the body is proportional to the amount present in the gastro-intestinal tract and the rate of elimination is proportional to the amount present in the body. It follows that the rate of change in the plasma concentrations of the drug and its metabolites is proportional to the total amount of each present in the body, assuming that the volumes in which they are distributed remain constant. Based on these assumptions the following can be estimated :-

(a) Apparent Plasma Elimination Half-Life: When absorption and distribution are complete the plasma concentrations decline such that $C_t = (\text{Const.})\ e^{-\beta t}$ ....(1) (Gibaldi and Perrier, 1975) where C_t is the plasma concentration at time t and β describes the elimination rate of the drug.

Therefore $\ln C_t = \ln(\text{Const.}) - \beta t$ ....(2)

A plot of $\ln C_t$ against time gives a straight line with slope $-\beta$.

The apparent plasma elimination half-life ($t_{\frac{1}{2}}$) is the time taken for the concentration to fall by one half

and is obtained from the formula

$$t_{\frac{1}{2}} = \frac{\ln 2}{\beta} \quad \ldots\ldots (3)$$

The method of least squares was used to estimate β from semilogarithmic plots of drug concentration against time. Similar relationships also hold for metabolites.

(b) Area under the Plasma Concentration - Time Curve (AUC):

The AUC is a measure of the amount of drug reaching the systemic circulation (Gibaldi, 1971).

Areas were calculated by the trapezoidal rule from points on a linear plot of plasma concentration against time after ingestion.

(c) Urinary Excretion: Assuming that the urinary excretion of drugs and metabolites is proportional to their plasma concentration, a plot of ln (excretion rate) against time gives a linear terminal phase with slope equal to the apparent elimination constant , as in equation (2).

The excretion rate was estimated from plots of the hourly excretion of paracetamol and its conjugates at the mid-point of each urine collection period. The urinary elimination half-life was estimated according to equation (3) above, using the method of least squares to estimate the slope.

(d) Renal Clearance: The renal clearance of paracetamol

and its sulphate and glucuronide conjugates were calculated as follows:-

$$\text{Renal Clearance} = \frac{\text{Total compound excreted in each urine collection period}}{\text{Area under plasma concentration-time curve in the same period}} \quad \ldots (4)$$

Values were calculated from the hourly urinary excretion of each compound and its plasma concentration at the sampling times described on p 114 . Plasma concentrations at 5 and 7 hours were interpolated semilogarithmically from the 4 and 6 hour concentrations and the 6 and 8 hour concentrations respectively. This enabled areas under the plasma concentration-time curves to be calculated for each hourly urine collection up to 8 hours after paracetamol ingestion.

(e) Recovery of Paracetamol and its Metabolites in Urine:

The urinary recovery of paracetamol and its sulphate, glucuronide, cysteine and mercapturic acid conjugates was determined up to 24 hours after paracetamol ingestion. Amounts of the metabolites were expressed as the paracetamol equivalent.

(f) Statistical Analysis: Mean values are given with the standard deviation (S.D) except where stated otherwise. Student's t-test for paired data was used for comparisons.

Chapter 3

Results

1) Ingestion of Paracetamol Alone

a) Plasma Concentrations of Paracetamol and its Conjugates

(i) Paracetamol: Table 12 and Figure 23 show the mean plasma concentrations of paracetamol and its sulphate and glucuronide conjugates after ingestion of 20 mg/kg of paracetamol in the eight subjects. The cysteine and mercapturic acid conjugates were not measurable (< 1μg/ml). Individual values for peak concentrations, times to reach peak concentrations and apparent elimination half-lives are shown in Tables 13 and 14.

TABLE 12

Mean Plasma concentrations of Paracetamol and its Sulphate and Glucuronide Conjugates following oral ingestion of 20 mg/kg Paracetamol in 8 healthy subjects.

Time after dose (hr)	Paracetamol mean* ± S.D.	Paracetamol Sulphate mean* ± S.D.	Paracetamol Glucuronide mean* ± S.D.
0.25	15.7 ± 5.1	2.3 ± 0.8	1.5 ± 1.3
0.5	18.7 ± 3.1	4.4 ± 1.0	3.7 ± 2.3
0.75	18.7 ± 3.1	5.4 ± 1.0	6.1 ± 3.9
1.0	18.0 ± 1.9	6.1 ± 1.1	8.2 ± 4.9
1.5	15.3 ± 1.3	6.0 ± 1.3	11.2 ± 5.8
2.0	13.7 ± 2.0	5.7 ± 0.8	12.3 ± 5.1
3.0	10.0 ± 1.8	4.8 ± 1.0	11.8 ± 4.6
4.0	7.5 ± 1.6	4.3 ± 0.9	10.7 ± 3.3
6.0	3.9 ± 1.3	2.8 ± 0.9	6.8 ± 1.9
8.0	2.5 ± 0.9	1.8 ± 0.7	4.6 ± 1.4

* Concentrations are μg/mℓ plasma

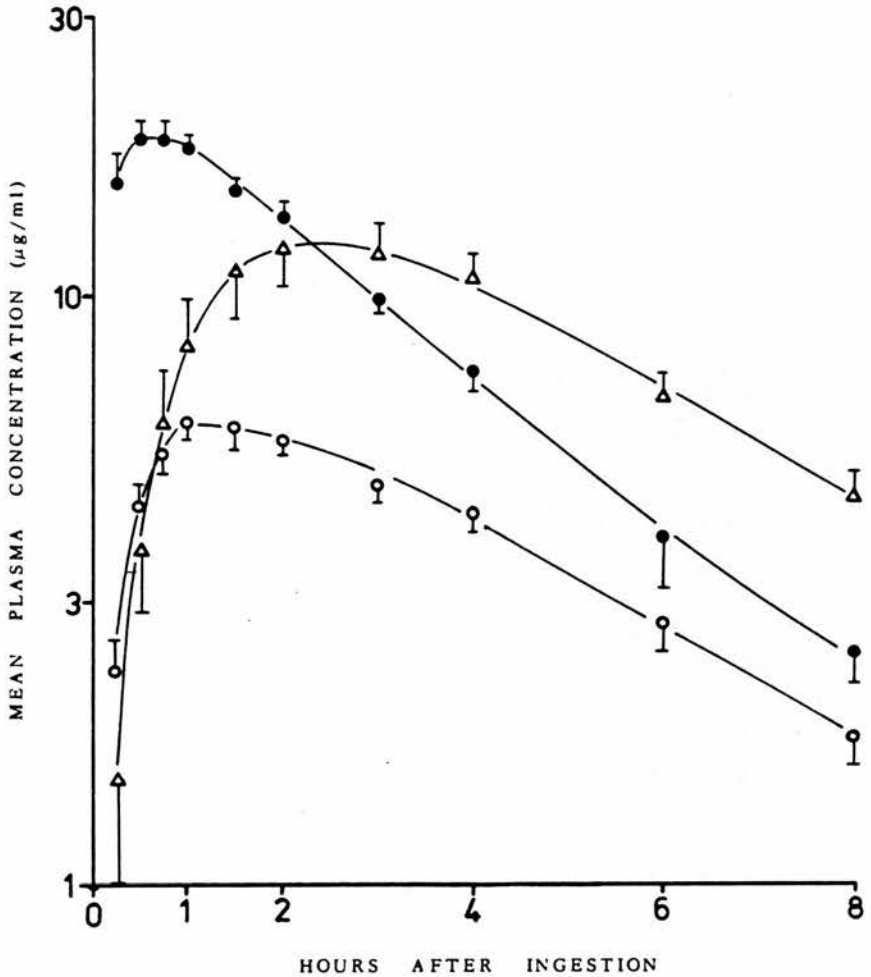


Fig 23: Plasma concentrations of paracetamol and its sulphate and glucuronide conjugates following a 20 mg/kg oral dose of paracetamol in 8 healthy subjects.
Values are means ± s.e.m (paracetamol equivalent).

Key: ● Paracetamol;
○ Paracetamol Sulphate;
△ Paracetamol Glucuronide.

TABLE 13

Paracetamol and its Sulphate and Glucuronide conjugates in plasma - individual peak concentrations and time to reach peak concentrations in 8 healthy subjects after oral ingestion of 20 mg/kg Paracetamol.

Subject	Peak Paracetamol		Peak Paracetamol Sulphate		Peak Paracetamol Glucuronide	
	Time (hr)	Conc^{n} (μg/mℓ)	Time (hr)	Conc^{n} (μg/mℓ)	Time (hr)	Conc^{n} (μg/mℓ)
PA	0.75	19.2	1	6.03	2	7.10
AB	0.5	18.4	2	5.92	2	14.5
JF	0.75	23.0	1	7.26	3	9.73
IK	1.0	15.3	1.5	6.30	4	7.66
AP	0.75	21.0	1	4.21	3	16.4
JP	0.5	21.6	2	5.91	2	13.3
LP	0.25	23.1	1	6.99	1.5	23.7
HR	0.75	16.2	1.5	8.07	2	9.73
Mean ± S.D. (s.e.m.)	0.66±0.23 (0.08)	19.7±3.0 (1.1)	1.4±0.4 (0.15)	6.34±1.15 (0.41)	2.4±0.8 (0.29)	12.8±5.5 (1.9)

TABLE 14

Apparent elimination half-life of Paracetamol and its Sulphate and Glucuronide conjugates from plasma in 8 healthy subjects after a 20 mg/kg oral dose of Paracetamol.

Subject	Apparent plasma elimination half-life (hr)		
	Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide
PA	2.67	5.03	2.70
AB	2.03	2.51	2.74
JF	2.22	3.85	3.05
IK	2.56	4.46	3.60
AP	2.31	4.11	3.78
JP	2.32	3.41	3.54
LP	1.47	2.40	2.45
HR	2.78	3.60	3.62
Mean±S.D. (s.e.m.)	2.30 ± 0.41 (0.15)	3.67 ± 0.90 (0.32)	3.19 ± 0.51 (0.18)

Paracetamol absorption was rapid with a mean individual peak concentration of 19.7± 3.0 μg/ml occurring at 0.66 ± 0.23 hours after ingestion. Thereafter levels appeared to decline monophasically in all subjects with a mean apparent elimination half-life of 2.30 ± 0.41 hours.

(ii) Metabolites: Paracetamol sulphate and glucuronide conjugates were present in plasma in all subjects at 15 min.. The peak concentration of the sulphate conjugate generally occurred within an hour of the peak concentration of the parent drug (mean 1.4 ± 0.4 hours after ingestion) but occurred somewhat later (mean 2.4 ± 0.7 hours) with the glucuronide. The concentrations of paracetamol sulphate were considerably lower than those of the free drug. In contrast, paracetamol glucuronide concentrations usually rose steadily, eventually exceeding those of the free drug (between 0.8 and 5 hours). In one subject (PA) paracetamol glucuronide concentrations were particularly low throughout. The mean individual peak concentrations of paracetamol sulphate and paracetamol glucuronide were 6.34 ± 1.15 μg/ml and 12.8 ± 5.5 μg/ml respectively.

The apparent elimination half-lives of the conjugates were longer than those of the free drug, being 3.67 ± 0.90 hours and 3.19 ± 0.51 hours for paracetamol sulphate and glucuronide respectively. Both conjugates were easily

measurable at 8 hours, (mean concentration of 1.8 ± 0.7 μg/ml and 4.6 ± 1.4 μg/ml for paracetamol sulphate and glucuronide respectively).

(b) Urinary Excretion of Paracetamol and its Conjugates

The mean urinary excretion rates of paracetamol and its major conjugates in collections up to 24 hours after paracetamol ingestion are shown in Table 19 and Fig. 24. The mean urinary elimination half-lives calculated from individual plots are shown in Table 20.

(i) Paracetamol: Urinary excretion rates of paracetamol were low in all subjects with a mean peak value of 11.3 ± 3.5 mg/hr occurring in the second hour after ingestion. After 4 - 5 hours the mean excretion rate of paracetamol was lower than each of the major conjugates and remained so. Individual plots of ln (excretion rate) against time were usually linear after 2 hours but sometimes fluctuated considerably. The mean urinary elimination half-life was 2.86 ± 0.91 hours (range 2.15 - 4.92 hours).

(ii) Paracetamol Sulphate: The mean excretion rate of paracetamol sulphate was higher than that of any of the other compounds during the first hour after ingestion (41.5 ± 11.9 mg/hr). The peak mean excretion rate (60.8 ± 14.2 mg/hr) occurred during the second hour after ingestion, as did the individual peak in 7 of the 8 subjects. In most subjects the plots of ln (excretion

TABLE 19

Mean hourly excretion of Paracetamol and its major conjugates in the urine of 8 healthy subjects following a 20 mg/kg oral dose of Paracetamol

Time after ingestion (hr)	Mean Excretion Rate (mg ± S.D. per hr) Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide	Paracetamol Mercapturate	Paracetamol Cysteine
0-1	9.8 ± 4.4	41.5 ± 11.9	34.6 ± 18.1	1.2 ± 0.7	1.2 ± 0.6
1-2	11.3 ± 3.5	60.8 ± 14.2	84.7 ± 40.7	4.7 ± 1.7	3.6 ± 1.1
2-3	9.6 ± 3.8	51.9 ± 13.8	96.0 ± 34.2	7.5 ± 2.7	5.3 ± 1.7
3-4	7.3 ± 2.8	41.4 ± 11.8	83.4 ± 26.1	6.8 ± 1.7	5.1 ± 1.1
4-5	4.8 ± 1.7	36.1 ± 10.2	72.3 ± 21.1	6.4 ± 1.9	5.1 ± 1.4
5-6	4.0 ± 1.6	32.8 ± 11.4	63.7 ± 14.2	5.1 ± 1.9	4.8 ± 1.7
6-7	2.8 ± 0.9	25.7 ± 11.7	47.4 ± 10.0	3.7 ± 1.1	4.0 ± 1.6
7-8	2.9 ± 1.0	20.2 ± 8.2	37.6 ± 10.1	3.3 ± 0.8	3.2 ± 1.6
8-9	1.5 ± 0.6	15.3 ± 6.8	29.3 ± 6.9	2.7 ± 0.8	2.5 ± 1.0
9-10	1.5 ± 0.6	13.6 ± 5.2	25.9 ± 7.4	2.6 ± 0.9	2.3 ± 1.1
10-11	1.5 ± 0.6	11.9 ± 5.7	21.1 ± 5.3	2.0 ± 0.7	2.0 ± 1.1
11-12	1.3 ± 0.5	10.3 ± 5.0	17.7 ± 5.6	1.6 ± 0.6	1.7 ± 1.0
12-24	0.4 ± 0.2*	4.0 ± 1.6*	6.6 ± 2.6*	0.6 ± 0.2*	0.5 ± 0.4*

* Mean of hourly excretion rate between 12 and 24 hours after ingestion.

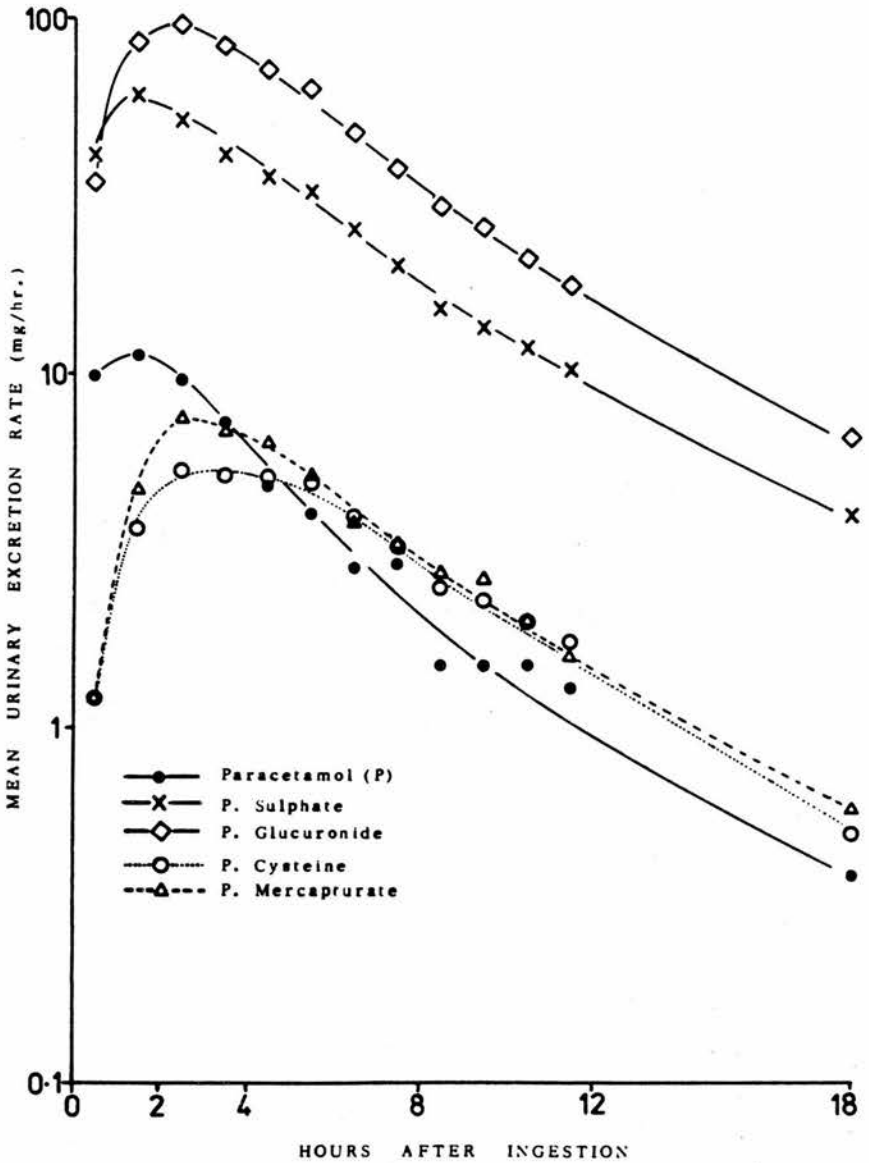


Fig. 24: Mean urinary excretion rates of paracetamol and its conjugates following 20 mg/kg paracetamol orally in 8 healthy subjects.

TABLE 20

Apparent urinary elimination half-life of Paracetamol and its conjugates in 8 healthy subjects following oral ingestion of 20 mg/kg Paracetamol

Compound	Apparent urinary elimination half-life (hour)	
	mean ± S.D.	(range)
Paracetamol	2.86 ± 0.91	(2.15 - 4.92)
Paracetamol Sulphate	3.66 ± 0.91	(2.65 - 4.95)
Paracetamol Glucuronide	3.39 ± 0.52	(2.66 - 3.96)
Paracetamol Mercapturate	3.88 ± 0.98	(2.48 - 4.88)
Paracetamol Cysteine	4.72 ± 2.38	(1.97 - 9.75)

rate) against time declined linearly after 2 - 3 hours and the mean individual urinary elimination half-life was the same as the plasma elimination half-life (3.66 ± 0.91 hours, range 2.65 - 4.95 hours).

(iii) Paracetamol Glucuronide: Excretion rate-time plots of paracetamol glucuronide were generally smooth curves with maxima 2 - 3 hours after ingestion. The maximum mean excretion rate (96.0 ±34.2 mg/hr) also occurred at 2 - 3 hours. From the second hour the mean excretion rate of paracetamol glucuronide exceeded that of the other compounds. However, in one subject (PA) excretion of the sulphate conjugate exceeded that of the glucuronide for most of the 24 hour study period. The mean individual urinary elimination half-life was 3.39 ± 0.52 hours (range 2.66 - 3.96 hours) which was similar to the value estimated from plasma concentrations.

(iv) Paracetamol Cysteine and Mercapturic Acid Conjugates Both of these compounds were present in the first (1 hour) urine sample of all subjects. The excretion rates were initially very low but increased steadily to reach a maximum usually between 2 and 4 hours after ingestion. The maximum mean excretion rates of the mercapturic acid and cysteine conjugates were 7.5 ± 2.7 and 5.3 ± 1.7 mg/hr respectively.

The mean estimated urinary elimination half-lives were 3.88 ± 0.98 (range 2.48 - 4.88 hr) and 4.72 ± 2.38 hr

(range 1.97 - 9.75 hr) for the mercapturic acid and cysteine conjugates respectively.

(c) Recovery of Paracetamol and its Conjugates in Urine:

The percentages of the dose recovered in 24 hour urine collections as paracetamol and its conjugates in individual subjects following ingestion of 20 mg/kg paracetamol are shown in Table 21.

Only small amounts were recovered as unchanged drug (mean 4.7 ± 1.1% of dose, range 3.2 - 6.3%), over half of which was excreted during the first 4 hours.

Excretion of the mercapturic acid and cysteine conjugates was slow at first but after 12 hours the recovery of each of these metabolites reached 87% of the 24 hour totals. After 24 hours, 4.0 ± 1.1% (range 2.5 - 5.6) and 3.4 ± 1.0% (range 2.0 - 5.1) of the dose was recovered as paracetamol mercapturate and paracetamol cysteine respectively. The individual combined recovery of these conjugates accounted for between 4.7 and 9.2% of the dose (mean 7.4 ± 1.7%).

Excretion of the sulphate and glucuronide conjugates reached 48% and 43% of the respective 24 hour recoveries after 4 hours and the amounts excreted after 12 hours were 88% of the 24 hour totals for both conjugates. After 24 hours, paracetamol sulphate and glucuronide accounted for 30.3 ± 9.0 and 50.3 ± 12.3 per cent of

TABLE 21

Percentage of dose recovered in 24 hour urine collections as Paracetamol (P) and its Sulphate (S), Glucuronide (G), Mercapturic acid (M) and Cysteine (C) conjugates following a 20 mg/kg oral dose of Paracetamol in 8 healthy subjects.

Subject	Percentage of dose recovered as:-						
	P	S	G	M	C	M+C	Total
PA	4.3	42.7	34.6	5.6	3.6	9.2	90.8
AB	6.3	23.9	49.4	4.4	3.1	7.5	87.1
JF	5.6	34.2	45.9	3.3	5.1	8.5	94.1
IK	5.8	35.5	38.9	5.4	3.8	9.1	89.3
AP	3.2	19.3	65.9	3.7	3.7	7.4	95.7
JP	4.7	26.4	55.3	4.0	3.8	7.8	94.2
LP	3.6	20.1	69.0	3.2	2.0	5.2	97.9
HR	4.1	40.0	43.7	2.5	2.2	4.7	92.6
Mean ±S.D.	4.7 ±1.1	30.3 ±9.0	50.3 ±12.3	4.0 ±1.1	3.4 ± 1.0	7.4 ±1.7	92.7 ±3.5

the dose respectively. Although mean recovery of the glucuronide was considerably greater than that of the sulphate conjugate, individual values varied widely, with ranges of 19.3 - 42.7 and 34.6 - 69.0% of the dose recovered as sulphate and glucuronide conjugates respectively. There was a statistically significant inverse correlation between the 24 hour recoveries of the two conjugates in each individual. In terms of amounts recovered, $r = -0.81$ ($p < .02$) whereas in terms of % of dose recovered, $r = 0.92$ ($p < .005$). In one subject (PA), 24 hour recovery of the sulphate was considerably higher than that of the glucuronide conjugate (42.7% and 34.6% of the dose respectively).

The apparent plasma paracetamol half-life appeared to be related to the excretion of the sulphate and glucuronide conjugates. A positive correlation was obtained between the percentage of dose excreted as paracetamol sulphate in 24 hours and the plasma paracetamol half-life ($r = 0.77$; $p < .025$). However, the correlation obtained between the percentage of dose excreted as paracetamol glucuronide in 24 hours and the plasma paracetamol half-life was negative ($r = -0.75$; $p < .05$). No relationship was found between the plasma paracetamol half-life and excretion of either the unchanged drug or combined excretion of the mercapturic acid and cysteine conjugates.

Overall, 87.1 - 97.9 per cent of the dose was recovered in 24 hours as paracetamol and its sulphate, glucuronide, mercapturic acid and cysteine conjugates in the 8 subjects (mean 92.7 ± 3.5 per cent).

(d) Proportional Excretion of Paracetamol and its Conjugates:

The change in the pattern of renal excretion of paracetamol and its metabolites with time is demonstrated by considering the amounts of each compound excreted relative to the total amount of all five compounds excreted in each sampling interval. Fig. 25 shows this pattern in a typical subject (JP) and Table 22 shows the mean individual values of all 8 subjects.

Sulphate conjugation accounted for a large proportion of the total drug excreted initially (mean 47.6 ± 10.3% in the first hour) but by 3 hours this fell to 31.2 ± 9.4%. The proportion excreted as paracetamol sulphate then remained fairly constant until 12 hours, after which it increased slightly. Glucuronide conjugation followed the opposite course, accounting for an increasing proportion of the total drug excreted during the first 3 hours (initially 38.6 ± 12.7%, up to 55.5 ± 12.0% in the third hour), followed by a decrease after 11 - 12 hours.

Excretion of unchanged drug accounted for a higher proportion of total excretion during the first 2 or 3

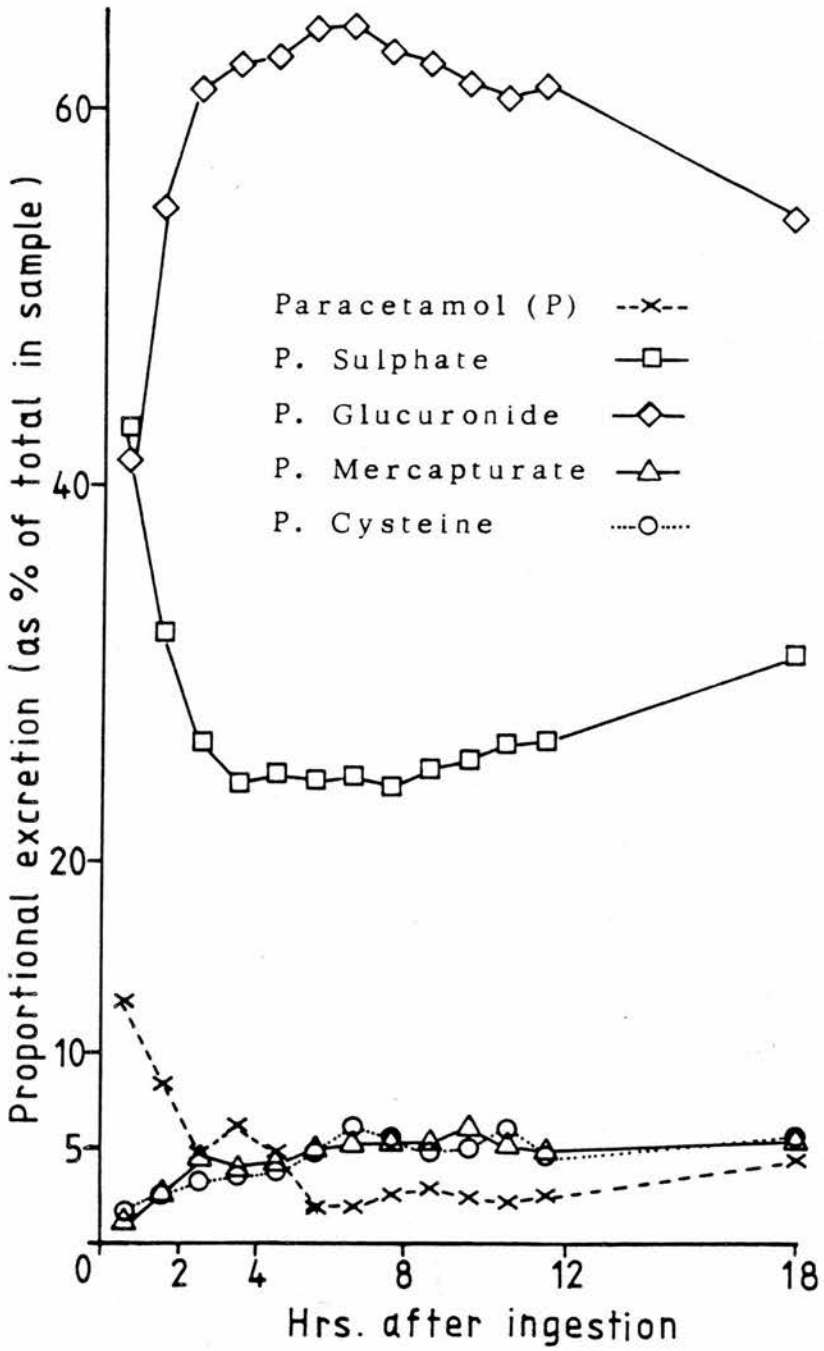


Fig 25: Urinary excretion of paracetamol and its conjugates after 20 mg/kg paracetamol orally in subject JP. Amounts are expressed as percentages of the total drug excreted in each sample.

TABLE 22

Urinary excretion of Paracetamol and its conjugates expressed as percentages of the total drug recovered in each sample following 20 mg/kg Paracetamol orally in 8 healthy subjects (values are mean ± S.D.)

Time after ingestion (hr)	Percentage of total in sample excreted as:-				
	S	G	P	M	C
0-1	47.6 ±10.3	38.6 ±12.7	11.1 ±4.3	1.3 ±0.6	1.4 ±0.6
1-2	38.1 ±10.8	49.6 ±13.0	7.0 ±2.3	2.9 ±1.2	2.3 ±0.8
2-3	31.2 ± 9.4	55.5 ±12.0	5.7 ±2.0	4.5 ±1.6	3.1 ±0.9
3-4	29.3 ± 9.3	57.1 ±11.0	5.1 ±1.9	4.9 ±1.7	3.6 ±0.7
4-5	29.5 ± 9.4	57.4 ±10.7	3.8 ±1.4	5.2 ±1.9	4.1 ±0.9
5-6	29.9 ±10.2	57.7 ±11.5	3.6 ±1.3	4.5 ±1.5	4.3 ±1.2
6-7	30.2 ±11.2	57.3 ±11.4	3.4 ±1.3	4.5 ±1.1	4.7 ±1.7
7-8	29.6 ±11.5	56.8 ±12.2	4.1 ±1.2	4.9 ±1.1	4.7 ±1.8
8-9	30.0 ±11.5	56.4 ±12.5	3.3 ±1.3	5.5 ±1.4	4.9 ±2.1
9-10	30.0 ±11.3	56.1 ±11.6	3.3 ±1.6	5.7 ±1.7	4.9 ±2.0
10-11	30.3 ±11.3	55.5 ±12.0	4.0 ±2.1	5.2 ±1.7	5.1 ±2.4
11-12	31.1 ±11.5	54.7 ±11.8	4.3 ±2.4	4.9 ±2.6	5.0 ±2.7
12-24	33.7 ±12.2	53.0 ±12.1	4.1 ±2.0	5.2 ±2.8	4.0 ±2.3

KEY:

P = Paracetamol
S = Paracetamol sulphate
G = Paracetamol glucuronide
M = Paracetamol mercapturate
C = Paracetamol cysteine

hours than subsequently (mean 11.1 ± 4.3% in the first hour down to 5.1 ± 1.9% in the fourth hour). This presumably reflects the higher plasma concentrations of paracetamol in the 3 hours following paracetamol ingestion.

Only 1.3% and 1.4% (mean values) of the total excreted in the first hour was in the form of the mercapturic acid and cysteine conjugates respectively. However, the mean combined excretion of these conjugates accounted for 8.5 - 10.6% of the total excreted after 3 hours. On average, slightly more was excreted as the mercapturic acid than as the cysteine conjugate.

(e) Renal Clearance of Paracetamol and its Sulphate and Glucuronide Conjugates:

Table 23 shows the renal clearance of paracetamol and its sulphate and glucuronide conjugates (mean ± SD of 8 subjects) during the first eight hours after paracetamol ingestion. Renal clearance of paracetamol was relatively low during the first hour (10.7 ± 5.2 ml/min), mainly due to very low values in 2 subjects who produced very low urine volumes in the first hour. Values then increased to a mean which remained relatively constant (14 - 17 ml/min) from 2 - 8 hours after ingestion. The overall mean value was 14.1 ± 6.1 ml/min and there was wide individual variation (range of individual means 8.1 - 20.6 ml/min). In contrast, the renal clearances

TABLE 23

Renal clearance of Paracetamol (P) and its Sulphate (S) and Glucuronide (G) conjugates during the first 8 hours after oral ingestion of 20 mg/kg Paracetamol in 8 healthy subjects (values are means ± S.D.)

Time after ingestion (hr)	Mean renal clearance (mℓ/min)			Urine flow rate (mℓ/min)	Urine pH
	P	S	G		
0-1	10.7 ± 5.2	182 ± 39	157 ± 30	1.2 ± 1.0	5.8 ± 0.6
1-2	12.2 ± 3.9	172 ± 31	134 ± 16	1.5 ± 1.3	6.2 ± 0.4
2-3	13.8 ± 5.3	163 ± 36	136 ± 26	1.8 ± 1.1	6.2 ± 0.6
3-4	14.5 ± 5.0	153 ± 26	128 ± 15	2.3 ± 1.8	6.4 ± 0.6
4-5	13.5 ± 6.8	156 ± 23	127 ± 12	1.0 ± 0.5	6.3 ± 0.6
5-6	16.3 ± 9.2	180 ± 42	144 ± 29	1.1 ± 0.5	6.6 ± 0.5
6-7	14.8 ± 7.3	173 ± 42	133 ± 22	1.0 ± 0.4	6.7 ± 0.7
7-8	17.3 ± 5.2	164 ± 34	126 ± 10	1.5 ± 1.0	6.7 ± 0.6
Overall mean (n=64)	14.1 ± 6.1	167 ± 34	135 ± 22	1.4 ± 1.1	6.4 ± 0.6

of the sulphate and glucuronide conjugates were both high with little variation. The respective mean values for the renal clearance of paracetamol sulphate and glucuronide were 167 ± 34 and 135 ± 22 ml/min (ranges of individual means 141 - 214 and 112 - 151 ml/min respectively).

The relationship between renal clearance and urine flow rate was studied using linear regression analysis. The correlation coefficient with paracetamol was $r = 0.33$ ($p < .01$; $n = 64$ values) suggesting that the renal clearance is dependent on urine flow rate. The respective values for the sulphate and glucuronide conjugates were $r = -0.09$ and $r = 0.08$, indicating no correlation between renal clearance and urine flow rate.

Similar analysis of the relationship between renal clearance and urine pH showed no significant correlations with any of the 3 compounds (the respective r values were 0.09, 0.03 and -0.14 for paracetamol and its sulphate and glucuronide conjugates).

2) Paracetamol with N-Acetylcysteine

(a) Plasma Concentrations of Paracetamol and its Conjugates

N-acetylcysteine (NAC) had little effect on the elimination of paracetamol and its metabolites from plasma in three subjects (Fig. 26; Table 24).

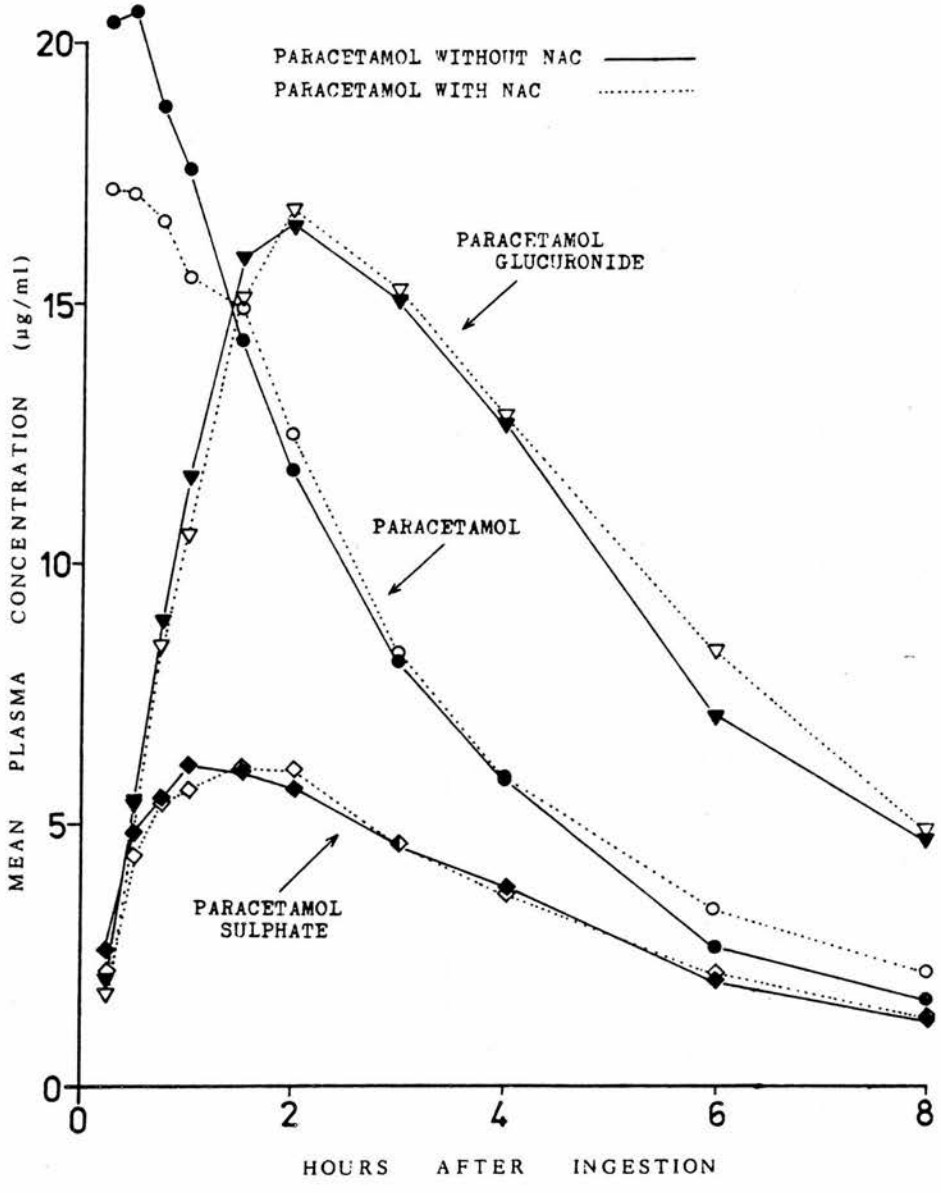


Fig 26:. Mean plasma concentrations of paracetamol and its sulphate and glucuronide conjugates in 3 healthy subjects after oral ingestion of 20 mg/kg paracetamol with and without N-acetylcysteine.

TABLE 24

Plasma concentrations of Paracetamol and its Sulphate and Glucuronide conjugates following Oral Ingestion of Paracetamol in 3 healthy subjects (Dose 20mg/kg Paracetamol with and without N-acetylcysteine).

Time after dose (hr)	Paracetamol		Paracetamol Sulphate		Paracetamol Glucuronide	
	*P - (µg ± S.D./mℓ)	†P + (µg ± S.D./mℓ)	P - (µg ± S.D./mℓ)	P + (µg ± S.D./mℓ)	P - (µg ± S.D./mℓ)	P + (µg ± S.D./mℓ)
0.25	20.4±3.3	17.2±4.3	2.6±0.4	2.2±1.2	2.0±1.4	1.7±1.8
0.5	20.6±1.9	17.1±2.9	4.8±0.6	4.3±1.6	5.4±2.9	5.3±4.8
0.75	18.8±2.1	16.6±0.3	5.5±0.7	5.4±1.2	8.9±5.3	8.4±6.0
1.0	17.6±0.8	15.5±1.3	6.1±0.8	5.6±1.2	11.7±6.5	10.5±8.1
1.5	14.3±0.8	14.9±2.9	6.0±0.5	6.1±0.7	15.9±6.8	15.1±7.8
2.0	11.8±1.8	12.5±2.4	5.7±0.5	6.0±0.4	16.5±4.5	16.8±8.0
3.0	8.1±1.5	8.3±1.8	4.6±0.2	4.6±0.4	15.1±4.3	15.3±5.4
4.0	5.8±1.5	5.9±1.4	3.8±0.5	3.6±0.5	12.7±2.0	12.9±3.8
6.0	2.6±1.0	3.4±1.2	2.0±0.5	2.1±0.3	7.1±2.1	8.3±1.6
8.0	1.6±0.6	2.2±1.5	1.3±0.4	1.4±0.7	4.7±1.0	4.9±0.9

* Plasma concentrations after ingesting Paracetamol alone

† Plasma concentrations after ingesting Paracetamol with N-acetylcysteine

The only obvious difference was that individual peak free drug concentrations were slightly lower (18.9 ± 2.7 μg/ml compared to 21.0 ± 2.4 μg/ml) and occurred later with NAC (0.83 ± 0.63 hr compared to 0.42 ± 0.14 hr, Table 25). The apparent plasma elimination half-lives of paracetamol and its sulphate and glucuronide conjugates were not significantly altered by NAC (Table 26).

(b) Areas Under the Plasma Concentration - Time Curves of Paracetamol and its Sulphate and Glucuronide Conjugates:

The areas under the plasma concentration-time curves (AUC) of paracetamol and its sulphate and glucuronide conjugates were measured in three subjects following paracetamol ingestion with and without NAC (Table 27). Intersubject variation was considerable, especially for paracetamol glucuronide. However, the values obtained for each compound with and without NAC were very similar for each individual. Overall, there were no significant changes in the AUC of any of the three compounds following ingestion of paracetamol with NAC.

(c) Urinary Excretion of Paracetamol and its Conjugates:

NAC had little effect on the urinary excretion of paracetamol and its major conjugates in the same 3 subjects (Fig. 27, Tables 28 a and b). There was a significant decrease in the excretion of unchanged drug

TABLE 25

Paracetamol and its Sulphate and Glucuronide conjugates in plasma - individual peak concentrations and time to reach peak concentrations in 3 healthy subjects following ingestion of 20 mg/kg Paracetamol orally with and without N-acetylcysteine.

Subject	Peak Paracetamol				Peak Paracetamol Sulphate				Peak Paracetamol Glucuronide			
	time (hr)		conc.(μg/mℓ)		time (hr)		conc.(μg/mℓ)		time(hr)		conc.(μg/mℓ)	
	- *	+	-	+	-	+	-	+	-	+	-	+
AB	0.5	0.75	18.4	16.6	2	2	5.9	5.5	2	2	14.5	14.7
JP	0.5	1.5	21.6	18.2	2	1.5	5.9	6.2	2	3	13.3	11.3
LP	0.25	0.25	23.1	21.8	1	1	7.0	7.0	1.5	2	23.7	25.6
Mean ± S.D.	0.42	0.83	21.0	18.9	1.7	1.5	6.3	6.3	1.8	2.3	17.2	17.2
	±0.14	±0.63	±2.4	±2.7	±0.6	±0.5	±0.6	±0.8	±0.3	±0.6	±5.7	±7.5

* + and - refer to Paracetamol ingestion with and without N-acetylcysteine respectively

TABLE 26

Apparent plasma elimination half-lives of Paracetamol and its Sulphate and Glucuronide conjugates in 3 healthy subjects following oral ingestion of 20 mg/kg Paracetamol with and without N-acetylcysteine.

Subject	Apparent plasma elimination half-life (hr)					
	Paracetamol		Paracetamol Sulphate		Paracetamol Glucuronide	
	* -	+	-	+	-	+
AB	2.03	1.99	2.51	2.63	2.74	2.99
JP	2.32	2.26	3.41	3.09	3.54	3.50
LP	1.47	1.73	2.40	2.19	2.45	2.62
Mean ± S.D.	1.94 ±0.43	1.99 ±0.27	2.77 ±0.55	2.64 ±0.45	2.91 ± 0.56	3.04 ± 0.44

* + and - refer to Paracetamol ingestion with and without N-acetylcysteine respectively

TABLE 27

Areas under the plasma concentration - time curves of Paracetamol and its sulphate and glucuronide conjugates in 3 healthy subjects following oral ingestion of 20 mg/kg Paracetamol with and without N-acetylcysteine

Subject	Area under the plasma concentration - time curve ($\mu g.\ hr.\ m\ell^{-1}$)					
	Paracetamol		Paracetamol Sulphate		Paracetamol Glucuronide	
	-	* +	-	+	-	+
AB	63.0	56.8	27.0	24.8	64.5	71.9
JP	68.0	73.6	30.9	30.0	73.7	61.4
LP	52.7	53.2	27.1	30.0	107.0	117.4
Mean ± S.D.	61.2 ± 7.8	61.2 ± 10.9	28.3 ± 2.2	28.3 ± 3.0	81.7 ± 22.4	83.6 ± 29.8

* - and + refer to ingestion of Paracetamol with and without N-acetylcysteine respectively

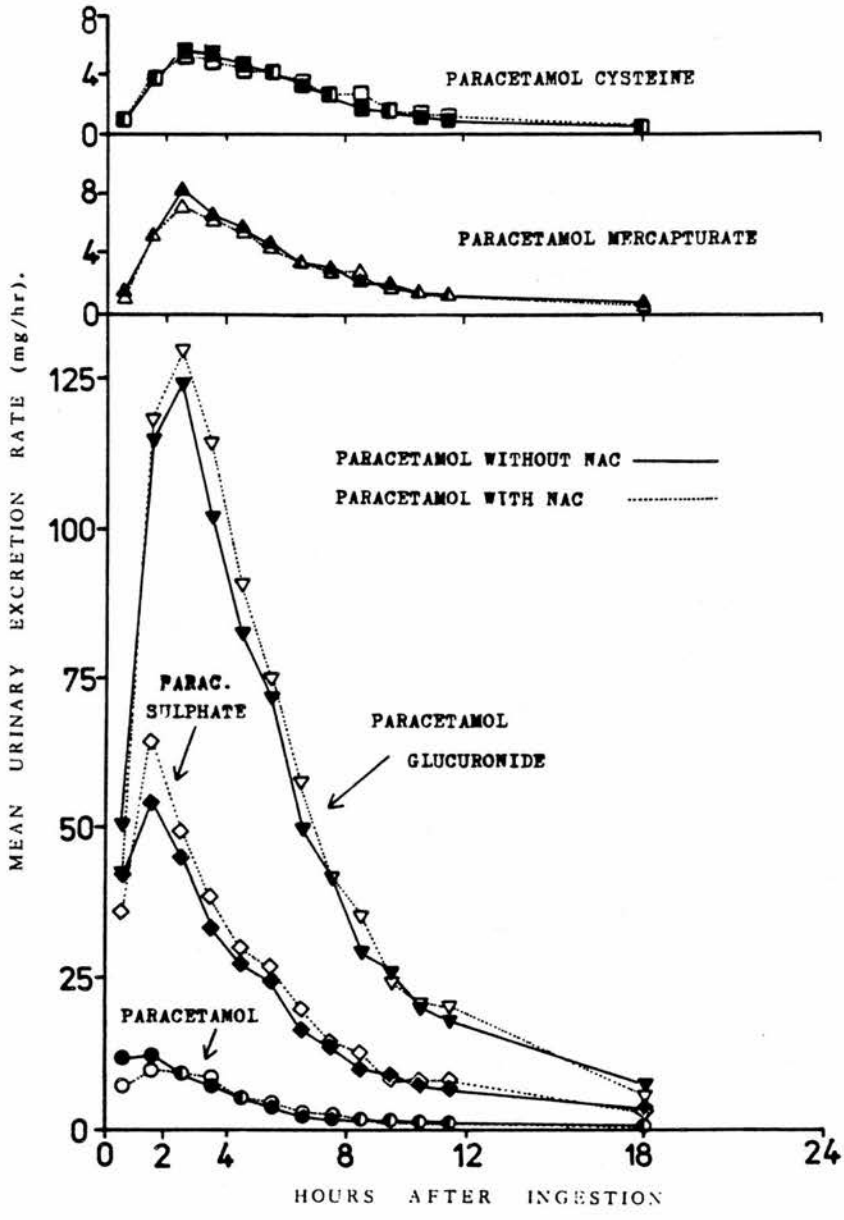


Fig 27: Mean urinary excretion rates of paracetamol and its conjugates following 20 mg/kg paracetamol orally with and without N-acetylcysteine in 3 healthy subjects.

TABLE 28(a)

Mean hourly excretion of Paracetamol and its Sulphate and Glucuronide conjugates in the urine of 3 healthy subjects following oral ingestion of 20 mg/kg Paracetamol with and without N-acetylcysteine

Time after dose (hr)	Paracetamol P − † (mg ± S.D./hr)	Paracetamol P + †† (mg ± S.D./hr)	Paracetamol Sulphate P − (mg ± S.D./hr)	Paracetamol Sulphate P + (mg ± S.D./hr)	Paracetamol Glucuronide P − (mg ± S.D./hr)	Paracetamol Glucuronide P + (mg ± S.D./hr)
0-1	11.9±1.3 ◇	6.4±0.8	41.8±1.6	35.8±7.3	50.7±20.9	42.1±20.6
1-2	12.4±0.9	9.8±2.9	53.8±4.5 ⊗	64.6±5.5	115±52	119±47
2-3	9.3±4.6	9.5±4.2	44.7±11.7	49.2±9.4	125±37	130±23
3-4	7.2±2.5	8.7±2.9	33.5±4.2	38.5±3.9	102±30	115±25
4-5	5.3±1.1	5.3±1.4	27.4±1.8	30.0±4.1	82.3±21.0	90.1±16.9
5-6	3.7±2.2	4.5±1.6	24.4±4.8	26.9±6.3	71.8±6.3	74.9±6.9
6-7	2.3±1.1	3.3±1.1	16.4±1.5	20.1±4.1	49.6±14.1	57.8±7.6
7-8	2.0±0.4	2.9±0.7	13.4±5.1	14.3±2.0	41.6±10.8	41.6±10.1
8-9	1.6±0.9	1.8±0.8	9.8±2.0	13.1±5.2	29.3±2.7	35.3±4.3
9-10	1.6±0.6	1.3±0.4	9.0±2.4	8.0±2.4	26.1±4.9	24.1±4.6
10-11	1.4±0.9	1.2±0.5	7.4±2.5	7.9±2.9	20.1±3.8	21.2±1.2
11-12	1.3±0.7	1.3±0.4	6.7±2.9	8.2±4.1	17.8±5.5	20.6±6.3
12-24	0.7±0.3*	0.5±0.1*	3.5±1.1*	2.7±0.8*	7.6±1.0*	5.8±1.0*

† Excretion rates after ingesting Paracetamol alone

†† Excretion rates after ingesting Paracetamol with N-acetylcysteine

* Mean of total excreted between 12 and 24 hours after ingestion

◇ $p < .02$

⊗ $p < .05$

TABLE 28 (b)

Mean hourly excretion of Paracetamol Mercapturic acid and Cysteine conjugates in the urine of 3 healthy subjects following oral ingestion of 20 mg/kg Paracetamol with and without N-acetylcysteine

Time after dose (hr)	Paracetamol Mercapturate (mg ± S.D./hr)		Paracetamol Cysteine (mg ± S.D./hr)	
	P - †	P + ††	P -	P +
0-1	1.5 ± 0.4	0.9 ± 0.2	1.1 ± 0.5	1.0 ± 0.3
1-2	5.2 ± 1.2	5.2 ± 1.7	3.7 ± 1.4	3.7 ± 0.7
2-3	8.4 ± 1.9	7.2 ± 1.1	5.5 ± 2.3	5.3 ± 0.4
3-4	6.6 ± 0.6	6.4 ± 1.5	5.4 ± 0.4	4.7 ± 1.0
4-5	5.8 ± 0.6	5.6 ± 0.9	4.7 ± 0.7	4.4 ± 0.2
5-6	4.9 ± 1.0	4.3 ± 1.1	4.1 ± 1.2	4.0 ± 0.3
6-7	3.5 ± 0.3	3.6 ± 0.6	3.4 ± 1.0	3.6 ± 0.4
7-8	3.1 ± 0.9	2.8 ± 0.6	2.7 ± 1.4	2.8 ± 0.5
8-9	2.3 ± 0.7	2.9 ± 0.8	1.7 ± 0.5	2.8 ± 0.6
9-10	2.0 ± 0.7	1.8 ± 0.5	1.6 ± 0.7	1.5 ± 0.4
10-11	1.4 ± 0.5	1.3 ± 0.6	1.2 ± 1.0	1.5 ± 0.2
11-12	1.3 ± 0.6	1.3 ± 0.6	1.0 ± 0.7	1.4 ± 0.5
12-24	0.7 ± 0.3*	0.4 ± 0.1*	0.5 ± 0.3*	0.5 ± 0.1*

† Excretion rates after ingesting Paracetamol alone

†† Excretion rates after ingesting Paracetamol with N-acetylcysteine

* Mean of total excreted between 12 and 24 hours after ingestion

in the first hour (6.4 $\pm$ 0.8 mg/hr compared with 11.9 $\pm$ 1.3 mg/hr without NAC; $p < .02$) followed by an increase in excretion of the sulphate conjugate during the second hour (64.6 $\pm$ 5.5 mg/hr compared with 53.8 $\pm$ 4.5 mg/hr without NAC; $p < .05$). However, in terms of the proportions of each compound excreted relative to the total drug recovery in each sample, the differences were not statistically significant although they were still considerable. The percentage excreted unchanged relative to the total drug recovered in the first hour decreased from 11.5 $\pm$ 3.0% without NAC to 8.1 $\pm$ 3.8% with NAC and the respective increase in paracetamol sulphate excretion in the second hour was from 29.4 $\pm$ 6.0% without NAC to 33.2 $\pm$ 7.2% with NAC.

The discrepancies noted above were due to a lower urinary recovery of paracetamol and its conjugates in the first hour followed by a slightly higher recovery in the second hour with NAC. The recoveries obtained were 7.5 $\pm$ 1.0 and 6.1 $\pm$ 1.5 per cent of the dose in the first hour after paracetamol without and with NAC respectively. The values for the second hour were 13.3 $\pm$ 3.0 and 14.2 $\pm$ 2.8 per cent of the dose respectively.

The initially lower excretion rate of unchanged drug reflected the lower concentrations of plasma paracetamol seen during the first hour after ingestion with NAC. However, the differences in plasma paracetamol sulphate concentrations were not as marked as the differences in

urinary excretion of the sulphate conjugate.

Excretion of paracetamol and its conjugates in all 8 subjects up to 2, 4, 8 and 24 hours after ingestion and also between these times is shown in Figs. 28 and 29 and Tables 29 and 30. The only appreciable effect of NAC was to decrease the excretion of paracetamol during the first two hours (mean 15.3 ± 4.3 mg with NAC and 21.1 ± 7.3 mg without NAC; $p < .05$). No other statistically significant differences were found.

(d) Total Recovery of Paracetamol and its Conjugates in Urine:

The 24 hour recoveries of paracetamol and its conjugates in all 8 subjects following ingestion of paracetamol with NAC are shown in Table 31. The mean value was 91.0 ± 4.3 per cent of the dose (range 84.1 - 96.4%) which was very similar to the recovery following paracetamol alone (mean 92.7 ± 3.5% Dose, range 87.1 - 97.9%). The mean recoveries of the individual compounds were also very close to those obtained following paracetamol alone (compare Tables 21 and 31) and no statistical differences were observed either separately or overall.

The inter-subject variation in the recovery of each compound was as wide as after paracetamol alone. Also, it was particularly notable that recoveries of the sulphate and glucuronide conjugates after paracetamol

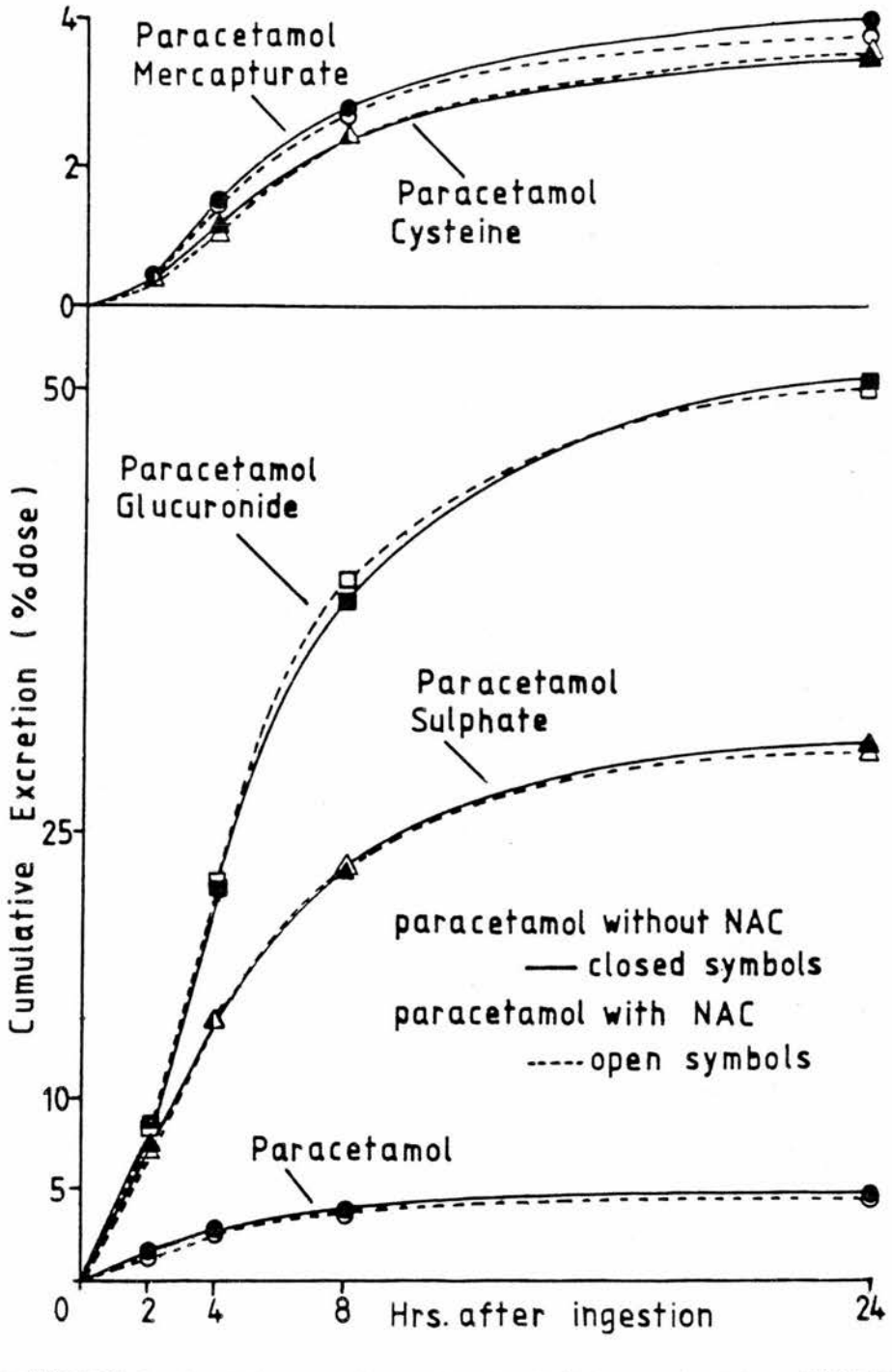


Fig 28: Mean Cumulative excretion of paracetamol and its conjugates in urine following 20 mg/kg paracetamol orally with and without N-acetylcysteine in 8 healthy subjects.

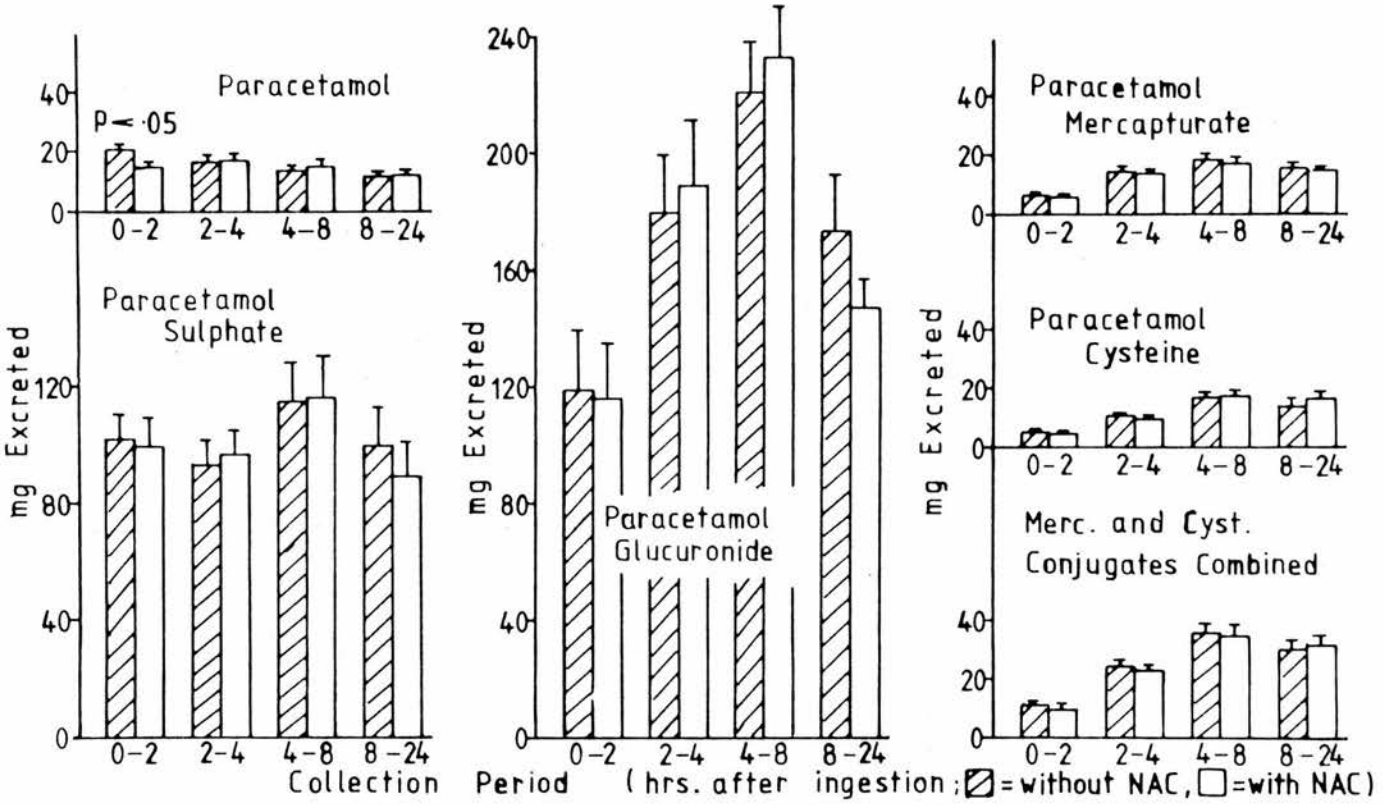


Fig.29: Urinary excretion of paracetamol and its conjugates following 20 mg/kg paracetamol orally with and without N-acetylcysteine. Values are mean ± s.e.m. of 8 subjects.

TABLE 29

Cumulative excretion of Paracetamol and its major conjugates in the urine of 8 healthy subjects following oral ingestion of 20 mg/kg Paracetamol with and without N-acetylcysteine (NAC)

Compound and Collection Period (hrs. after dose)		Mean cumulative excretion (mg ± S.D.) - NAC	+ NAC
Paracetamol	0-2	21.1 ± 7.3	* 15.3 ± 4.3
	0-4	38.0 ± 11.5	32.9 ± 9.6
	0-8	52.4 ± 14.5	48.7 ± 14.4
	0-24	64.3 ± 16.0	60.9 ± 18.1
Paracetamol Sulphate	0-2	102 ± 23	99 ± 28
	0-4	196 ± 46	196 ± 49
	0-8	310 ± 82	313 ± 84
	0-24	410 ±113	402 ± 115
Paracetamol Glucuronide	0-2	119 ± 58	116 ± 55
	0-4	299 ± 114	305 ± 117
	0-8	520 ± 159	537 ± 164
	0-24	694 ± 199	685 ± 187
Paracetamol Mercapturate	0-2	5.9 ±2.4	5.3 ± 2.1
	0-4	20.2 ±6.6	18.8 ± 5.6
	0-8	38.6 ±10.6	36.3 ± 9.9
	0-24	54.6 ±12.9	51.4 ± 12.7
Paracetamol Cysteine	0-2	4.8 ±1.5	4.1 ± 1.2
	0-4	15.2 ±3.7	13.7 ± 3.5
	0-8	32.4 ±8.0	31.2 ± 9.3
	0-24	46.8 ±15.1	48.3 ± 14.7
Merc. and Cysteine conjugates combined	0-2	10.7 ±3.6	9.4 ± 3.2
	0-4	35.4 ±9.4	32.5 ± 8.6
	0-8	71.0 ±15.8	67.5 ± 17.6
	0-24	101.4 ±22.5	99.7 ± 23.6

* $p < 0.05$

TABLE 30

Excretion of Paracetamol and its major conjugates in urine during different intervals after oral ingestion of 20 mg/kg Paracetamol with and without N-acetylcysteine (NAC) in 8 healthy subjects.

Compound and Collection Period (hrs. after dose)		Mean amount excreted (mg ± S.D.) - NAC	+ NAC
Paracetamol	0-2	21.1 ± 7.3	*15.3 ± 4.3
	2-4	16.9 ± 2.1	17.6 ± 6.1
	4-8	14.4 ± 4.2	15.8 ± 5.4
	8-24	11.9 ± 4.2	12.2 ± 4.0
Paracetamol Sulphate	0-2	102 ± 23	99.4 ± 28.3
	2-4	93.3 ± 24.1	96.7 ± 24.2
	4-8	115 ± 39	117 ± 39
	8-24	99.8 ± 38.4	88.9 ± 33.2
Paracetamol Glucuronide	0-2	119 ± 58	116 ± 55
	2-4	180 ± 57	189 ± 64
	4-8	221 ± 52	233 ± 51
	8-24	174 ± 53	147 ± 28
Paracetamol Mercapturate	0-2	5.9 ± 2.4	5.3 ± 2.1
	2-4	14.3 ± 4.3	13.5 ± 4.0
	4-8	18.4 ± 5.1	17.5 ± 5.0
	8-24	16.0 ± 4.4	15.1 ± 4.0
Paracetamol Cysteine	0-2	4.8 ± 1.5	4.1 ± 1.2
	2-4	10.4 ± 2.4	9.6 ± 2.6
	4-8	17.2 ± 5.8	17.5 ± 6.6
	8-24	14.5 ± 8.2	17.1 ± 7.0
Merc. and Cysteine conjugates combined	0-2	10.7 ± 3.6	9.4 ± 3.2
	2-4	24.7 ± 6.0	23.1 ± 6.2
	4-8	35.6 ± 9.4	35.0 ± 10.6
	8-24	30.5 ± 11.0	32.2 ± 9.4

* $p < 0.05$

TABLE 31

Percentage of dose recovered in 24 hour urine collections as Paracetamol (P) and its Sulphate (S), Glucuronide (G), Mercapturic acid (M) and Cysteine (C) conjugates following a 20 mg/kg oral dose of Paracetamol with N-acetylcysteine in 8 healthy subjects

Subject	Percentage of dose recovered as:-						
	P	S	G	M	C	M+C	Total
PA	4.9	42.0	34.9	4.6	3.6	8.2	90.0
AB	4.4	26.8	57.6	3.0	3.0	6.0	94.8
JF	5.2	32.7	46.4	2.9	4.9	7.8	92.2
IK	5.3	34.7	37.2	4.9	3.6	8.4	85.6
AP	1.9	14.9	57.3	5.1	4.8	9.9	84.1
JP	5.9	28.6	52.6	3.6	3.2	6.8	93.8
LP	2.8	19.4	67.4	3.8	3.0	6.8	96.4
HR	5.4	37.9	43.7	2.4	2.1	4.4	91.4
Mean ±S.D.	4.5 ±1.4	29.6 ±9.2	49.7 ± 11.1	3.8 ±1.0	3.5 ±0.9	7.3 ±1.7	91.0 ±4.3

with NAC were very similar to those obtained in each subject following paracetamol alone.

The apparent relationship between sulphate and glucuronide conjugate excretion noted after ingestion of paracetamol alone was maintained after paracetamol with NAC, although the inverse correlation was slightly weaker. In terms of amounts excreted as each conjugate, $r = -0.72$ ($p < .05$) and in terms of per cent of the dose excreted, $r = -0.88$ ($p < .005$).

3) Summary of Results

1) Absorption of an oral dose of paracetamol was rapid but may have been delayed by NAC. After paracetamol alone the mean peak paracetamol concentration was 19.7 µg/ml and occurred 0.66hr after ingestion (mean of 8 subjects). In 3 subjects the mean peak paracetamol concentration was reduced from 21.0 to 18.9 µg/ml and was delayed from 0.42 to 0.83 hr after co-administration of NAC. Absorption was essentially complete as judged by the mean 24 hour urinary recovery of 92.7% of the ingested dose (91.0% with NAC)as paracetamol and its major conjugates.

2) Paracetamol was eliminated almost entirely by metabolism since only 1.9 - 6.3% of the dose was recovered unchanged in the urine in 24 hours. The mean plasma half-life was 2.30 ± 0.41 hours. The urinary half-life was more variable but not significantly different from the plasma value (2.86 ± 0.91 hr).

3) The sulphate and glucuronide conjugates were formed rapidly and both were present in the plasma 15 minutes after paracetamol ingestion. Plasma concentrations of paracetamol sulphate reached peak values of 4.2 - 8.1 µg/ml usually within 1 hour after those of the parent drug. Plasma paracetamol glucuronide concentrations increased slowly to maxima of 7.1 – 23.7 µg/ml 1.5 - 4 hours after paracetamol ingestion, usually exceeding the

parent drug concentrations.

4) The mercapturic acid and cysteine conjugates were not measurable in plasma following a 20 mg/kg oral dose of paracetamol. The limit of detection for both conjugates was below 1 μg/ml.

5) NAC (total dose 150 mg/kg) given with paracetamol did not significantly affect plasma concentrations of parent drug or its metabolites, although paracetamol concentrations were lowered during the first hour.

6) The sulphate and glucuronide conjugates were rapidly excreted in the urine. Sulphate conjugation accounted for a mean of 47.6% of the total excreted during the first hour but means of only 29 - 34% after the second hour. In contrast, excretion of the glucuronide conjugate increased from a mean of 38.6% of the total in the first hour to means of 53 - 58% after the second hour. In 24 hours, individual totals of 15 - 43 and 35 - 69 per cent of the dose were recovered in urine as the sulphate and glucuronide conjugates respectively.

7) Urinary excretion of the mercapturic acid and cysteine conjugates was initially very low and always accounted for a minor portion of the total drug excreted (maximum 10.6% combined). The 24 hour urinary recoveries of these conjugates were 2.4 - 5.6 and 2.0 - 5.1 per cent of the dose respectively.

8) NAC given with paracetamol had little effect on the metabolism and urinary elimination of paracetamol.

9) Individual variability in the metabolism of paracetamol was wide but reproducible.

10) The renal clearance of paracetamol (mean value 14.1 ml/min) was dependent on urine flow rate, suggesting tubular reabsorption in the kidney. In contrast, the renal clearance of the sulphate and glucuronide conjugates (mean values 167 and 135 ml/min respectively) were consistent with active tubular secretion. Urine pH did not affect the renal excretion of any of these compounds. There was no limitation of renal clearance at the higher plasma concentrations of each compound following the 20 mg/kg paracetamol dose.

Chapter 4 : Discussion and Summary

(a) Paracetamol

The present study indicates that the absorption and elimination of paracetamol in man are rapid and essentially complete following a single oral dose of 20 mg/kg. The mean peak plasma concentration of parent drug, which occurred at 0.66 hours, was high relative to those of the metabolites and urinary excretion of the unchanged drug and its sulphate, glucuronide, mercapturic acid and cysteine conjugates accounted for a mean of 92.7% of the dose in 24 hours. The minor metabolites, conjugated dihydroxy derivatives reported by Mrochek et al (1974) and Andrews et al (1976) and the thiomethyl derivative reported by Klutch et al (1978) probably contribute to the fraction of the dose not accounted for in the present study. Paracetamol and its metabolites are unlikely to be excreted to any extent by other routes.

Absorption of paracetamol after oral dosage occurs mostly after gastric emptying into the duodenum (Heading et al, 1973) and this is followed by passage through the liver and then to the systemic circulation. Significant metabolism is unlikely to occur in the gastro-intestinal tract. Josting et al (1976) observed only about 4% conjugation of paracetamol in rat intestine and gut mucosal cell preparations after 30 minutes incubation,

by which time most of a dose would be absorbed. However, significant "first pass" metabolism of paracetamol occurs, presumably mostly in the liver (Rawlins et al, 1977; Perucca and Richens, 1979).

The rate of paracetamol metabolism is affected by its rate of delivery to the liver. In the present study, the mean proportion of unchanged drug relative to total drug excreted in urine fell from 11.1 ± 4.3% in the first hour, when drug delivery rates were relatively high, to 5.7 ± 2.0% in the third hour after ingestion, by which time absorption was complete. The data of Heading et al (1973) show that more than double the proportion of unchanged to conjugated drug was excreted in 4 hour urine collections from subjects with normal gastric emptying rates (and hence drug delivery rates) compared to those with slow emptying.

This effect is also reflected in studies on the proportion of the dose reaching the systemic circulation (ie the bioavailability) after initial passage through the liver. Rawlins et al (1977) found that the bioavailability of a 0.5g oral dose of paracetamol was considerably lower than that of 1g and 2g doses, indicating that drug reaching the liver in low concentrations from small doses is more efficiently removed than in higher concentrations. Their observation that this difference was not maintained beyond a 1g dose suggests that this limitation of the ability of the liver to

eliminate paracetamol is probably transient and related to the short period of peak drug delivery.

In the present study, a mean of 89% of the total drug recovered in the first hour was in the form of metabolites, almost all as the sulphate and glucuronide conjugates. Sulphate conjugation appears to be particularly important early after paracetamol ingestion. Mean plasma concentrations of paracetamol sulphate were higher than those of the glucuronide during the first 30 minutes or so and the sulphate conjugate accounted for a relatively high proportion of total drug excreted during the first two hours. It seems likely, therefore, that this route is primarily responsible for the first pass metabolism of paracetamol. If glucuronidation, the other major route for paracetamol elimination, contributes significantly, low first pass metabolism would be expected in subjects with low glucuronide-conjugating ability (Gilbert's Syndrome). This was not found by Douglas et al (1978) who noted that the first pass metabolism of paracetamol was unimpaired in such subjects.

Elimination by glucuronidation rapidly becomes more prominent once paracetamol absorption is complete. Mean plasma concentrations of paracetamol glucuronide continued to rise for about an hour after those of the sulphate conjugate had begun to fall. Also, the mean peak concentration of the glucuronide (12.8 $\pm$ 5.5

μg/ml) was twice as high as that of the sulphate conjugate (6.3 ± 1.2 μg/ml). These differences were not due to renal clearance (which was similar for both compounds) and were reflected in the urinary excretion pattern.

Other workers have suggested that sulphate conjugation of paracetamol in man is easily saturated, even at therapeutic doses (Levy and Yamada, 1971; Davis et al, 1976). Weitering et al (1979) observed decreasing sulphation and increasing glucuronidation with increasing doses of phenol in rats and concluded that different relative affinities of the two enzyme systems for the substrate rather than limited availability of sulphate accounted for this.

A minor but significant proportion of the dose (mean 7.4 ± 1.7%) was excreted as the mercapturic acid and cysteine conjugates. Neither conjugate was measurable in plasma (with a limit of detection of less than 1 μg/ml) following the 20 mg/kg dose and their urinary excretion rates increased slowly relative to those of the other metabolites. This may be explained by the findings with rat cell preparations that these compounds are formed in liver and kidney cells from the glutathione conjugate. The glutathione conjugate is hydrolysed to the cysteine conjugate, some of which is excreted as such and some is acetylated to paracetamol mercapturate (Moldeus et al, 1978). It seems, therefore, that the rate of elimination of the mercapturic acid and cysteine

conjugates depends on the rate of formation of glutathione conjugate. From 3 to 24 hours, after absorption and first pass effects were complete, these conjugates combined accounted for a fairly constant 8.5 - 10.6% (mean values, Table 22) of the total drug excreted in urine.

The change in the pattern of urinary excretion of paracetamol with time was similar in all the subjects but the differences in metabolism and excretion by each conjugation pathway were considerable. The variation in the urinary recovery of the sulphate and glucuronide conjugates was each about two fold and the individual recoveries of these compounds were inversely related. In addition, the plasma paracetamol half-life was negatively and positively correlated with the urinary recoveries of the glucuronide and sulphate conjugates respectively. These relationships imply that paracetamol elimination is primarily dependent on glucuronide conjugation and that in subjects with relatively low glucuronidating activity more dependence is placed on sulphate conjugation. The variation in the combined urinary recovery of the mercapturic acid and cysteine conjugates was again about two fold but this did not appear to be related to the elimination of the other compounds or the paracetamol half-life at the 20 mg/kg dose level.

In the present study, a mean of less than 5% of the dose was recovered unchanged in the urine in 24 hours, indicating that the elimination of paracetamol depends almost entirely on metabolism. However, elimination was rapid as judged by its mean plasma elimination half-life, regardless of individual metabolic variation. The mean plasma half-life of paracetamol (determined during the first 8 hours after ingestion) was 2.30 $\pm$ 0.41 hours. This was considerably less than the values of 3.67 $\pm$ 0.90 and 3.19 $\pm$ 0.51 hours for the sulphate and glucuronide conjugates respectively. A mean of 69.6% of the dose was excreted in urine in the first 8 hours and 92.7% in 24 hours. This indicates that long-term distribution of paracetamol into poorly-perfused tissues is negligible after a single oral dose and that paracetamol may be distributed into short-term, well-perfused tissue sites (eg liver and kidney) during the first 8 hours. This is followed by fairly rapid release and elimination from these sites subsequently. Gwilt et al (1963) showed in dogs that paracetamol distributes fairly evenly into most tissues except fat. Duggin and Mudge (1976) produced evidence for renal concentration of paracetamol using dog kidneys and Douglas et al (1978) discussed the possibility that the liver may act as a storage compartment via the glucuronyl transferase system.

(b) Paracetamol with NAC:

NAC produced little effect on the overall elimination

of paracetamol at the doses given (20 mg/kg paracetamol and 150 mg/kg NAC). The areas under the plasma concentration-time curves of paracetamol and its sulphate and glucuronide conjugates in 3 subjects were not altered after NAC. Also, the 24 hour urinary excretion of the unchanged drug and its conjugates was almost unaltered in all 8 subjects. Peak plasma paracetamol concentrations were lower and later in 3 subjects, possibly indicating a delaying effect on paracetamol absorption due to nausea and delayed gastric emptying produced by the large dose of NAC. This is supported by the fact that the mean urinary recovery of paracetamol and its metabolites was lower in these 3 subjects during the first hour than after paracetamol alone. However, neither of these findings was statistically significant.

It is also possible that NAC might affect the metabolism of paracetamol. There was a decrease in excretion of unchanged drug during the first hour followed by a transient increase in the urinary output of paracetamol sulphate in the second hour. This may indicate increased first pass sulphate conjugation. Sulphate conjugation depends on the availability of sulphate and drugs such as salicylamide competitively inhibit paracetamol sulphate formation (Levy and Yamada, 1971). This inhibition can be reversed by cysteine which presumably acts as a precursor for sulphate conjugation. It might therefore be expected that NAC

would act similarly and enhance paracetamol sulphate production, thus increasing first pass paracetamol metabolism. However, the differences described above may also be explained by the probability that sulphate conjugation is the most easily limited metabolic route early after ingestion. The lower rate of delivery of paracetamol with NAC may allow a greater proportion to be metabolised by sulphation, regardless of extra availability of sulphate. Small increases in the mean proportion of the drug excreted as paracetamol sulphate were observed in the first and second hours in the three subjects studied in detail, but the differences were not significant. Taking all 8 subjects, the increase was minimal.

It is also possible that NAC might have increased the formation of the mercapturic acid and cysteine conjugates, either directly (Chasseaud, 1974) or via glutathione (Strubelt et al, 1974). There was no evidence of this in the urinary excretion studies. However, sulphate conjugation is saturated with doses of paracetamol higher than 20 mg/kg (Levy and Yamada, 1971; Davis et al, 1976). NAC may have a significant effect on paracetamol metabolism in such circumstances.

(c) Renal Clearance of Paracetamol and its Conjugates:

The very low mean value for the renal clearance of paracetamol indicates extensive renal tubular reabsorption.

The likelihood of this is confirmed by the dependence of its renal clearance on urine flow rate. The low renal clearance of paracetamol contrasts with the values obtained for the sulphate and glucuronide conjugates. The mean values were higher than the normal glomerular filtration rate in each case, suggesting active tubular secretion. Also, there was no correlation between renal clearance and urine flow rate with the conjugates. These compounds are highly hydrophilic and, together with their active secretion, are presumably not significantly reabsorbed at low urine flow rates.

The lack of effect of the urine pH on the renal clearance of all 3 compounds is not surprising. Paracetamol is only weakly acidic (pKa = 9.5) and the two conjugates are both hydrophilic and therefore the water/lipid partition characteristics of all 3 compounds are hardly altered over the range of physiological pH. The renal clearances of all 3 compounds did not change significantly with time, implying no effect of plasma concentration on their renal clearance after a 20 mg/kg dose of paracetamol.

The absence of measurable concentrations of the mercapturic acid and cysteine conjugates in plasma has already been discussed (p163). This fact and the fact that the mean urinary excretion rates of these compounds were similar to that of paracetamol after about 3 hours suggest that their renal clearance is high

and that they are actively secreted in a similar manner to the sulphate and glucuronide conjugates.

Overall, changes in renal clearance have no discernable influence on paracetamol elimination after therapeutic doses. Only the renal clearance of unchanged drug could be influenced (by urine flow rate) and this only accounts for a few per cent of the dose removed via the kidneys.

The renal clearance of paracetamol and its sulphate and glucuronide conjugates has been studied in dogs (Duggin and Mudge, 1975, 1976) and in patients with paracetamol overdosage (Prescott and Wright, 1973). Their findings were similar to those of the present study with regard to the relationships between urine flow rate and pH and the renal clearance of the 3 compounds. Duggin and Mudge (1975) found that the renal clearance of the conjugates was reduced at high plasma concentrations in dogs, suggesting saturation of the renal tubular transport system. The plasma concentrations of the sulphate and glucuronide conjugates ranged from 3 to 26 and 15 to 90 μg/ml respectively in their study, about 3 times higher than those encountered in the present study. However, the renal clearance values for these conjugates combined reported by Prescott and Wright (1973) in patients with paracetamol poisoning were very similar to those obtained in the present study for the separate conjugates. This suggests that saturation

of the renal tubular transport of the sulphate and glucuronide conjugates is unlikely to occur in man. The renal clearance of these conjugates and paracetamol at high plasma concentrations is discussed further in the following section on paracetamol overdosage.

(d) Other Factors Influencing Paracetamol Metabolism:

The present study throws little light on the influence of many factors which affect paracetamol metabolism. A notable finding in comparing paracetamol metabolism with and without NAC was the consistent metabolic pattern in each subject contrasting with great inter-subject variation. The extent of individual variation in the 24 hour urinary recovery of each compound was very similar, as was the relationship between the percentage of the dose recovered as the glucuronide and sulphate conjugates in each individual. This consistency in the wide variation between individuals has also been noted by other workers (Levy and Yamada, 1971). The explanation for such wide but consistent differences in paracetamol metabolism in normal subjects of similar age and weight is not clear but is presumably related to environmental and genetic factors.

Age appears to influence paracetamol metabolism considerably. In young children and neonates sulphate conjugation plays the major role in eliminating paracetamol whereas glucuronide formation appears to be limited

compared with young adults (Levy et al, 1975; Miller et al, 1976). The same workers found that the mean urinary recovery of paracetamol and its sulphate and glucuronide conjugates was relatively low in neonates (63 and 70% of the dose respectively) and tended to increase with older children. The urinary elimination half-life tended to be high (mean values of 3.5 hr reported by Levy et al, 1975, and 4.9 hr by Miller et al, 1976) indicating that overall metabolism of paracetamol may be slow in the very young, although slow gastric emptying in such subjects may partially explain this finding.

Studies in elderly subjects (mean age above 65 years) have revealed marginally slower paracetamol elimination relative to young adults (age range 20 - 40 years; Briant et al, 1976). There was very wide individual variation in both subject groups and the plasma half-life values overlapped considerably (0.95 - 2.79 hours in young adults and 1.43 - 3.45 hours in the elderly). No studies on paracetamol metabolite excretion in the elderly have been reported.

Sex has little effect on paracetamol elimination. No differences between the sexes were noted in the urinary elimination of paracetamol in children and young adults after a 10 mg/kg oral dose (Miller et al, 1976). Also, in young adults and elderly subjects, the plasma half-life of paracetamol was no different follow-

ing a 1g dose orally (Briant et al, 1976). These workers noted that the apparent volume of distribution of paracetamol was slightly lower in females but not significantly so. In a more closely observed comparison following 1g paracetamol orally (Wojcicki et al, 1979), the apparent volume of distribution was significantly lower in 19- 21 year old women, particularly in the luteal phase of the menstrual cycle, compared to men of similar age and weight. Paracetamol metabolism appeared to be slower in women, particularly in the luteal phase. However, the differences in the plasma paracetamol half-life were not significant either with respect to sex or the stage of the menstrual cycle.

Alteration of paracetamol elimination by agents which induce metabolic enzyme activity has been reported. Mitchell et al (1974) studied paracetamol elimination in healthy subjects before and after phenobarbital pretreatment and noted that this pretreatment produced an increase in the amount of paracetamol mercapturate excreted after a 1.2g dose. Perucca and Richens (1979) found that metabolically-induced patients eliminated a significantly higher proportion of a 1g oral dose by first pass metabolism than normal subjects. The overall urinary elimination of unchanged paracetamol and its sulphate and glucuronide conjugates was no different and the plasma half life was shorter but not significantly so in these induced patients. Mitchell et

al (1974) found no increase in the overall paracetamol elimination rate following enzyme induction in normal subjects.

Metabolism of paracetamol is dose-dependent. Mitchell et al (1974) noted an increase in the proportion of the dose excreted as paracetamol mercapturate following 0.9, 1.2 and 1.8g doses of paracetamol in healthy subjects. Davis et al (1976) found similar results with doses of 1 - 4g and also noted limited paracetamol sulphate excretion at the higher doses. Differences in the affinities and capacities of the different conjugation pathways and their relationship with the cytochrome P-450-dependent oxidation of paracetamol probably account for this dose-dependent metabolism.

Once sulphate conjugation is limited, a larger proportion of the remaining drug appears to be conjugated via glucuronidation and oxidised via cytochrome P-450-dependent metabolism. This results in larger proportions being excreted as the glucuronide and the mercapturic acid and cysteine conjugates respectively. These two pathways do not appear to become saturated at paracetamol doses up to 4g since the 24 hour urinary recovery of the drug is not decreased at the higher doses (Davis et al, 1976). The effect of very large doses of paracetamol on its metabolism is discussed in the following section on overdosage.

Comparisons with Previous Studies in Normal Subjects

i) Paracetamol Absorption and Elimination: Previous reports on the elimination of paracetamol in normal adults after therapeutic doses are summarised with the present data in Table 32. The mean peak plasma paracetamol concentration and time to reach peak values obtained in the present study were comparable with other studies, taking into account the dosage variation. Similarly, the range and mean values for the plasma and urinary paracetamol half-life in the present study agree well with other reports. After oral doses of 0.5 - 2g in fasting subjects, the mean paracetamol half-life ranged from 2.0 - 2.9 hours in plasma (Prescott et al, 1968; McGilveray and Mattok, 1972; Richter and Smith, 1974; Rawlins et al, 1977; Perucca and Richens, 1979). This compares with the value of 2.3 hours obtained in the present study. The values obtained in urine were 2.0 - 3.6 hours (Nelson and Morioka, 1963; Cummings et al, 1967; Levy and Regårdh, 1971; McGilveray and Mattok, 1972; Miller et al, 1976) compared with 2.9 hours in the present study. Similar results were obtained after intravenous paracetamol (Rawlins et al, 1977; Perucca and Richens, 1979).

ii) Elimination of Paracetamol Metabolites

There have been no previous detailed investigations of plasma concentrations of paracetamol sulphate and

TABLE 32

Comparison of estimations of Paracetamol elimination half-life ($t_{\frac{1}{2}}$) and peak plasma Paracetamol concentrations (C_{max}) in normal subjects.

Authors	Dose and Route	No. of Subjects	$t_{\frac{1}{2}}$ (hr)	measured in:-	C_{max} (µg/mℓ)
Nelson & Morioka (1963)	1g oral	5 (N=9)	1.6-2.8	Urine	-
Cummings et al (1967)	12 mg/kg oral	4	2.0-2.6	Urine	-
Prescott et al (1968)	1.8g oral	8	1.3-3.0	Plasma	10-52 (1-2hr)
Levy & Regårdh (1971)	1g oral	5	1.6-2.8	Urine	-
McGilveray & Mattok (1972)	1g oral	4	2.0-2.7 2.7-3.1	Plasma Urine	19-28 (0.33hr)
Richter & Smith (1974)	2g oral	8x6 (N=48)	1.8-2.6	Plasma	18-58 (0.5hr)
Miller et al (1976)	10 mg/kg oral	4	3.6±0.1 (S.E.)	Urine	-
Rawlins et al (1977)	0.5,1,2g oral 1g IV.	6 each dose 6	means 2.8, 2.7, 2.3 2.2-3.3	Plasma Plasma	- -
Perucca & Richens (1979)	1g IV.	6	2.5±0.25 (S.E.)	Plasma	-
Present Study	20 mg/kg oral	8	1.5-2.8 2.1-4.9	Plasma Urine	15-23 (0.25-1.0hr)

glucuronide in normal subjects, although their combined measurement has been reported (Brodie and Axelrod, 1948a, 1949; Lowenthal et al 1976). As noted in the present study, Lowenthal et al (1976) found that the combined plasma concentrations of these conjugates became greater than that of the parent drug soon after ingestion and that their combined elimination rate was similar to that of paracetamol. The present study also showed that the glucuronide usually accounted for most of the conjugates present in plasma. Mrochek et al (1974) reported plasma concentrations of paracetamol glucuronide similar to and paracetamol sulphate somewhat lower than those observed in the present study. These workers also noted the absence of the cysteine conjugate in plasma and paracetamol mercapturate was not reported in plasma or urine.

The urinary excretion rates of paracetamol and its sulphate and glucuronide conjugates measured by Cummings et al (1967) and Levy and Yamada (1971) were comparable to those measured in the present study. The data of Cummings et al (1967) suggested that the urinary elimination half-lives of the sulphate and glucuronide conjugates were similar to that of the unchanged drug. In the present study, metabolite urinary half-lives were usually longer than that of paracetamol, as was also observed by Miller et al (1976) and suggested by the data of Levy and Yamada (1971). Levy and Yamada (1971)

also noted a plateau effect in paracetamol sulphate excretion which was not seen in any of the subjects in the present study, although similar doses were administered.

iii) Recovery of Paracetamol and its Conjugates in Urine

Studies concerning the recovery of paracetamol and its conjugates in urine have been reported by many authors. A representative selection is summarised in Table 33, together with data from the present study. Paracetamol dosage varied from 0.65g to 4g but the percentage of dose recovered as the unchanged drug and its sulphate and glucuronide conjugates was fairly consistent between studies. Fewer workers have measured the cysteine and mercapturic acid conjugates and there is much greater variability in recovery. In most reports less than 6% of the dose was recovered as each of these 2 compounds and their combined total was 10% or less.

There have been occasional notable exceptions to the general range of values obtained. Davis et al (1976) reported very low recovery of parent drug and very high combined recovery of the mercapturic acid and cysteine conjugates in 3 subjects taking 1 - 4g of paracetamol orally. Their analytical method included TLC and quantitation by densitometry, an approach which was found to be unworkable in this laboratory (Howie, 1977). Most studies, including the present one, report mean recoveries

TABLE 33

Estimations of 24 hour recovery of Paracetamol and its metabolites in urine after oral Paracetamol ingestion in normal subjects (values are % of dose).

AUTHORS	Subjects and dose	Parac.	Conjugate					TOTAL
			Sulph.	Gluc.	Merc.	Cyst.	Other	
Cummings et al (1967)	4; 12mg/kg	3.0-4.1	22-26	42-59	-	-	-	69-89*
Jagenburg et al(1968)	3; 1.5-2g	3.0-4.5	18-34	31-59	4.5-6.1	0.4-5.9	-	82-91
Levy & Yamada (1971)	3; 1 or 2g	3.1-3.7	20-31	58-65	-	-	-	86-98
Mrochek et al (1974)	2; 23mg/kg	1.5	29	59	-	approx. 1	four dihydroxy derivs.(1-5% each)	101
Mitchell et al (1974)	2 and 10 0.9-1.8g	1.9	47	38	approx.4 (12 subjs.)	trace	-	90
Thomas et al (1975)	5;0.65g	6.8	28	63	1.7	1.9	-	100**
Davis et al (1976)	3;1-4g	0-2	20-30	45-55	15-25 (combined)		-	85-92
Andrews et al (1976)	?; 2g	approx. 80 (combined)			approx. 5-10 (combined)		Catechol derivs. (5-10% combined)	-
Present study	8;20mg/kg do.+ NAC	3.1-6.3 1.9-5.9	19-43 15-42	35-69 35-67	2.5-5.6 2.4-5.1	2.0-5.1 2.1-4.9	- -	87-98 84-96

* Extrapolated from 0-15 hour collection.

** Recovery complete by 12 hours.

of the glucuronide to be considerably higher than those of the sulphate conjugate. However, Mitchell et al (1974) used ^{3}H-labelled paracetamol in 2 subjects and recovered a mean of 47% and 38% of a 1.2g dose in 24 hours as the sulphate and glucuronide conjugates respectively. Quantitation relied on lyophilisation, elution and TLC prior to scintillation counting of the separated compounds. Recovery of the sulphate exceeded that of the glucuronide conjugate in only 1 out of 3 subjects studied by Jagenburg et al (1968) and 1 out of 8 in the present study.

Overall, the 24 hour urinary recoveries were 70 - 100%, although in one study in which only the combined sulphate and glucuronide conjugates and parent drug were measured (Nelson and Morioka, 1963), 92 - 113% was recovered. An average of 77% was recovered from 4 subjects as paracetamol and its sulphate and glucuronide conjugates by Cummings et al (1967) following a mean dose of 0.82g whereas Thomas et al (1975) recovered 98% of a 0.65g dose as these compounds in 12 hours with 5 subjects.

Summary

The absorption, metabolism and elimination of paracetamol was studied in 8 healthy male subjects following a 20 mg/kg oral dose of paracetamol with and without N-acetylcysteine (NAC; dose 150 mg/kg). Paracetamol absorption was rapid and essentially complete but may have been delayed with NAC. Metabolism was also rapid and almost complete, showing wide but reproducible individual variation. Metabolism via sulphate conjugation was important in the first 1 - 2 hours after ingestion but the overall order of importance was glucuronidation > sulphation > glutathione conjugation (as mercapturic acid and cysteine conjugates). Only a few per cent of the dose was excreted unchanged. The mercapturic acid and cysteine conjugates were not measurable in plasma (with a limit of less than 1.0 μg/ml). The overall effect of NAC on paracetamol metabolism at the above doses was minimal.

The plasma paracetamol half-life (mean value 2.3 hrs) appeared to be negatively and positively correlated with the urinary excretion of the glucuronide and sulphate conjugates respectively. The renal clearance of all the conjugates was rapid whereas the unchanged drug appeared to be extensively reabsorbed in the kidney.

The results obtained in the present study agree well with previous reports on paracetamol metabolism in normal subjects.

SECTION V

PARACETAMOL METABOLISM IN OVERDOSAGE

PARACETAMOL METABOLISM IN OVERDOSAGE

Chapter 1: Introduction

The effect of paracetamol taken in overdosage is a clinical problem which has been studied extensively in recent years. Early reports describing paracetamol overdosage (Davidson and Eastham, 1966; Thomson and Prescott, 1966; MacLean et al, 1968) recognised the danger of hepatotoxicity. However, effective ways of preventing liver damage have since been developed using various sulphydryl compounds (Prescott et al, 1974, 1976, 1977, 1979; Crome et al, 1976; Peterson and Rumack, 1977).

Paracetamol is probably absorbed rapidly and completely (Heading et al, 1973), even in overdosage (Prescott et al, 1971), although factors such as the drug formulation, and the presence of food and other drugs may affect the rate of absorption (Richter and Smith, 1974; McGilveray and Mattok, 1972; Nimmo et al 1973a, 1975). There is probably little likelihood of significant metabolism in the gut (Josting et al, 1976) and kidney (Jones et al, 1979) therefore the rate of delivery of paracetamol to its major site of metabolism and toxicity, the liver, is likely to be dose related.

In the previous section (pp 134 - 137) paracetamol metabolism was observed to be related to the time after ingestion, with sulphate conjugation showing signs of limitation following the high initial rate of drug

delivery. Saturation of sulphate conjugation was observed even with therapeutic doses of paracetamol (Levy and Yamada, 1971; Davis et al, 1976) and was more notable with larger doses (Davis et al, 1976; Howie et al 1977). This saturation is probably related to the depletion of inorganic sulphate (Houston and Levy, 1976; Galinsky et al, 1979) and since this appears to occur readily, the relationship between paracetamol sulphate formation and the time after ingestion is likely to be important in overdosage. There is also indirect evidence that the major pathway for paracetamol elimination, via glucuronidation, may also be saturable (Levy and Yamada, 1971). This has been demonstrated in isolated rat hepatocytes (Moldeus, 1978) and has been reported to occur with overdoses in man (Davis et al, 1976; Slattery and Levy, 1979), although this may be a result of toxicity (Prescott and Wright, 1973).

The mechanism whereby paracetamol causes liver and renal toxicity and the nature of the intermediate metabolite involved have already been discussed (pp 17 - 24). The potentially toxic pathway is active at all dose levels but with therapeutic doses the toxic metabolite is inactivated by conjugation with reduced glutathione (Mitchell et al, 1974). The glutathione conjugate is eventually excreted in the urine as the cysteine and mercapturic acid conjugates (Moldeus et al, 1978). Paracetamol overdosage results in a shift in metabolsm via this pathway, with an increase

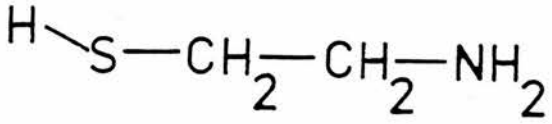
in the proportions of drug excreted as the cysteine and mercapturic acid conjugates (Davis et al, 1976; Howie et al, 1977). This shift in metabolism is related to the extent of liver damage sustained and probably reflects the increased conversion of paracetamol to the toxic intermediate. Renal toxicity occurs in a similar manner to hepatotoxicity (Mitchell et al, 1977; Healey et al, 1978).

Other metabolites of paracetamol (Mrochek et al, 1974; Andrews et al, 1976; Knox and Jurand, 1977, 1978; Klutch et al, 1978), which account for a minor proportion of therapeutic doses, are an unknown quantity in overdosage. However, their contribution to the overall elimination of paracetamol is likely to remain small. (Andrews et al, 1976).

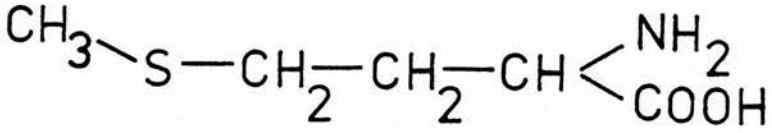
Other factors have been shown to affect paracetamol elimination at therapeutic doses. Age has little effect beyond childhood and there are only minor sex differences (Miller et al, 1976; Levy et al, 1975; Briant et al, 1976; Wojcicki et al, 1979). However, it is possible that more marked differences in metabolism related to these factors may occur with overdoses of paracetamol, although there are no detailed reports on paracetamol metabolism after overdosage with respect to age or sex. The ingestion of more than one drug in overdosage is a common occurrence (Matthew, 1971). This may affect paracetamol elimination by competitive metabolism (Levy and Yamada, 1971; Dybing,

1976), by altering its rate of absorption (Nimmo et al, 1973a, 1975) and by enzyme induction (Mitchell et al, 1974; Perucca and Richens, 1979). Such effects may be particularly relevant in overdosage. Wright and Prescott (1973) found that potentially induced patients developed significantly more severe liver damage than non-induced patients following paracetamol overdosage. Impaired renal function does not alter the plasma half-life of paracetamol (Lowenthal et al, 1976) but the reduced renal excretion of paracetamol conjugates results in their accumulation in the plasma (Prescott, 1969; Lowenthal et al, 1976). Thus renal function is important in assessing the metabolic effects of paracetamol overdosage.

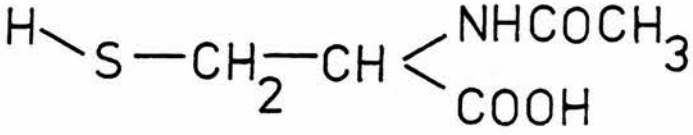
The 3 compounds investigated in the present study were L-methionine, N-acetyl-L-cysteine (NAC) and cysteamine (Fig. 30). The hepatoprotective effect of all 3 compounds following paracetamol overdosage has been demonstrated in animals (see pp 33-35). Methionine probably acts by repleting hepatic glutathione (McLean and Day, 1975; McLean and Nuttall, 1978), cysteamine appears to inhibit the microsomal oxidation of paracetamol to its toxic intermediate (Prescott et al, 1974; Harvey and Levitt, 1976) and NAC may be first converted to cysteine (Chasseaud, 1974) which then probably repletes hepatic glutathione (Strubelt et al, 1974). Recently, Buckpitt et al (1979), using mouse liver microsomal preparations, have shown that NAC can be conjugated directly with the reactive metabolite formed from



Cysteamine



Methionine



N-acetylcysteine

Fig 30: Molecular structures of cysteamine, methionine and N-acetylcysteine.

paracetamol, as can cysteamine to a much lesser extent. Also, high concentrations of cysteamine reduced the rate of paracetamol metabolism. Methionine formed no conjugate directly and did not alter the rate of paracetamol metabolism.

In the present study, the metabolism of paracetamol was investigated in relation to overdosage in patients with and without hepatotoxicity and with and without specific treatment. Serial measurements of paracetamol and its conjugates were made in the plasma and urine of patients admitted to the Edinburgh Regional Poisoning Treatment Centre following paracetamol overdosage. In addition, the renal clearance of paracetamol and its sulphate and glucuronide conjugates was calculated. Varying degrees of liver damage were observed, from absent to mild and severe, and some of the latter died. Comparisons were made using data from untreated patients, patients treated with methionine, cysteamine or NAC and healthy volunteers given a therapeutic dose of paracetamol.

It was hoped that such studies might throw light on the relationship between paracetamol metabolism and toxicity and the mechanism of hepatoprotection of the 3 sulphydryl compounds.

Chapter 2 Patients and Methods

Patients admitted on more than one occassion were considered as separate cases.

Assessment of the Severity of Overdosage

Ingestion of the drug was confirmed by rapid estimation of plasma paracetamol on admission using a spectro-photometric method (Gibson, 1972). Information concerning the time of ingestion and amount claimed to have been ingested was obtained from the patients' records.

b) Treatment

Patients admitted within 4 hours of paracetamol ingestion were normally given gastric aspiration and lavage in order to remove any unabsorbed drug. The need for specific treatment was assessed from the admission plasma paracetamol concentration in relation to the time of ingestion (Prescott et al, 1974, 1978, 1979 ; Fig 5 , p 31). Patients whose admission plasma paracetamol concentrations were above a line joining 200 µg/ml at 4 hrs and 50 µg/ml at 12 hrs on a semilogarithmic graph of plasma paracetamol concentration against time after ingestion were considered to be at risk of developing liver damage (Prescott et al, 1974, 1978, 1979).
All treatments were administered intravenously according to the following regimes.

(i) Cysteamine (as the hydrochloride) was infused in an initial loading dose of 2.0g (free base equivalent)

over 10 minutes, followed by 0.8g over 4 hours and 0.4g in each of two subsequent 8 hour periods. The total dose was 3.6g cysteamine base over 20 hours (Prescott et al, 1974).

(ii) L-Methionine was given in a loading dose of 5g infused over 10 minutes, followed by 5g in the next 4 hrs and 5g in each of two subsequent 8 hour periods (total 20g in 20 hours).

(iii) N-acetyl-L-Cysteine (NAC) was infused in a loading dose of 150 mg/kg over 15 minutes. This was followed by 50 mg/kg during the next 4 hours and 100 mg/kg over the subsequent 16 hours (total 300 mg/kg in 20 hours).

Patients not treated with specific agents (subsequently referred to as "untreated" patients) were given supportive therapy which included intravenous fluids and vitamin K_1 where necessary (Prescott et al, 1971). In subsequent comparisons, patients were divided into those treated within 10 hours of paracetamol ingestion (early-treated) and those treated after 10 hours (late-treated). The efficacy of specific treatment has been shown to diminish markedly when given more than 10 hours after overdosage (Prescott, 1978; Prescott et al, 1979).

c) Clinical Assessment of Liver Damage

Plasma concentrations of aspartate and alanine aminotransferases (AST, ALT) are thought to be reliable indicators of hepatic damage after paracetamol overdosage

(Prescott et al, 1971; Stewart and Simpson, 1973). These enzymes were estimated daily for up to 5 days after admission using standard methods of enzyme reaction rate analysis (Kessler et al, 1975). Normal values were below 40 iu/L. Patients with liver damage were graded as trivial, mild and severe cases according to the maximum AST or ALT values recorded (40 - 99, 100 - 1000 and > 1000 iu/L respectively).

d) Collection of Samples

Blood was usually taken 4 to 8 hourly for at least 24 hours after admission and the plasma stored at -20°c prior to analysis. Urine was collected for 2 - 5 days. The volumes and pH were measured and 15 ml aliquots stored at -20°c prior to analysis.

e) Analytical Methods

The concentration of paracetamol and its sulphate and glucuronide conjugates in plasma and urine and the concentrations of the cysteine and mercapturic acid conjugates in urine were measured as described in Section III. Plasma paracetamol concentrations had been measured by gas liquid chromatography (Prescott, 1971b) in some patients who were admitted before this study was undertaken. Quantities of the paracetamol metabolites are expressed as the parent drug equivalent throughout.

f) Paracetamol Disposition

Various aspects of paracetamol disposition used to compare the metabolism and elimination of paracetamol are described in each appropriate chapter.

g) Statistical Methods

Mean values are given with standard deviations unless stated otherwise. Comparisons were made by using Student's t-test for unpaired or paired data or the χ^2 test.

Chapter 3

Clinical Details of Overdosage Patients

The clinical details relating to patients in this section are shown in Table 34. Unless otherwise stated, renal function was normal as judged by urine output and plasma creatinine and urea concentrations.

The metabolism and elimination of paracetamol was studied in 22 untreated patients and 61 patients given methionine, cysteamine or NAC. Their mean ages were 28 ± 9 and 30 ± 10 respectively. Severe liver damage occurred in 25 of the 83 patients and one (U1) died as a result of fulminant hepatic failure. The ratio of females to males was high in the untreated group and the groups given cysteamine and methionine (15 to 7, 7 to 3 and 12 to 5 respectively) but the disproportion was small in patients given NAC (19 females, 15 males). More than half the patients (46) had taken other drugs either with or regularly before paracetamol overdosage, the most common being heavy consumption of alcohol (28). This was particularly common in the patients given NAC and cysteamine (12 of 34 and 7 of 17 respectively). The ingestion-to-admission and ingestion-to-treatment intervals varied considerably (from 1.8 to 32 and 3.8 to 20.3 hours respectively). The lack of control introduced by these factors together with other factors related to sample collection (discussed later) limited the scope of the present study.

TABLE 34

Clinical details of paracetamol overdosage patients *

Patient and Treatment	Age and Sex	Other Drugs Taken **	Ingestion - Admission Interval (hour)	Ingestion - Treatment Interval (hour)	Max. AST/ALT (iu/L)	Plasma Paracetamol conc. (µg/mℓ) Admission	4 hour	Plasma Paracetamol half-life (hour)	Total Urinary Paracetamol (g)
Untreated									
U1 †	54F	I	4.0	-	3840	627	627	10.7	21.7
U2	30F	-	3.3	-	23	269	208	2.5	11.6
U3	25M	I	5.5	-	6000	189	245	3.7	17.0
U4	20F	-	3.9	-	49	301	287	2.5	15.8
U5	40F	-	2.5	-	684	354	290	4.5	13.1
U6	34F	ID	10.0	-	62	69	295	2.3	10.3
U7	28M	D	7.2	-	9940	153	213	3.6	13.3
U8	18F	-	6.1	-	19	144	241	3.0	9.8
U9	24F	-	2.5	-	576	200	144	3.0	8.8
U10	29M	-	3.5	-	18	195	176	3.6	8.9
U11	32F	-	3.5	-	23	123	103	2.3	9.1
U12	19F	-	3.5	-	5840	145	128	4.1	8.1
U13	34F	D	4.0	-	33	142	142	2.4	5.8
U14	24M	I	17	-	9120	146	461	7.9	8.6
U15	27M	-	32	-	> 4000	26	587	6.2	6.9
U16	24M	I	18	-	4800	149	420	9.4	9.4
U17	16F	-	2.8	-	23	246	200	4.2	-

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TABLE 34(continued)

Clinical details of paracetamol overdosage patients *

Patient and Treatment	Age and Sex	Other Drugs Taken **	Ingestion - Admission Interval (hour)	Ingestion - Treatment Interval (hour)	Max. AST/ALT (iu/L)	Plasma Paracetamol (μg/mℓ) conc. Admission	Plasma Paracetamol (μg/mℓ) 4 hour	Plasma Paracetamol half-life (hour)	Total Urinary Paracetamol (g)
Untreated									
U18	36F	B	7.2	-	18	104	220	3.2	-
U19	22F	-	3.5	-	15	201	194	2.5	-
U20	20F	M	3.5	-	20	192	185	4.2	-
U21	36F	BI	13	-	>1100	68	168	6.9	-
U22	24M	I	12	-	>1000	153	378	6.3	-
Methionine									
M1	25M	-	2.0	8.0	21	289	229	2.8	15.1
M2	17F	-	5.0	9.0	78	171	288	2.3	14.2
M3	18F	-	3.0	5.7	70	359	311	3.3	16.0
M4	32F	-	3.3	7.5	13	309	292	2.3	13.0
M5	24M	I	4.5	6.5	43	218	227	2.8	13.6
M6	35F	I	5.6	8.8	9200	298	357	9.2	17.7
M7	27F	-	6.0	9.5	3200	181	252	4.5	11.4
M8	20F	-	4.0	4.0	61	206	206	2.9	14.7
M9	26M	-	2.0	6.3	161	550	464	6.8	17.6
M10	19F	-	8.8	10.0	12000	186	353	5.9	12.1

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TABLE 34 (continued)

Clinical details of paracetamol overdosage patients *

Patient and Treatment	Age and Sex	Other Drugs Taken **	Ingestion - Admission Interval (hour)	Ingestion - Treatment Interval (hour)	Max. AST/ALT (iu/L)	Plasma Paracetamol conc. (μg/mℓ) Admission	Plasma Paracetamol conc. (μg/mℓ) 4 hour	Plasma Paracetamol half-life (hour)	Total Urinary Paracetamol (g)
Cysteamine									
C1	27F	-	7.0	8.5	16	189	339	3.4	21.0
C2	21F	DB	3.8	3.8	23	274	259	3.5	9.6
C3	45F	I	2.5	6.2	602	330	301	3.1	15.0
C4	35F	I	2.3	5.8	153	444	339	2.3	13.5
C5	25F	I	1.8	6.3	960	537	375	2.7	21.6
C6	41F	-	5.5	6.8	22	166	323	1.6	11.9
C7	27F	BM	3.8	7.8	19	284	275	3.4	13.0
C8	38M	I	8.3	10.0	66	144	354	3.3	20.6
C9	24F	-	3.5	5.7	29	244	230	3.5	13.5
C10	24M	I	6.5	7.5	91	258	356	5.0	19.9
C11	28F	I	4.7	6.3	40	198	205	3.1	7.8
C12	27F	-	3.8	3.8	17	470	455	3.6	-
C13	24M	I	13.0	13.3	> 6400	190	314	10.7	-
C14	36F	B	17.0	19.5	6240	89	252	8.3	-
C15	39M	I	12.0	13.0	6960	132	360	5.3	-
C16	20M	-	2.0	11.5	1900	276	214	5.5	-
C17	45F	-	3.7	5.7	231	312	310	3.7	-

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TABLE 34 (continued)

Clinical details of paracetamol overdosage patients. *

Patient and Treatment	Age and Sex	Other Drugs Taken **	Ingestion - Admission Interval (hour)	Ingestion - Treatment Interval (hour)	Max. AST/ALT (iu/L)	Plasma Paracetamol (μg/mℓ) conc. Admission	4 hour	Plasma Paracetamol half-life (hour)	Total Urinary Paracetamol (g)
NAC									
N1	64M	D	2.0	4.5	34	526	461	4.6	14.2
N2	20F	I	6.0	7.7	35	314	366	2.7	16.4
N3	16F	-	7.0	9.0	19	147	264	2.3	11.0
N4	22M	M	6.5	9.5	3990	288	342	6.4	18.0
N5	40F	BMI	4.0	6.3	25	633	633	2.4	22.2
N6	29M	I	5.5	7.8	56	196	327	2.1	13.2
N7	21F	-	3.8	5.3	27	259	245	2.9	8.8
N8	44F	D	3.0	4.7	23	311	319	3.0	11.3
N9	40F	-	4.7	5.8	38	423	463	3.6	18.6
N10	26M	IM	6.0	9.5	81	283	343	3.2	11.6
N11	24M	D	7.8	9.5	35	204	446	2.5	17.0
N12	27F	I	2.5	6.8	37	285	230	2.9	12.0
N13	41F	-	6.5	8.5	36	265	412	2.3	16.2
N14	27F	BI	5.5	8.5	570	327	403	3.3	24.1
N15	25M	-	8.2	10.0	270	229	407	3.9	18.2
N16	52F	DI	4.0	10.2	10400	307	307	3.2	11.0
N17	25M	-	10.0	11.7	10300	106	210	7.1	9.1

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TABLE 34 (continued)

Clinical details of paracetamol overdosage patients.

Patient and Treatment	Age and Sex	Other Drugs Taken **	Ingestion - Admission Interval (hour)	Ingestion - Treatment Interval (hour)	Max. AST/ALT (iu/L)	Plasma Paracetamol (µg/mℓ) conc. Admission	Plasma Paracetamol (µg/mℓ) 4 hour	Plasma Paracetamol half-life (hour)	Total Urinary Paracetamol (g)
NAC									
N18 ††	23M	I	6.0	8.2	24	245	351	3.5	18.1
N19 ††	15F	-	3.3	5.5	24	253	241	2.0	9.8
N20	28M	-	8.0	8.5	43	98	427	2.1	9.8
N21	26M	BI	19.5	20.3	5225	103	604	5.9	17.7
N22	24M	I	13.0	15.7	9075	133	288	8.1	-
N23	26M	-	18.0	19.0	10200	139	442	9.2	-
N24	33F	D	15.0	16.5	5575	180	513	5.6	-
N25	20F	-	9.5	10.3	3125	178	491	3.8	-
N26	15F	M	4.0	7.8	46	215	215	3.7	-
N27	45F	-	4.0	6.0	26	344	344	1.9	-
N28	27F	MI	4.5	7.5	40	284	322	3.3	-
N29	24M	I	4.8	6.0	47	249	267	4.4	-
N30	48F	DI	2.5	5.4	28	502	432	2.6	-
N31	44M	I	7.0	7.8	50	221	352	2.8	-
N32	28M	DBM	5.3	7.3	68	343	412	3.2	-
N33	34F	-	8.3	8.4	50	182	368	2.1	-
N34	53F	BM	12.5	13.5	1440	353	-	5.1	-

†Patient died in hepatic coma; †† Treated with L-cysteine

** I = metabolic inducers (e.g. alcohol, barbiturates), D = Distalgesic, B = Benzodiazepines; M = miscellaneous.

Chapter 4

Plasma Paracetamol Concentration at 4 Hours after Ingestion

a) Relationship with Liver Damage

The relationship between the 4 hour plasma paracetamol concentration and the maximum plasma transaminase values (which reflect the severity of poisoning and liver damage respectively; Prescott et al, 1971) was investigated. The 4 hour plasma paracetamol concentration was calculated by semilogarithmic extrapolation or interpolation of measured values.

Data were assessed (χ^2 test) from 78 untreated patients (25 males, 53 females, mean age 29 ± 14) and also 103 treated patients (38 males, 65 females, mean age 30 ± 12), 33 of whom were treated more than 10 hours after paracetamol ingestion.

Results

In the untreated patients, there was a trend towards increasing liver damage with increasing 4 hour plasma paracetamol concentrations (χ^2 = 40.5, $p < .0001$, Table 35; Fig 31). Every untreated patient with a 4 hour plasma paracetamol concentration above 300 µg/ml sustained severe liver damage, as did 6 out of 33 untreated patients in the 200 - 300 µg/ml group.

TABLE 35

Relationships between 4 hour plasma paracetamol concentrations, liver damage and early and late treatment following paracetamol overdosage.

Patient group and number in group	4 hour plasma paracetamol conc. (μg/mℓ)	Extent of liver damage; *** Number of patients with:- None	Trivial	Moderate	Severe	Comment
Untreated (78)	<200	16	3	3	4	Highly significant relationship between 4 hour plasma paracetamol conc. and liver damage ($\chi^2 = 40.5$; $p<.0001$)
	200-300	9	7	6	11	
	>300	0	0	0	19	
* Early Treated (70)	200-300	13	7	3	1	No relationship between 4 hour plasma parac. conc. and liver damage ($\chi^2 = 3.8$; NS)
	>300	15	15	13	3	
** Late Treated (33)	⩾200	4	4	7	18	Outcome of late treatment is significantly worse than with early treatment ($\chi^2 = 34.0$; $p<.0001$)

* treated ⩽ 10 hours after paracetamol ingestion;

** treated > 10 hours after paracetamol ingestion.

*** defined according to max.plasma AST/ALT conc. (see page 190).

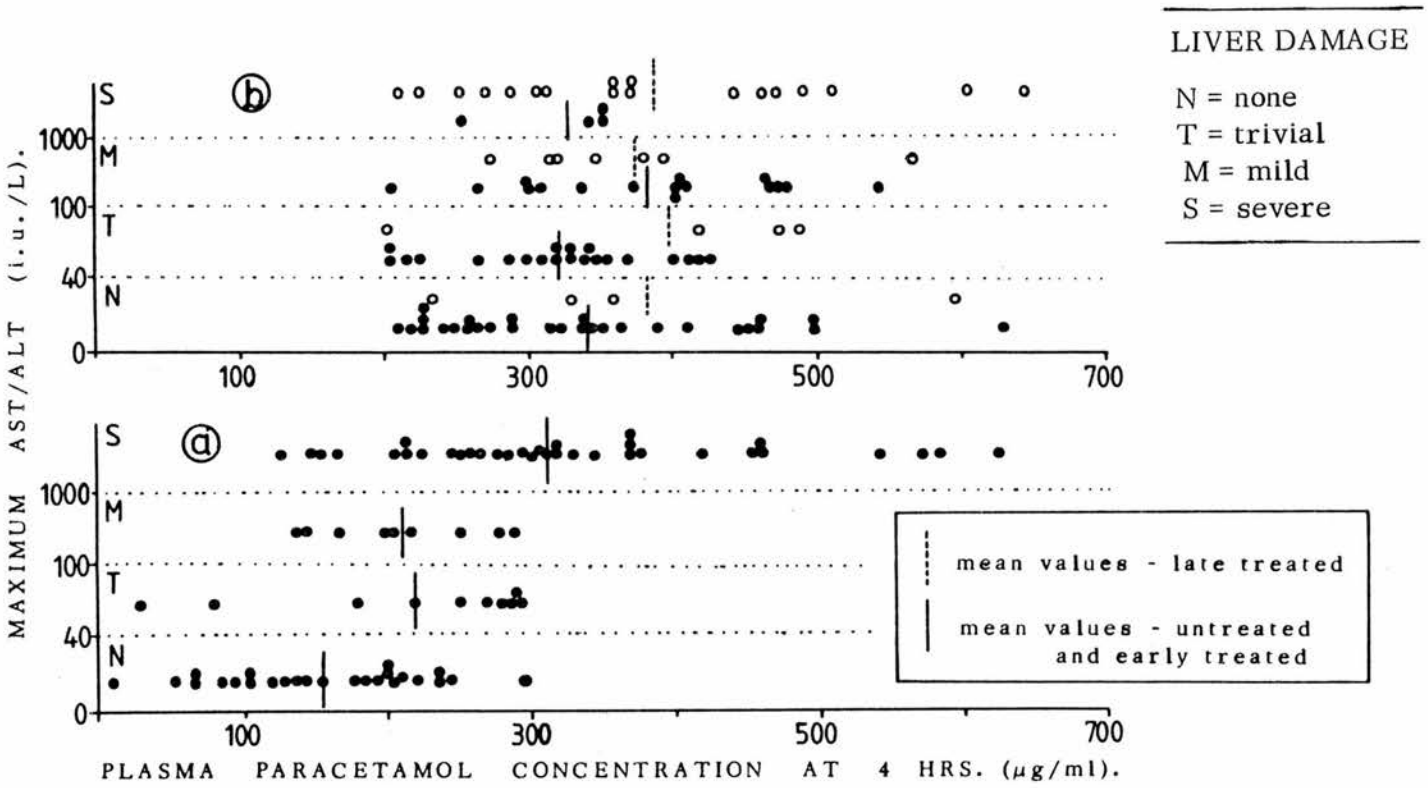


Fig. 31: Relationship between the 4 hour plasma paracetamol concentration and liver damage following paracetamol overdosage with and without treatment.

(a) Untreated patients (b) Patients treated with NAC, cysteamine or methionine.

(In (b) closed circles = patients treated within 10 hours of paracetamol ingestion, ie early-treated, and open circles = patients treated more than 10 hours after paracetamol ingestion, ie late-treated).

In the early-treated patients, the trend towards severe liver damage at high 4 hour plasma paracetamol concentrations was broken (χ^2 = 3.8, N.S.; Table 35) suggesting the efficacy of early treatment. Across the whole range of 4 hour plasma paracetamol concentrations, 16 and 4 of these 70 patients developed mild and severe liver damage respectively. However, 7 and 18 of the 33 late-treated patients sustained mild and severe liver damage respectively, illustrating the relative lack of effect of treatment if geven more than 10 hours after paracetamol ingestion. The outcome of late treatment was significantly worse than that of early treatment (χ^2 = 34.0, $p < .0001$; Table 35), even though the mean 4 hour plasma paracetamol concentrations in the two groups were not significantly different (343 ± 90 and 384 ± 119 µg/ml in the early and late treated groups respectively).

b) Relationship with Total Urinary Recovery of Paracetamol and its Metabolites

The relationship between the 4 hour plasma paracetamol concentration and the total urinary recovery of paracetamol and its conjugates was studied in 16 untreated, 11 cysteamine-treated, 9 methionine-treated and 15 NAC-treated patients (U1 - 16, C1 - 11, M1 - 9 and N1 - 15 respectively, Table 34). The data were assessed by linear regression analysis.

Results

The 16 untreated patients were admitted between 2.2 and 32 hours after ingestion and the correlation between the total urinary recovery of paracetamol and its conjugates and the 4 hour plasma paracetamol concentration was not significant ($r = 0.35$; N.S.). However, if only patients admitted up to 10 hours after ingestion are considered ($n = 13$), the correlation was highly significant ($r = 0.84$; $p < .001$). The total urinary recovery of paracetamol and its conjugates in the 3 patients admitted more than 10 hours after ingestion was very low relative to their respective 4 hour plasma paracetamol concentrations. This is expected because of loss of urine samples prior to admission. The treated patients were all admitted and treated within 10 hours of ingestion and in each group there was a very significant correlation between total urinary recovery of paracetamol and its conjugates and the 4 hour plasma paracetamol concentration (correlation coefficients $r = 0.82$, $p < .005$; $r = 0.68$, $p < .025$ and $r = 0.74$, $p < .005$ for the cysteamine, methionine and NAC treated groups respectively).

Four hour plasma paracetamol concentrations in the treated patients were all greater than 200 μg/ml. In the 8 untreated patients with similar values the correlation between total urinary recovery of paracetamol and its conjugates and the 4 hour plasma paracetamol concentration was still significant ($r = 0.76$; $p < .05$).

c) Discussion

The comparisons described above indicate that the severity of paracetamol poisoning, as judged by the 4 hour plasma paracetamol concentration, is reflected in the total urinary recovery of paracetamol and its conjugates in patients from whom little or no urine is lost following overdosage. Also, the 4 hour plasma paracetamol concentration gives some indication of the likelihood of liver damage occurring in relation to the severity of poisoning.

Chapter 5

Plasma Concentrations of Paracetamol and its Conjugates

Plasma concentrations of paracetamol and its sulphate and glucuronide conjugates were measured in 6 untreated, 10 NAC-treated and 4 cysteamine-treated patients without severe liver damage (patients U8, 10, 17 - 20; N12, 13, 26 - 33; C9, 10, 12, 17 respectively, Table 34). Four untreated, 5 NAC-treated and 4 cysteamine-treated patients with severe liver damage were studied similarly (patients U7, 12, 21, 22; N21 - 25; C13 - 16 respectively, Table 34). All the treated patients without severe liver damage were given treatment within 10 hours of paracetamol ingestion (mean 7.2 ± 1.1 and 5.7 ± 1.5 hours in the NAC and cysteamine-treated groups respectively). All the treated patients with severe liver damage were treated more than 10 hours after paracetamol ingestion (mean 16.4 ± 3.9 and 14.3 ± 3.5 hours in the NAC and cysteamine-treated groups respectively).

If necessary, paracetamol concentrations were interpolated (on a semilogarithmic scale) to specific times after overdosage (see results, Tables 36 - 38, pp 207,210,212) so that mean values at these times could be calculated for each group. Plasma concentrations of the sulphate and glucuronide conjugates were interpolated on a linear scale. The plasma paracetamol half-life in each individual patient was determined by a least squares fit of the measured plasma paracetamol concentrat-

ions. Concentrations of the cysteine and mercapturic acid conjugates were too low to measure.

Results

a) The first blood sample was often obtained more than 4 hours after ingestion and therefore the absorption and distribution phases were missed. Paracetamol absorption after overdosage was usually complete within 4 hours since plasma concentrations nearly always declined rapidly thereafter.

i) Untreated Patients

Fig. 32 and Table 36 show the mean interpolated concentrations of paracetamol and its sulphate and glucuronide conjugates in the plasma of untreated patients with and without severe liver damage. Patients with severe liver damage were generally admitted later than other patients and therefore no values before 12 hours are included for this group. Mean plasma paracetamol concentrations showed a linear exponential decline in both groups. However, the mean individual plasma paracetamol half-life in the group with severe liver damage (5.2 ± 1.6 hours) was significantly longer than in the group without severe liver damage (3.5 ± 0.7 hours; $p < .05$). Both these values were significantly longer than the value of 2.3 ± 0.4 hours obtained in healthy volunteers taking a therapeutic dose ($p < .001$ and $p < .005$ with respect to the groups with and without

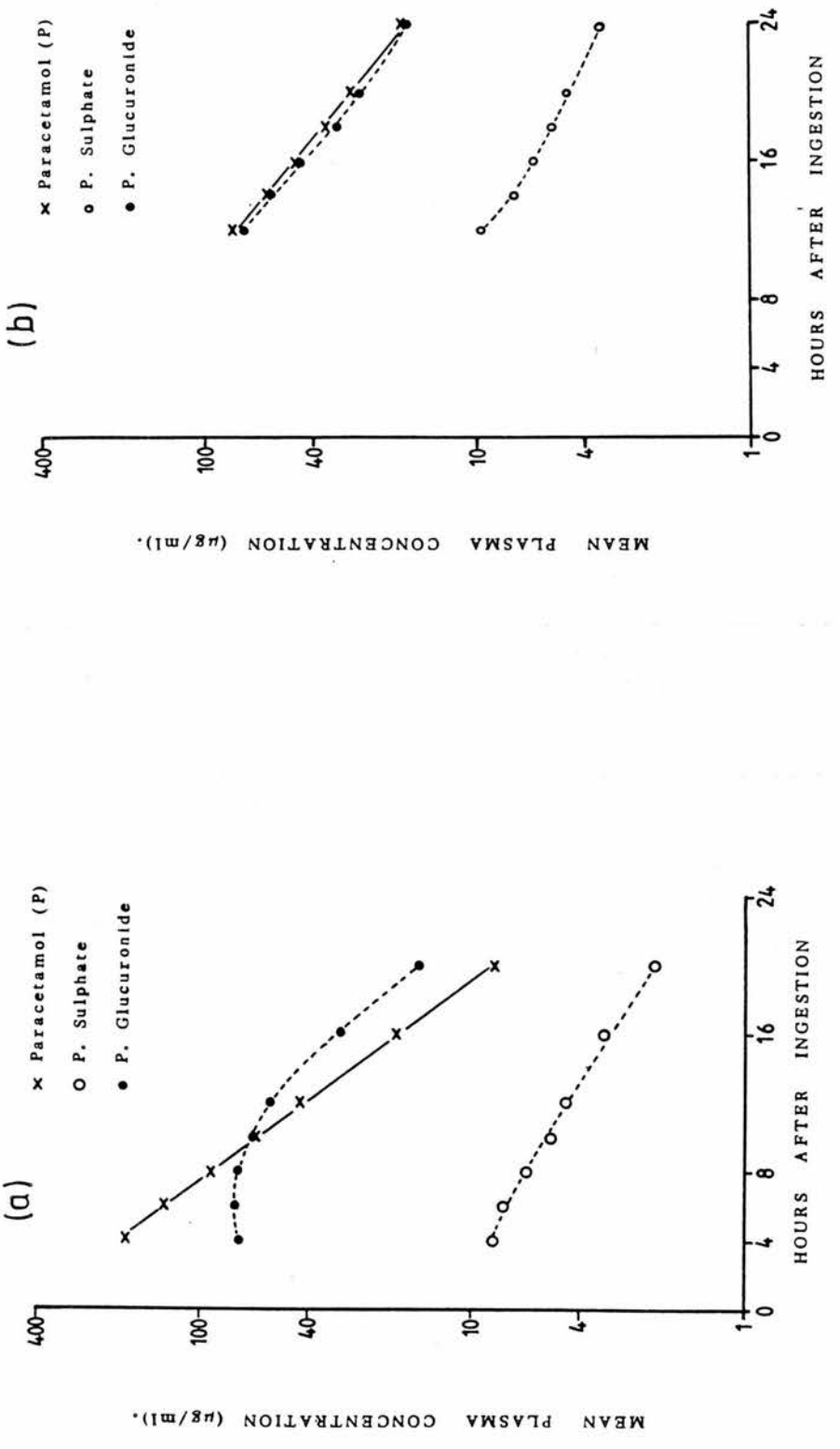


Fig. 32: Mean plasma concentrations * of paracetamol and its sulphate and glucuronide conjugates in untreated patients who (a) did not and (b) did develop severe liver damage following paracetamol overdosage.
(* Interpolated where necessary.)

TABLE 36

Mean plasma concentrations of paracetamol and its Sulphate and Glucuronide conjugates following paracetamol overdosage in untreated patients who did and did not develop severe liver damage.

Time after ingestion (hr)	Number of Patients	Mean Plasma Concentration * (µg/mℓ ± S.D.) Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide
WITHOUT SEVERE LIVER DAMAGE				
4	4	186 ± 9	8.2 ± 2.5	71 ± 19
6	5	132 ± 14	7.6 ± 1.4	73 ± 17
8	6	90 ± 15	6.2 ± 1.1	72 ± 22
10	6	62 ± 13	5.0 ± 1.6	64 ± 19
12	6	43 ± 12	4.5 ± 1.5	55 ± 14
16	6	19 ± 9	3.3 ± 1.1	30 ± 12
20	4	8.2 ± 4.1	2.1 ± 0.4	16 ± 5
WITH SEVERE LIVER DAMAGE				
12	3	79 ± 80	9.7 ± 6.4	72 ± 30
14	4	59 ± 55	7.3 ± 5.2	58 ± 26
16	4	46 ± 45	6.2 ± 4.6	45 ± 27
18	4	36 ± 37	5.3 ± 3.9	33 ± 24
20	4	29 ± 30	4.7 ± 3.3	27 ± 21
24	4	19 ± 19	3.5 ± 2.2	18 ± 14

* Interpolated where necessary - see page 204

severe liver damage respectively).

In the group without severe liver damage, the relationship between mean plasma concentrations of paracetamol glucuronide and those of the parent drug was similar to that found with therapeutic doses (Fig. 23, p 122) although mean concentrations of the glucuronide conjugate did not exceed those of paracetamol until about 10 hours after ingestion, compared with about 2.5 hours after therapeutic dosage. In the group with severe liver damage, mean plasma concentrations of the glucuronide conjugate were similar to but slightly lower than those of the parent drug. Mean plasma concentrations of paracetamol sulphate were very low relative to those of paracetamol and paracetamol glucuronide in both overdosage groups and were not far above those observed following therapeutic dosage. However, a complete comparison was not possible since no early plasma samples were obtained after overdosage.

ii) Treated Patients

Fig. 33 and Table 37 show the mean interpolated plasma concentrations of paracetamol and its sulphate and glucuronide conjugates in the groups treated early with NAC and cysteamine who did not sustain severe liver damage. Similar data in the late treated groups with severe liver camage are shown in Fig 34 and Table 38.

Mean plasma paracetamol concentrations showed an

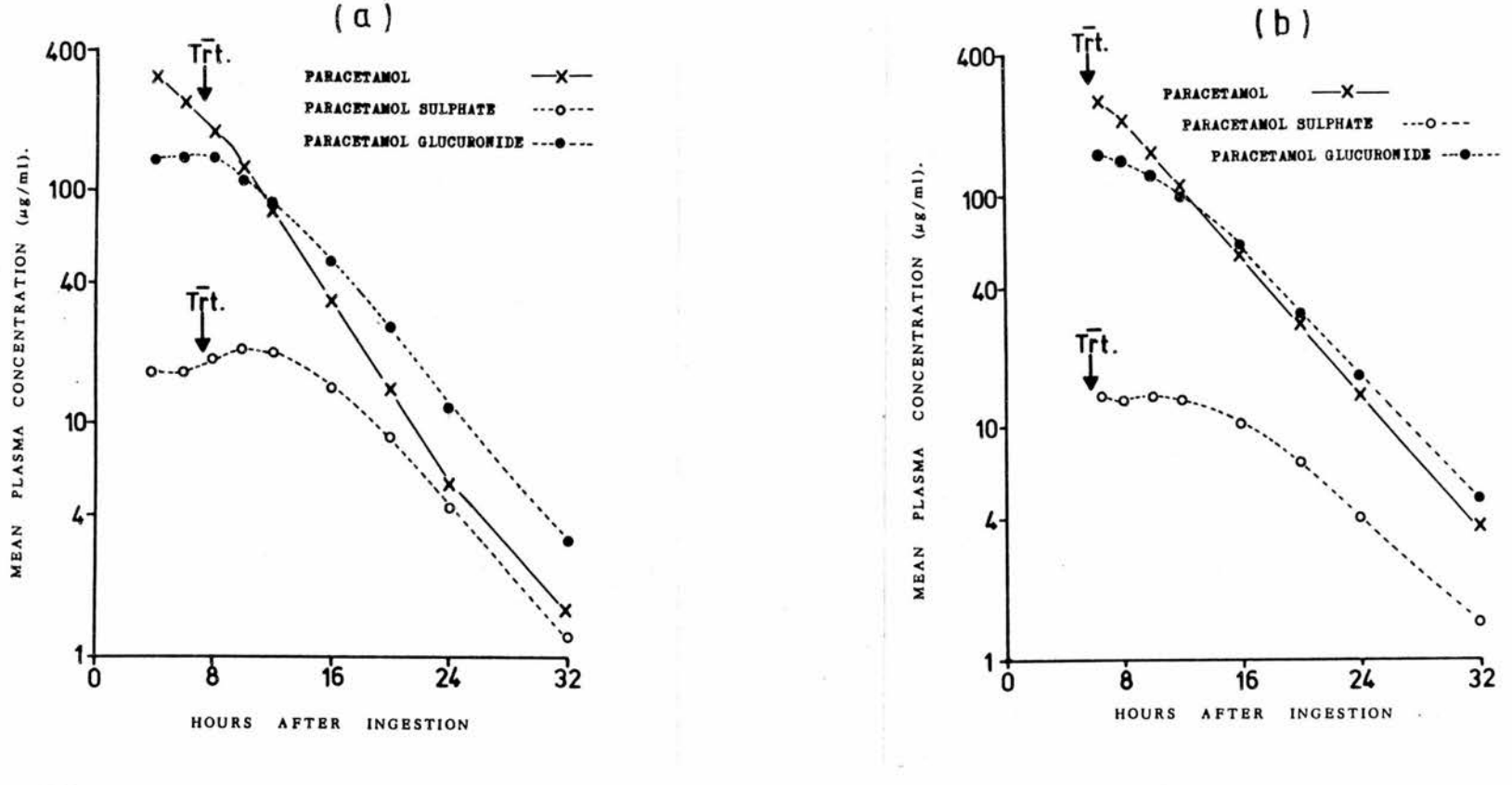


Fig. 33: Mean plasma concentrations * of paracetamol and its sulphate and glucuronide conjugates in patients who did not develop severe liver damage after treatment with (a) NAC and (b) cysteamine within 10 hours of paracetamol overdosage. (* Interpolated where necessary.)

T̄rt. denotes the mean ingestion-to-treatment interval in each group.

TABLE 37

Mean plasma concentrations of paracetamol and its sulphate and glucuronide conjugates in NAC and Cysteamine-treated patients who did not develop severe liver damage following paracetamol overdosage. (All patients were treated within 10 hours of ingestion).

Time after ingestion (hr)	Number of patients	Mean Plasma Concentration * (μg/mℓ ± S.D.) Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide
NAC-treated Patients				
4	4	305 ± 102	16.5 ± 6.3	134 ± 58
6	7	235 ± 71	16.5 ± 7.5	138 ± 50
8	9	177 ± 55	18.8 ± 9.4	139 ± 57
10	10	124 ± 42	20.7 ± 11.2	111 ± 48
12	10	82 ± 29	19.9 ± 8.9	90 ± 35
16	10	34 ± 15	14.3 ± 5.2	50 ± 19
20	10	14 ± 7	8.6 ± 3.5	26 ± 10
24	10	5.4 ± 2.6	4.3 ± 2.1	12 ± 6
32	8	1.6 ± 0.6	1.2 ± 0.7	3.1 ± 1.2
Cysteamine-treated Patients				
6.5	4	261 ± 90	13.6 ± 4.4	150 ± 44
8	4	210 ± 75	13.1 ± 2.0	141 ± 36
10	4	155 ± 58	13.5 ± 1.1	122 ± 27
12	4	110 ± 45	13.0 ± 1.4	100 ± 23
16	4	56 ± 29	10.3 ± 2.2	60 ± 22
20	4	28 ± 18	7.0 ± 1.8	31 ± 16
24	4	13 ± 11	4.0 ± 1.1	16 ± 9
32	4	3.7 ± 3.3	1.4 ± 0.7	4.8 ± 2.5

* Interpolated where necessary - see page 204 .

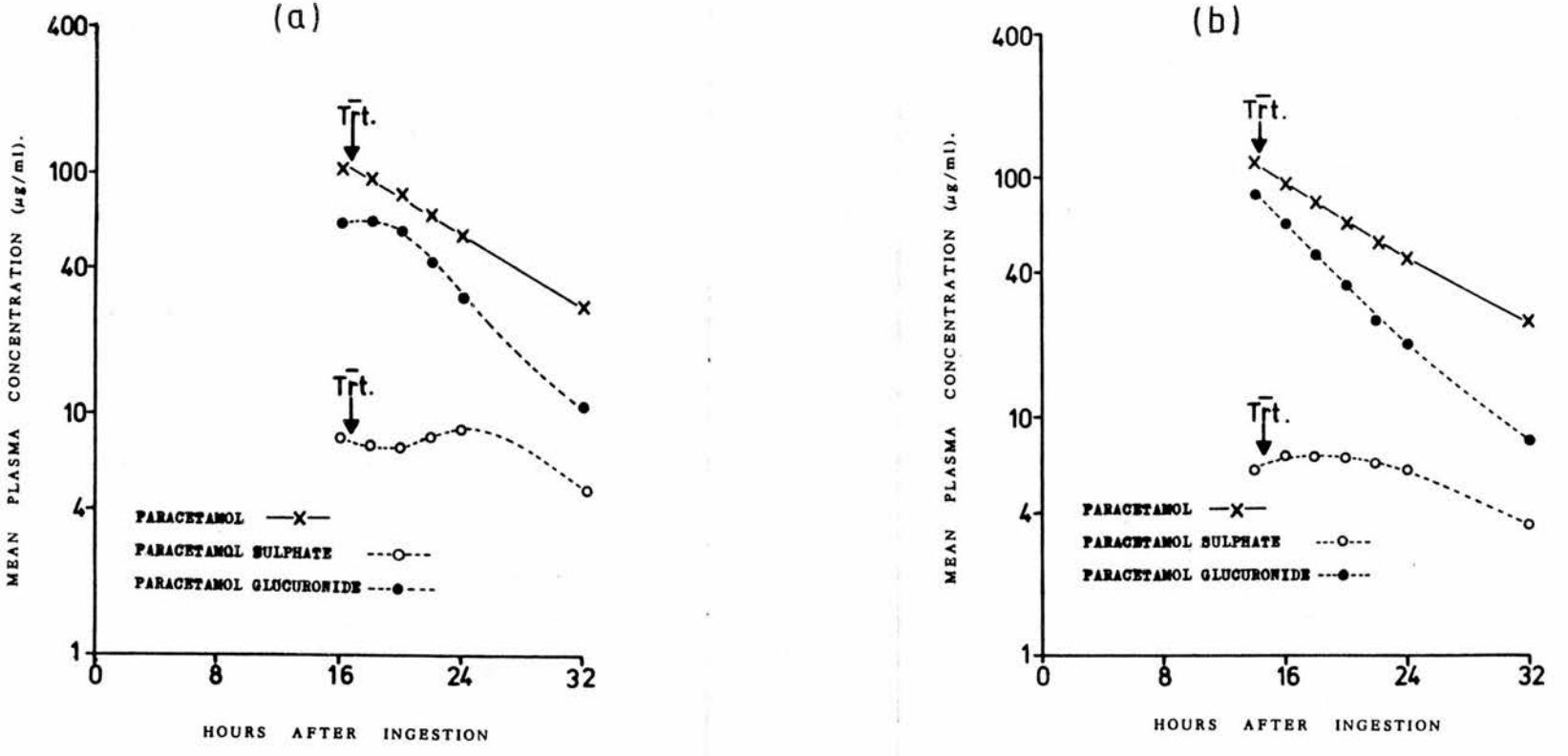


Fig. 34: Mean plasma concentrations * of paracetamol and its sulphate and glucuronide conjugates in patients who developed severe liver damage after treatment with (a) NAC and (b) cysteamine more than 10 hours after paracetamol overdosage. (* Interpolated where necessary.)

$\bar{T}rt.$ denotes the mean ingestion-to-treatment interval in each group.

TABLE 38

Mean plasma concentrations of paracetamol and its sulphate and glucuronide conjugates in NAC and Cysteamine-treated patients who developed severe liver damage following paracetamol overdosage. (All patients were treated more than 10 hours after ingestion.)

Time after ingestion (hr)	Number of patients	Mean Plasma Concentration * (μg/mℓ ±S.D.) Paracetamol	Paracetamol Sulphate	Paracetamol Glucuronide
NAC-treated Patients				
16	3	106 ± 53	7.9 ± 7.7	63 ± 22
18	4	97 ± 46	7.4 ± 5.6	64 ± 38
20	5	84 ± 36	7.3 ± 3.1	59 ± 34
22	5	68 ± 31	8.0 ± 1.2	44 ± 27
24	5	56 ± 27	8.5 ± 1.7	31 ± 19
32	5	28 ± 16	4.9 ± 1.8	11 ± 6
Cysteamine-treated Patients				
14	3	115 ± 61	6.0 ± 2.1	86 ± 37
16	3	93 ± 54	6.9 ± 1.5	64 ± 26
18	4	78 ± 39	6.9 ± 1.7	47 ± 15
20	4	64 ± 35	6.7 ± 0.9	35 ± 12
22	4	53 ± 31	6.4 ± 1.0	25 ± 8
24	4	46 ± 30	6.0 ± 1.4	20 ± 6
32	4	25 ± 21	3.5 ± 1.2	8.0± 1.0

* Interpolated where necessary - see page 204.

increase in the rate of decline in the NAC treated group without severe liver damage shortly after the mean ingestion-to-treatment interval. The mean individual plasma paracetamol half-life was initially 4.5 ± 1.0 hours and this subsequently decreased to 2.8 ± 0.6 hours ($p < .001$) after a mean initial period of 10.5 ± 2.6 hours. There was little evidence for enhanced paracetamol elimination in the cysteamine-treated group without severe liver damage and the mean individual plasma paracetamol half-life in this group was 4.0 ± 0.7 hours, longer than the value of 3.5 hours in untreated patients without severe liver damage. The mean plasma paracetamol half-life in the NAC-treated group without severe liver damage was between the value in the corresponding untreated group and the value obtained after therapeutic dosage (2.3 ± 0.4 hours).

The elimination of paracetamol from the plasma of both treated groups with severe liver damage was slower than in the respective untreated group and much slower than in the early-treated groups. The mean individual plasma paracetamol half-lives were 6.5 ± 2.2 hours and 7.5 ± 2.6 hours in the groups treated late with NAC and cysteamine respectively. Late treatment with NAC showed little evidence for the enhanced elimination of paracetamol observed after early treatment.

As with the untreated patients without severe liver

damage, mean concentrations of paracetamol glucuronide eventually exceeded those of the parent drug in both early-treated groups, although this occurred later and to a lesser extent in the cysteamine-treated group. In both late-treated groups, mean concentrations of paracetamol glucuronide were considerably lower than those of the parent drug at all times, indicating that glucuronide conjugation was apparently more impaired than in the untreated group with severe liver damage.

A marked rise in mean plasma paracetamol sulphate concentrations was observed in the group treated early with NAC. The maximum occurred at 10 hours, shortly after the mean ingestion-to-treatment interval and at about the same time as the mean paracetamol half-life began to decrease. There was a less marked rise in plasma paracetamol sulphate concentrations in the group treated early with cysteamine and the rise occurred later in relation to the mean ingestion-to-treatment interval. The pattern of plasma paracetamol sulphate elimination in the two early-treated groups (Fig.33) contrasts with the steady decline and considerably lower mean plasma concentrations observed in the untreated groups (Fig. 32) and therapeutic dose group (Fig.23, p 122). In the two late-treated groups, small increases in mean plasma paracetamol sulphate concentrations were observed.

The data described above are summarised with the mean 4 hour plasma paracetamol concentrations in each group,

including extrapolated values where necessary (Table 39). The severity of poisoning, as judged from the mean 4 hour plasma paracetamol concentration, was similar in the untreated groups although the outcome of overdosage was very different. All the treated groups appear to have been more severely poisoned than the untreated patients particularly the group treated late with NAC. In the cysteamine-treated patients, the plasma paracetamol half-life was considerably longer in those with severe liver damage than in those without, even though poisoning was apparently less severe in the former group.

Fig. 35 shows the changes in the ratios of the concentrations of paracetamol to its sulphate and glucuronide conjugates in plasma in each of the 3 groups without and with severe liver damage. The enhancement of sulphate conjugation produced by NAC and cysteamine is apparent in the lowered ratios of paracetamol to paracetamol sulphate following treatment, even in the groups with severe liver damage. Glucuronide conjugation in the cysteamine-treated groups appeared to be more limited than in the other groups whether severe liver damage was present or not.

b) Discussion

The decline in plasma paracetamol concentrations in overdosage was usually exponential, implying that saturation kinetics do not apply to the elimination of large doses of paracetamol. Galinsky et al (1979) have

TABLE 39

Elimination of paracetamol and its sulphate and glucuronide conjugates from plasma after overdosage with and without treatment and after a therapeutic dose. (Values are mean ± S.D.)

Group	Liver Damage (number in group)	Mean 4 hour Plasma Paracetamol concentration (μg/mℓ)	Mean Ingestion to Treatment Interval (hour)	Mean Plasma Paracetamol half-life (hour)	Mean Conjugate concentrations in relation to Paracetamol (P) (G = paracetamol glucuronide S = paracetamol sulphate)
Therapeutic	None (8)	7.5 ± 1.6	-	2.3 ± 0.4**	G > P after 2.5 hours; S < P and declined steadily after 4 hours
Untreated	None (6)	198 ± 21	-	3.5 ± 0.7	G > P after 10-12 hours; S<<P and declined steadily
"	Severe (4)	242 ± 119	-	5.2 ± 1.6 *	G ≃ P; S <<P and declined steadily
NAC-treated	None (10)	335 ± 77	7.2 ± 1.1	4.5 ± 1.0,* 2.8 ± 0.6 † after 10.5 hr.	G > P after 10-12 hours; S increased +++ after treatment
"	Severe (5)	468 ± 116	16.4 ± 3.9	6.5 ± 2.2 ††	G < P; S increased + after treatment
Cysteamine-treated	None (4)	363 ± 128	5.7 ± 1.5	4.0 ± 0.7	G > P after 12-16 hours; S increased ++ after treatment
"	Severe (4)	294 ± 80	14.3 ± 3.5	7.5 ± 2.6 ††	G < P; S increased + after treatment

† not significantly different
* p<.05; ††p<.01; ** p<.005

} in relation to untreated group without severe liver damage

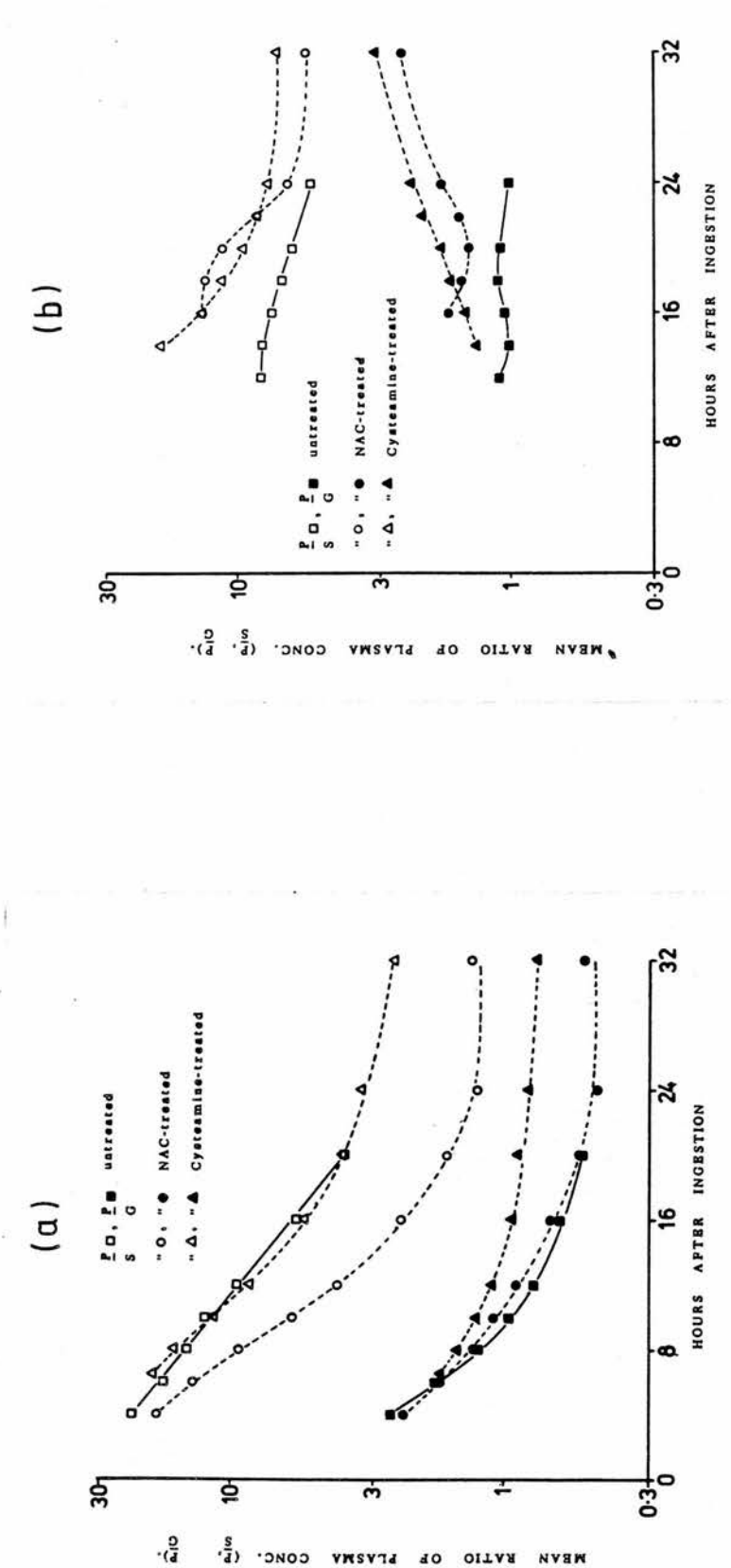


Fig. 35: Changes in the mean plasma concentration ratios of paracetamol to its sulphate and glucuronide conjugates with time in treated and untreated patients (a) without and (b) with severe liver damage following paracetamol overdosage.

suggested otherwise, although supporting evidence was not provided. Judging from the 4 hour plasma paracetamol concentrations, the plasma paracetamol half-life was dose-dependent and was particularly prolonged with severe liver damage. Paracetamol glucuronide was always the major metabolite in plasma, eventually exceeding the parent drug in concentration if no liver damage was present. Also, plasma concentrations of paracetamol sulphate, although always low, were particularly low in relation to the parent drug in patients with severe liver damage (Fig. 35). Thus liver damage appears to strongly influence paracetamol metabolism and elimination. Similar observations were reported by Prescott et al (1971) and Prescott and Wright (1973).

The low plasma concentrations of paracetamol sulphate suggest limitation of the sulphate conjugation pathway. This could result from either enzyme saturation or depletion of the cofactors required for sulphate conjugation or both. The former explanation was favoured by Weitering et al (1979). They found that "high" doses of phenol in rats did not deplete plasma concentrations of inorganic sulphate, although the proportion excreted as phenyl sulphate decreased with increasing doses. However, the highest dose given was only equivalent to 40 mg/kg paracetamol. Overdoses in man are often more than 150 mg/kg (more than 10g, see total urinary paracetamol, Table 34) and serum concentrations of

inorganic sulphate in rats are much higher than in man (Weitering et al, 1979). Thus depletion of inorganic sulphate may well occur in man following paracetamol overdosage.

In favour of inorganic sulphate depletion, Houston and Levy (1976) found that sodium sulphate could reverse the competitive effect of ascorbic acid on sulphate conjugation with paracetamol. Also, Galinsky et al (1979) observed enhanced paracetamol elimination and increased plasma paracetamol sulphate concentrations following the administration of sodium sulphate to rats given a 150 mg/kg dose of paracetamol.

Treatment of paracetamol overdosage with NAC and cysteamine had significant but different effects on paracetamol metabolism and elimination (Table 39). After early treatment with NAC, plasma paracetamol half-lives were significantly reduced to values which were not much longer than those observed after therapeutic dosage, even though the apparent dose ingested by the NAC-treated patients was high even for overdosage. The enhanced elimination of paracetamol was associated with increased production of paracetamol sulphate. Thus NAC appears to be acting in a similar manner to sodium sulphate (Galinsky et al, 1979) in aiding the supply of the necessary cofactors required for sulphate conjugation. This was also observed with the related compound, cysteine, in reducing the competitive inhibition of paracetamol

sulphate conjugation with salicylamide (Levy and Yamada, 1971).

Early treatment with cysteamine had less effect on sulphate conjugation than NAC and did not enhance paracetamol elimination significantly. In addition, cysteamine may have inhibited glucuronide conjugation. Late treatment with either compound did not protect against liver damage although a small enhancement of sulphate conjugation was apparent. Thus the hepatoprotective action of these two compounds appears to be related to early treatment and the mechanisms whereby protection is afforded appear to differ.

In addition to the effects on sulphate conjugation described above, NAC has also been shown to conjugate directly with the toxic metabolite of paracetamol and reduce its covalent binding to macromolecules in mouse liver microsomal preparations (Buckpitt et al, 1979). The hepatoprotective action of cysteamine may be similar to that of piperonyl butoxide, which inhibits both the glucuronidation and microsomal oxidation and also the covalent binding of paracetamol in hamsters (Jollow et al, 1974; Potter et al, 1974). Apparent inhibition of the hepatic microsomal oxidation of paracetamol and a reduced rate of metabolism of paracetamol have been reported with cysteamine in isolated perfused rat liver (Harvey and Levitt, 1976) and mouse liver microsomal preparations (Buckpitt et al, 1979).

Chapter 6

Total Urinary Recovery of Paracetamol and its Conjugates

The total urinary recovery of paracetamol and its conjugates was studied in 13 untreated and 9, 11, and 15 patients treated with methionine, cysteamine and NAC respectively (patients U1 - 13, M1 - 9, C1 - 11 and N1 - 15 respectively in Table 34). The patients were all admitted and/or treated within 10 hours of paracetamol ingestion and there was a significant correlation between the 4 hour plasma paracetamol concentration and the total drug recovered in urine within each group (see pp 201-203). The 4 hour plasma paracetamol concentrations in the treated patients were all above 200 μg/ml. The clinical details for each group are shown in Table 40.

Pooled urine samples were analysed and the total recovery of each compound and the percentage of the total drug recovered as each compound (expressed as the paracetamol equivalent) were compared in relation to the overall recovery of drug, liver damage and treatment.

One NAC-treated patient (N8) apparently excreted an abnormally high proportion of drug unchanged (41.8%, 4.73g) together with an abnormally low proportion of paracetamol glucuronide (28.8%, 3.3g; Tables 44d and 45d, pp 234,238). This was probably the result of bacterial hydrolysis of the glucuronide conjugate prior to freezing the urine. Often many hours elapsed between the collection

TABLE 40

Clinical particulars of the patient groups used in comparisons of the overall urinary excretion of paracetamol and its conjugates. (Values are mean ± S.D.)

Treatment	Number of Patients	Age (year)	Ingestion-Admission Interval (hour)	Ingestion-Treatment Interval (hour)	4 hour Plasma Paracetamol concentration (μg/mℓ)	Total urinary recovery of Paracetamol (g)
Untreated (any 4 hour plasma paracetamol concentration)	13	30±10	4.6 ± 2.1	-	238 ± 133	11.8 ± 4.3
Untreated (4 hour plasma paracetamol concentration ≥ 200 μg/mℓ)	8	31±12	5.3 ± 2.4	-	301 ± 136	14.1 ± 4.0
Methionine	9	25±6	3.9 ± 1.5	7.3 ± 1.8	292 ± 80	14.8 ± 2.1
Cysteamine	11	30±8	4.5 ± 2.1	6.8 ± 1.6	305 ± 56	15.2 ± 4.8
NAC	15	31±13	5.3 ± 1.9	7.6 ± 1.9	377 ± 103	15.5 ± 4.3

of urine samples from patients and processing of the samples in the laboratory (for instance with evening collections). In such cases some of the glucuronide conjugate could be hydrolysed to paracetamol by bacterial β-glucuronidase. Patient N8 was excluded from comparisons involving paracetamol and its glucuronide conjugate.

a) Results

i) Untreated Patients: Table 41a and b shows the amounts of each compound recovered and their proportions relative to the overall urinary recovery of paracetamol and the maximum AST/ALT concentrations in the untreated group. The recovery of each compound generally reflected the total recovery of the drug and its metabolites except in the case of paracetamol sulphate, where there was no relationship with the total recovery of drug and metabolites (Table 42). Also, a wide range of recoveries was observed for all compounds except the sulphate conjugate (Table 42).

The proportions excreted as each compound relative to the total urinary recovery of drug showed wide individual variation, especially with the cysteine conjugate (Table 42). The proportion excreted as paracetamol sulphate was inversely related to the total recovery of drug and metabolites, whereas no similar relationships were observed with the other compounds (Table 42).

TABLE 41(a)

Amounts of paracetamol and its conjugates recovered in urine following paracetamol overdosage in 13 untreated patients.

Patient No.	Max. AST/ALT (iu/L)	Amount (g) recovered in urine as:- P	S	G	M	C	M+C	TOTAL (g)
U1	3840	1.88	1.37	14.8	1.25	2.41	3.66	21.7
U2	23	1.45	0.80	8.9	0.30	0.14	0.45	11.6
U3	6000	1.96	1.21	12.1	1.10	0.68	1.78	17.0
U4	49	1.32	0.84	13.0	0.33	0.25	0.58	15.8
U5	684	1.04	0.94	10.0	0.49	0.56	1.05	13.1
U6	62	0.38	1.43	7.3	0.60	0.54	1.14	10.3
U7	9940	0.60	1.40	9.4	0.96	0.92	1.88	13.3
U8	19	0.66	0.95	7.2	0.56	0.42	0.97	9.8
U9	576	1.44	0.80	6.1	0.29	0.22	0.51	8.8
U10	18	0.74	1.00	6.6	0.34	0.27	0.60	8.9
U11	23	0.76	1.10	6.6	0.26	0.37	0.63	9.1
U12	5840	0.54	0.62	5.9	0.38	0.61	0.99	8.1
U13	33	0.27	0.74	4.4	0.24	0.18	0.42	5.8
Mean ±S.D.	⩽1000 (n=9)	0.90 ± 0.44	0.96 ± 0.21	7.8 ± 2.5	0.38 ± 0.14	0.33 ± 0.15	0.70 ± 0.27	10.4 ± 2.9
Mean ±S.D.	>1000 (n=4)	1.25 ± 0.78	1.15 ± 0.36	10.6 ± 3.8	0.92* ± 0.38	1.15 ± 0.85†	2.08* ± 1.13	15.0 ± 5.8

* Significantly different from patients with max. AST/ALT ⩽ 1000, $p<.005$

† " " " " " " " " , $p<.02$.

KEY:-

P - Paracetamol; S, G, M, C - sulphate, glucuronide, mercapturic acid and cysteine conjugates respectively.

TABLE 41(b)

Urinary recovery of Paracetamol and its conjugates expressed as percentages of the total drug recovered in urine following Paracetamol overdosage in 13 untreated patients.

Patient No.	Percentage of the total recovered as:- P	S	G	M	C	M+C	Max. AST/ALT (iu/L)
U1	8.7	6.3	68.2	5.7	11.1	16.8	3840
U2	12.5	6.9	76.8	2.6	1.2	3.8	23
U3	11.6	7.1	70.8	6.5	4.0	10.5	6000
U4	8.4	5.3	82.6	2.1	1.6	3.7	49
U5	8.0	7.2	76.8	3.8	4.3	8.1	684
U6	3.7	13.9	71.4	5.8	5.2	11.0	62
U7	4.5	10.5	70.8	7.2	6.9	14.1	9940
U8	6.7	9.7	73.6	5.7	4.2	9.9	19
U9	16.3	9.0	68.9	3.2	2.5	5.7	576
U10	8.3	11.2	73.7	3.8	3.0	6.8	18
U11	8.3	12.4	72.3	2.9	4.1	7.0	23
U12	6.7	7.7	73.4	4.7	7.5	12.2	5840
U13	4.6	12.7	75.6	4.1	3.1	7.2	33

(See Table43 for comparisons with respect to liver damage)

KEY:-

P - Paracetamol; S, G, M, C - sulphate, glucuronide, mercapturic acid and cysteine conjugates respectively.

TABLE 42

Recovery of paracetamol and its conjugates in relation to the total recovery of paracetamol and its conjugates in the urine of 13 untreated patients following paracetamol overdosage.

Compound	Recovery Range		Correlation (r) between total recovery of paracetamol and conjugates and:-	
	Amt. (g)	% of Total	Amt. (g)	% of Total
Paracetamol	0.27 - 1.96	3.7 - 16.3	0.76 ($p<.005$)	0.16 (NS)
Paracetamol Sulphate	0.62 - 1.43	5.3 - 13.9	0.53 (NS)	-0.65 ($p<.02$)
Paracetamol Glucuronide	4.4 - 14.8	68.2 - 82.6	0.90 ($p<.001$)	-0.12 (NS)
Paracetamol Mercapturate	0.24 - 1.25	2.1 - 7.2	0.79 ($p<.005$)	0.29 (NS)
Paracetamol Cysteine	0.14 - 2.41	1.2 - 11.1	0.75 ($p<.005$)	0.45 (NS)
Combined Mercapturate and Cysteine conjugates	0.42 - 3.66	3.7 - 16.8	0.80 ($p<.005$)	0.43 (NS)
TOTAL	5.8 - 21.7	-	-	-

The mean recovery of each compound was higher in the patients with severe liver damage than in those without, due mainly to the considerably higher mean total recovery of drug in the former group than in the latter (Table 41a). However, the mean amount of drug excreted as the mercapturic acid and cysteine conjugates was very much higher with severe liver damage (2.08 ± 1.13g) than without (0.70 ± 0.27g; $p < .005$, Table 41a). The same was true of the mean proportion of drug recovered as these conjugates (13.4 ± 2.7% and 7.4 ± 2.9% in the patients with and without severe liver damage respectively; $p < .005$, Table 43). The mean proportions excreted as unchanged paracetamol and its sulphate and glucuronide conjugates were not significantly different between the 2 groups.

The pattern of urinary excretion after overdosage (with or without severe liver damage) differed markedly from that observed after therapeutic dosage (Table 43). The mean proportions of the total urinary recovery excreted as unchanged paracetamol and its glucuronide conjugate were each significantly higher after overdosage, irrespective of liver damage, than after a 20 mg/kg dose whereas the mean proportion recovered as paracetamol sulphate was very much lower ($p < .001$; Table 43). The mean proportion excreted as the mercapturic acid and cysteine conjugates was little different in overdosage without severe liver damage (7.0 ± 2.5%) compared with therapeutic dosage (8.1 ± 2.9%). However, in the group

TABLE 43

Recovery of paracetamol and its conjugates in relation to total urinary recovery of drug following overdosage and therapeutic dosage with paracetamol.

Compound	Mean % of total recovered in urine ± S.D.			
	overdose without severe liver damage (n=9)	overdose with severe liver damage (n=4)	therapeutic dose (20 mg/kg; n=8)	
Paracetamol	8.5 ± 3.9	7.9 ± 3.0	5.1 ± 1.4	(d)
Paracetamol Sulphate	9.8 ± 3.0	7.9 ± 1.8	32.8 ± 10.3	(e)
Paracetamol Glucuronide	74.6 ± 3.9	70.8 ± 2.1	54.0 ± 11.7	(f)
Paracetamol Mercapturate	3.8 ± 1.3 (a)	6.0 ± 1.1	4.4 ± 1.2	(g)
Paracetamol Cysteine	3.2 ± 1.3 (b)	7.4 ± 2.9	3.7 ± 1.1	(h)
Mercapturate and Cysteine conjugates combined	7.0 ± 2.5 (c)	13.4 ± 2.7	8.1 ± 1.9	(i)

(a); (b); (c) - $p<.02$; $p<.005$; $p<.005$ respectively, comparing overdosage with and without severe liver damage.

(d); (e) - $p<.05$; $p<.001$ respectively, comparing therapeutic dosage with each overdosage group.

(f) - $p<.001$ and $p<.02$ comparing therapeutic dosage with overdosage, without and with severe liver damage respectively.

(g); (h); (i) - $p<.05$; $p<.01$; $p<.005$ respectively, comparing therapeutic dosage with overdosage resulting in severe liver damage.

with severe liver damage, proportional excretion of these conjugates was significantly higher than after therapeutic dosage (13.4 ± 2.7% compared with 8.1 ± 2.9%; $p < .005$). Excretion of the mercapturic acid and cysteine conjugates considered separately generally reflected their combined excretion (Tables 41a, 43).

ii) Treated Patients: Tables 44 and 45 (a - d) show the total recovery of paracetamol and its conjugates in comparable treated and untreated patients (see p 221).

The mean total drug recoveries according to liver damage and treatment (Table 46) show that similar mean amounts were recovered from all 4 treatment groups. Untreated patients without severe liver damage excreted a somewhat smaller mean quantity of paracetamol overall than all the other groups (although the difference was significant only with respect to the methionine-treated group). Patients with severe liver damage tended to excrete larger quantities than those without severe liver damage.

A larger mean amount of paracetamol was excreted unchanged by untreated patients with severe liver damage than by those without but this was not so with treated patients (Table 47). The proportions of total paracetamol recovery excreted unchanged were similar in patients with and without severe liver damage. Unchanged paracetamol accounted for a higher mean percentage of overall drug recovery in the NAC-treated group than in

TABLE 44(a)

Amounts of Paracetamol and its conjugates recovered in urine following Paracetamol overdosage in 8 untreated patients (4 hour plasma Paracetamol concentrations were above 200 μg/mℓ in each patient).

Patient No.	Amount (g) recovered in urine as:- P	S	G	M	C	M+C	TOTAL (g)	Max. AST/ALT (iu/L)
U1	1.88	1.37	14.8	1.25	2.41	3.66	21.7	3840
U2	1.45	0.80	8.9	0.30	0.14	0.45	11.6	23
U3	1.96	1.21	12.1	1.10	0.68	1.78	17.0	6000
U4	1.32	0.84	13.0	0.33	0.25	0.58	15.8	49
U5	1.04	0.94	10.0	0.49	0.56	1.05	13.1	684
U6	0.38	1.43	7.3	0.60	0.54	1.14	10.3	62
U7	0.60	1.40	9.4	0.96	0.92	1.88	13.3	9940
U8	0.66	0.95	7.2	0.56	0.42	0.97	9.8	19

KEY:-

P - Paracetamol; S, G, M, C - Paracetamol sulphate, glucuronide, mercapturic acid and cysteine conjugates respectively.

TABLE 44(b)

Amounts of Paracetamol and its conjugates recovered in urine following Paracetamol overdosage in 11 patients treated with Cysteamine within 10 hours of Paracetamol ingestion.

Patient No.	Amount (g) recovered in urine:- P	S	G	M	C	M+C	TOTAL (g)	Max. AST/ALT (iu/L)
C1	1.23	1.96	16.9	0.38	0.47	0.86	21.0	16
C2	1.02	1.27	6.7	0.25	0.30	0.55	9.6	23
C3	0.93	1.57	11.3	0.68	0.58	1.26	15.0	602
C4	1.74	1.40	9.9	0.26	0.21	0.47	13.5	153
C5	3.24	1.59	15.5	0.47	0.84	1.31	21.6	960
C6	0.74	0.99	9.4	0.36	0.41	0.77	11.9	22
C7	1.11	1.98	9.3	0.20	0.37	0.57	13.0	19
C8	1.32	2.21	15.5	0.84	0.77	1.61	20.6	66
C9	0.77	1.31	10.6	0.41	0.41	0.82	13.5	29
C10	1.78	2.85	13.2	1.16	0.93	2.09	19.9	91
C11	0.69	1.35	5.3	0.22	0.21	0.43	7.8	40

No patient treated with cysteamine within 10 hours of paracetamol ingestion developed severe liver damage.

KEY:-

P - Paracetamol; S, G, M, C - Paracetamol sulphate, glucuronide, mercapturic acid and cysteine conjugates respectively.

TABLE 44(c)

Amounts of Paracetamol and its conjugates recovered in urine following Paracetamol overdosage in 9 patients treated with Methionine within 10 hours of Paracetamol ingestion.

Patient No.	Amount (g) recovered in urine						TOTAL (g)	Max. AST/ALT (iu/L)
	P	S	G	M	C	M+C		
M1	0.96	1.31	11.8	0.67	0.33	1.00	15.1	21
M2	0.67	0.82	12.0	0.32	0.36	0.68	14.2	78
M3	1.31	2.11	11.3	0.70	0.57	1.27	16.0	70
M4	1.85	1.08	9.3	0.44	0.33	0.77	13.0	13
M5	1.18	1.72	9.0	0.95	0.75	1.70	13.6	43
M6	1.73	1.79	11.4	1.08	1.70	2.77	17.7	9200
M7	0.67	1.24	8.3	0.58	0.60	1.17	11.4	3200
M8	1.37	1.34	11.0	0.61	0.32	0.93	14.7	61
M9	1.08	2.20	12.2	1.21	0.85	2.06	17.6	161

KEY:-

P - Paracetamol; S, G, M, C - Paracetamol sulphate, glucuronide, mercapturic acid and cysteine conjugates respectively.

TABLE 44(d)

Amounts of Paracetamol and its conjugates recovered in urine following Paracetamol overdosage in 15 patients treated with NAC within 10 hours of Paracetamol ingestion.

Patient No.	Amount (g) recovered in urine:- P	S	G	M	C	M+C	TOTAL (g)	Max. AST/ALT (iu/L)
N1	1.17	2.26	8.6	1.22	0.98	2.20	14.2	34
N2	1.85	1.82	11.6	0.59	0.57	1.16	16.4	35
N3	0.81	1.24	8.0	0.41	0.59	1.00	11.0	19
N4	1.63	1.78	12.2	1.32	1.06	2.38	18.0	3990
N5	3.48	1.75	15.8	0.62	0.55	1.16	22.2	25
N6	2.10	1.62	8.8	0.37	0.37	0.74	13.2	56
N7	1.49	1.25	5.2	0.50	0.46	0.96	8.8	27
N8	4.73*	2.05	3.3*	0.59	0.69	1.28	11.3	23
N9	2.77	2.53	11.4	0.78	1.08	1.86	18.6	38
N10	1.29	2.73	6.8	0.40	0.31	0.72	11.6	81
N11	0.97	3.52	10.6	1.27	0.70	1.97	17.0	35
N12	1.36	1.77	8.1	0.42	0.33	0.75	12.0	37
N13	0.73	2.09	12.2	0.50	0.66	1.15	16.2	36
N14	4.43	2.31	13.3	2.09	1.93	4.03	24.1	570
N15	1.28	3.09	11.3	1.24	1.33	2.56	18.2	270

* Patient N8 not included in mean values of P and G - see page 221 for explanation.

KEY:-

P - Paracetamol; S, G, M, C - Paracetamol sulphate, glucuronide, mercapturic acid and cysteine conjugates respectively.

TABLE 45(a)

Urinary recovery of paracetamol and its conjugates expressed as percentages of the total drug recovered in urine following paracetamol overdosage in 8 untreated patients. (4 hour plasma paracetamol concentrations were above 200 µg/ml in each patient.)

Patient	Percentage of the total recovered as:-						Max. AST/ALT (iu/L)
	P	S	G	M	C	M+C	
U1	8.7	6.3	68.2	5.7	11.1	16.8	3840
U2	12.5	6.9	76.8	2.6	1.2	3.8	23
U3	11.6	7.1	70.8	6.5	4.0	10.5	6000
U4	8.4	5.3	82.6	2.1	1.6	3.7	49
U5	8.0	7.2	76.8	3.8	4.3	8.1	684
U6	3.7	13.9	71.4	5.8	5.2	11.0	62
U7	4.5	10.5	70.8	7.2	6.9	14.1	9940
U8	6.7	9.7	73.6	5.7	4.2	9.9	19

KEY:-

P - Paracetamol; S, G, M, C - Paracetamol sulphate, glucuronide, mercapturic acid and cysteine conjugates respectively.

TABLE 45(b)

Urinary recovery of paracetamol and its conjugates expressed as percentages of the total drug recovered in urine following paracetamol overdosage in 11 patients treated with Cysteamine. (Treatment commenced within 10 hours of paracetamol ingestion.)

Patient No.	Percentage of the total recovered as:- P	S	G	M	C	M+C	Max. AST/ALT (iu/L)
C1	5.9	9.3	80.7	1.8	2.3	4.1	16
C2	10.7	13.3	70.2	2.6	3.2	5.8	23
C3	6.2	10.4	75.0	4.5	3.9	8.4	602
C4	12.8	10.4	73.3	1.9	1.5	3.5	153
C5	14.9	7.3	71.7	2.2	3.9	6.0	960
C6	6.2	8.3	78.9	3.1	3.4	6.5	22
C7	8.6	15.3	71.8	1.5	2.9	4.4	19
C8	6.4	10.7	75.1	4.1	3.7	7.8	66
C9	5.7	9.7	78.5	3.1	3.1	6.1	29
C10	8.9	14.3	66.2	5.8	4.7	10.5	91
C11	8.9	17.3	68.4	2.8	2.6	5.5	40

No patient treated with cysteamine within 10 hours of paracetamol ingestion developed severe liver damage.

KEY:-

P - Paracetamol; S, G, M, C - Paracetamol sulphate, glucuronide, Mercapturic acid and cysteine conjugates respectively.

TABLE 45(c)

Urinary recovery of paracetamol and its conjugates expressed as percentages of the total drug recovered in urine following paracetamol overdosage in 9 patients treated with Methionine. (Treatment commenced within 10 hours of paracetamol ingestion)

Patient No.	Percentage of the total recovered as:- P	S	G	M	C	M+C	Max. AST/ALT (iu/L)
M1	6.4	8.6	78.4	4.4	2.2	6.6	21
M2	4.7	5.8	84.7	2.3	2.5	4.8	78
M3	8.1	13.2	70.8	4.4	3.6	7.9	70
M4	14.2	8.3	71.6	3.4	2.5	5.9	13
M5	8.7	12.7	66.1	7.0	5.5	12.5	43
M6	9.8	10.1	64.4	6.1	9.6	15.7	9200
M7	5.9	10.9	72.9	5.1	5.2	10.3	3200
M8	9.3	9.1	75.2	4.1	2.2	6.3	61
M9	6.2	12.5	69.6	6.9	4.8	11.7	161

KEY:-

P - Paracetamol; S, G, M, C - Paracetamol sulphate, glucuronide, mercapturic acid and cysteine conjugates respectively.

TABLE 45(d)

Urinary recovery of paracetamol and its conjugates expressed as percentages of the total drug recovered in urine following paracetamol overdosage in 15 patients treated with NAC. (Treatment commenced within 10 hours of paracetamol ingestion.)

Patient No.	Percentage of the total recovered as:- P	S	G	M	C	M+C	Max. AST/ALT (iu/L)
N1	8.2	15.9	60.4	8.6	6.9	15.5	34
N2	11.3	11.1	70.6	3.6	3.5	7.1	35
N3	7.3	11.2	72.3	3.7	5.4	9.1	19
N4	9.1	9.9	67.8	7.4	5.9	13.2	3990
N5	15.7	7.9	71.2	2.8	2.5	5.2	25
N6	15.9	12.3	66.3	2.8	2.8	5.6	56
N7	16.8	14.1	58.3	5.6	5.2	10.8	27
N8	41.8*	18.1	28.8*	5.2	6.1	11.3	23
N9	14.9	13.6	61.5	4.2	5.8	10.0	38
N10	11.1	23.6	59.0	3.5	2.7	6.2	81
N11	5.7	20.7	62.1	7.4	4.1	11.5	35
N12	11.3	14.8	67.6	3.5	2.8	6.2	37
N13	4.5	12.9	75.4	3.1	4.1	7.1	36
N14	18.4	9.6	55.3	8.7	8.0	16.7	570
N15	7.0	17.0	61.9	6.8	7.3	14.1	270

* Patient N8 not included in mean values of P and G - see page 221 for explanation.

KEY:-

P - Paracetamol; S, G, M, C - Paracetamol sulphate, glucuronide, mercapturic acid and cysteine conjugates respectively.

TABLE 46

Mean total urinary recovery of paracetamol and its conjugates in relation to liver damage following paracetamol overdosage in treated and untreated patients. (Treatment commenced within 10 hours of paracetamol ingestion in all specifically-treated patients.)

Treatment	mean total drug recovered in urine (g) ± S.D. and number of patients (n)		
	without severe liver damage	with severe liver damage	TOTAL
Untreated	12.1 ± 2.4 (5)	17.3 ± 4.3 (3)	14.1 ± 4.0 (8)
Cysteamine	15.2 ± 4.8 (11)	-	15.2 ± 4.8 (11)
Methionine	14.9 ± 1.6* (7)	14.6 (2)	14.8 ± 2.1 (9)
NAC	15.3 ± 4.4 (14)	18.0 (1)	15.5 ± 4.3 (15)
All specific Treatments	15.2 ± 4.0 (32)	15.7 ± 3.7 (3)	15.2 ± 4.0 (35)
TOTAL	14.8 ± 4.0 (37)	16.5 ± 3.7 (6)	15.0 ± 3.9 (43)

* $p < .05$ compared with untreated patients without severe liver damage.

TABLE 47

Mean amounts and percentages of paracetamol recovered unchanged in urine following paracetamol overdosage in treated and untreated patients. (Treatment commenced within 10 hours of paracetamol ingestion in all specifically-treated patients.)

Treatment	Mean amount recovered in urine (g) ± S.D. and number of patients (n)			Mean % of total recovered in urine ± S.D. and number of patients (n)		
	Without severe liver damage	With severe liver damage	All	Without severe liver damage	With severe liver damage	All
Untreated	0.97 ± 0.45 (5)	1.48 ± 0.76 (3)	1.16 ± 0.59 (8)	7.9 ± 3.2 (5)	8.3 ± 3.6 (3)	8.0 ± 3.1 (8)
Cysteamine	1.32 ± 0.73 (11)	-	1.32 ± 0.73 (11)	8.7 ± 3.1 (11)	-	8.7 ± 3.1 (11)
Methionine	1.20 ± 0.37 (7)	1.20 (2)	1.20 ± 0.41 (9)	8.2 ± 3.1 (7)	7.9 (2)	8.1 ± 2.8 (9)
NAC*	1.83 ± 1.11 (13)	1.63 (1)	1.81 ± 1.07 (14)	11.4 ± 4.6 (13)	9.1 (1)	11.2 ± 4.5 (14)
All specific treatments	1.51 ± 0.88 (31)	1.34 ± 0.59 (3)	1.49 ± 0.85 (34)	9.7 ± 4.0 (31)	8.3 ± 2.1 (3)	9.6 ± 3.8 (34)
TOTAL	1.43 ± 0.85 (36)	1.41 ± 0.61 (6)	1.43 ± 0.82 (42)	9.5 ± 3.9 (36)	8.3 ± 2.6 (6)	9.3 ± 3.7 (42)

* Not including patient N8

each of the other groups but the difference was not significant in any case.

Specific treatment was associated with a significant increase in the mean amounts and proportions of drug excreted as paracetamol sulphate (mean values 1.83 ± 0.61g, 12.3 ± 3.9% in all treated patients and 1.12 ± 0.26g 8.4 ± 2.8% in untreated patients; $p < .005$ and $p < .02$ respectively, Table 48). Considering each treatment separately, the increase was highly significant with NAC (2.12 ± 0.64g, 14.2 ± 4.3%; $p < .001$ and $p < .005$ respectively), significant with cysteamine (1.68 ± 0.53g, 11.5 ± 3.1%; $p < .02$ and $p < .05$ respectively) but not significant with methionine. Patients with severe liver damage usually excreted a smaller proportion of the total recovery as paracetamol sulphate than those without severe liver damage, although the differences were generally minor (overall mean values 9.1 ± 1.9% and 12.0 ± 4.1% respectively, Table 48).

The mean amounts of paracetamol glucuronide excreted by each treatment group were similar (Table 49). However, the mean proportions of total drug recovered as paracetamol glucuronide in patients given NAC was significantly lower than in each of the other treatment groups, both in patients without severe liver damage and overall (Table 49). There were no consistent differences in terms of the amounts excreted in relation to liver damage, although generally lower proportions of drug were excreted as paracetamol glucuronide by patients with severe liver damage.

TABLE 48

Mean amounts and percentages of drug recovered as paracetamol sulphate following paracetamol overdosage in treated and untreated patients. (Treatment commenced within 10 hours of paracetamol ingestion in all specifically-treated patients.)

Treatment	Mean amount recovered in urine (g) ± S.D. and number of patients (n)			Mean % of total recovered in urine ± S.D. and number of patients (n)		
	Without severe liver damage	With severe liver damage	All	Without severe liver damage	With severe liver damage	All
Untreated	0.99 ± 0.25 (5)	1.33 ± 0.10 (3)	1.12 ± 0.26 (8)	8.6 ± 3.4 (5)	8.0 ± 2.2 (3)	8.4 ± 2.8 (8)
Cysteamine	1.68 ± 0.53** (11)	-	1.68 ± 0.53** (11)	11.5 ± 3.1 (11)	-	11.5 ± 3.1* (11)
Methionine	1.51 ± 0.52 (7)	1.52 (2)	1.51 ± 0.47 (9)	10.0 ± 2.8 (7)	10.5 (2)	10.1 ± 2.4 (9)
NAC	2.15 ± 0.66 †† (14)	1.78 (1)	2.12 ± 0.64◊ (15)	14.5 ± 4.3** (14)	9.9 (1)	14.2 ± 4.3†† (15)
All specific treatments	1.85 ± 0.63 † (32)	1.60 ± 0.32 (3)	1.83 ± 0.61†† (35)	12.5 ± 4.0* (32)	10.3 ± 0.53 (3)	12.3 ± 3.9** (35)
TOTAL	1.73 ± 0.66 (37)	1.47 ± 0.26 (6)	1.69 ± 0.63 (43)	12.0 ± 4.1 (37)	9.1 ± 1.9 (6)	11.6 ± 4.0 (43)

* $p<.05$; ** $p<.02$; † $p<.01$; †† $p<.005$; ◊ $p<.001$ - each in comparison with the respective untreated group

TABLE 49

Mean amounts and percentages of drug recovered as paracetamol glucuronide following paracetamol overdosage in treated and untreated patients. (Treatment commenced within 10 hours of paracetamol ingestion in all specifically-treated patients.)

Treatment	Mean amount recovered in urine (g) ± S.D. and number of patients (n)			Mean % of total recovered in urine ± S.D. and number of patients (n)		
	Without severe liver damage	With severe liver damage	All	Without severe liver damage	With severe liver damage	All
Untreated	9.3 ± 2.4 (5)	12.1 ± 2.7 (3)	10.4 ± 2.7 (8)	76.2 ± 4.2 †† (5)	70.0 ± 1.5 (3)	73.9 ± 4.6†† (8)
Cysteamine	11.2 ± 3.7 (11)	-	11.2 ± 3.7 (11)	73.6 ± 4.5** (11)	-	73.6 ± 4.5** (11)
Methionine	10.9 ± 1.3 (7)	9.9 (2)	10.7 ± 1.5 (9)	73.8 ± 6.2† (7)	68.7 (2)	72.6 ± 6.2 † (9)
NAC*	10.1 ± 2.9 (13)	12.2 (1)	10.3 ± 2.8 (14)	64.8 ± 6.2 (13)	67.8 (1)	65.0 ± 6.0 (14)
All specific treatments	10.7 ± 2.9 (31)	10.6 ± 2.1 (3)	10.7 ± 2.8 (34)	69.9 ± 7.1 (31)	68.4 ± 4.3 (3)	69.8 ± 6.9 (34)
TOTAL	10.5 ± 2.9 (36)	11.4 ± 2.3 (6)	10.6 ± 2.8 (42)	70.8 ± 7.1 (36)	69.2 ± 3.0 (6)	70.6 ± 6.6 (42)

* Not including patient N8; † $p<.01$; †† $p<.005$; ** $p<.001$ - each in comparison with the respective NAC-treated group

The combined recovery of paracetamol mercapturic acid and cysteine conjugates was markedly higher in the groups with severe liver damage than in those without (Table 50). Considering the 4 treatment groups combined, patients without severe liver damage excreted a mean of 1.21 ± 0.72g (8.0 ± 3.4%) compared with 2.27 ± 0.87g (13.4 ± 2.7%) in patients with severe liver damage; $p < .005$ and $p < .001$ with respect to the amounts and proportions respectively. In the patients without severe liver damage, those given NAC excreted a larger mean amount and proportion of total drug recovery as the mercapturic acid and cysteine conjugates than each of the other groups. In relation to the cysteamine-treated group, the difference in these proportions was significant ($p < .02$; Table 50). Considering all the patients in each group, the cysteamine-treated group excreted a significantly smaller mean percentage as the mercapturic acid and cysteine conjugates than each of the other treatment groups (Table 50). A mean of 6.2 ± 2.0% was excreted as these compounds by the patients given cysteamine, compared with 9.7 ± 4.6% in the untreated group ($p < .05$), 9.1 ± 3.7% in the patients given methionine ($p < .05$) and 10.0 ± 3.7% in the NAC-treated group ($p < .01$).

The observations noted above for the excretion of the mercapturic acid and cysteine conjugates combined generally reflected the situation with the individual

TABLE 50

Mean amounts and percentages of drug recovered as paracetamol mercapturic acid and cysteine conjugates combined following paracetamol overdosage in treated and untreated patients. (Treatment commenced within 10 hours of paracetamol ingestion in all specifically-treated patients.)

Treatment	Mean amount recovered in urine (g) ± S.D. and number of patients (n)			Mean % of total recovered in urine ± S.D. and number of patients (n)		
	Without severe liver damage	With severe liver damage	All	Without severe liver damage	With severe liver damage	All
Untreated	0.84 ± 0.30 (5)	2.44 ± 1.06 (c) (3)	1.44 ± 1.03 (8)	7.3 ± 3.4 (5)	13.8 ± 3.2 (a) (3)	9.7 ± 4.6 (f) (8)
Cysteamine	0.98 ± 0.53 (11)	-	0.98 ± 0.53 (11)	6.2 ± 2.0 (11)	-	6.2 ± 2.0 (11)
Methionine	1.20 ± 0.51 (7)	1.97 (2)	1.37 ± 0.69 (9)	8.0 ± 3.0 (7)	13.0 (2)	9.1 ± 3.7 (f) (9)
NAC	1.54 ± 0.92 (14)	2.38 (1)	1.59 ± 0.91 (15)	9.7 ± 3.8 (g) (14)	13.2 (1)	10.0 ± 3.7 (h) (15)
All specific treatments	1.27 ± 0.75 (32)	2.11 ± 0.83 (3)	1.34 ± 0.78 (35)	8.1 ± 3.4 (32)	13.1 ± 2.7 (b) (3)	8.6 ± 3.6 (35)
TOTAL	1.21 ± 0.72 (37)	2.27 ± 0.87 (d) (6)	1.36 ± 0.82 (43)	8.0 ± 3.4 (37)	13.4 ± 2.7 (e) (6)	8.8 ± 3.8 (43)

(a) $p<.05$; (b) $p<.025$; (c) $p<.02$; (d) $p<.005$; (e) p .001 - compared with respective group without severe liver damage.

(f) $p<.05$; (g) $p<.02$; (h) $p<.01$ - compared with respective cysteamine-treated group.

compounds (Tables 44 and 45 a-d). The excretion of both compounds was equally limited following cysteamine treatment. In addition, proportionately more cysteine conjugate than mercapturic acid conjugate was excreted by patients with severe liver damage than by those without in the untreated and methionine-treated groups. The mean ratios of cysteine to mercapturic acid conjugate excreted by these groups combined were 1.22 ± 0.52 and 0.77 ± 0.21 in patients with and without severe liver damage respectively ($p < .02$).

b) Summary

1) The pattern of urinary excretion of paracetamol after overdosage was considerably different from the pattern after therapeutic dosage.

In relation to the total drug recovered in urine:-

(a) The proportion recovered as paracetamol sulphate was significantly reduced and was inversely proportional to the total recovery of paracetamol.

(b) The proportions recovered unchanged and as paracetamol glucuronide were significantly increased.

(c) The proportions recovered as the cysteine and mercapturic acid conjugates were not significantly different, except in patients who developed severe liver damage.

2) Significantly larger amounts and proportions of drug (about double) were excreted as the mercapturic acid and

cysteine conjugates by patients with severe liver damage than by those without.

3) Excretion of unchanged paracetamol and its sulphate and glucuronide conjugates was not significantly different in untreated patients with and without severe liver damage.

4) Treatment with cysteamine and particularly NAC was associated with significantly increased excretion of paracetamol sulphate. The increase observed with methionine was not statistically significant.

5) A significantly reduced percentage of paracetamol was recovered as the glucuronide conjugate in patients given NAC.

6) Treatment with cysteamine significantly reduced the proportion of paracetamol excreted as its mercapturic acid and cysteine conjugates. In patients without severe liver damage, a larger mean amount and proportion of drug was recovered as these conjugates in those given NAC than in each of the other groups.

c) Discussion

The change in the urinary excretion pattern of paracetamol and its metabolites observed with increased dosage is consistent with saturation of sulphate conjugation and compensation by increased glucuronide conjugation (as also noted by Davis et al, 1976) and increased renal excretion of unchanged drug, as observed

by Prescott and Wright (1973). The larger amounts of the mercapturic acid and cysteine conjugates produced following paracetamol overdosage indicate that the production of the toxic intermediate increased with the dose. As expected, the amounts and proportions of drug excreted as these conjugates were larger in patients who developed severe liver damage, as also noted by Davis et al, 1976 and Howie et al, 1977. In addition, patients without severe liver damage excreted similar proportions of drug as the mercapturic acid and cysteine conjugates as did healthy volunteers after a therapeutic dose, whereas in patients with severe liver damage, the proportions were significantly higher.

In treated patients, protection against liver damage was apparent despite ingestion of large doses of paracetamol, although this was less obvious after treatment with methionine. The most consistent metabolic effect of specific treatment was an increase in the amount and proportion of paracetamol excreted as the sulphate conjugate. This finding was in keeping with the observation of increased plasma paracetamol sulphate concentrations following NAC and cysteamine treatment (pp 214-215). Similar increases were observed with the related compound, cysteine, after sulphate conjugation of paracetamol had been competitively inhibited with salicylamide (Levy and Yamada, 1971).

The excretion of the mercapturic acid and cysteine

conjugates was affected in different ways by the different treatments. The results of treatment with methionine were similar to those in the untreated patients with regard to liver damage and excretion of these conjugates. This suggests that methionine has little or no effect on the production or detoxification of the toxic intermediate metabolite, as also observed in vitro by Buckpitt et al (1979). Cysteamine appeared to reduce the excretion of the mercapturic acid and cysteine conjugates. Therefore, a possible explanation for the hepato-protective action of cysteamine is that it inhibits the formation of the toxic intermediate which causes liver damage (Harvey and Levitt, 1976; Buckpitt et al, 1979). However, large quantities of the reactive intermediate must already have been formed before treatment was given. In contrast, NAC appeared to increase the excretion of these conjugates but it still protected against liver damage. Thus NAC might act as a direct precursor of glutathione or it might undergo direct conjugation with the toxic metabolite (Strubelt et al, 1974; Buckpitt et al, 1979).

By far the largest proportion of paracetamol was recovered as the glucuronide conjugate, irrespective of treatment or liver damage. However, treatment with NAC significantly lowered the proportion excreted as this conjugate. This was probably due to the enhanced sulphate conjugation and possibly increased excretion of

the mercapturic and cysteine conjugates. With cysteamine, sulphate conjugation was increased and glutathione conjugation was decreased resulting in little overall effect on glucuronide conjugation.

Chapter 7

Changes in the Urinary Excretion of Paracetamol and its Conjugates with Time

Paracetamol and its conjugates were measured in sequential urine samples from treated and untreated patients. The data were assessed graphically by comparing individual excretion patterns with time and also the excretion patterns in different treatment groups during various intervals after overdosage. Mean individual excretion rates and the proportions of the total drug excreted as each compound were plotted at the mean mid-points of the urine collection intervals. In some cases plots were constructed using data from 3 urine collection intervals only (approximately 0 - 15, 15 - 24 and > 24 hours after paracetamol ingestion).

Four untreated patients who developed only trivial liver damage and 2 untreated patients who developed severe liver damage were studied in detail. Five treated patients who developed trivial liver damage (3 given NAC and 2 given cysteine, within 10 hours of paracetamol ingestion) and 2 patients treated late with NAC who developed severe liver damage were studied similarly (Table 51). Two other groups were given cysteamine and methionine within 10 hours of paracetamol ingestion (Table 51).

TABLE 51

Changes in the urinary excretion of paracetamol and its conjugates with time - details of patients studied.

Treatment and Outcome	Patient Nos. *	Ingestion - treatment interval (hour)	Total urinary recovery of Paracetamol (g)
Untreated, no severe liver damage	U6, 8 10 and 11	-	9.5 ± 0.6
Untreated, severe liver damage	U1	-	21.7
	U12	-	8.1
NAC/Cysteine,** no severe liver damage	N2, 3, 20† N18, 19 ††	7.8 ± 1.4	13.1 ± 3.9
NAC, severe liver damage	N16	10.2	11.0
	N17	11.7	9.1
Cysteamine,** no severe liver damage	C7, 8, 10	8.4 ± 1.4	17.8 ± 4.2
Methionine,** severe liver damage	M6, 7, 10	9.4 ± 0.6	13.7 ± 3.5

* Full details in Table 34

** All treated within 10 hours of paracetamol ingestion

† given NAC

†† given cysteine

Values are mean ± S.D.

a) Results

i) Untreated Patients: In the patients without severe liver damage, the mean excretion rate of unchanged paracetamol declined from the outset with a half-life of 3.6 hours (Fig 36a, Table 52). Excretion of the sulphate and glucuronide conjugates was maximal in the first period but declined slowly at first. Excretion of the mercapturic acid and cysteine conjugates was relatively slow at first, peaking in the second interval and subsequently paralleling that of the unchanged drug.

The excretion rate-time patterns of the paracetamol metabolites in patients U1 and U12 (Fig 37, Tables 53 and 54) followed that of the unchanged drug in a similar manner to that observed in the patients without severe liver damage. However, the urinary half-life of paracetamol was prolonged in patient U1 but short in patient U12. Renal function in patient U1 was not impaired until 40 hours after paracetamol ingestion. The maximum excretion rate of paracetamol sulphate was similar (60 - 70 mg/hr) in all the untreated patients but the decline was most rapid in patient U12. Paracetamol glucuronide was excreted at a very high rate compared to patients without severe liver damage, particularly by patient U1. In both patients large amounts of the mercapturic acid and the cysteine conjugates were excreted, especially in patient U1.

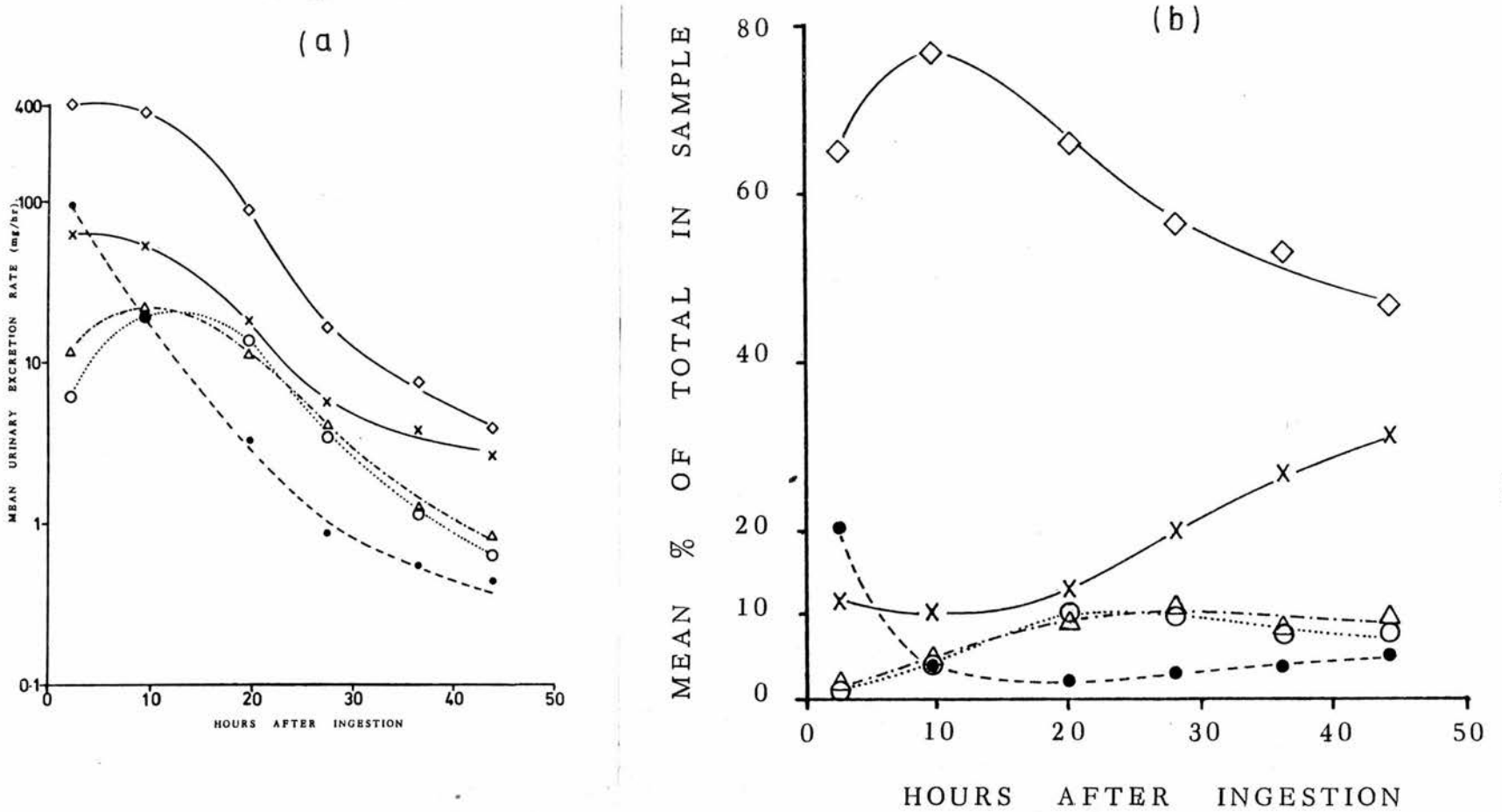


Fig. 36: Urinary excretion of paracetamol and its conjugates following paracetamol overdosage in 4 untreated patients who sustained no liver damage subsequently. (The mean overall recovery was 9.5 ± 0.6 g paracetamol equivalent).

(a) Changes in mean idividual urinary excretion rates with time.

(b) Changes in mean individual proportional excretion with time.

● = Paracetamol (P); X = P. Sulphate; ◇ = P. Glucuronide; △ = P. Mercapturate; O = P. Cysteine.

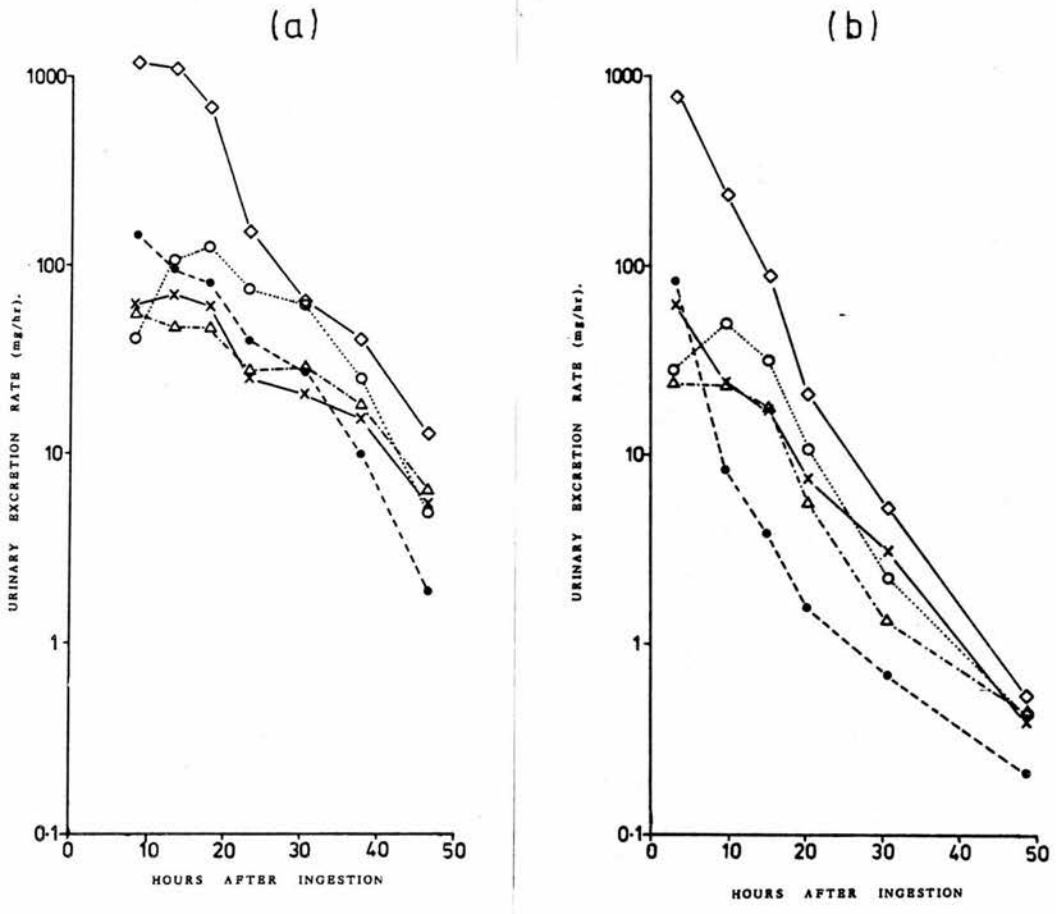
TABLE 52

Mean individual urinary excretion rates and proportional excretion of paracetamol and its conjugates in 4 untreated patients who sustained no liver damage following paracetamol overdosage. The mean overall recovery of paracetamol was 9.5 ± 0.6 g.

Mean urine collection period (hr)	Mean mid-point time of urine collection (hr) (and number of patients)		Mean individual urinary excretion rate (mg/hr)					Mean individual percentage of the total in each urine collection excreted as:-				
			P	S	G	M	C	P	S	G	M	C
0-4.6	2.3	(3)	95	62	406	12	6	20.4	11.7	65.2	1.7	1.0
4.6-15.6	9.5	(4)	19	53	369	22	19	3.9	10.5	76.8	4.7	4.2
15.6-24	20	(4)	3.3	18	90	11	14	2.3	12.9	65.8	9.0	10.0
24-31	28	(4)	0.86	5.6	17	4.1	3.5	3.0	20.1	56.4	10.6	10.0
31-41	36	(2)	0.55	3.9	7.7	1.3	1.2	3.7	26.8	53.2	8.5	7.8
41-47	44	(2)	0.45	2.7	3.9	0.83	0.63	5.2	31.2	46.4	9.7	7.4

KEY:

P = Paracetamol; S, G, M, C = Paracetamol sulphate, glucuronide, mercapturic acid and cysteine conjugates respectively.



Key:
Paracetamol ..... --●--
Paracetamol Sulphate ..... —×—
Paracetamol Glucuronide... —◇—
Paracetamol Mercapturate .. ·-·△·-·
Paracetamol Cysteine ..... ·····○·····

Fig.37: Changes in the urinary excretion rates of paracetamol and its conjugates with time in 2 untreated patients who developed severe liver damage following paracetamol overdosage.

(a) Patient U1: Total urinary recovery = 21.7g paracetamol equivalent. (This patient subsequently died in hepatic failure).

(b) Patient U12: Total urinary recovery = 8.1g paracetamol equivalent.

TABLE 53

Urinary excretion rates and proportional excretion of paracetamol and its conjugates in an untreated patient (U1) who developed severe liver damage following paracetamol overdosage. The overall recovery of paracetamol was 21.7 g and the patient subsequently died in hepatic failure

Urine collection period (hr)	Mid-point time of urine collection (hr)	Urinary excretion rate (mg/hr)					Percentage of the total in each urine collection excreted as:-				
		P	S	G	M	C	P	S	G	M	C
6.7-10	8.4	147	61	1219	56	40	9.7	4.0	80.1	3.7	2.6
10-16.8	13.4	97	70	1098	46	106	6.8	5.0	77.5	3.3	7.5
16.8-19	17.9	81	61	700	46	126	8.0	6.1	69.0	4.5	12.4
19-27	23	40	26	151	28	76	12.4	8.2	47.1	8.7	23.6
27-33	30	28	21	66	29	63	13.4	10.0	31.9	13.9	30.7
33-42	38	10	16	41	19	26	9.1	14.0	37.2	16.6	23.1
42-51	47	1.9	5.5	13	6.5	5.0	5.9	17.5	40.2	20.6	15.8

KEY:

P = Paracetamol; S, G, M, C = paracetamol sulphate, glucuronide, mercapturic acid and cysteine conjugates respectively.

TABLE 54

Urinary excretion rates and proportional excretion of paracetamol and its conjugates in an untreated patient (U12) who developed severe liver damage following paracetamol overdosage. The overall recovery of paracetamol was 8.1 g.

Urine collection period (hr)	Mid-point time of urine collection (hr)	Urinary excretion rate (mg/hr)					Percentage of the total in each urine collection excreted as:-				
		P	S	G	M	C	P	S	G	M	C
0-5.5	2.8	84	63	789	24	28	8.5	6.4	79.8	2.4	2.9
5.5-13.3	9.4	8.4	24	237	24	50	2.5	7.1	69.1	6.9	14.6
13.3-16.5	14.9	3.9	17	87	17	32	2.5	11.0	55.5	11.1	20.0
16.5-24	20	1.6	7.6	21	5.6	11	3.3	16.3	45.2	12.0	23.2
24-38	31	0.69	3.2	5.2	1.3	2.2	5.5	25.1	41.1	10.6	17.7
38-60	49	0.21	0.39	0.53	0.44	0.43	10.4	19.4	26.5	22.3	21.5

KEY:

P = Paracetamol; S, G, M, C = Paracetamol sulphate, glucuronide, mercapturic acid and cysteine conjugates respectively

The proportion of drug excreted unchanged in the patients without severe liver damage was initially high (mean 20%; Fig.36b, Table 52) but only 5% or less was recovered unchanged after 10 hours or so. The proportion excreted as paracetamol sulphate was about 12% initially and after falling slightly this increased steadily to 31% after 40 hours. In contrast, the proportion excreted as the glucuronide conjugate peaked at 77% in the second collection period and then declined steadily to 46%. The mercapturic acid and cysteine conjugates each accounted for less than 2% of the total drug excreted initially, increasing to 7 - 10% each subsequently.

The proportional excretion patterns in patients U1 and U12 (Tables 53 and 54, Fig 38) differed in detail from that of the patients without severe liver damage. Notable differences were the very low proportion of drug excreted as paracetamol sulphate initially and the dramatic rise in the proportions excreted as the mercapturic acid and the cysteine conjugates.

ii) Treated Patients: In the group without severe liver damage the mean excretion rate of all 5 compounds increased initially (Fig 39a, Table 55). Subsequently, unchanged paracetamol was excreted with a half-life of 2.8 hours and the mean excretion rate of paracetamol glucuronide declined from a peak of 733 mg/hr in parallel with unchanged drug. In contrast, the mean excretion rate of paracetamol sulphate remained near the peak of

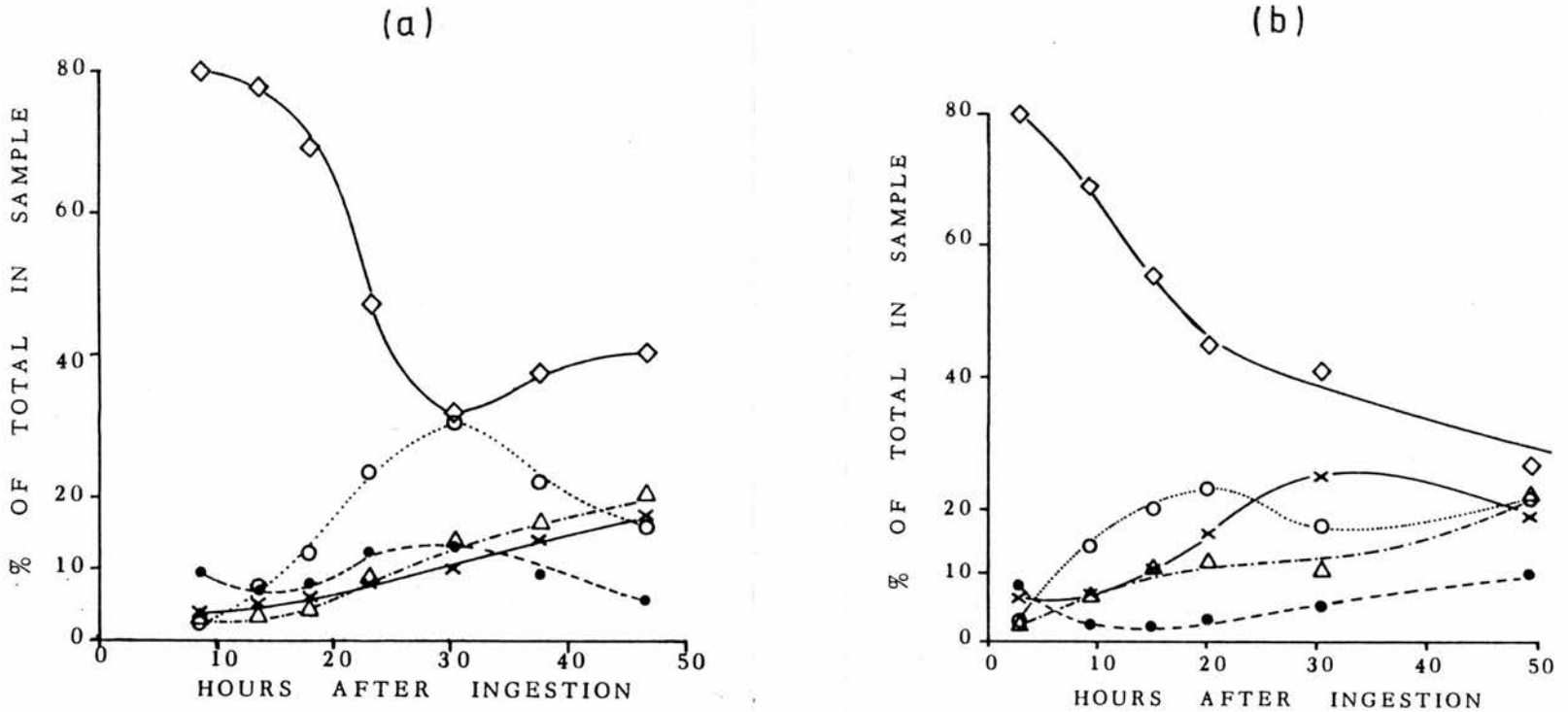


Fig. 38 : Changes in the proportional urinary excretion of paracetamol and its conjugates with time in 2 untreated patients who developed severe liver damage following paracetamol overdosage.

(a) Patient U1: Total urinary recovery 21.7g paracetamol equivalent. (This patient subsequently died in hepatic failure).

(b) Patient U12 : Total urinary recovery 8.1g paracetamol equivalent.

Key: ●= Paracetamol (P); ×= P. Sulphate; ◇ = P. Glucuronide; △ = P. Mercapturate; O = P. Cysteine.

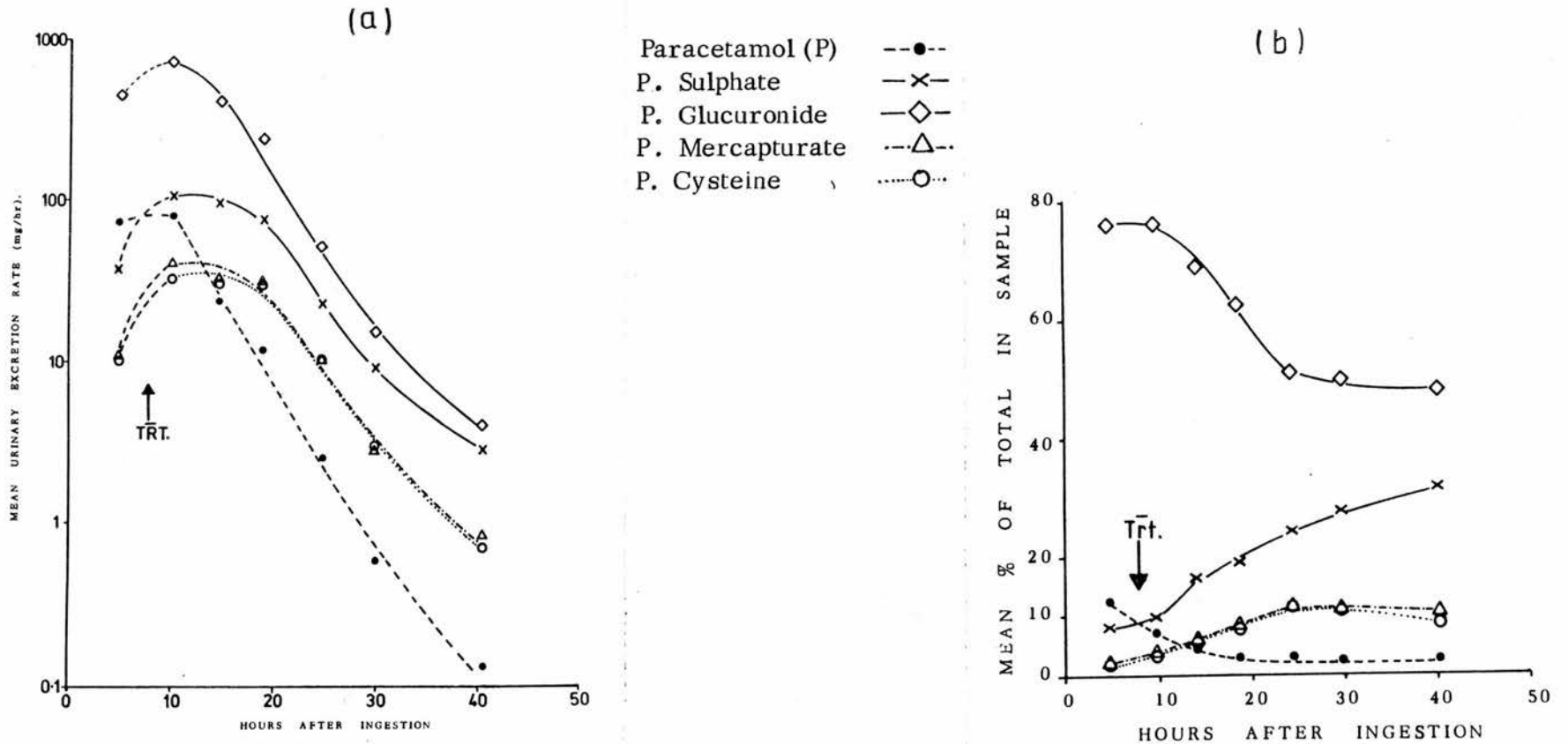


Fig. 39: Urinary excretion of paracetamol and its conjugates following paracetamol overdosage in 5 patients without severe liver damage following treatment with NAC (3) and cysteine (2) within 10 hours of paracetamol ingestion. (The mean total recovery was 13.1 ± 3.9g paracetamol equivalent).

(a) Changes in mean individual urinary excretion rates with time.

(b) Changes in mean individual proportional excretion with time.

$\bar{T}rt$. denotes the mean ingestion-to-treatment interval.

TABLE 55

Mean individual urinary excretion rates and proportional excretion of paracetamol and its conjugates in 5 patients treated with NAC (3) and cysteine (2) following paracetamol overdosage. All patients were treated within 10 hours of paracetamol ingestion (mean 7.8 ± 1.4 hours) and none sustained liver damage. The mean overall recovery of paracetamol was 13.1 ± 3.9 g.

Mean urine collection period (hr)	Mean mid-point time of urine collections (hr) (and number of patients)	Mean individual urinary excretion rate (mg/hr)					Mean individual percentage of the total in each urine collection excreted as:-				
		P	S	G	M	C	P	S	G	M	C
0? -8.7	4.9 ? * (5)	72	38	457	11	10	12.3	8.0	75.9	2.0	1.8
8.7-11.6	10.1 (5)	81	104	733	41	33	7.1	9.8	76.3	3.8	3.1
11.6-17.5	14.6 (5)	25	98	423	34	30	4.1	16.2	68.8	5.7	5.3
17.5-20.6	18.9 (4)	12	77	246	32	31	2.8	18.8	62.5	8.2	7.6
20.6-29.1	25 (5)	2.6	24	54	11	11	2.7	24.1	50.9	11.1	11.2
29.1-32.7	30 (4)	0.58	9.4	16	2.8	3.0	1.2	27.5	49.8	11.0	10.6
32.7-47.3	41 (4)	0.13	2.9	4.1	0.84	0.70	1.4	31.7	48.0	10.5	8.3

KEY:

P = Paracetamol; S, G, M, C = Paracetamol sulphate, glucuronide, mercapturic acid and cysteine conjugates respectively.

* = urine collections probably incomplete

104 mg/hr for about 15 hours before declining rapidly and was more than double the rate observed in the untreated group between 12 and 28 hours (Figs 36a and 39a).

The pattern of urinary excretion of the mercapturic acid and cysteine conjugates was similar to that of the sulphate conjugate. The mean excretion rates(maximum 41 and 33 mg/hr for the mercapturic acid and cysteine conjugates respectively) were almost double the rates observed in the untreated group (Figs. 36a and 39a).

The urinary excretion of paracetamol and its conjugates in the patients with severe liver damage following late treatment with NAC was relatively rapid in one (patient N16; Fig. 40a, Table 56) but prolonged in the other (patient N17; Fig. 40b, Table 57). Renal function was normal. The urinary half-life of unchanged drug was 3.5 hours in patient N16 and 7.5 hours in patient N17. The initial excretion rate of paracetamol sulphate in both patients was comparable with the early-treated group. The predominance of paracetamol cysteine over paracetamol mercapturate resembled that seen in the 2 untreated patients with severe liver damage.

The mean proportion of paracetamol excreted unchanged by the early-treated group was initially high (12%, Fig. 39b Table 55), falling to less than 5%. However, the mean proportion excreted as paracetamol sulphate was very low at first (8%) but rose progressively to 32%. Paracetamol glucuronide followed the opposite course, falling rapidly

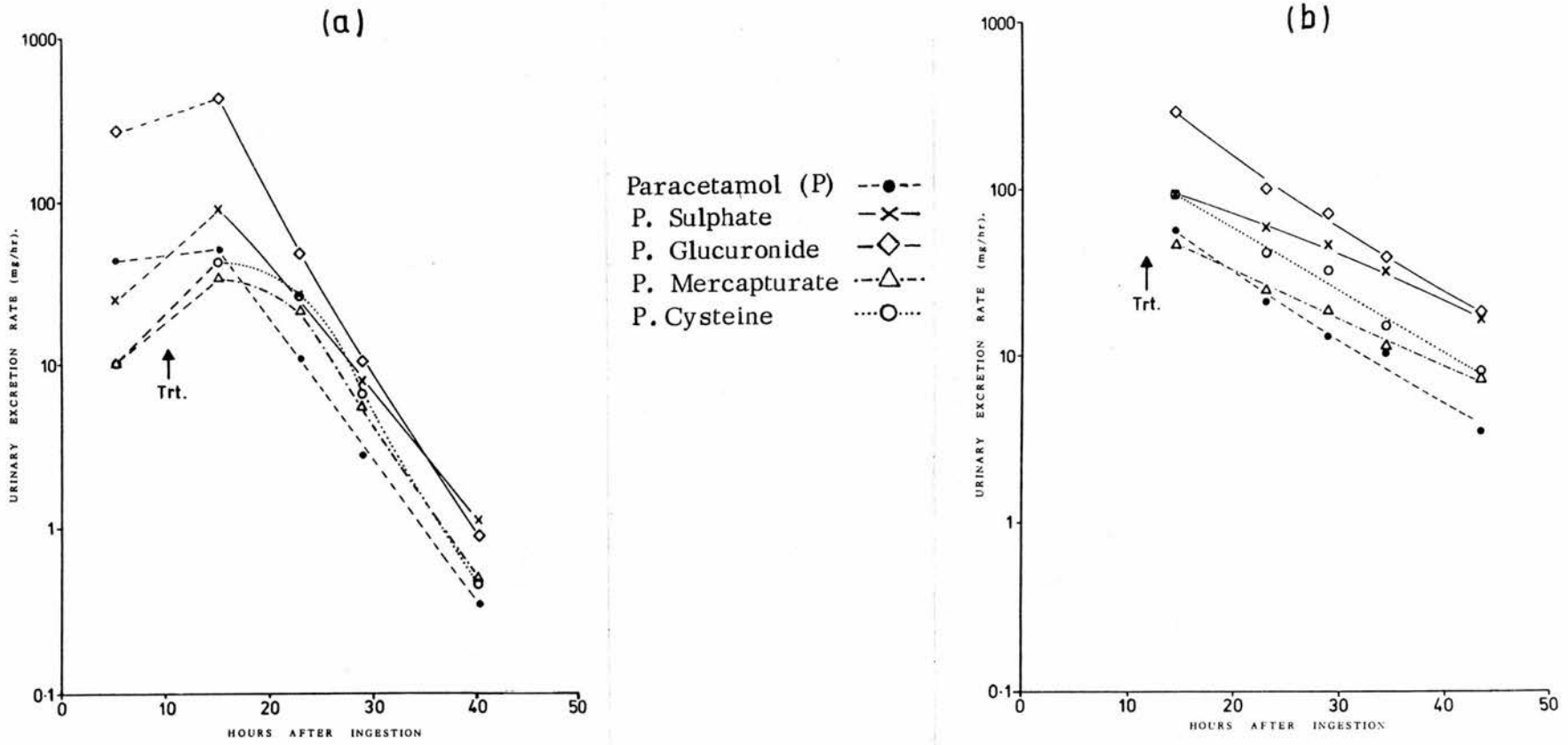


Fig. 40: Urinary excretion rates of paracetamol and its conjugates in 2 patients who were treated with NAC more than 10 hours after paracetamol overdosage.

(a) Patient N16: 11.0g paracetamol equivalent recovered overall.

(b) Patient N17: 9.1g paracetamol equivalent recovered overall.

Treatment (Trt.) commenced 10.2 hrs and 11.7 hrs after paracetamol ingestion in patients N16 and N17 respectively.

TABLE 56

Urinary excretion rates and proportional excretion of paracetamol and its conjugates in a patient (N16) who was treated with NAC 10.2 hours after paracetamol overdosage. The patient developed severe liver damage and 11.0 g. of paracetamol was recovered overall.

Urine collection period (hr)	Mid-point time of urine collection (hr)	Urinary excretion rate (mg/hr)					Percentage of the total in each urine collection excreted as:-				
		P	S	G	M	C	P	S	G	M	C
0? -10.6	5.3 ? *	44	25	269	10	10	12.2	7.0	75.2	2.3	2.8
10.6-19.8	15.2	51	91	439	34	43	7.7	13.8	66.8	5.2	6.6
19.8-26	23	11	27	48	21	28	7.8	20.1	35.4	15.9	20.8
26-32	29	2.8	7.9	10	5.4	6.6	8.3	24.0	31.4	16.3	20.0
32-49	40	0.34	1.1	0.91	0.59	0.55	9.9	31.1	26.1	17.1	15.8

KEY:

P = Paracetamol; S, G, M, C = Paracetamol sulphate, glucuronide, mercapturic acid and cysteine conjugates respectively

* = Urine collection probably incomplete

TABLE 57

Urinary excretion rates and proportional excretion of paracetamol and its conjugates in a patient (N17) who was treated 11.7 hours after paracetamol overdosage. The patient developed severe liver damage and 9.1 g. of paracetamol was recovered overall.

Urine collection period (hr)	Mid-point time of urine collection (hr)	Urinary excretion rate (mg/hr)					Percentage of the total in each urine collection excreted as:-				
		P	S	G	M	G	P	S	G	M	C
0-10.5	Sample lost	-	-	-	-	-	-	-	-	-	-
10.5-18.8	14.6	58	95	293	46	95	9.8	16.2	50.0	7.9	16.1
18.8-28	23	21	60	104	25	42	8.4	23.8	41.4	9.9	16.5
28-30	29	13	48	75	19	33	7.1	25.7	39.7	10.1	17.4
30-38	34	10	33	40	12	15	9.4	29.9	36.4	10.6	13.8
38-49	44	3.6	17	19	7.4	8.2	6.5	30.7	34.4	13.4	15.0

KEY:

P = Paracetamol; S, G, M, C = Paracetamol sulphate, glucuronide, mercapturic acid and cysteine conjugates respectively

from 76% to about 50% at 30 hours. The mean proportions excreted as the mercapturic acid and cysteine conjugate increased steadily from 2% to 11% each over the same period.

The proportional excretion pattern in patient N16 (Fig. 41a) was similar to that observed in the early-treated group (Fig 39b). However, unchanged paracetamol accounted for a higher proportion and the proportion excreted as paracetamol glucuronide declined more sharply (to about 30%). The proportions excreted as the mercapturic acid and cysteine conjugates rose more rapidly to much higher levels (more than 16% each). The pattern in patient N17 (Fig. 41b) was similar to patient N16 but the changes were less dramatic.

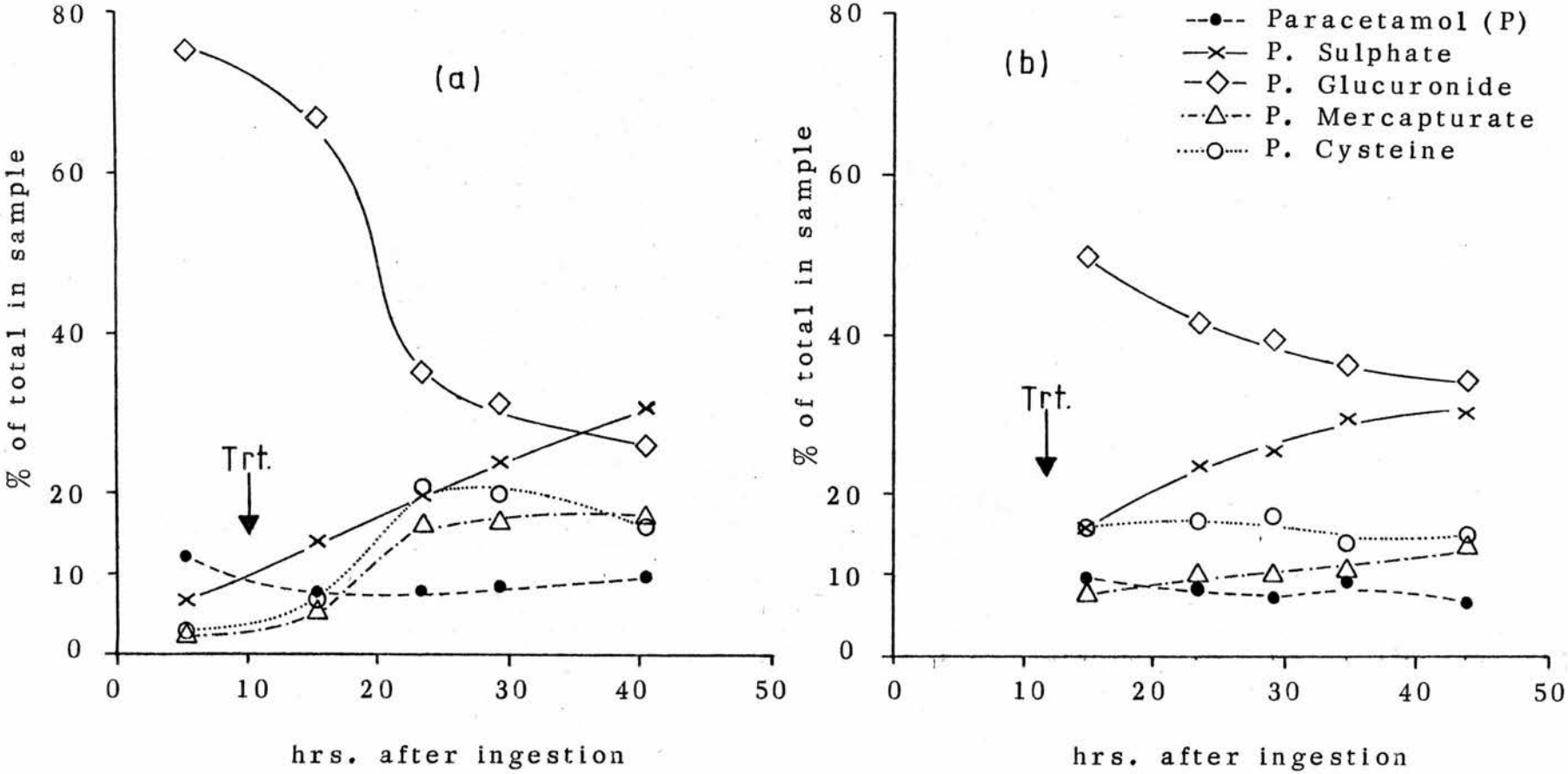
iii) Urinary Excretion of Paracetamol and its Conjugates at Intervals After Overdosage

Important differences between the groups were observed in the urinary excretion of paracetamol and its conjugates in the 3 intervals studied (Fig. 42, Table 58a and b). In the group given cysteamine, the proportional excretion of the mercapturic acid and cysteine conjugates was particularly low at all times. Less than 5% was excreted as each compound compared with 10% or more in the other groups. The amounts excreted were comparable in all 3 groups without severe liver damage.

Fig.41: Proportional excretion of paracetamol and its conjugates in the urine of 2 patients who were treated with NAC more than 10 hours after paracetamol overdosage. Both patients developed severe liver damage.

(a) Patient N16: treatment (Trt) commenced 10.2 hrs after paracetamol ingestion; 11.0 g paracetamol equivalent recovered overall.

(b) Patient N17: treatment (Trt) commenced 11.7 hrs after paracetamol ingestion; 9.1 g paracetamol equivalent recovered overall.



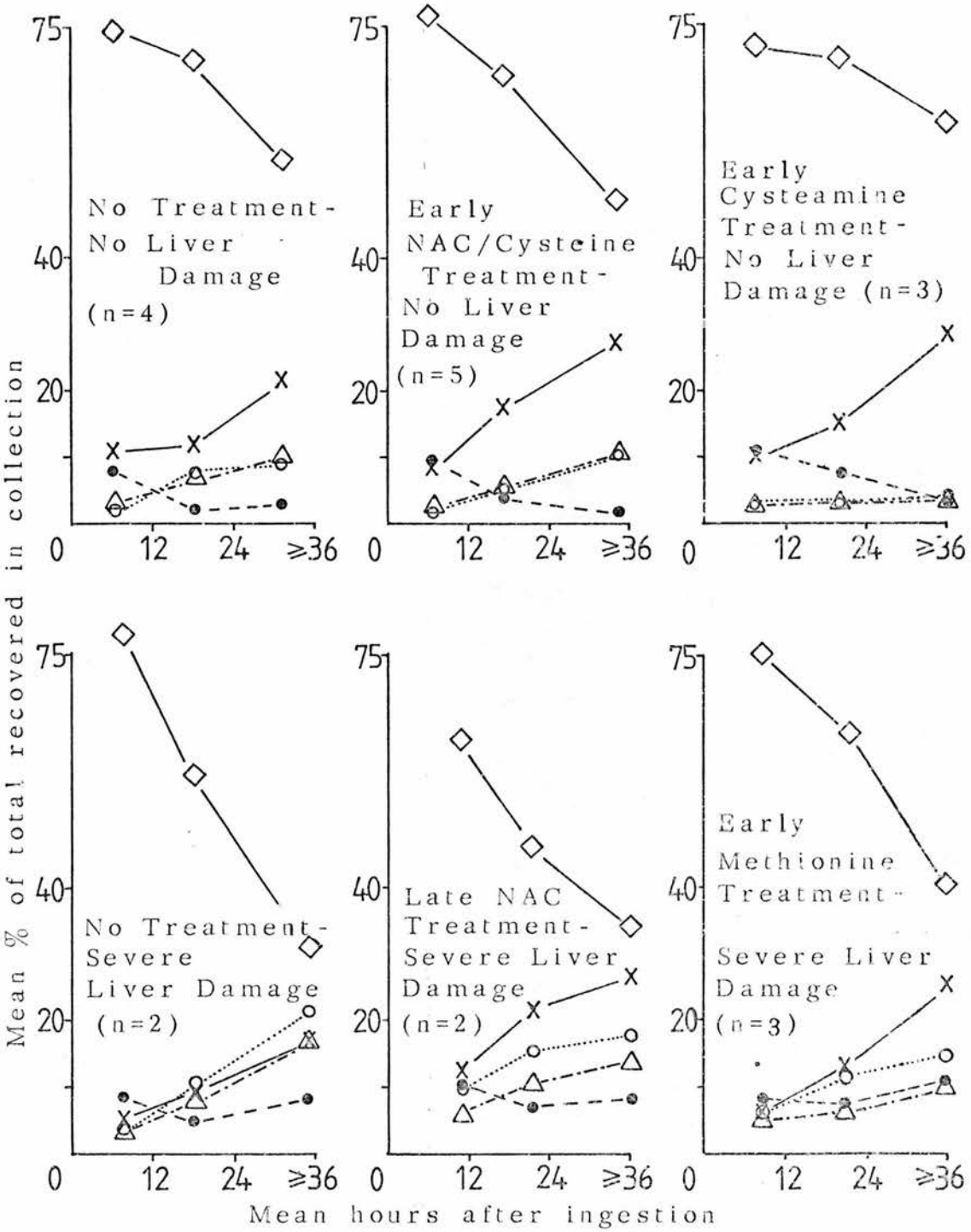


Fig.42: Change in the proportional excretion of paracetamol and its conjugates in urine collected at different intervals after paracetamol overdosage, in treated and untreated patients.

● = Paracetamol (P); X = P. Sulphate; O = P. Cysteine;
◇ = P. Glucuronide; △ = P. Mercapturate

TABLE 58(a)

Urinary excretion of paracetamol and its conjugates at intervals following paracetamol overdosage in treated and untreated patients who developed no more than trivial liver damage subsequently. (Specific treatment commenced within 10 hours of paracetamol ingestion.)

Treatment and number of patients	Mean urine collection interval (hour)	Mean amount excreted (mg)					% of mean overall recovery	Mean individual % of total excreted as:-				
		P	S	G	M	C		P	S	G	M	C
Untreated (n=4)	up to 12	524	743	4911	235	184	70	8.4	11.0	74.6	3.4	2.6
	12 to 24	62	267	1725	165	185	26	2.6	12.1	69.6	7.2	8.5
	> 24	11	71	177	40	34	4	3.2	21.6	55.3	10.3	9.6
NAC/Cysteine (n=5)	up to 11	713	556	5297	195	162	53	9.6	8.6	76.9	2.6	2.2
	11 to 23	218	999 -	3812	323	308	43	3.8	17.3	67.6	5.7	5.5
	> 23	12	140	232	59	59	4	2.2	27.5	48.5	11.0	10.9
Cysteamine (n=3)	up to 15	724	882	6710	276	282	50	11.1	10.8	71.7	3.2	3.3
	15 to 24	569	981	4632	340	278	38	7.8	15.3	69.8	3.5	3.5
	> 24	64	483	1319	80	95	12	3.1	28.8	60.5	3.3	4.4

KEY:- P = Paracetamol; S, G, M, C = Paracetamol sulphate, glucuronide, mercapturate and cysteine respectively.

TABLE 58(b)

Urinary excretion of paracetamol and its conjugates at intervals following paracetamol overdosage in treated and untreated patients who developed severe liver damage subsequently.

Treatment and number of patients	Mean urine collection interval (hour)	Mean amount excreted (mg)					% of mean overall recovery	Mean individual % of total excreted as:-				
		P	S	G	M	C		P	S	G	M	C
Untreated (n=2)	up to 12	471	320	4531	203	250	39	8.4	5.3	78.7	3.5	4.0
	12 to 24	542	438	5197	333	804	49	5.2	9.3	61.8	7.8	15.8
	> 24	199	236	656	280	453	12	8.5	17.1	35.9	17.0	21.5
NAC, >10 hours after ingestion (n=2)	up to 17	641	705	4046	302	497	62	10.3	12.4	61.5	5.6	10.2
	17 to 26	136	435	950	213	311	20	6.9	21.4	46.1	10.3	15.3
	> 26	144	485	682	202	290	18	8.3	26.4	34.2	13.7	17.4
Methionine, ≤10 hours after ingestion (n=3)	up to 16	744	576	6991	443	577	68	7.8	5.8	75.7	4.6	6.1
	16 to 26	232	342	1913	173	379	22	7.1	13.0	62.5	5.9	11.5
	> 26	153	336	547	118	191	10	10.6	25.1	40.4	9.5	14.4

KEY:- P = Paracetamol; S, G, M, C = Paracetamol sulphate, glucuronide, mercapturate and cysteine respectively.

The methionine-treated group who developed severe liver damage despite early treatment showed a similar excretion pattern to the other groups with severe liver damage (Table 58b, Fig. 42). In all 3 groups the mean proportion of unchanged drug was always more than 5% and large proportions and amounts were excreted as the mercapturic acid and particularly the cysteine conjugates.

Most of the drug and metabolites were recovered in the first collection period in all groups except the untreated patients with severe liver damage (Table 58a and b) and 80 - 95% of the total was usually recovered in the first 24 hours. Elimination was delayed relatively in patients with severe liver damage.

b) Summary

1) There was a common pattern in the urinary elimination of paracetamol and its metabolites following overdosage.
a) The initial relatively high proportion excreted as unchanged drug (about 10%) fell to less than 5%.
b) The proportion excreted as paracetamol glucuronide fell from 70 - 80% initially to about 50% after 24 hours, whereas paracetamol sulphate (less than 12% initially) accounted for increasing proportions subsequently (up to 30%).
c) The mercapturic acid and cysteine conjugates each accounted for very small proportions (usually 3% or less) initially but eventually accounted for 10% or more.

2) This basic pattern was modified by liver damage and treatment as follows :-

a) Patients with severe liver damage continued to excrete relatively large amounts of unchanged drug. The proportions excreted as paracetamol glucuronide fell more markedly and the proportions and amounts excreted as paracetamol sulphate were initially lower . Paracetamol mercapturate and particularly paracetamol cysteine were excreted in larger amounts and proportions at all times.

b) Treatment with NAC and cysteine and, to a lesser extent, cysteamine was associated with an increase in the amounts and proportions excreted as paracetamol sulphate.

c) The proportions excreted as the mercapturic acid and cysteine conjugates were reduced following treatment with cysteamine. NAC and cysteine apparently increased the excretion rate of these conjugates.

d) Little alteration in paracetamol elimination was observed in the patients who developed severe liver damage following treatment with methionine.

3) Elimination of paracetamol was rapid regardless of liver damage and treatment (> 80% excreted in 24 hours).

4) The pattern of urinary excretion of paracetamol with time differed markedly from that following therapeutic doses, as follows :-

a) A higher proportion was excreted unchanged initially.

b) The proportions excreted as the sulphate and glucuronide conjugates were much smaller and larger respectively

c) The proportions excreted as the mercapturic acid and cysteine conjugates were eventually about double those observed after therapeutic doses.

c) Discussion

The urinary excretion pattern of paracetamol and its metabolites after overdosage and therapeutic doses indicated early saturation or limitation of the sulphate conjugation pathway. Similar observations were made by Houston and Levy (1976) who compared the urinary elimination of 1g doses of paracetamol with and without ascorbic acid, a competitive inhibitor of sulphate conjugation. The maximum excretion rate of paracetamol sulphate appeared to be about 70 mg/hr. Levy and Yamada (1971) reported similar findings with a 2g dose of paracetamol. The renal clearance of paracetamol sulphate is not dose-dependent, and is not reduced after overdosage in the absence of renal failure (see following chapter, pp 277-281 The shortfall in sulphate conjugation was largely compensated for by glucuronide conjugation until most of the drug had been eliminated. The increased production and excretion of paracetamol sulphate following treatment with NAC suggests that availability of inorganic sulphate may be a limiting factor.

The peak excretion rates of the mercapturic acid and cysteine conjugates occurred later than with the other metabolites. This suggests that these conjugates

and/or their precursor (paracetamol glutathione conjugate) are not totally eliminated from the body immediately after formation. Biliary excretion of the glutathione conjugate (which has been demonstrated in isolated perfused rat liver; Harvey and Levitt, 1976) followed by recirculation may account for this. In patients with severe liver damage, very small proportions of the drug were eliminated by sulphate conjugation initially, and more must have been diverted to the other routes of elimination, including the potentially toxic oxidative route. In such cases relatively high proportions of drug continued to be excreted unchanged rather than as conjugates. This finding together with the observation that the plasma paracetamol half-life is prolonged from the outset indicates early impairment of hepatic metabolism of paracetamol. This is confirmed by the reduced plasma concentrations of paracetamol sulphate and glucuronide conjugates in patients with severe liver damage (Chap 5). Similar observations were reported by Prescott and Wright (1973).

NAC (and cysteine) appeared to modify the metabolism of paracetamol. The maximum excretion rate of paracetamol sulphate in patients given NAC or cysteine was much higher than in untreated patients. Less effect was seen in patients who developed severe liver damage after late treatment with NAC. It seems unlikely that the differences in paracetamol sulphate excretion were due to differences in the amount of paracetamol absorbed

or incomplete urine collections since similar effects were seen with plasma concentrations of paracetamol sulphate following early and late treatment with NAC.

In the patients treated early with NAC or cysteine, the mean excretion rates of the mercapturic acid and cysteine conjugates were high but none developed severe liver damage. The excretion rates of these compounds were also high with late treatment but severe liver damage occurred. It appears that early treatment with NAC or cysteine protected against liver damage in spite of the increased production of the mercapturic acid and cysteine conjugates. This contrasts with the increased excretion of these conjugates associated with severe liver damage in other patients who were either not treated, treated late or treated with methionine. These findings may be explained in terms of repletion of hepatic glutathione following NAC and cysteine treatment (Strubelt et al, 1974) and/or direct conjugation with the toxic metabolite of paracetamol (Buckpitt et al, 1979), a reaction which apparently does not occur with methionine.

Cysteamine treatment was associated with the reduced excretion of the mercapturic acid and cysteine conjugates. In addition, the overall elimination rate of paracetamol was slower relative to the other groups without severe liver damage. Thus cysteamine apparently reduced the rate of conversion of paracetamol to its reactive metabolite, therefore giving protection to the liver,

while also reducing the overall elimination rate of paracetamol. Similar observations were made by Buckpitt et al (1979) in mouse liver microsomal preparations.

Chapter 8

Renal Clearance of Paracetamol and its Conjugates

The renal clearance of paracetamol and its sulphate and glucuronide conjugates was measured at different plasma concentrations. The plasma concentrations of the 3 compounds at the beginning and end of the appropriate urine collection periods were estimated by interpolation as in Chap. 5, p 204 and the renal clearances determined as in Sec. IV, p 119 . The relationships between renal clearance and plasma concentration, urine flow rate and urine pH were investigated using linear regression analysis.

Twelve urine collections from 9 patients were analysed. Renal function was normal in all patients, as judged by urine flow rate and plasma creatinine concentrations(Table 59).

a) Results

Table 59 shows the renal clearances of paracetamol and its sulphate and glucuronide conjugates in the 9 patients. In the three patients who developed severe liver damage, the renal clearances of all 3 compounds were comparable with those in the other patients. The correlations between renal clearance and plasma concentrations, urine flow rate and urine pH are summarised in Table 60. The low mean renal clearance of paracetamol

TABLE 59

Renal clearance of paracetamol (P) and its sulphate (S) and glucuronide (G) conjugates at high plasma concentrations following paracetamol overdosage.

Patient number *	Plasma Creatinine Conc. ** (μMol/L)	Urine Flow Rate (mℓ/min)	Urine pH	Mean plasma concentrations (μg/mℓ)			Renal Clearance (mℓ/min)			Max. AST/ALT (iu/L)
				P	S	G	P	S	G	
N12	62	2.8	6.2	222	12	122	8.7	87	77	37
"	"	0.6	5.6	123	12	111	5.1	109	95	"
"	"	1.1	5.2	44	14	58	6.1	114	96	"
N13	86	2.0	5.2	108	25	143	8.1	106	114	36
N15	70	1.7	5.4	94	13	56	11.4	223	206	270
"	"	0.7	5.9	30	7.9	22	8.8	207	160	"
N23	99	1.0	5.5	74	11	40	10.2	122	118	10200
N24	76	1.3	5.7	72	8.6	32	7.6	179	158	5575
N26	76	4.5	5.5	39	14	38	24.0	163	142	46
N27	66	1.8	5.0	94	13	167	11.5	139	114	26
N34	91	1.8	5.7	116	10	74	8.4	169	126	1440
C10	66	4.1	6.3	262	15	91	15.1	158	147	91
Mean ± S.D.	-	1.9 ± 1.3	5.6 ± 0.4	106 ± 71	13 ± 4	79 ± 47	10.4 ± 5.0	148 ± 42	129 ± 35	-

* All patients were treated with NAC except patient C10 (given Cysteamine).

** Measured on same day as urine sample; normal range 55-150 μMol/L.

TABLE 60

Relationships between renal clearance and plasma concentration, urine flow-rate and urine pH for paracetamol and its sulphate and glucuronide conjugates at high plasma concentrations following paracetamol overdosage.

Compound	Mean renal Clearance ±S.D. and range (mℓ/min)	Plasma Concentration Mean ± S.D. and range (μg/mℓ)	Plasma Concentration Correlation with renal Clearance*	Urine Flow Rate Mean ± S.D. and range (mℓ/min)	Urine Flow Rate Correlation with renal Clearance*	Urine pH Mean ± S.D. and range	Urine pH Correlation with renal Clearance*	Mean renal Clearance after 20 mg/kg Paracetamol (and range of plasma conc.)
Paracetamol	10.4 ± 5.0 (5.1 - 24.0)	106 ± 71 (30 - 262)	r = 0.01 (NS)	1.9 ± 1.3 (0.6 - 4.5)	r = 0.84 (p<.001)	5.6 ± 0.4 (5.0 - 6.3)	r = 0.08 (NS)	14.1 ± 6.1 mℓ/min (0.9 - 23 μg/mℓ)
Paracetamol Sulphate	148 ± 42 (87 - 223)	13 ± 4 (7.9 - 25)	r = -0.42 (NS)	"	r = -0.001 (NS)	"	r = 0.29 (NS)	167 ± 34 mℓ/min (1 - 8.1 μg/mℓ)
Paracetamol Glucuronide	129 ± 35 (77 - 206)	79 ± 47 (22 - 167)	r = -0.53 (NS)	"	r = 0.08 (NS)	"	r = 0.04 (NS)	135 ± 22 mℓ/min (0.4 - 24 μg/mℓ)

* n = 12 values; NS = not significant.

(10.4 ± 5.0 ml/min.) indicated extensive tubular reabsorption and the high values for the sulphate and glucuronide conjugates (148 ± 42 and 129 ± 35 ml/min. respectively) suggested active secretion. The mean renal clearance values of all 3 compounds following overdosage were similar to the values obtained following a 20 mg/kg dose (Table 60).

There were no significant correlations between renal clearance and plasma concentrations of any of the compounds (Table 60). The correlation between renal clearance and urine flow rate was only significant with paracetamol ($r = 0.84$; $p < .001$) and there was no correlation between the renal clearance of any compound and urine pH (Table 60). Similar observations were made at therapeutic dosage (pp 137 - 139).

b) Discussion

Following overdosage, the renal clearance of paracetamol and its glucuronide and sulphate conjugates and the correlations with urine flow rate and pH were similar to those observed following therapeutic doses (Sec 1V, pp167-170). Unchanged paracetamol apparently underwent extensive renal tubular reabsorption and its sulphate and glucuronide conjugates were apparently actively secreted. Active tubular secretion of the mercapturic acid and cysteine conjugates probably occurs also since their plasma concentrations were too low to measure despite high urinary excretion rates.

Thus these findings show that the renal tubular transport of the glucuronide and sulphate conjugates is not saturated following paracetamol overdosage. The studies in dogs reported by Duggin and Mudge (1975, 1976), although in broad agreement with the present study, indicated saturation of the renal clearance of these conjugates at high plasma concentrations. The concentration ranges encountered in their studies were 8 - 150, 3 - 26 and 15 - 19 μg/ml for paracetamol and its sulphate and glucuronide conjugates respectively, compared with 30 - 262, 8 - 25 and 22 - 167 μg/ml respectively in the present study. It would appear that the renal transport mechanism is more easily saturated in dogs than in man. The renal clearance measurements made by Prescott and Wright (1973) involved plasma concentrations similar to those of the present study and similar results were obtained.

Chapter 9

Summary of Results

1) The plasma concentration of paracetamol 4 hours after overdosage showed a highly significant relationship ($p < .0001$) with the degree of liver damage sustained in untreated patients. No such relationship was observed after treatment within 10 hours of ingestion with intravenous methionine, cysteamine or N-acetylcysteine (NAC), indicating that such treatment is highly effective in reducing liver damage. Treatment more than 10 hours after overdosage had little or no effect.

2) The 4 hour plasma paracetamol concentration correlated significantly with the total urinary recovery of paracetamol and its conjugates for patients admitted within 10 hours of overdosage.

3) The actual paracetamol dose absorbed could not be accurately determined owing to loss of urine samples and possible formation of unidentified metabolites.

4) The absorption of paracetamol in overdosage was rapid, with peak plasma concentrations occurring within 4 hours of ingestion.

5) Paracetamol was eliminated rapidly, almost entirely by metabolism irrespective of liver damage or treatment with NAC, cysteamine or methionine. In 13 untreated patients, from whom a mean of 11.8g was recovered as

parent drug and its 4 major conjugates (sulphate, glucuronide, mercapturic and cysteine conjugate), only 8.3% was unchanged drug.

6) The plasma paracetamol half-life was significantly prolonged in poisoned patients without severe liver damage (3.5 ± 0.7 hours) relative to therapeutic dosage (2.3 ± 0.4 hours). The half-life was longer in patients with severe liver damage (5.2 ± 1.6 hours). Early treatment with NAC reduced the half-life in 10 patients, from 4.5 ± 1.0 hours to 2.8 ± 0.6 hours but the effect of cysteamine was much less marked (mean plasma paracetamol half-life of 4.0 ± 0.7 hours). Treatment more than 10 hours after overdosage did not alter the plasma paracetamol half-life.

7) In patients with normal renal function, plasma concentrations of the glucuronide, and particularly the sulphate conjugate, were low in relation to those of the parent drug after overdosage compared with therapeutic doses. Treatment with NAC (and to a lesser extent cysteamine) increased the plasma concentrations of paracetamol sulphate, although this effect was less marked in patients treated more than 10 hours after overdosage.

8) Plasma concentrations of the mercapturic acid and cysteine conjugates were too low to measure.

9) In overdosage, an initially high proportion of paracetamol was excreted unchanged, together with a low proportion as the sulphate conjugate. Overall elimination was prolonged compared with therapeutic dosage.

10) Urinary excretion of the mercapturic acid and cysteine conjugates after overdosage was very low at first and peak excretion rates occurred much later than after therapeutic doses (about 10 hours and 2 - 3 hours after ingestion respectively).

11) The proportions excreted as the mercapturic acid and cysteine conjugates in overdosage were similar to the proportions excreted after therapeutic dosage except in patients with severe liver damage. Patients with severe liver damage excreted significantly larger proportions as these conjugates, with the cysteine conjugate predominating.

12) Treatment of overdosage with intravenous NAC was associated with a significant increase in the amount and proportion of drug excreted as paracetamol sulphate. Also, large amounts of the mercapturic acid and cysteine conjugates were excreted without the liver damage usually associated with production of such large quantities of these compounds.

13) Treatment of overdosage with intravenous cysteamine was associated with a significant increase in the proportion of drug excreted as paracetamol sulphate but a decreased

proportion excreted as the mercapturic acid and cysteine conjugates.

14) Intravenous methionine resulted in an insignificant increase in the proportion of drug excreted as paracetamol sulphate following paracetamol overdosage and there was no change in the excretion of the cysteine and mercapturic acid conjugates. Methionine was the least effective of the 3 specific treatments in preventing liver damage after overdosage.

15) The renal clearances of paracetamol and its sulphate and glucuronide conjugates at high, toxic plasma concentrations were not significantly different from those at therapeutic concentrations. As observed with therapeutic dosage, the renal clearance of paracetamol was very low (mean 10.4 ml/min) and dependent on urine flow rate, indicating extensive renal tubular reabsorption. The renal clearances of the sulphate and glucuronide conjugates however, were high (mean 148 and 129 ml/min respectively) and independent of urine flow rate, suggesting active tubular secretion. Urine pH had no effect on the renal clearance of any of the 3 compounds.

Chapter 10

Discussion and Summary

The present study indicates that elimination of paracetamol taken in overdosage is rapid. Similar findings were reported by Prescott et al (1971).

The three specific treatments studied in detail were effective in preventing liver damage after paracetamol overdosage, particularly NAC and cysteamine. Efficacy of treatment was paralleled by effects on the metabolism and elimination of paracetamol. The treatment which had the least effect on paracetamol metabolism (methionine) was also the least effective in preventing liver damage.

i) Assessment of Overdosage and Liver Damage

The relative lack of control in drug overdosage studies greatly hinders interpretation of the results obtained. This problem cannot readily be overcome since one cannot ethically conduct a controlled clinical trial. For this reason it was necessary to assess the severity of poisoning using the 4-hour plasma paracetamol concentration, overall urinary recovery and maximum plasma AST/ALT values for comparison of paracetamol dispositon and the effects of treatment.

Accurate 4 hour plasma paracetamol values depend on accurate knowledge of the time of drug ingestion and speed of absorption. The general unreliability of

overdosage patients (Matthew, 1971) undoubtedly leads to errors in the estimation of this value. The 4 hour plasma paracetamol concentration (which was calculated by extrapolating later plasma concentrations back to 4 hours) is subject to additional error if the half-life changes. This is very important in the case of patients admitted many hours after paracetamol ingestion. Further, where the half-life was shortened by treatment, values would be overestimated. In practice, most patients were admitted within 10 hours of drug ingestion and sufficient samples were collected to estimate the 4 hour plasma paracetamol concentration before significant changes in the half-life occurred. Patients with severe liver damage tended to be admitted late, therefore it is possible that their 4 hour plasma paracetamol concentrations may have been underestimated.

The "total urinary recovery" of paracetamol only referred to the unchanged drug and its sulphate, glucuronide, cysteine and mercapturic acid conjugates. Other minor metabolites of paracetamol are known (Mrochek et al, 1974; Andrews et al, 1976; Klutch et al, 1978) and Knox and Jurand (1977, 1978) reported at least one other metabolite (methoxyparacetamol) in urine after overdosage. Also, some paracetamol may remain covalently bound to liver (Jollow et al, 1973). The contribution of these other compounds to the total urinary recovery after overdosage is not known but is unlikely to be large (Andrews et al, 1976) and in any

case no assumptions have been made regarding the actual dose ingested. Completeness of urine collections could never be guaranteed and despite the exclusion of patients admitted more than 10 hours after paracetamol ingestion, some losses were known to have occurred. Nevertheless, the significant correlation between 4 hour plasma paracetamol concentrations and the total urinary recovery of paracetamol in the 4 treatment groups suggested that the groups were comparable.

The definition of liver damage is arbitary with respect to the cut-off values of the peak serum concentrations of the aminotransferases (AST and ALT). Prescott et al (1971) found that the peak serum concentrations of the transaminases (and also bilirubin and prothrombin time ratio) correlated well with the 4 hour plasma paracetamol concentrations and plasma paracetamol half-life. This indicates the association between the extent of liver damage sustained (as estimated by these measurements) and the amount of paracetamol absorbed and its elimination rate.

James et al (1975) compared AST, bilirubin and prothrombin time ratio measurements with estimations of liver damage in liver biopsy samples from patients with paracetamol overdosage. They noted that serum AST concentrations were a good indicator of severe histological damage whereas serum bilirubin concentrations and the prothrombin time ratio often changed little in cases of

moderate cell damage. They are alone in considering that peak AST concentrations of greater than 400 iu/L indicated severe liver damage. Maximum AST/ALT concentrations above 1000 iu/L have been used as and index of severe liver damage by most workers (Prescott et al, 1976; Crome et al, 1976; Smith et al, 1978) and this level was adopted in the present study.

Of the 48 patients studied in detail, 22 were clinically normal with respect to ALT/AST measurements (less than 40 iu/L) compared with 29 by serum bilirubin concentrations and 28 by prothrombin time ratio (upper normal limits 1.0 mg/100 ml and 1.3 respectively).

ii) Limitations

Many factors other than dose, liver damage and treatment can affect paracetamol metabolism and some of these factors could have influenced the results of the present study.

Age: The patients investigated in the present study showed a wide age range with a bias towards young adults (20 - 30 years). Briant et al, (1976) noted a wide variation in the elimination rate of a 1g dose of paracetamol in adults although there was a tendency towards slower elimination in the elderly. Children appear to eliminate paracetamol more slowly than adults and rely on sulphate conjugation in particular (Levy et al, 1975; Miller et al, 1976). However, Miller et al

noted that the urinary excretion pattern of paracetamol and its sulphate and glucuronide conjugates in 12 year olds was similar to that in adults. The youngest patient in the present study was 16 years old.

Sex: There were many more females than males in the present study and the ratio varied considerably between the patient groups. Some workers (Briant et al, 1976; Wojcicki et al, 1979) have found small sex differences in the kinetics of elimination of the parent drug following a 1g dose in young adults. However, Miller et al (1976) noted no sex differences in either the urinary half-life of paracetamol or the overall excretion of the unchanged drug and its sulphate and glucuronide conjugates after a 10 mg/kg dose in both young adults and children.

Other Drugs: Many of the patients treated for paracetamol overdosage had also ingested other drugs and many were regular consumers of large quantities of alcohol. This may well have affected both the clinical outcome and the metabolism of paracetamol in some of the patients due to the induction of liver microsomal enzymes (Kater et al, 1969; Soloman et al, 1971; Wright and Prescott 1973). Studies in rats and mice havd shown that microsomal enzyme induction with phenobarbital potentiates paracetamol hepatotoxicity (Mitchell et al, 1973 a,b). Induction was also associated with a higher proportion of the paracetamol dose being recovered as the mercapturic

acid conjugate in animals (Jollow et al, 1974). In man, Wright and Prescott (1973) observed that potentially induced patients developed significantly more severe liver damage than non-induced patients after paracetamol overdosage. Also, Mitchell et al (1974) showed that volunteers excreted a higher proportion of a 1.2g dose of paracetamol as the mercapturic acid conjugate following pre-treatment with phenobarbital.

Dextropropoxyphene, a narcotic analgesic, is now commonly taken in overdosage with paracetamol (in a combination preparation,"Distalgesic"). Narcotics delay gastric emptying and hence absorption of paracetamol (Nimmo et al, 1975) and this may have occurred in patients who took Distalgesic. Delayed absorption will probably result in an unreliable estimate of the 4 hour plasma paracetamol concentration but the pattern of urinary excretion is unlikely to be altered (Slattery and Levy, 1979). However, delayed absorption followed by specific treatment may result in altered urinary excretion since a larger proportion of the dose absorbed will be exposed to the metabolic effects of treatment.

iii) Paracetamol Metabolism and Liver Damage

The oxidation of paracetamol via cytochrome P-450 to the potentially toxic intermediate and its subsequent inactivation with reduced glutathione are particularly important. The present study showed that the metabolism

of paracetamol in overdosage and therapeutic dosage differs. The prolonged elimination half-life, together with substantially reduced sulphate conjugation and more extensive cytochrome P-450-dependent oxidation, is consistent with saturation of sulphate conjugation with increased production of the reactive intermediate. These effects of paracetamol metabolism were most marked in patients who developed severe liver damage. However, even in these patients paracetamol elimination was rapid and most of the drug recovered was excreted within 24 hours.

In the 13 untreated patients, the mean overall urinary recovery of paracetamol and its metabolites in the 4 patients who developed severe liver damage was 15.0g compared with 10.4g for those without severe liver damage. These values were similar to the pharmacokinetically-derived estimates of the dose ingested in two similar groups of 4 patients (Prescott and Wright, 1973). Their estimates of the mean dose in patients with and without liver damage were 18.8g and 12.7g respectively. The amount of oxidised paracetamol covalently bound to liver proteins probably accounts for only a small proportion of drug absorbed (Jollow et al, 1973; Davis et al, 1976a).

The overall urinary recoveries following overdosage reported by Davis et al (1976) were also similar to those observed in the present study. However, these workers reported that the proportions of dose excreted

as the glutathione-derived conjugates were far larger (up to 50%) and the proportion excreted unchanged much smaller (up to 2%) than in the present study. These differences were probably attributable to the unsatisfactory analytical methods they used (Howie, 1977). The lack of a clear association between dose and toxicity of paracetamol was also noted by Prescott and Wright (1973) and Davis et al (1976).

Jollow et al (1974) showed in animals that the proportions excreted as the sulphate and glucuronide conjugates decreased and increased respectively and the unchanged proportion increased with increasing paracetamol dosage (as found in the present study). Also, as the dose of paracetamol increased, the proportions of overall recovery represented by the oxidative metabolites began to decrease, reflecting glutathione depletion, and this stage was associated with the occurrence of liver damage. The doses encountered in the present study (up to at least 300 mg/kg) were comparable with those used in animal studies (mostly 100 - 1000 mg/kg). Considerable species differences exist in the metabolism of paracetamol and in the susceptibility to its toxic effects (Mitchell et al, 1973 a; Jollow et al, 1974; Davis et al, 1974). The proportion recovered as the mercapturate and cysteine conjugates in the present study were always low during the first 10 hours or so and particularly immediately after ingestion, during which time most of the drug is metabolised. It is therefore possible that the excretion

pattern during this period may reflect the depletion of glutathione to an extent likely to cause liver damage in some individuals.

The delayed appearance of the mercapturic acid and cysteine conjugates in urine may be due in part to biliary excretion and recirculation of these compounds and/or their precursor, the glutathione conjugate (Harvey and Levitt, 1976). However, in overdosage the maximum excretion rates of these compounds occurred 7 hours or more later than after therapeutic dosage, which may reflect the period during which glutathione depletion occurs.

Dose-related changes in paracetamol metabolism were also found by other workers. Prescott and Wright (1973) noted the rapid excretion of unchanged drug shortly after ingestion, and the higher proportion excreted unchanged overall. They also noted, in patients with liver damage, the high ratio of parent drug to conjugates in plasma and the high proportion of unchanged drug excreted at all times after overdosage. Davis et al (1976) observed that overall excretion of the glucuronide conjugate was limited with paracetamol doses above 10g whereas that of paracetamol sulphate was limited with doses of less than 4g. Production of the glutathione-derived conjugates was not limited with increasing dosage and correlated with the incidence of liver damage. Similar observations were noted in the present study although glucuronide

conjugation did not appear to be limited to the same extent.

Slattery and Levy (1979) studied the data of other workers from overdosage patients and subjects taking therapeutic doses of paracetamol and obtained similar results to the present study. They predicted saturable metabolism via glucuronide and particularly sulphate conjugation and non-saturated oxidative metabolism. This was verified using computer-aided pharmacokinetic analysis which also revealed the dose-related plasma paracetamol half-life reported in the present study. They also commented on an apparent lack of influence of liver damage on the plasma half-life of paracetamol. Their predictions for the proportions of dose excreted as the various compounds in overdosage were based on the data of Davis et al (1976) and therefore differed somewhat from the present study.

iv) Effects of Specific Treatment on Paracetamol Metabolism

In the present study cysteamine, methionine and NAC were shown to produce various effects on the metabolism of paracetamol taken in overdosage and these effects could be related to the efficacy of treatment in preventing hepatotoxicity. The use of these treatments in man was based on the results of their hepato-protective effect in animals given large doses of paracetamol (Mitchell et al, 1973b, 1974; Strubelt et al, 1974; McLean and Day, 1975; Piperno and Berssenbruegge, 1976). The clinical efficacy

of these agents in man has since been reported (Prescott et al, 1974, 1976, 1977, 1979; Crome et al, 1976; Peterson and Rumack, 1977).

However, there are no published studies relating to metabolic effects of these treatments on paracetamol overdosage in man except for the limited investigation by Howie et al(1977). In this study it was noted that paracetamol metabolism showed greater differences in relation to liver damage than in relation to treatment with cysteamine or methionine, a finding which was confirmed in the present study. Howie et al also observed that excretion of the mercapturic acid and cysteine conjugates was halved during a 6 hour infusion of cysteamine (1g) in one volunteer who took a 1.5g dose of paracetamol. This suggested that cysteamine limits the cytochrome P-450-dependent oxidation of paracetamol in man. The findings in the present study support this hypothesis even though the 11 patients studied were given cysteamine on average 6.8 hours after paracetamol overdosage.

A major additional finding in the present study was the enhancement of sulphate conjugation with these treatments. Levy and Yamada (1971) noted a similar effect (after therapeutic doses of paracetamol) with cysteine, which reversed the limitation of paracetamol sulphate production caused by salicylamide. Slattery and Levy (1979) investigated the effect of "unsaturating" sulphate conjugation on the elimination rate of paracetamol following

large and small doses using pharmacokinetic analysis. They predicted that little effect would be found with therapeutic doses but a pronounced enhancement of paracetamol elimination should be seen with doses of 10g or more. This was precisely as observed with NAC in the present study. Prescott et al (1974) found that plasma paracetamol half-lives were significantly reduced following cysteamine treatment.

Piperno et al (1978) did not find this effect with NAC in mice. "Early" administration of NAC did not change the plasma half-life of paracetamol. Paracetamol (1200 mg/kg orally) followed 1 hour later by 1200 mg/kg NAC orally resulted in a reduction in the urinary excretion of paracetamol sulphate and an increase in the excretion of the mercapturic acid and cysteine conjugates. They concluded that the protective effect of NAC was not associated with either enhanced elimination or major changes in the urinary excretion pattern of paracetamol. However, the metabolism and elimination of paracetamol overdoses is considerably more rapid in mice (half-life about 1 hour) than in man (half-life 3 hours or more).

McLean and Nuttall (1978) showed that the fall in free sulphydryl concentrations following the incubation of rat liver slices with paracetamol could be reversed with methionine and cysteine, which is consistent the replenishment of glutathione stores. Harvey and Levitt

(1976) studied the effects of various agents on paracetamol metabolism in the isolated perfused rat liver. Cysteamine was found to markedly inhibit the formation of the glutathione conjugate whereas cysteine and methionine did not. They concluded that methionine and cysteine protect the liver by supplying precursors for glutathione synthesis whereas cysteamine inhibited the cytochrome P-450-dependent oxidation of paracetamol rather than becoming incorporated into paracetamol conjugates.

Buckpitt et al (1979) investigated the effects of various sulphydryl compounds on the oxidation and sulphydryl conjugation of paracetamol in mouse liver microsomal preparations. They found that cysteamine in high concentrations decreased both the rate of paracetamol metabolism and the rate of covalent binding but in low concentrations it was capable of forming an adduct with paracetamol. Both cysteine and NAC inhibited covalent binding and both formed conjugates with paracetamol, NAC particularly so. Cysteine appeared to decrease the rate of paracetamol metabolism also. On the other hand, methionine did not form an adduct with paracetamol and had no significant effect on the rates of paracetamol metabolism and covalent binding.

These findings are in complete agreement with the present study. Methionine did not inhibit or enhance the formation of the oxidative metabolites and its

hepatoprotective effect was inferior to the other treatments. Cysteamine reduced the formation of the oxidative metaboites and also appeared to decrease the rate of metabolism of paracetamol while at the same time preventing liver damage. NAC (and cysteine) treatment resulted in larger amounts of the oxidative metabolites being excreted without the otherwise expected liver damage. In man, the enhanced elimination of paracetamol probably contributed to the reduced incidence of liver damage with NAC.

v) Summary

The metabolism and elimination of paracetamol was studied in patients with paracetamol poisoning and was related to disposition of the drug following therapeutic dosage. The effects of liver damage and specific treatment on paracetamol disposition were also studied. The scope of the investigation was limited by the uncontrolled nature of drug overdosage, ingestion of other drugs, difficulty in obtaining complete urine collections and the variation in times of admission and treatment.

The metabolism and elimination of paracetamol was related to dosage and hepatotoxicity. However, the drug was always extensively metabolised. Sulphate conjugation was readily saturated and inorganic sulphate was probably depleted. Oxidative metabolism did not appear to be limited and this may account for toxicity

at higher doses.

Paracetamol toxicity was related to the plasma concentration in relation to the time after ingestion although individual susceptibility varied widely. Specific treatment with methionine, cysteamine and N-acetylcysteine (NAC) within 10 hours of paracetamol ingestion reduced toxicity whereas later treatment was ineffective. The two most effective treatments, intravenous NAC and cysteamine, apparently had different effects on paracetamol metabolism. NAC enhanced the elimination of paracetamol via sulphate conjugation and increased the urinary excretion of the mercapturic acid and cysteine conjugates. Cysteamine increased sulphate conjugation to a lesser degree and seemed also to inhibit the formation of the oxidative metabolite, as shown by a decrease in the proportions of drug excreted as the mercapturic acid and cysteine conjugates. Intravenous methionine had no obvious effect on the metabolism of paracetamol.

Acknowledgements

I thank Professor RH Girdwood and Dr LF Prescott of the Department of Therapeutics and Clinical Pharmacology of the Royal Infirmary, Edinburgh, for providing me with the opportunity and facilities to carry out this work.

I am particulary grateful to Dr LF Prescott, my supervisor, for his invaluable advice and constructive criticism. I also thank Professor JH Knox and Dr J Jurand of the Wolfson Liquid Chromatography Unit, University of Edinburgh for their helpful suggestions concerning liquid chromatography. I am obliged to Dr RS Andrews of Sterling-Winthrop Ltd., Newcastle-upon-Tyne and Dr W Madison of McNeil Laboratories Inc., Fort Washington, USA for providing reference compounds.

I am indebted to the nursing and junior medical staff of the Regional Poisoning Treatment Centre of the Royal Infirmary, Edinburgh for their help with sample collection. I am also grateful to Lindsay Brown for technical assistance, Ken Marwick for photographing the figures and Jan Hollingdale for typing the tables.

Especial thanks are due to my wife, Liz, for her constant support and encouragement and also for typing the text.

This study was made possible by a grant from McNeil laboratories Inc., Fort Washington, USA.

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COMMUNICATIONS

Paracetamol metabolism following overdosage: application of high performance liquid chromatography

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There has been renewed interest in the metabolism of paracetamol since Mitchell, Thorgeirsson & others (1974) described the mechanisms of hepatic necrosis induced by this drug. In particular, the significance of the cysteine and mercapturic acid conjugates has been established in relation to the formation of a hepatotoxic intermediate metabolite (Mitchell & others, 1974; Andrews, Bond & others, 1976; Davis, Simmons & others, 1976). Sulphydryl donors such as cysteamine and L-methionine protect against severe liver damage following overdosage (Prescott, Park & others, 1976), but the mechanisms involved are unknown.

The urinary excretion of paracetamol and its conjugates (as measured by high performance liquid chromatography, h.p.l.c.) was studied in 5 males and 5 females admitted to the Regional Poisoning Treatment Centre, Edinburgh, following severe paracetamol poisoning. Their ages ranged from 19–54 years (mean 30·4). The mean plasma paracetamol concentration 4 h after ingestion was 371 μg ml^{-1} and the mean total urinary recovery of paracetamol ranged from 11·8–24·2 g (mean 18·3 g). Serial blood samples were taken and urine collected for 3–5 days for estimation of paracetamol and its metabolites. Blood was also taken daily for 5 days for estimation of aspartate and alanine aminotransferases, bilirubin and prothrombin time ratio to obtain a composite 'liver damage score' as described previously (normal value $< 2{\cdot}4$) (Prescott & others, 1976). Four patients were treated with L-methionine, 3 with cysteamine, 1 with L-cysteine and 2 with supportive therapy only. Treatment was begun in all within 10 h of ingestion (Prescott & others, 1976). Severe liver damage (aminotransferases > 1000 i.u. litre^{-1}) occurred in 3 patients given L-methionine and the two receiving supportive therapy, one of whom died in hepatic failure. None of the patients developed renal failure.

The pattern of urinary excretion of paracetamol metabolites did not appear to be influenced by the treatment given, but was related to the severity of liver damage (Table 1). The proportion of the total excreted as the sulphate conjugate was significantly less in the patients with severe liver damage than in those without, and was inversely related to the liver damage score ($r = 0{\cdot}76$, $P = < 0{\cdot}01$). In contrast, the proportion excreted as the cysteine conjugate was significantly higher in the patients with severe liver damage than in those without, and was directly related to the liver damage score ($r = 0{\cdot}96$, $P = <0{\cdot}0001$). A similar but less marked trend was observed with the mercapturic acid conjugate. In all patients there was a marked change in the proportional excretion of paracetamol conjugates with time. During the first 10–20 h after overdosage very little paracetamol was excreted as the sulphate (5–15%) and the glucuronide was the major metabolite (> 80%). After 50–60 h however, the proportions excreted as sulphate and glucuronide were similar and each accounted for about 40% of the total. The proportion excreted as cysteine and mercapturic acid conjugates tended to increase with time, and reached a peak at about 30 h in some patients with liver damage.

The urinary excretion of paracetamol metabolites was also studied in a healthy volunteer given single oral doses of 1·5 g of paracetamol with and without cysteamine (1·0 g infused intravenously over the first 6 h). In the control study 18·6% was excreted in 24 h as the sulphate, 76% as glucuronide, 3·1% as mercapturic acid, 1·0% as cysteine conjugates, and 3·3% as unchanged paracetamol. Cysteamine reduced the urinary excretion of mercapturic acid and cysteine conjugates. In the control study 2·0% of the dose was excreted as

Table 1. *Urinary excretion of paracetamol (P) and its sulphate (S), glucuronide (G), mercapturic acid (M) and cysteine (C) conjugates in relation to severity of liver damage following overdosage.*

Patients	4 h plasma P (μg ml^{-1})	Liver damage score	Urinary excretion (%) P	S	G	M	C
Liver damage (n = 5)	402 (140)	22·3 (8·2)	7·2 (1·5)	10·1 (4·0)	70·1 (5·2)	5·0 (0·9)	7·6 (2·0)
No liver damage (n = 5)	341 (80)	2·8** (0·8)	6·3 (0·8)	14·7* (1·7)	71·6 (3·4)	4·0 (1·8)	3 4** (1·0)

Values given are means with s.d. * $P = <0{\cdot}05$, ** $P = <0{\cdot}005$.

* Correspondence.

these metabolites in the first 6 h while the corresponding value during the infusion of cysteamine was only 1·0%.

Taken together, these observations are consistent with the hypothesis that the protective agents act by inhibiting the formation of the hepatotoxic metabolites of paracetamol. Had they acted by replacing hepatic glutathione (Strubelt, Siegers & Schütt, 1974), an increased excretion of mercapturic acid and cysteine conjugates would be expected in the patients without liver damage. No new metabolites of paracetamol were detected in the treated patients, and the excretion of the cysteine conjugate was directly related to the severity of liver damage irrespective of treatment. The initial very low proportional excretion of paracetamol sulphate is consistent with early saturation of sulphate conjugation (Levy & Yamada, 1971), although it is possible that available sulphur is diverted to vital glutathione synthesis in the face of impending hepatic necrosis. Generally similar findings were reported by Davis & others (1976) in untreated patients with different degrees of liver damage following paracetamol overdosage. However, they reported a much greater excretion of mercapturic acid and cysteine conjugates, ranging from 21% in healthy volunteers given high therapeutic doses to 39% in patients with severe liver damage. These differences may be related to methodology since our results are similar to those reported by Mitchell & others (1974).

The estimation of urinary cysteine and mercapturic acid conjugates of paracetamol has been a problem. Davis & others (1976) used two dimensional thin-layer chromatography and scanning densitometry which in our hands lacked reproducibility and had poor sensitivity. Other methods required the use of radiolabelled paracetamol (Mitchell & others, 1974) or analysis times of 21–45 h (Mrochek, Katz & others, 1974) and did not measure both the cysteine and mercapturic acid conjugates. Reported h.p.l.c. methods for the estimation of plasma paracetamol are complex, do not use an internal standard, and give no details of reproducibility (Riggin, Schmidt & Kissinger, 1975; Mrochek & others, 1974; Wong, Solomonraj & Thomas, 1976).

High performance liquid chromatographic methods have now been developed for (A) the simultaneous estimation of paracetamol and its sulphate, glucuronide, cysteine and mercapturic acid conjugates in urine and, (B) unchanged paracetamol in plasma.

H.p.l.c. system A assembly and running conditions

Orlita Model AE 10-4 pump, Cecil Model 212 ultraviolet detector (set at 250 nm, 10 μl flow cell), Honeywell Model 194 recorder, Hewlett-Packard Model 3370A integrator. Column: internally polished stainless steel tube, 170 × 4·9 mm. i.d., packed with octadecylsilane-bonded spherical silica, particle size 10 μm (Spherisorb 10-ODS, Phase Separations, Clwyd), with a septum injector. Mobile phase: 1% aqueous acetic acid –methanol–ethyl acetate (90:15:0·1) at 4·5 MNm^{-2} (650 lb in^{-2}); flow rate 1·6 ml min^{-1}. Dilute urine sample up to 50-fold with distilled water if necessary. To 0·8 ml add internal standard solution (4-fluorophenol: 0·2 ml of a 20 mg ml^{-1} solution in water). Mix and inject 2–4 μl. For less concentrated urine samples (e.g. those collected several hours after a therapeutic dose of drug) reduce internal standard concentration to 4 mg ml^{-1}. Run appropriate aqueous paracetamol standards with each set of unknowns.

H.p.l.c. method B

Apparatus: as in A with a short column (90 × 4·5 mm i.d.) and the same reverse-phase packing. Mobile phase: water–acetic acid–ethyl acetate (98:1:1) at 2·75 MNm^{-2} (400 lb in^{-2}); flow rate 3 ml min^{-1}. To 1 ml of plasma containing 25–500 μg ml^{-1} of paracetamol in a 10 ml glass tube add slowly 1·0 ml of 25% (w/w) aqueous trichloroacetic acid containing 5 mg ml^{-1} of 4-fluorophenol with agitation on a vortex mixer. Centrifuge off the precipitated protein and inject clear supernatant. For samples containing less than 25 μg ml^{-1} of paracetamol, add 100 μl of a solution containing 7 mg ml^{-1} of 4-fluorophenol in 75% (w/w) aqueous trichloroacetic acid to 1·0 ml of plasma and inject up to 25 μl of supernatant.

Symmetrical peaks are obtained for all compounds (Fig. 1) and calibration graphs of the peak area ratios of paracetamol to 4-fluorophenol plotted against paracetamol concentrations are linear and pass through the origins for both assays. The responses to paracetamol and its sulphate, glucuronide, cysteine and

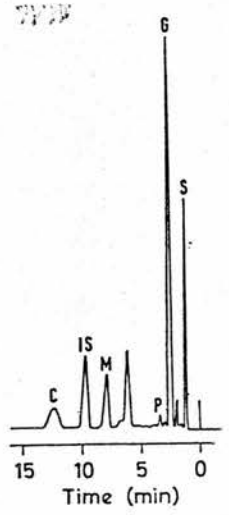

FIG. 1. High performance liquid chromatogram from a urine sample obtained 3–4 h after ingestion of 1·5 g of paracetamol in a healthy volunteer (2 μl injection). S = paracetamol sulphate, G = paracetamol glucuronide, P = paracetamol, M = paracetamol mercapturic acid, C = paracetamol cysteine, IS = internal standard (4-fluorophenol).

mercapturic acid conjugates in urine are linear over the ranges of 3–1600, 5–2600, 50–10 000, 2–800 and 2–1100 μg ml^{-1} respectively. The concentrations of conjugates are expressed as 'paracetamol equivalents' since their molar extinction coefficients are essentially the same as for paracetamol. Replicate analyses of urine samples containing different amounts of paracetamol and its conjugates yielded standard deviations of about 1, 2, 4 and 9% at concentrations of 1000, 200, 50 and 10 μg ml^{-1} respectively. Interference by other drugs has not been encountered in either assay, but interference by other glucuronide and sulphate conjugates in urine has not been excluded. Paracetamol and its conjugates in urine are stable at —20° for at least 9 months, but 4-fluorophenol solutions should be freshly made weekly.

The plasma paracetamol assay can be completed within 15 min. The retention times of the drug and internal standard were 2·1 and 3·7 min respectively, there were no interfering peaks with drug-free plasma and the limit of detection was about 0·1 μg ml^{-1}. The standard deviations of replicate analyses of samples containing 500–50 and 30–1 μg ml^{-1} were 3·7 and 5·8% respectively using peak height ratios. There was a highly significant correlation between the concentrations of paracetamol in 125 plasma samples assayed once by the above method and once by gas-liquid chromatography (Prescott, 1971) (Fig. 2).

We gratefully acknowledge advice and guidance given by Dr J. Jurand and Professor J. H. Knox, Edinburgh University, Department of Chemistry, and we thank Dr R. Andrews, Sterling Winthrop, Newcastle-upon-Tyne, and Dr W. Madison, McNeil Laboratories, Fort Washington, Pa., U.S.A. for reference samples of metabolites. This work was supported by grants from the Scottish Home and Health Department and McNeil Laboratories. February 7, 1977

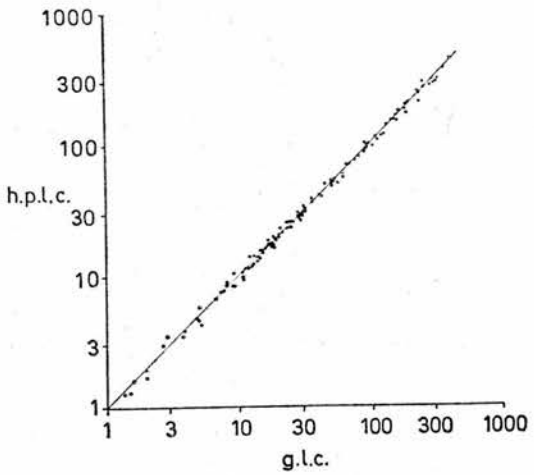

FIG. 2. Comparison of results of 125 plasma paracetamol estimations (μg ml^{-1}) by high performance liquid chromatography h.p.l.c. and gas-liquid chromatography g.l.c.

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sensitised red blood-cells, and by some antibodies which react with lymphocyte surface antigens.[2]

In transplant patients, there are at least three possible sources of immune complexes. Firstly, they could result from the reaction of host antibodies with graft antigens. The development of alloantibodies may result from previous pregnancy or blood-transfusion or from the antigenic stimulation of the renal transplant. Release of alloantigens into the blood may be a feature of normal cells and tissues, since HLA antigenic material has been detected in normal human serum.[3] However, the variability of the results of Fc-rosette inhibition tests performed before rejection, and the consistently positive results during rejection episodes, suggest that release of antigen in amounts sufficient for the formation of detectable immune complexes in the blood occurs at the time of rejection. The injury caused by the rejection process may itself be responsible for antigen release, just as ischæmic injury is presumably responsible for release of antigen from recently placed renal transplants in dogs.[4] Secondly, most types of glomerulonephritis are regarded as being the result of immune-complex disease, which can presumably remain active after renal failure and transplantation. This could not, however, explain the positive tests observed in those of our patients whose renal failure was attributable to pyelonephritis. Thirdly, immune complexes might result from the various infections which are so common in immunosuppressed transplant patients. All three of these sources of complexes may have contributed to the result observed for group-I patient 2, in whom the test was positive during rejection and remained positive after removal of the transplant: this patient's renal failure was due to glomerulonephritis and during rejection cytomegalovirus infection developed which persisted after removal of the transplant.

Antibodies capable of Fc-rosette inhibition do not seem to have conventional HLA specificity.[2] As lymphocytes bearing Fc receptors are mostly B cells and K cells (which are probably of B-cell origin[5]), Fc-rosette inhibition may well be a property of those antibodies which react preferentially with B-cell antigens, perhaps determined by the HLA-D locus.[6] By using a cytoxicity test on suspensions enriched with B cells, Ettenger et al.[7] detected such antibodies in the serum of some transplant patients and noted their association with rejection. We are aware of only one report on the use of an Fc-rosette inhibition test in transplant patients:[8] inhibition was observed with the serum of 6 transplant patients showing signs of rejection, while sera from 4 non-rejecting patients were negative. The serum was ultracentrifuged before testing and the inhibitory effect was attributed to donor-specific alloantibodies, but the work is reported as an abstract and details are not available.

Evidence for the occurrence of immune complexes in the blood of transplant patients is largely indirect, but such complexes may be important in immunological enhancement .[9,10] Palosuo et al.[11] have reported the use of a platelet-aggregation test for immune complexes: the test was positive in 3 of 16 transplant patients, but no correlation with rejection was demonstrated.

In conclusion, we found that inhibition of Fc-rosette formation by the serum of renal allograft patients provides useful confirmation of rejection, although its value as a predictive test remains doubtful.

The roles of immune complexes and alloantibodies in Fc-rosette inhibition also require elucidation. In preliminary experiments, the effects of alkylation and reduction suggest that both factors have contributed to our findings. The inhibitory activity of some normal sera accords with similar results with other tests purporting to demonstrate immune complexes.[12,13]

It is unlikely that positive results were due to anti-rejection therapy (1 g prednisolone intravenously), since only 1 of the acute-rejection cases (group Ic) had received such therapy within the preceding five days, whereas the same therapy was used routinely at the time of transplantation and 6 non-rejecting cases in group-I with negative tests had received therapy within the twelve days preceding (3 of them within five days) testing.

We thank Dr Heather Dick for permission to quote the results of tests for alloantibodies in the patients' serum.

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TREATMENT OF PARACETAMOL (ACETAMINOPHEN) POISONING WITH N-ACETYLCYSTEINE

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Summary Fifteen patients with paracetamol (acetaminophen) poisoning were treated with intravenous N-acetylcysteine (300 mg/kg given over 20 h). Mean admission and 4 h plasma-paracetamol concentrations were 262 and 369 μg/ml, respectively. Liver-function tests remained normal or were only slightly disturbed in 11 of 12 patients treated within 10 h of paracetamol ingestion. Severe liver damage developed in the other patient and in the three in whom treatment was started more than 10 h after paracetamol ingestion. In contrast to cysteamine, N-acetylcysteine was

very well tolerated and has the advantage of being available as a pharmaceutical preparation in a 20% sterile solution.

INTRODUCTION

CURRENT treatment of paracetamol (acetaminophen) poisoning is unsatisfactory. Although cysteamine can prevent severe liver damage if given intravenously in adequate dosage within 10 h of ingestion, it causes unpleasant and occasionally alarming side-effects.[1,2] Furthermore, it is not available commercially as a pharmaceutical product. Other available agents such as dimercaprol, penicillamine, and methionine are less effective and do not always prevent severe liver damage.[1-3] Some time ago we suggested that N-acetylcysteine might be a useful alternative to cysteamine.[4] N-acetylcysteine has the advantage of being available as a sterile 20% aqueous solution ('Airbron', Duncan Flockhart) and, although intended for intrabronchial administration, as much as 36 g daily has been given orally and rectally without incident.[5] Investigations in mice have shown that N-acetylcysteine is less toxic than cysteamine and more effective in protecting against paracetamol hepatotoxicity.[6] N-acetylcysteine is rapidly hydrolysed to cysteine in vivo and presumably protects by the same mechanisms.[7]

METHOD

We used N-acetylcysteine to treat fifteen patients with admission plasma-paracetamol concentrations above a line joining 200 μg/ml at 4 h and 60 μg/ml at 12 h on a semilogarithmic plot.[1] Paracetamol was taken as 'Distalgesic' (dextroproxyphene hydrochloride and paracetamol) by patients 1, 4, 9, and 13, and as 'Para-Hypon' (paracetamol, caffeine, codeine phosphate, and phenolphthalein) by patient 7 (table I). Otherwise, no other drugs were taken at the time of overdosage. Three patients who took distalgesic were in deep coma (grade IV) on admission including patient 1 who was apnœic, hypotensive, and had several convulsions. Adequate doses of naloxone reversed the effects of the dextropropoxyphene in every case. Five patients were regarded as being at particular risk of severe hepatic necrosis because of alcoholism or previous consumption of microsomal-enzyme inducing drugs.[8] An initial dose of 150 mg/kg of N-acetylcysteine (airbron) was given directly intravenously over 15 min. followed by infusion of 50 mg/kg in 500 ml of 5% dextrose in 4 h, and 100 mg/kg in a litre of 5% dextrose over the next 16 h (total 300 mg/kg in 20 h).

Hepatic, cardiac, and renal function was monitored daily for at least 5 days and severe liver damage was defined as an increase in serum-aspartate-aminotransferase (A.S.T.) or serum-alanine-aminotransferase (A.L.T.) above 1000 I.U./l. Serial blood and urine samples were collected for estimation of plasma-paracetamol and total urinary recovery of drug by gas liquid or high-performance liquid chromatography.[9,10] The severity of overdosage was assessed by the actual or extrapolated plasma-paracetamol concentration 4 h after ingestion.[1]

RESULTS AND DISCUSSION

Mean admission and 4 h plasma-paracetamol concentrations were 262 and 369 μg/ml, respectively, and despite incomplete urine collections the mean urinary recovery of paracetamol was 14·9 g (range 8·8–24·9 g). Twelve patients were treated with N-acetylcysteine within 10 h of ingestion (mean 7·2 h) (table I). Liver-function tests remained normal, or were only slightly disturbed in all except patient 12. This patient had taken other unknown drugs and a rodenticide contain-

TABLE I—CLINICAL DETAILS AND LABORATORY INVESTIGATIONS IN PATIENTS WITH PARACETAMOL POISONING TREATED WITH N-ACETYLCYSTEINE

Patient	Age and sex	Ingestion-treatment interval (h)	4 h plasma-paracetamol (μg/ml)	Maximum A.S.T. (I.U./l)	Maximum A.L.T. (I.U./l)	Maximum bilirubin (mg/dl)	Maximum prothrombin-time ratio
1	64M	4·5	461	34	17	0·8	1·3
2	16F	9·0	264	19	16	0·5	1·3
3	20F*	7·7	366	33	35	0·6	1·4
4	61F	9·5	299	34	51	0·8	1·3
5	40F*	6·3	633	64	81	0·7	1·3
6	28M	8·5	427	36	43	0·7	1·2
7	29M*	7·8	327	56	34	1·1	1·2
8	21F	5·3	245	27	16	0·9	1·4
9	44F	4·7	320	23	20	0·8	1·3
10	31F*	9·0	393	45	28	0·6	1·4
11	40F	5·8	463	90	38	0·9	1·3
12	22M	7–9·5	342	2520	3260	1·4	2·7
13	52F*	10·2	307	10 400	5020	3·2	2·0
14	53M	23·5	474	3960	13 600	5·3	2·4
15	25M	11·7	210	1608	10 300	3·5	1·6

*Previous heavy consumption of ethanol or drugs causing microsomal enzyme induction A.S.T.=serum-asparate-aminotransferase. A.L.T.=serum-alanine-aminotransferase.

TABLE II—FREQUENCY OF SEVERE LIVER DAMAGE* IN PATIENTS WITH PARACETAMOL POISONING TREATED WITH SUPPORTIVE THERAPY ONLY, AND CYSTEAMINE, L-METHIONINE OR N-ACETYLCYSTEINE GIVEN WITHIN 10 HOURS

4 h plasma-paracetamol (μg/ml)	Supportive therapy		Methionine		Cysteamine		N-acetylcysteine	
	No. of patients	No. with severe liver damage	No. of patients	No. with severe liver damage	No. of patients	No. with severe liver damage	No. of patients	No. with severe liver damage
200–300	33	11	8	1	9	0	3	0
>300	19	19	7	2	17	0	9	1

*Severe liver damage=A.S.T. or A.L.T. >1000 I.U./l.

ing warfarin during the previous week and the prothrombin-time ratio was 2·7 on admission. Patient 1 died from a cerebrovascular accident 9 days after admission.

Three patients (13–15) were treated after 10 h. All sustained severe liver damage without hepatic encephalopathy and in patient 14 acute renal failure also developed.

The frequency of severe liver damage after paracetamol overdosage in patients receiving supportive therapy only, cysteamine, methionine,[1] or N-acetylcysteine within 10 h of ingestion is shown in table II. Although the numbers are too small for statistical comparison, N-acetylcysteine given within 10 h seems to protect most patients from severe liver damage. It failed in one patient treated within this time and in all the patients treated more than 10 h after ingestion. Larger doses of N-acetylcysteine might have given better protection, but the results of treatment after 10 h remain to be established. The efficacy of N-acetylcysteine could perhaps be increased by oral administration since most of the dose should pass through the liver. However, in our experience nausea and vomiting usually develop within 10 h of overdosage in the most severely poisoned patients.

In contrast to cysteamine, N-acetylcysteine was very well tolerated. Eight patients had nausea with occasional retching and vomiting mostly during the first hour or so of treatment, but these symptoms had usually been present before treatment. There was a minor transient rise in blood-pressure in three patients, but no arrhythmias were observed during treatment. N-acetylcysteine had no obvious effects on the central nervous system. The availability of sterile N-acetylcysteine as a pharmaceutical preparation is a further advantage which may make it the treatment of choice for paracetamol poisoning.

We thank the nursing staff of the Regional Poisoning Treatment Centre for their help and Mrs L. Brown for technical assistance. This work was supported in part by McNeil Laboratories Inc., Fort Washington, Pennsylvania, U.S.A.

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Hypothesis

POSSIBLE EFFECTS OF GROWTH HORMONE ON DEVELOPMENT OF ACUTE LYMPHOBLASTIC LEUKÆMIA

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Summary Growth hormone (G.H.) or a G.H.-dependent somatomedin may be involved in the process of acute lymphoblastic leukæmia (A.L.L.). Growth hormone has a trophic effect on lymphoid tissue and also specific receptors on lymphocytes, most probably T cells. Hypophysectomy in rats with induced T-cell leukæmia can suppress the leukæmic process. Resting concentrations of G.H. and somatomedin activity are raised in some children with A.L.L. and may be reduced after remission is achieved. It is suggested that control of G.H. and/or somatomedin concentrations may be necessary for adequate treatment of some cases of A.L.L. in children.

ARE humoral regulators which are out of control involved in the pathogenesis of leukæmia? Erythropoietin, colony-stimulating factor, and thymic humoral factor were not clearly shown to influence the leukæmia process.[1] Hormonal substances are involved in the proliferation of neoplasms such as breast cancer and prostatic cancer. Evidence is accumulating that growth hormone (G.H.) or a G.H.-dependent factor influences the development or maintenance of acute lymphoblastic leukæmia (A.L.L.). G.H.-dependent factors, known as somatomedins,[2] are peptides under partial control of G.H. Although they are not insulin, they are insulin-like in their action and stimulate cell growth in numerous tissues.

What evidence is available to implicate G.H. or its end organ operator in the pathogeneses of leukæmia? Firstly, G.H. has a trophic effect on lymphoid tissues—either directly, or indirectly via somatomedin. Simultaneous administration of G.H. prevented thymic cortex involution and spleen follicular atrophy after hypophysectomy in animals.[3,4] Thymic size in mice was reduced by G.H. antibodies.[5] Reduced ^{3}H-thymidine incorporation into D.N.A. of thymocytes and splenocytes of hypophysectomised rats was corrected by the administration of G.H.[6]

G.H. receptors are present on circulating human lymphocytes,[7] bovine thymocytes,[8] and cultured human lymphocytes.[9,10] This was demonstrated by incubating the cells with radiolabelled G.H. and measuring specific binding by displacement of the labelled growth hormone by unlabelled human G.H. Circulating lymphocytes with the receptor are probably T cells, as demonstrated by the alteration in electrophoretic mobility which was produced by the binding of G.H. to a specific subpopulation of T cells.[6]

Human G.H. also had an effect on lymphocyte transformation in cell-culture.[11,12] Incubation of cultured lymphocytes with human G.H. increased the percentage of cells undergoing blastogenic transformation. Morphologically the stimulated blast forms were typical lymphoblasts and not immunoblasts, suggesting increased stimulation of T cells rather than B cells. Human lymphocyte transformation as measured by ^{3}H-thymidine uptake is enhanced by the addition of G.H., and transformation by phytohæmagglutinin stimulation plus G.H. exceeds that of phytohæmagglutinin alone.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ESTIMATION OF PARACETAMOL METABOLITES IN PLASMA

There has been considerable interest in the metabolism of paracetamol following the elucidation of the mechanisms of hepatotoxicity after overdosage (Mitchell, Thorgeirsson, Potter, Jollow & Keiser, 1974). Only one method for the estimation of paracetamol metabolites in plasma has been published (Mrochek, Katz, Christie & Dinsmore, 1974) but this procedure takes at least 24 h and the mercapturic acid conjugate, a metabolite of major toxicological significance, was not identified. We describe here a simple, rapid method for the simultaneous assay of paracetamol and its sulphate, glucuronide, cysteine and mercapturic acid conjugates in plasma using high performance liquid chromatography.

An Orlita pump (Model AE 10-4) was used with a Waters model 440 U.V. absorbance detector (254 nm filter) and peak areas were measured with a Hewlett-Packard HP 3370A integrator. The columns were internally-polished stainless steel tubes 100 mm × 4.9 mm i.d. slurry packed with Hypersil-ODS (Shandon Southern Products) and fitted with a septum injector. The mobile phase was 0.1 M potassium dihydrogen phosphate, 98% formic acid and isopropanol (100:0.1:1.7 v/v/v). Stock solutions of internal standard/protein precipitant were prepared containing 360 μg/ml of N-propionyl-4-aminophenol in 6% aqueous perchloric acid and were stored deep frozen prior to use. Aliquots (1 ml) of plasma from patients with paracetamol overdosage were pipetted into glass tubes which were placed on a vortex mixer while 1.0 ml of internal standard/perchloric acid solution was added slowly. The tubes were centrifuged and up to 5 μl of the clear supernatant injected directly onto the column. Samples containing lower concentrations of paracetamol and metabolites (e.g. after therapeutic doses) were assayed by the same procedure except that the internal standard/protein precipitant solution contained 300 μg/ml N-propionyl-4-aminophenol in 30% aqueous perchloric acid and 100 μl was added to 1.0 ml plasma.

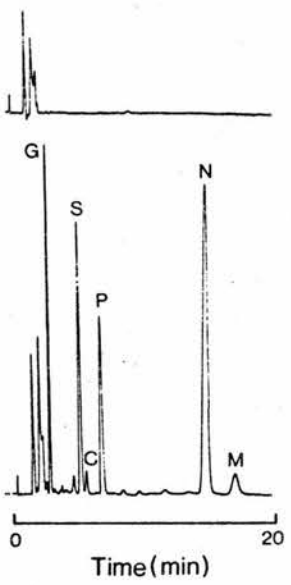

Figure 1 Chromatograms obtained from drug-free plasma (above) and plasma obtained 60 h after severe paracetamol overdosage (below). The peaks are: paracetamol glucuronide (G), sulphate (S), cysteine conjugate (C), free drug (P), internal standard (N) and mercapturic acid conjugate (M).

With a solvent flow of 0.9 ml/min (650 p.s.i.) the glucuronide, sulphate and cysteine conjugates, paracetamol, internal standard and mercapturic acid conjugate eluted in 2.5, 4.8, 5.3, 6.5, 14.3 and 16.5 min respectively. The peaks were sharp and well resolved with no interference from endogenous compounds (Figure 1) and samples could be injected every 18 min. The limit of measurement with a 5 μl injection was less than 1 μg/ml for all compounds. Plots of the peak area ratios of paracetamol and the sulphate and glucuronide conjugates to internal standard against concentration were linear and passed through the origin. These three compounds normally account for practically all the paracetamol-related material in plasma. However, in patients developing severe liver damage after overdosage, easily measurable concentrations of the cysteine and mercapturic acid conjugates may be present (Figure 1). The extinction coefficients of the glucuronide and sulphate conjugates relative to paracetamol and the recoveries from plasma relative to water differ somewhat, but using the appropriate correction factors (0.84 for glucuronide, and 1.03 for sulphate at 254 nm) it is possible to calibrate the assay with plasma standards containing paracetamol only. Insufficient authentic mercapturic acid and cysteine conjugate was available for the preparation of plasma standards and the linear relationship previously demonstrated for the corresponding assay in urine (Howie, Adriaenssens & Prescott, 1977) was assumed to hold for plasma. The standard deviation of replicate assays of paracetamol and its glucuronide and sulphate conjugates was less than 3% (mean 1.0%) for each compound with concentrations ranging from 1–250 μg/ml (paracetamol equivalent). Day-to-day reproducibility was 3–4%. Despite the direct injection

of deproteinized plasma, the columns have lasted for many months with daily use.

Figure 1 shows chromatograms of drug-free plasma and a sample obtained 60 h after paracetamol overdosage in a patient who developed severe liver damage.

The method has been used together with a previously described high performance liquid chromatographic assay for paracetamol metabolites in urine (Howie *et al.*, 1977) in studies of paracetamol metabolism following therapeutic doses as well as overdosage of paracetamol.

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Received March 15, 1978

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DOUBLE-BLIND TRIAL OF INDORAMIN IN DIGITAL ARTERY DISEASE

Trophic changes in the fingers resulting from digital artery disease secondary to scleroderma or other connective tissue disorders are difficult to treat (Rowell, 1972), and no wholly satisfactory form of therapy has been suggested. Vasoconstrictor tone in the periphery is controlled by α-adrenoceptor sympathetic innervation, and blockade of this control leads to peripheral vasodilation. Indoramin (3-[2-(4-benzamidopiperid-1-yl)ethyl]indole hydrochloride) has been shown to possess potent α-adrenoceptor blocking activity in all species so far studied (Alps, Hill, Johnson & Wilson, 1970; Alps, Hill, Johnson & Wilson, 1972; Collis & Alps, 1973) including man (Royds & Lockhart, 1972; Royds & Lockhart, 1974), and intravenous indoramin has been shown to be effective in increasing cutaneous blood flow to affected digits of patients with Raynaud's disease (Fares & Milliken, 1974). There is also a suggestion from other studies that indoramin may provide subjective improvement in both primary and secondary Raynaud's phenomenon (Alps *et al.*, 1972; Fares & Milliken, 1974). This preliminary study was undertaken to assess the effect of oral indoramin on digital blood flow, and on the symptoms of Raynaud's phenomenon associated with digital vascular insufficiency resulting from scleroderma and other connective tissue diseases.

Sixteen patients suffering from digital artery disease in association with trophic changes in the digits were selected for study, eleven of whom were suffering from scleroderma with sclerodactyly as its most prominent manifestation and were receiving no other therapy during the trial. The primary disorder in the other patients, all but two of whom were female, was either rheumatoid arthritis, systemic lupus erythemetosus or polymyositis, and these patients continued to receive their maintenance dose of prednisolone and/or azathioprine throughout the study. Apart from four subjects who continued to receive therapy for hypertension and two subjects who were receiving anti inflammatory propionic acid derivatives for arthritic pain, all other agents were discontinued before the trial commenced and the dose of existing therapy which was not changed had been stabilized for at least 6 months before the period of assessment.

Patients were randomly allocated to two groups and the study was conducted in double-blind manner. Group A received indoramin 90 mg daily in three divided doses for 3 weeks followed by placebo for 3 weeks. Group B received placebo for the first 3 week period followed by 3 weeks receiving 90 mg indoramin in divided doses. Each patient entered a daily record of the number of attacks of Raynaud's phenomenon, and an overall estimate of the discomfort suffered in the day on a five point scale. Additional comments related to unusual physical activity, marked climatic changes

Annals of Clinical Biochemistry, 1979, 16, 89-95

Inappropriate methods for the emergency determination of plasma paracetamol

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SUMMARY Methods for the estimation of plasma paracetamol which depend on acid hydrolysis to *p*-aminophenol without a prior extraction step also measure inactive metabolites which are present in high concentrations.

The extent of the overestimate obtained with such methods was determined using 24 samples from patients after paracetamol overdosage. There was a positive error of between 40 and 700% compared with a high-performance liquid chromatographic reference method which measured only unchanged paracetamol.

These non-specific methods should not be used to determine the need for specific therapy in patients with paracetamol poisoning.

Since the observation by Davidson and Eastham (1966) that paracetamol poisoning may lead to fatal hepatic damage, there have been numerous subsequent reports (Proudfoot and Wright, 1970; Prescott *et al.*, 1971; Clark *et al.*, 1973). Paracetamol poisoning is an increasingly common problem; the number of admissions to the Edinburgh Regional Poisoning Treatment Centre in which paracetamol was implicated rose from 15 in 1967 to 247 in 1977 (10·4% of all admissions in 1977).

There is much individual variation in the susceptibility to the hepatotoxicity of paracetamol. In particular, patients whose liver microsomal enzymes have been previously induced by alcohol or drugs such as barbiturates are liable to be more severely affected than others (Wright and Prescott, 1973).

The severity of paracetamol poisoning cannot be determined on clinical grounds alone since paracetamol does not cause coma, nor are there any specific early signs or symptoms. Clinical evidence of liver damage does not become apparent for many hours, and abnormal liver function tests (in particular aminotransferase) may not be noted until 24 hours or more after ingestion while maximum abnormalities may be delayed for several days (Stewart and Simpson, 1973).

The use of sulphydryl compounds to protect against liver damage was suggested by the work of Mitchell and his colleagues (Jollow *et al.*, 1973; Mitchell *et al.*, 1973a, b; Potter *et al.*, 1973), who showed that hepatic necrosis was associated with depletion of glutathione and covalent binding of a reactive intermediate metabolite of paracetamol to hepatic macromolecules. Glutathione itself is relatively ineffective but a variety of sulphydryl donors has been investigated. Intravenous (iv) cysteamine is effective (Prescott *et al.*, 1974; Douglas *et al.*, 1976; Prescott *et al.*, 1976; Hughes *et al.*, 1977) but it causes unpleasant and prolonged side effects. The use of oral and iv methionine has been advocated by Crome *et al.* (1976) and Solomon *et al.* (1977) and may reduce the severity of liver damage in some cases. Currently, iv N-acetyl cysteine (NAC) is considered to be the treatment of choice. It is effective and is available as a pharmaceutical preparation; no important side-effects have been encountered (Prescott *et al.*, 1977). Whatever agent is used, treatment must be started within 10 hours of ingestion of paracetamol (Prescott, 1978), and in particularly susceptible patients the time available for treatment may be further reduced (Scott and Stewart, 1975). The decision whether to start treatment depends on knowledge of the plasma paracetamol concentration and the time after ingestion, as this is the only known guide to the likelihood of subsequent liver damage (Prescott *et al.*, 1974). In practice, the observed plasma paracetamol concentration is interpreted in relation to a 'treatment line' (Fig. 1). Patients with concentrations above the line are treated, since they have at least a 60% chance of developing severe liver damage (AST or ALT > 1000 IU/l), a 10% risk of acute renal failure, and a 5% chance of death in hepatic failure (Prescott, 1978).

Simple, rapid, and accurate methods are therefore

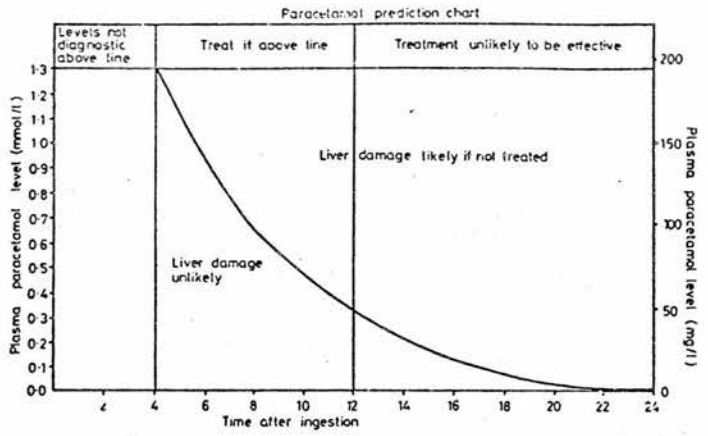

Fig. 1 *Treatment line for use in cases of paracematol poisoning (modified from Prescott* et al. *(1974)).*

required for the emergency estimation of plasma paracetamol. Many methods have been described, and the subject has recently been reviewed by Wiener (1978). However, some methods which have become popular on the grounds of simplicity and speed are non-specific and give rise to serious errors since they also measure the inactive glucuronide and sulphate conjugates of paracetamol which are present in plasma in amounts which may greatly exceed the concentration of the unchanged drug (Prescott and Wright, 1973). These metabolites of paracetamol are very polar and therefore do not interfere with methods involving extraction of paracetamol into an organic solvent. However, they are readily hydrolysed to paracetamol and are thus included in methods based on colorimetric assay of *p*-aminophenol produced by the acid hydrolysis of paracetamol without prior extraction of the paracetamol.

A comparative study of different methods for the determination of paracetamol was therefore undertaken using plasma from 24 patients admitted with paracetamol poisoning. The non-specific acid-hydrolysis methods of Welch and Conney (1965) and Wilkinson (1976) were compared with the simple colorimetric nitration method of Glynn and Kendal (1975) and a reference HPLC method (Adriaenssens and Prescott, 1978) which also simultaneously determined the concentrations of paracetamol sulphate and glucuronide.

Methods

SPECIMENS

Blood samples were obtained between 5 and 47 hours after paracetamol overdosage from 24 patients admitted to the Edinburgh Regional Poisoning Treatment Centre. The plasma was separated, stored frozen, and subsequently divided for analysis by each of the methods detailed below.

STANDARDS

Paracetamol standards were prepared by serial dilution of an aqueous stock solution of paracetamol with horse serum (Wellcome) or pooled human plasma. The solutions were divided into aliquots and stored at −20°C.

Paracetamol glucuronide and sulphate were obtained from Sterling-Winthrop Laboratories, Newcastle upon Tyne. Standard solutions of metabolites were prepared as above in human plasma.

QUALITY CONTROL

Specimens prepared and stored as above were used. The concentrations were 0·5 mmol/l for the colorimetric methods and 0·33 and 1·65 mmol/l for high-performance liquid chromatography (HPLC).

Analytical methods

(1) HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (ADRIAENSSENS AND PRESCOTT, 1978)

Internal standard (1 ml of 2·16 mmol/l N-propionyl-*p*-aminophenol in 0·33 M perchloric acid) was added to 1 ml plasma, mixed, and centrifuged. A 5 μl sample of the clear supernatant was injected on to a 100 mm × 4·6 mm ID stainless steel column packed with Hypersil ODS (Shandon) (5 μM octadecyl bonded silica microspheres). The solvent was 0·1 M KH_2PO_4: 98% formic acid: propan-2-ol (100:0·1:1·7 v/v/v).

(2) COLORIMETRIC NITRATION METHOD (GLYNN AND KENDAL, 1975), MODIFIED BY MACE AND WALKER (1976))

Aqueous trichloroacetic acid (2 ml of a 0·6 M

solution) was added to 1 ml plasma, mixed, and centrifuged. To 2 ml of supernatant were added 1 ml 6 M HCl and 2 ml 1·44 M sodium nitrite (freshly prepared). After 2 minutes, 2 ml 1·54 M sulphamic acid were added slowly, followed by 5 ml of 2·5 M NaOH. The yellow colour was read at 450 nm against a reagent blank.

(3) COLORIMETRIC METHOD OF WELCH AND CONNEY (1965)

The colour reagent was prepared freshly for each batch by mixing 80 ml 0·2 M NaOH, 10 ml of 9·3 M phenol, and 10 ml of a mixture of 15 ml bromine water in 100 ml 1 M Na_2CO_3. To 0·5 ml plasma was added 0·5 ml 10% w/v aqueous trichloroacetic acid solution. After mixing and centrifugation 0·5 ml of the supernatant were added to 2 ml 4 M HCl. The tubes were loosely stoppered and heated at 100°C in a water bath for 1 hour. After cooling to room temperature the contents were diluted to 5·0 ml with water, and 0·5 ml of this solution was added to 5·0 ml colour reagent. The contents of the tubes were mixed thoroughly and allowed to stand for 40 minutes; the blue colour was read at 620 nm against a reagent blank.

(4) COLORIMETRIC METHOD OF WILKINSON (1976)

To 0·5 ml plasma was added 4·5 ml 0·33 M perchloric acid in a conical centrifuge tube. After mixing and centrifugation, 1·0 ml of the supernatant solution was pipetted into a 10 ml tube, loosely stoppered, and placed in a boiling water bath for 40 minutes. After cooling, 1 ml 0·1 M *o*-cresol was added, followed by 2·0 ml 4 M NH_4OH solution. The contents were mixed and the blue colour was read at 615 nm against a reagent blank.

Results

The precision and accuracy of the HPLC assay have been reported (Adriaenssens and Prescott, 1978) and the results obtained for unchanged paracetamol in specimens from poisoned patients have been shown to correlate well with a gas chromatographic method (Howie *et al.*, 1977). Values obtained by HPLC were therefore used as reference data. Since the data were non-Gaussian in distribution the results obtained using the different methods were compared with HPLC using the Wilcoxon matched pairs test of significance.

COLORIMETRIC NITRATION METHOD (GLYNN AND KENDAL, 1975)

There was good agreement between the values obtained using HPLC and the method of Glynn and Kendal ($r = 0·99$). The mean values did not differ significantly ($P = 0·9879$) and the correlation is shown in Figure 2. This colorimetric method is uninfluenced by the presence of paracetamol metabolites.

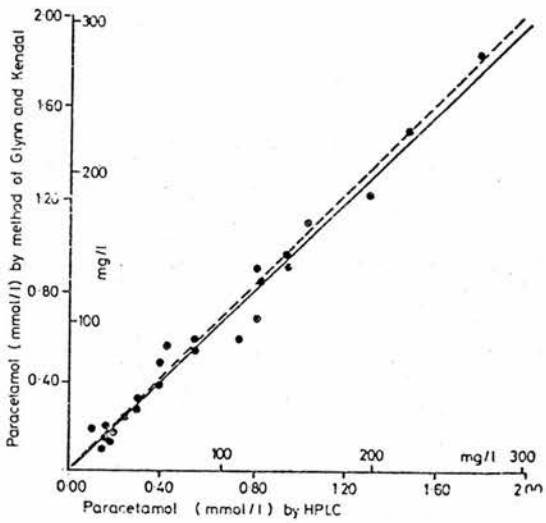

Fig. 2 *Comparison of plasma paracetamol concentrations measured by the colorimetric nitration method of Glynn and Kendal (1975) and the reference (HPLC) method: $n = 24$; $r = 0·99$;* ———— *45° line;* – – – – – – – *regression line.*

COLORIMETRIC METHOD OF WELCH AND CONNEY (1965)

In every case the values for 'paracetamol' obtained by this method greatly exceeded those obtained using HPLC (Fig. 3). The mean value was about three times higher and there was considerable variation in the extent of the overestimate with a maximum discrepancy of 700% (Table) ($P < 0·0001$).

To determine whether hydrolysis of the conjugates to *p*-aminophenol was complete, the values obtained for 'paracetamol' were compared with the sum of the paracetamol, paracetamol sulphate, and paracetamol glucuronide concentrations as estimated by HPLC in each sample. The results (Fig. 4 and Table) show close agreement for both the individual values and the mean value ($P > 0·9$). These observations confirm complete hydrolysis of paracetamol and its conjugates to *p*-aminophenol with this method.

COLORIMETRIC METHOD OF WILKINSON (1976)

The values obtained for paracetamol were again

Table *Plasma paracetamol concentrations in samples from poisoned patients assayed by different methods*

Method	*Mean plasma paracetamol concentration (24 samples) (mmol/l)*	*Maximum individual overestimate %*	*Mean of individual overestimates %*
HPLC paracetamol (reference)	0·608		
Colorimetric nitration (Glynn and Kendal, 1975)	0·603	107	0·5
Perchlorate hydrolysis (Wilkinson, 1976)	0·905	423	76
HCl hydrolysis (Welch and Conney, 1965)	1·393	700	275
HPLC—total paracetamol and metabolites	1·468	—	—

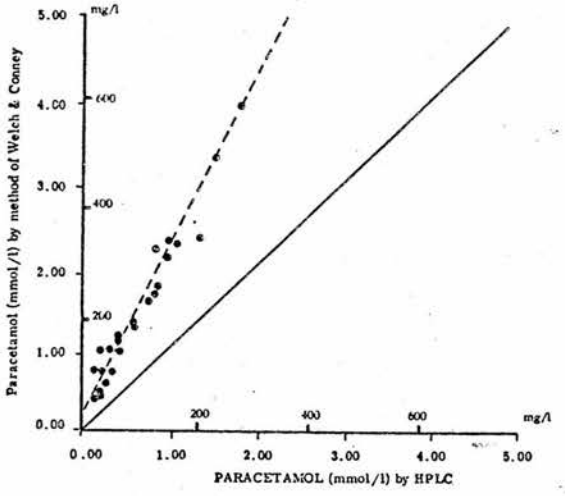

Fig. 3 *Comparison of plasma paracetamol concentrations obtained using HCl hydrolysis (Welch and Conney, 1965) with the reference (HPLC) method: n = 24; r = 0·98; ——— 45° line; – – – – – – – – regression line.*

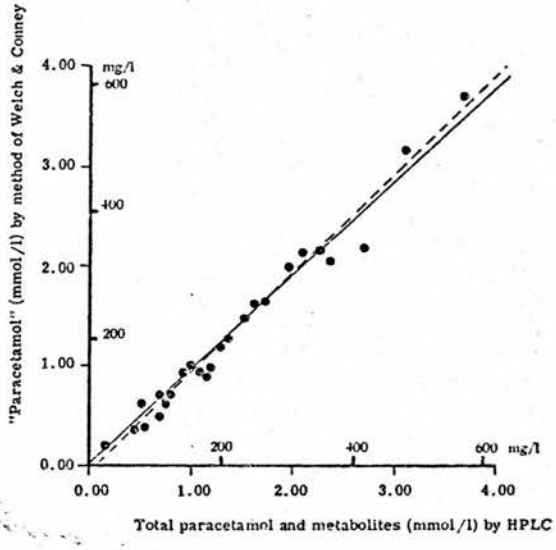

Fig. 4 *Comparison of plasma paracetamol concentrations obtained using HCl hydrolysis (Welch and Conney, 1965) with total paracetamol and metabolites as determined by HPLC: n = 24; r = 0·99; ——— 45° line; – – – – – – – – regression line.*

greater than the HPLC values (Fig. 5) ($P < 0.0001$). The mean value gave an average overestimate of 76% (Table) with a maximum error of 423%. When the paracetamol values obtained by this method were compared with the sum of the concentrations of paracetamol, paracetamol sulphate, and paracetamol glucuronide as determined by HPLC, they were always lower than the HPLC values for total paracetamol plus metabolites (Table) ($P < 0.0001$).

These results suggested either incomplete hydrolysis or loss of *p*-aminophenol. Aliquots of the perchloric acid mixture were therefore sampled at intervals during the 40 minute period of hydrolysis and analysed directly by HPLC. The sample taken after 10 minutes hydrolysis was of particular interest since this is the time advocated by Love (1977) in his modification of this method. The chromatograms obtained are shown (Fig. 6). Paracetamol sulphate had disappeared by 10 minutes, but even after 40 minutes hydrolysis both paracetamol and paracetamol glucuronide were still present in large amounts.

The actual 'paracetamol' concentration is overestimated to a variable degree as there is only partial hydrolysis of the conjugates. The results are therefore even more difficult to interpret than those from a method which gives complete hydrolysis.

Discussion

Wiener (1978) mentioned the problem of non-specificity of some methods in his review and quoted the work of White (1976), which indicated that paracetamol may be overestimated.

This study shows the extent to which methods that involve a hydrolysis step without prior separation of paracetamol from its polar conjugates overestimate paracetamol. The methods of Wilkinson (1976) and Love (1977) are particularly unsatisfactory since

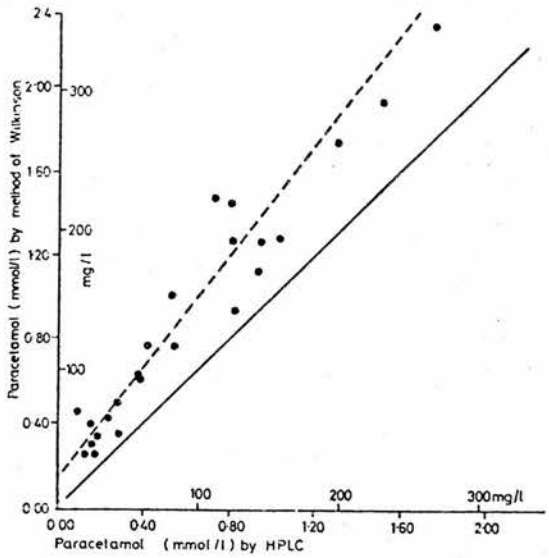

Fig. 5 *Comparison of plasma paracetamol concentrations obtained using perchlorate hydrolysis (Wilkinson, 1976) and the reference (HPLC) method: n = 24; r = 0·96;* ———— *45° line;* - - - - - - - - *regression line.*

hydrolysis is incomplete and variable. Although this serious disadvantage has been pointed out before (Stewart, 1974; White, 1976) yet another method with the same shortcomings was recently published (Plakogiannis and Saad, 1978).

Since the concentrations of paracetamol conjugates in plasma may exceed those of the unchanged drug in patients with paracetamol poisoning, it is not surprising that paracetamol may be overestimated by as much as 700% with non-specific methods. The highest ratios of conjugates to unchanged paracetamol occur in patients *without* severe liver damage (Prescott and Wright, 1973).

In practical terms, such serious errors are of major clinical significance. Misleadingly high values may lead to unnecessary treatment with toxic agents such as cysteamine, and a mistaken impression of the efficacy of treatment may be gained. Paracetamol concentrations as high as 7·4 mmol/l were reported by Jones and Thomas (1977) and were found on subsequent enquiry to have been estimated by the method of Wilkinson (1976). The results of a trial of cysteamine in patients presenting more than 12 hours after ingestion of paracetamol could not be interpreted since the estimations had been carried out

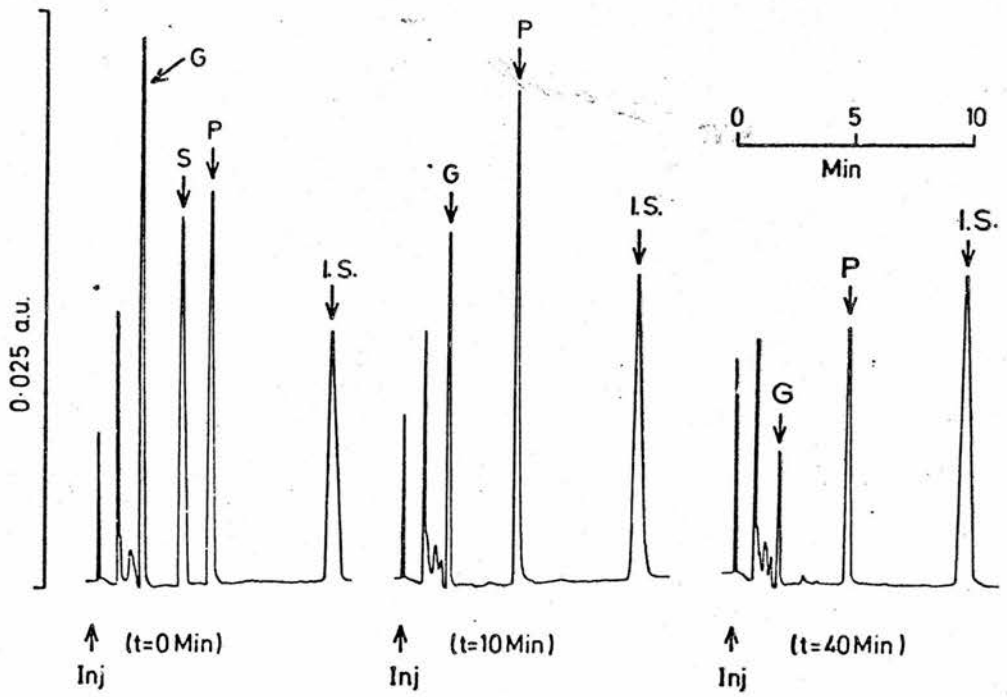

Fig. 6 *High-performance liquid chromatograms showing paracetamol (P) and its glucuronide (G) and sulphate (S) conjugates in plasma before and after hydrolysis in perchloric acid for 10 and 40 minutes. IS = internal standard (N-propionyl-p-aminophenol).*

using the non-specific method of Welch and Conney (Smith *et al.*, 1978; Prescott *et al.*, 1978a; Gilbertson *et al.*, 1978). In another report (Rigby *et al.*, 1978) unnecessary charcoal haemoperfusion was carried out as a result of a misleading high plasma paracetamol concentration measured by the method of Welch and Conney.

The proper interpretation of plasma paracetamol concentrations by the clinician depends on his understanding of published data. This is at present confusing, not only because of the multiplicity of units in use (Prescott *et al.*, 1978b), but also because of the continuing failure to appreciate the gross errors introduced by the use of non-specific analytical methods. Prognostic guides such as the treatment line in Fig. 1 are useless unless used in conjunction with accurate plasma paracetamol concentrations.

The failure of some laboratory analysts to appreciate the problem of non-specificity has been due, in part, to the use of internal quality control plasma containing weighed-in paracetamol. Such plasma contains no conjugates and does not give false high values with non-specific methods. For the same reason, non-specific methods correlate well with specific methods when synthetic, as opposed to patients', specimens are used (Wilkinson, 1976).

The proper management of paracetamol poisoning depends on the rapid availability of an accurate estimate of the plasma paracetamol concentration. Gas chromatographic and high-performance liquid chromatographic methods are highly specific and are among the most accurate by virtue of the use of internal standards. Unfortunately they are not always available and not the most convenient for emergency analysis.

There are two accurate methods currently available which may be carried out in small laboratories. The colorimetric method of Glynn and Kendal (1975) is not affected by paracetamol conjugates and correlates well with HPLC as shown above, and with GLC as reported by Chambers and Jones (1976). The single point showing a result 107% higher than the result by the reference method was close to the origin and probably is a reflection of precision at this level rather than specificity. The method is subject to interference by other drugs including salicylate, and the modification described by Mace and Walker (1976) should be used if salicylate is present.

The method of Routh *et al.* (1968) and its modification by Gibson (1972) are also adquate for emergency use in the majority of cases. The latter has been in use in the Royal Infirmary of Edinburgh for over seven years and in our hands gives a coefficient of variation of ±4·8%. The method suffers from interference from a small group of drugs, of which the barbiturates and phenylbutazone are the most troublesome. In our experience, an abnormal UV scan is obtained in 10% of specimens analysed; but in only 4% is the interference likely to cause a significant error.

According to Martin and Powell (1976), the method of Glynn and Kendal gives lower results than the method of Routh *et al.* (1968). We have not confirmed this and find that the UV method of Gibson (1972) correlates well with the method of Glynn and Kendal using samples from patients.

The methods of Routh *et al.*, Gibson, and Glynn and Kendal are prone to occasional interference from other drugs, whereas the methods of Wilkinson (1976), Love (1977), Welch and Conney (1965), and Plakogiannis and Saad (1978) consistently overestimate paracetamol. Methods that involve acid hydrolysis to *p*-aminophenol without prior separation of paracetamol from its conjugates should no longer be used in clinical chemistry laboratories.

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Accepted for publication 31 January 1979