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**Studies on the metabolism of paracetamol and aspirin.**

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Studies on the metabolism of paracetamol and aspirin

Submitted by Harriet G Oldham for the degree of Ph.D. of the University of Bath, 1983.

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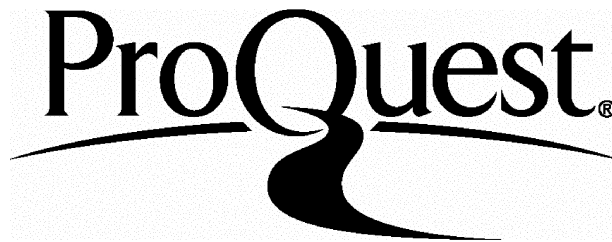
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## Summary

The metabolism of paracetamol has been studied in man following a therapeutic dose, in neonates who received the drug via breast milk, in patients with rheumatoid arthritis and hyperthyroidism, in the presence of salicylamide and after overdose. The finding of acid-labile prearomatic species in urine has led to the suggestion that these metabolites might arise from an epoxide.

The metabolism of aspirin in man was investigated in normal volunteers after a therapeutic dose, in patients with rheumatoid arthritis and after overdose.

An age effect was noted in the metabolism of paracetamol and aspirin in normal volunteers. The urinary metabolic profile of both paracetamol and aspirin in rheumatoid patients was different from that of normal volunteers. Recoveries of paracetamol and its metabolites in neonatal urine was different from normal volunteers. Predosing with salicylamide affected both the AUC and the urinary metabolic profile of paracetamol. Administration of L-methionine and/or N-acetyl cysteine to paracetamol poisoned patients appeared to replete both sulphate and glutathione stores. Aspirin overdosed patients showed a lower recovery of salicyluric acid in urine than normals.

The metabolism of paracetamol in mice and guinea pigs has been investigated in single and repeated doses in the presence of enzyme inducing agents and metabolic competing agents. The metabolism of paracetamol in the presence of L-methionine in rabbits has also been studied. Pretreatment with inducing agents, concurrent treatment with competing agents and repeated dosing were found to alter the metabolism of paracetamol when compared with a single dose of paracetamol alone. A large dose of paracetamol administered to rabbits showed a different metabolic profile from a small dose of paracetamol and from the large dose given concurrently with L-methionine.

### Acknowledgements

I should like to express my gratitude to the School of Pharmacy and Pharmacology at the University of Bath for the use of its facilities, to the Arthritis and Rheumatism Council for making available the grant and to Smith Kline and French Research Ltd for financing the typing. I should like to especially thank Dr P N Bennett M.D., F.R.C.P and Dr L J Notarianni M.Sc, Ph.D for their continuing interest, advice and stimulating comments throughout this project. My thanks also go to Dr A St.J Dixon M.D., F.R.C.P. who applied jointly with Dr Bennett for the grant and for his valuable assistance in gaining access to patients and in liason with the Royal National Hospital for Rheumatic Diseases in Bath and to Professor R T Parfitt, Dr A Ogunbona and Dr C C B Southgate for their very helpful advice. Finally I should like to thank Mrs S Humphries for collecting samples, my father for statistical advice and Mrs Gwen Seer for her typing.

Dedicated to:

My parents, my sister Sarah and my  
fiancé Clive with thanks for  
their continuing support and encouragement.



Abstract

- 1.i. The metabolism of paracetamol has been studied in man following a therapeutic dose, in neonates who received the drug via the breast milk, in patients with rheumatoid arthritis and thyroid disease, in normal volunteers in the presence of salicylamide and after overdose.
- 1.ii After a therapeutic dose of paracetamol administered to normal volunteers, the percentages of excreted material recovered as paracetamol cysteine were found to decrease and as paracetamol glucuronide were found to increase with increasing age.
- 1.iii After indirect administration of paracetamol in breast milk, neonates excreted larger proportions of unchanged paracetamol and lesser proportions of paracetamol sulphate than normal volunteers.
- 1.iv Patients with rheumatoid arthritis who took varying amounts of paracetamol excreted greater amounts of paracetamol glucuronide and lower amounts of unchanged paracetamol and paracetamol sulphate than did control subjects.
- 1.v The seven hyperthyroid patients showed a metabolic profile similar to subjects in the control study.
- 1.vi In volunteers pre-dosed with salicylamide, the percentage recovery of paracetamol as sulphate in 24 hour urine was lower than when paracetamol was given alone. It was also found that the area under the serum paracetamol concentration time curve was greater when salicylamide was taken than when paracetamol was taken alone.
- 1.vii After overdose, it was found that excretion of the sulphate and glutathione conjugates of paracetamol was lower than after a therapeutic dose. After administration of N-acetyl cysteine and/or L-methionine to these patients, it is suggested that repletion of both sulphate and glutathione stores may occur.

- 1.viii The finding of pre-aromatic species in urine which were converted to 3-hydroxy paracetamol and paracetamol cysteine plus mercapturic acid on incubation with acid, has led to the suggestion that these metabolites might arise from an epoxide. Thus it has been proposed that 4-hydroxy-2,3-dihydroacetanilide-2-3-oxide is likely to be an hepatotoxic metabolite of paracetamol in man.
  
2. The metabolism of paracetamol in mice and guinea pigs has also been investigated in the presence of enzyme inducing agents and metabolic competing agents and in single and repeated doses. The metabolism of paracetamol in the presence of L-methionine in rabbits has been studied.
  - 2.i Phenobarbitone, phenylbutazone and rifampicin were found to increase the recoveries of the cysteine and mercapturic acid metabolites in mice and guinea pigs indicating an increase in paracetamol oxidation.
  
  - 2.ii Repeated dosing with paracetamol 100mg/kg twice daily in guinea pigs resulted in a significantly lower recovery of paracetamol glucuronide and a greater recovery of unchanged paracetamol than did a single dose of paracetamol ( $p < 0.05$ ). Mice showed a decreased percentage recovery of paracetamol glucuronide with time after repeated doses of paracetamol 100mg/kg twice daily accompanied by an increased recovery of unchanged paracetamol. Percentage recoveries of the glutathione conjugates of paracetamol decreased with time up to 168 hours but then increased dramatically. Marked differences between single and repeated doses in mice were also observed.
  
  - 2.iii L-ascorbic acid, salicylamide and  $\alpha$ -tocopherol given concurrently with paracetamol 100mg/kg increased recoveries of paracetamol glucuronide and decreased paracetamol excreted unchanged in guinea pigs.

In mice,  $\alpha$ -tocopherol increased the percentage recoveries of paracetamol glucuronide and paracetamol cysteine and mercapturic acid and decreased the recovery of unchanged paracetamol. L-ascorbic acid and salicylamide did not alter the metabolic profile of paracetamol in mice.

- 2.iv Paracetamol 400mg/kg administered to rabbits caused a decrease in the percentage recoveries of paracetamol cysteine and mercapturic acid metabolites in urine compared with paracetamol 100mg/kg. L-methionine 400mg/kg administered with paracetamol 400mg/kg resulted in an increase in paracetamol cysteine and mercapturic acid recoveries but these percentages were still not as high as those found after paracetamol 100mg/kg.
3. The metabolism of aspirin in man was investigated in normal volunteers after a therapeutic dose, in patients with rheumatoid arthritis and after overdose.
- 3.i After a 600mg dose of aspirin, the percentage dose of aspirin excreted was found to decrease with increasing age and the percentage recoveries of salicylic acid and the salicyl glucuronides were also found to vary with age. Percentage recovery of salicylic acid in 0 - 8 hour urine was positively correlated with urinary pH.
- 3.ii In patients with rheumatoid arthritis who took varying amounts of aspirin the percentage recovery of salicyluric acid was greater and the recoveries of unchanged salicylate and salicyl glucuronides were lower than after a therapeutic dose of aspirin in normal volunteers.
- 3.iii In overdose patients, the major metabolite recovered initially was salicylic acid. The rate of excretion of each metabolite with time was found to vary markedly between individuals and it is suggested that maximal excretion rates of salicyluric acid and the salicyl glucuronides are not reached at such doses of aspirin as was previously thought.

## Glossary of Abbreviations

ASA -	acetyl salicylic acid
GA -	gentisic acid
HPLC-	high performance liquid chromatography
P -	paracetamol
PC -	paracetamol cysteine
PG -	paracetamol glucuronide
PM -	paracetamol mercapturic acid
PS -	paracetamol sulphate
SA -	salicylic acid
SG -	salicyl glucuronides
SUA -	salicyluric acid

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## Chapter 1

### INTRODUCTION

#### 1.1 General Introduction

##### 1.1.1 Analgesics - their history and use

Almost everyone at some time experiences minor or major pain. The most ancient pain-relieving substance, opium, which was originally smoked or drunk, relieves most types and degrees of pain but is characterized by significant tolerance, physical dependence and social abuse. Today, morphine is used mainly for the relief of severe pain, for example in cancer, postoperative pain or trauma. The relief of pain by the opioids is selective to the extent that other senses (touch, vibration, vision and hearing) are not affected but the level at which pain is perceived is raised and euphoria is induced so that the sufferer is more inclined to ignore the pain. The serious limitations of morphine and other narcotic analgesics stimulated the search for potent analgesics that would not lead to compulsive drug use.

This led to the discovery of the antipyretic, non-narcotic, mild analgesics such as aspirin and paracetamol which provide relief of moderate pain and are not associated with appreciable tolerance or physical dependence.

##### 1.1.2. History of aspirin and paracetamol

The Reverend Edward Stone of Chipping Norton first described the efficacy of willow bark in a letter to the President of the Royal Society, Lord Macclesfield, dated 25th April 1763. This began:

"My Lord,

Among the many useful discoveries which this age has made, there are few which better deserve the attention of the public than what I am going to lay before your Lordship. There is a bark of an English tree which I have found by experience to be a powerful astringent, and very efficacious in curing aguish (rheumatism) and

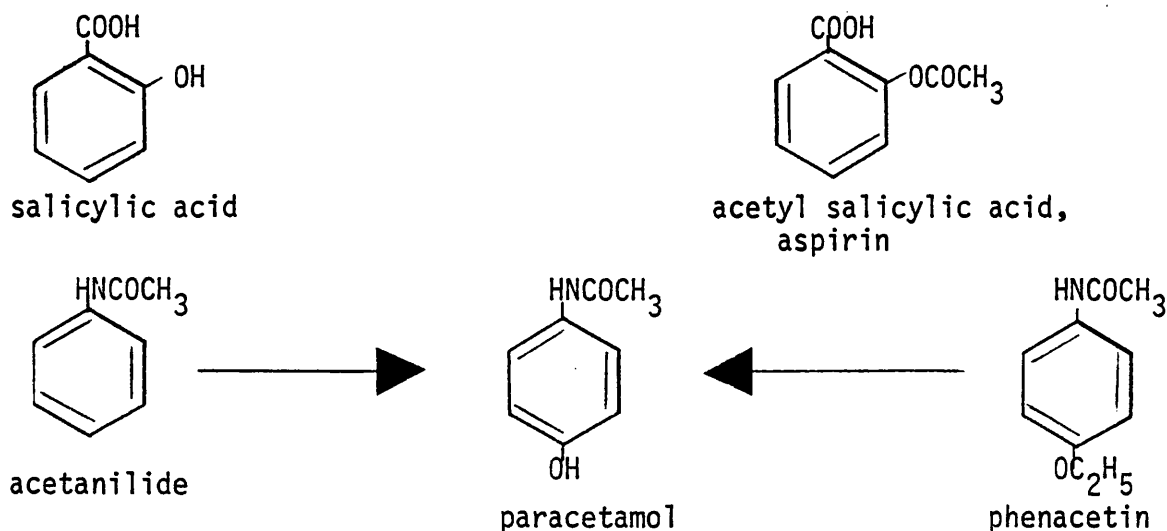
intermitting disorders (bouts of fever)" (taken from Fairley, 1978). Following publication of this letter, willow bark was frequently prescribed for fevers but usually as a substitute for the expensive and scarce Peruvian bark (Cinchona). An English pharmacist called White reported in 1798 "Since the introduction of this bark into practice in the Bath City Infirmary and Dispensary, as a substitute for the Cinchona, not less than 20 pounds a year have been saved to the Charity" (Fairley, 1978).

Salicylic acid was first synthesised chemically by Lowig in 1835 and its use as an anti-inflammatory and analgesic in rheumatic fever was reported by MacLagan in 1876. Unfortunately salicylic acid was found to be too great an irritant to the mouth, gullet and stomach to be of great use except in the most painful cases. In 1853 Von Gerhardt made a small amount of acetyl salicylic acid but the synthesis was so expensive and time consuming that it was not put into production. Forty years later, Felix Hoffmann became interested because his father had arthritis and benefited from salicylic acid but could not tolerate its effect on his stomach. Hoffman studied Gerhard's acetylation process and modified it so that the synthesis was practicable. Hoffman and a colleague, Dreser, both of whom worked for Bayer, established that salicylic acid was released from acetyl salicylic acid. Dreser and Hoffman decided to call the drug aspirin, the 'a' stood for acetyl, the 'spir' for the spiraea plant family (otherwise known as meadowsweet from which the salicylic acid was obtained) and the 'in' for good measure (Fairley, 1978). In 1899 aspirin was marketed by Bayer.

Dr. T.B. Begg of the Medical Research Council's Clinical Chemotherapy Research Unit wrote that "From 1876 until the present time, sodium salicylate and from 1899, acetyl salicylic acid ... have been the mainstay of the chemotherapy of acute rheumatism" (taken from Fairley, 1978).

Some years before aspirin came into general use in medicine, derivatives of phenyl and aniline (compounds derived from the benzene fraction of coal tar) were found to have an antipyretic action and the first of these compounds, acetanilide was introduced into medicine by accident when a pharmacist mistakenly dispensed acetanilide in place of naphthalene. Unfortunately acetanilide was found to cause cyanosis. Baron Joseph Von Mering searching for less toxic alternatives to acetanilide observed that acetanilide was converted in the body to para-aminophenol and (from derivatives of para-aminophenol) discovered phenacetin and paracetamol (which is actually the form in which phenacetin is excreted from the body). Phenacetin which had about the same analgesic potency as aspirin, was found however to be capable of causing severe renal necrosis and the sale of phenacetin was banned except when on prescription from 1st September 1974. Von Mering dismissed paracetamol as being too toxic and it did not really become popular until 1949 when it was recognized as the major active metabolite of both acetanilide and phenacetin (Brodie and Axelrod, 1949). Five years later, the drug was marketed in Britain under the trade-name Panadol, produced by Bayer Products Ltd whose German parent-company had originally patented aspirin (Fairley, 1978). Panadol became very popular in Britain at a time when aspirin's irritant effect on the gastrointestinal tract was being publicized in the newspapers and is even now used as an alternative to aspirin. Paracetamol was included in the British Pharmacopoeia from 1963 and became widely used for many minor painful and febrile conditions.

Structures of salicylic acid, aspirin, acetanilide, paracetamol and phenacetin



Aspirin and paracetamol as antipyretics and analgesics may be considered to be equipotent (Yaffe, 1981; Cooper, 1981) and both are often formulated with other drugs such as opiates, vasoconstrictors, anti-emetics, CNS stimulants etc. First reports of hepatic necrosis from overdose of paracetamol were published in 1966 (Davidson and Eastham, 1966; Thomson and Prescott, 1966). Despite their reported side-effects, since 1949 aspirin and paracetamol have been the two most commonly used drugs in the relief of mild to moderate pain.

### 1.1.3. Modes of action and uses of paracetamol and aspirin

The mechanisms of the inflammatory process are complex; contenders as mediators of inflammation so far include histamine (but not in the inflammation seen in rheumatoid arthritis), 5-hydroxy tryptamine, complement and more recently the prostaglandins. Prostaglandins when injected into the skin cause erythema and increased vascular permeability leading to oedema and hyperalgesia. It is thought that prostaglandins may act as modulators capable of increasing or decreasing the response to pain. Prostaglandin  $E_1$  is in addition a strong pyrogen when injected into the interior hypothalamus or cerebral ventricles and this effect is not blocked by antipyretic agents. Prostaglandins are also thought to be involved in the adhesion of platelets to blood vessel walls and there appear to be two different mechanisms for the metabolism of arachidonic acid (a precursor of the prostaglandins) in platelets and in the vessel wall. In platelets it is assumed that thromboxane  $A_2$  is formed which enhances aggregation and has a vasoconstrictory effect. In contrast, in vessel walls the same intermediate product gives rise to prostacyclin which has an anti-aggregant and vasodilator effect.

Aspirin and paracetamol have analgesic and antipyretic effects and both are effective in relieving pain of moderate intensity including headache, myalgia, arthralgia and dysmenorrhoea. Severe pain and that originating in the viscera are not alleviated by these drugs.



The analgesia caused by aspirin is considered to be due to both a central and a peripheral action. Ferreira and Vane (1974) suggested that salicylates act by inhibiting prostaglandin synthesis which occurs in inflamed tissues and thus may prevent sensitisation of pain receptors to chemicals such as bradykinin or histamine or to mechanical stimulation. These authors showed that paracetamol had almost the same potency as aspirin in inhibition of prostaglandin synthesis in the brain but found that paracetamol was much less effective than aspirin as an inhibitor of peripheral prostaglandin synthetase. Further to this McDonald-Gibson and Collier (1979) found that paracetamol in low concentrations (0.07 - 1.98mM) stimulated prostaglandin production in bull seminal vesicle homogenates perhaps by acting as a co-factor of cyclo-oxygenase. Higher concentrations (6.61mM) significantly depressed prostaglandin production. In addition these workers found that mixtures of paracetamol and acetylsalicylic acid exhibited more inhibitory potency than either drug alone. They concluded that this might be due to the action of paracetamol as a phenolic cofactor which increases the susceptibility of prostaglandin cyclo-oxygenase to acetyl salicylic acid. This raises the possibility that mixtures of paracetamol and aspirin may be more effective than each drug separately.

Aspirin is an efficient anti-inflammatory agent and is therefore of particular use in conditions in which inflammation plays a dominant role - notably rheumatoid arthritis. Aspirin may be especially effective as an analgesic therefore in inflamed tissues where prostaglandin synthesis is leading to pain. Mills (1974) suggested that the markedly different sensitivity of central and peripheral prostaglandin synthetases to paracetamol may partly explain its weak anti-inflammatory actions in clinical doses. More recently an anti-inflammatory effect of paracetamol has been reported and it may reduce pain and swelling in inflammatory conditions other than arthritis sometimes more effectively than aspirin (Cooper, 1981 and Skjelbred, Alburna and Lokken, 1977).

Aspirin lowers body temperature in febrile patients but not in patients with normal temperatures. This effect also appears to involve inhibition of prostaglandins of a PGE-like nature in the brain and this effect is inhibited by salicylates which suggests that PGE may be an endogenous pyrogen. Paracetamol has been shown to inhibit the action of endogenous pyrogens on the hypothalamic heat-regulating centres (Milton, 1976) and its antipyretic effect may stem from a direct action on these centres resulting in dissipation of heat.

The possible role of aspirin as an antithrombotic agent has recently evoked much interest (Aspirin Myocardial Infarction Study Group (1980), Barnett, 1978 and Persantine Aspirin Reinfarction Study Research Group, 1980). Since platelets are involved in thrombus formation it may be possible, by using a suitable dose of aspirin, selectively to inhibit the synthesis of thromboxane but not prostacyclin and thus prevent platelet aggregation. Clinical trials are in progress to ascertain the value of aspirin in the prevention of mortality after myocardial infarction and preliminary reports suggest that aspirin may be of benefit although the evidence is not clear-cut (Loew, 1977; Editorial, Lancet, 1979, 1356; Elwood 1980 and Aspirin Abstracts Nos. 4 and 5).

In summary, aspirin has an important place in the treatment of pain of most types, of fever and of inflammation of many causes including that encountered in the rheumatic diseases. Paracetamol has similar analgesic and antipyretic effects to those of aspirin but may possess only a weak anti-inflammatory action. Therefore it is used in mild to moderate pain which is not of visceral origin and which is not accompanied by significant inflammation. Paracetamol as yet appears to have no place as an antithrombotic agent.

#### 1.1.4. Toxicity of aspirin and paracetamol

Aspirin and paracetamol were in use long before the current system of legislation for the introduction of new medicines in the United Kingdom was in operation, but their wide consumption, duration of use and availability without prescription indicates that these drugs are considered to be safe in normal clinical doses.

#### 1.1.4.1. Toxicity in chronic use

Moderate doses of aspirin taken over a period of time have long been associated with occult and overt gastrointestinal bleeding and gastric ulcer (see Silviso, Ivey, Butt et al, 1979). However recently Rees and Turnberg (1980) concluded that although acute exposure of the gastric mucosa results in increased blood-loss, abnormal mucosal function and short lasting morphological damage, there was little evidence that low dose aspirin consumption results in either peptic ulcer or acute gastrointestinal bleeding. Frequent or heavy consumption of the analgesic seems to be associated with gastric ulcer and acute gastrointestinal bleeding but this may reflect a tendency for aspirin ingestion in response to pain from peptic ulcer causing gastrointestinal bleeding. Support for this hypothesis has been given by Piper, McIntosh, Ariotti, Fenton and MacLennan (1981) who found a strong positive correlation between heavy aspirin consumption (relative risk of 17.3) or heavy paracetamol consumption (relative risk of 24.4) with chronic gastric ulcer where relative risk is the ratio of the risk amongst that exposed to that amongst the unexposed. Unlike aspirin, paracetamol does not cause gastric mucosal injury (Loebl, Craig, Culic, Ridolfo, Falk and Schmid, 1977), Ivey, Silviso and Krause, (1978). However Langman, Edmond and Hardy (1979) found paracetamol consumption to be greater than aspirin consumption in 190 patients with upper gastrointestinal bleeding. This suggests that a causal relationship between aspirin intake and gastrointestinal bleeding may not necessarily exist.

Aspirin may also cause stomach upset including dyspepsia, heartburn, nausea and vomiting. It may also prolong the duration of pregnancy if taken regularly during the second and third trimester. Its role as a causative agent in Reye's syndrome has yet to be evaluated.

Aspirin and paracetamol have also been associated with nephrotoxicity. However paracetamol has to be given in extremely large doses to

experimental animals (except Gunn rats) to produce renal papillary necrosis and there have been few reports on paracetamol-induced nephropathy (Prescott, 1982). Aspirin has been shown to produce experimental renal necrosis, (Molland, 1978) and possible renal function impairment and analgesic nephropathy in man (Lee, 1979). In a recent study however, carried out by Akyol, Thomson and Kerr (1981) in 15 patients who had taken no other known nephrotoxin but aspirin over a mean of 16 years, no evidence of analgesic nephropathy was found. Thus while longterm analgesic abusers do develop a nephropathy, a causal relationship is difficult to prove for any specific analgesic. There may be an association between uroepithelial tumours and analgesic nephropathy and although phenacetin seems to be a common link, tumours have also been reported in patients taking salicylates without phenacetin (Prescott, 1982).

Aspirin may have adverse effects on the liver but these usually occur in association with diseases such as rheumatic fever, juvenile rheumatoid arthritis and systemic lupus erythematosus when aspirin is taken in large doses over a long period. There have been only a few reports of hepatotoxicity arising as a result of chronic ingestion of paracetamol (for example see Zimmerman, 1981 and Johnson and Tolman, 1977) despite its effect on the liver in acute overdose.

#### 1.1.4.2. Toxicity in acute overdose

High doses of aspirin cause tinnitus, deafness, nausea, abdominal pain, respiratory alkalosis (more common in adults), metabolic acidosis (may occur in children) and hepatotoxicity. Acute overdose with paracetamol is manifest as liver necrosis and, more recently recognised, nephrotoxicity (Cobden, Record, Wood and Kerr 1982). After severe paracetamol poisoning peak disturbance of hepatic function occurs 4 - 6 days after overdose and may be followed by frank liver failure and hepatic encephalopathy (Meredith and Goulding, 1980).

The toxicity of aspirin is related both to metabolic and pharmacokinetic factors and paracetamol toxicity is thought to arise from its mode of metabolism. A brief description of the ways in which drug biotransformation contributes towards overall elimination of a drug from the body and some of the factors influencing drug metabolism therefore follows.

## 1.2. Drug Metabolism

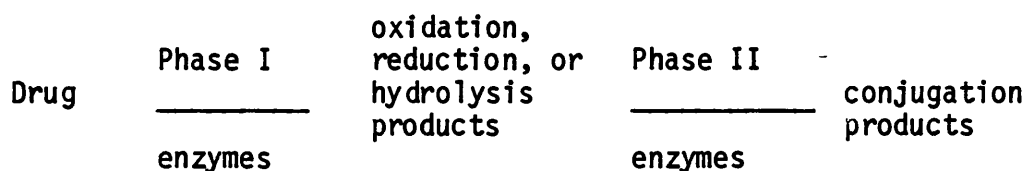
Termination of a drug's biological effect requires that it be removed from its site of action by metabolism and excretion. Since the products of metabolic transformations are usually more polar than their parent compounds and therefore more readily eliminated, it follows that the metabolism of a drug or xenobiotic may affect not only its duration of action but also its toxicity.

Williams (1972) summarised the possible fate of a drug in the body as follows:

- a drug can undergo an enzyme catalyzed transformation
- a drug can undergo a spontaneous reaction when given certain physical conditions such as pH e.g. thalidomide
- a drug can be excreted unchanged.

Most drugs undergo the first of these and enzymes that catalyse these metabolic changes are located mainly in the endoplasmic reticulum of the liver. These enzymes vary in nature and amount from species to species and variation in the duration of action of drugs between species may be explained by these differences (Brodie, 1956). Some extra-hepatic tissues (e.g. lung and kidney) are also capable of drug metabolism but these tend to be less active per unit weight than liver.

It is conventional to consider drug metabolism in two phases. Phase I reactions include oxidation, reduction and hydrolytic reactions which allow polar groups (e.g. OH, COOH, NH<sub>2</sub> and SH) to be introduced into the molecule. Phase II reactions are conjugation (synthetic) reactions which involve the addition of polar groups to the molecule.



Phase I reactions can lead to inactivation of the drug (e.g. oxidation of pentobarbitone), they can convert an inactive drug to an active drug (e.g. prontosil > sulphanilamide) or they may convert a pharmacologically active drug to an active metabolite (e.g. phenylbutazone > oxyphenbutazone). Phase II reactions generally result in termination of the activity of a drug. Some drugs which already possess a suitable (e.g. hydroxyl) group may only undergo a Phase II reaction. Phase II reactions give rise to very polar molecules which are rapidly excreted from the body (for example salicylic acid > salicyluric acid, paracetamol > paracetamol glucuronide + paracetamol sulphate) and involve the conjugation with an endogenous molecule (e.g. glucuronic acid, glycine, cysteine, methionine and sulphate) with the drug or its Phase I metabolite. A source of energy is also needed for the conjugation and this is usually supplied through adenosine triphosphate.

Some factors which may affect the rate of metabolism of drugs are listed in Table 1.1.

Table 1.1

Some factors that influence the rate of drug metabolism (from Williams, 1972)

species	other drugs or foreign compounds
strain	route of administration
age	season
chronic administration	diet
sex (mainly in rats)	gut flora
stress	altitude
temperature	biliary excretion and enterohepatic circulation
time of day	disease

These factors particularly alter the activities of microsomal drug-metabolising enzymes; the conjugation reactions are generally influenced to a lesser extent. As indicated in Table 1.1. activity of microsomal drug-oxidising enzymes may be altered by pathological states such as liver or kidney disease, hormonal disturbances, tumour bearing states and adjuvant arthritis (for a review see Kato, 1977). Examples of the effect of disease states on drug metabolism include the prolongation of hexobarbitone sleeping time in jaundiced rabbits (McLuen and Fouts, 1960) and the prolongation of the sleeping time of several barbiturates in rats with adjuvant induced arthritis (Querauviller, Chalchat, Brovilhet and Delberre, 1968). The kinetics of several common drugs have been reported to be altered in non-neoplastic liver disease. Half life was reported to be increased of diazepam (Klotz, Antonin, Brugel and Bieck, 1976); hexobarbitone (Breimer, Zilly and Richter, 1975); paracetamol

(Prescott, Wright, Roscoe and Brown, 1971); lignocaine (Thomson, Melmon, Richardson et al, 1973) and propranolol (Branch, Morgan, James and Read, 1976).

Chronic administration of a drug can decrease the pharmacological activity of another drug by stimulating its metabolic inactivation and thus reducing its concentration at its site of action. This effect seems to be produced by an increase in the amounts of drug metabolising enzymes (and in particular cytochrome P-450) and is referred to as enzyme induction. This term has been used specifically to describe a process which increases the rate of synthesis of an enzyme or decreases its rate of degradation (or both) relative to its rate of synthesis in the uninduced organism.

A number of drugs cause an increase in hepatic drug metabolising enzymes. These include polycyclic aromatic hydrocarbons such as 3,4-benzpyrene and 3-methyl cholanthrene which show considerable specificity, the barbiturates which are relatively non specific, rifampicin, phenylbutazone and ethyl alcohol. Many environmental chemicals can stimulate enzyme activity and effects of these must be allowed for in the design of experiments involving enzyme induction.

Factors which influence concentrations of drug metabolising enzymes may alter drug toxicity. Pretreatment of rats with enzyme inducers decreases the acute toxicity of strychnine, pentobarbitone and meprobamate because these drugs are converted rapidly to less active metabolites by microsomal enzymes (Conney, 1971). Prescott, Critchley, Balali-Mood and Pentland (1981) concluded that although the half life of paracetamol was decreased in patients taking anti-convulsants or rifampicin there did not appear to be increased conversion of paracetamol to its toxic intermediate metabolite.



### 1.3. Drug Elimination

Most drugs are excreted through the kidneys and drugs can be handled by the kidneys in three ways:

- i) the majority of drugs are eliminated mainly by glomerular filtration and all drugs of low molecular weight and all water soluble drugs are rapidly cleared by this fairly non-selective process. The rate of glomerular filtration depends upon the availability of the drug in an unbound form in the plasma and the rate at which the drug is converted to polar products by biotransformation.
  
- ii) lipid soluble drugs and metabolites which have entered the renal tubule by glomerular filtration diffuse back into the plasma by passive diffusion following a concentration gradient. This prolongs the duration of action of such drugs. The rate of reabsorption depends upon the lipid solubility of the drug but as most drugs are weak electrolytes, they may be present partly in the lipid soluble non-ionized form at physiological pH. The pH of urine can therefore affect the degree of ionisation of weak electrolytes and hence tubular reabsorption. This forms the basis of forced alkaline or, (less commonly) forced acidic diuresis for the treatment of poisoned patients.
  
- iii) Drugs, which are strong bases or acids are eliminated by a process of active secretion into kidney tubules.

Organic anions and cations are handled by separate active transport mechanisms which are energy dependent and blocked by metabolic inhibitors. The anionic group has been studied in detail and can be saturated at high concentrations. Each acid has its characteristic maximum tubular excretion rate ( $T_m$ ). The various anionic compounds compete for secretion, for example probenecid blocks the otherwise rapid excretion of penicillin thereby prolonging its duration of action.

Organic cations are excreted by a different energy dependent pathway for which they compete and which is exclusive to cations.

#### 1.4. Disposition and metabolism of paracetamol and aspirin

A more detailed description of the absorption, distribution, excretion and in particular the metabolism of paracetamol and aspirin follows.

##### 1.4.1. Paracetamol

###### 1.4.1.1. Absorption, distribution and excretion

Paracetamol is rapidly absorbed from the gastrointestinal tract and Hunt and Dunford in 1979 found that peak plasma concentrations occur from half to one hour after ingestion. They also found that gastric emptying time was a major variable in determining the rate but not the extent of paracetamol absorption. This study showed in addition that the mean plasma elimination half life was three hours (range 2.52 - 3.62h). Distribution of paracetamol is relatively uniform in most body fluids. Gazzard, Ford-Hutchinson, Smith and Williams (1973) found no plasma protein binding of paracetamol at plasma concentrations below 60 $\mu$ g/ml but at 280 $\mu$ g/ml, 15 - 21% was bound in humans and pigs. Hunt and Dunford (1977) found that prolongation of mean plasma half life in patients with severe liver disease was related to reduced plasma albumin although this could result from the liver disease per se. Following metabolism paracetamol is mainly eliminated by the kidneys and only a small percentage (about 5%) is excreted unchanged in urine.

#### 1.4.1.2 Metabolism

The biochemical mechanism of the hepatotoxicity of paracetamol was first suggested by Mitchell, Jollow, Potter, Davis, Gillette and Brodie in 1973. Studies on the mechanism of paracetamol-induced liver damage in laboratory animals showed that paracetamol is converted in the body to a chemically reactive arylating agent that covalently binds to vital hepatocellular macromolecules. In addition a direct relationship was demonstrated between the formation of a paracetamol glutathione conjugate, arylation of hepatic macromolecules and hepatic damage after paracetamol ingestion.

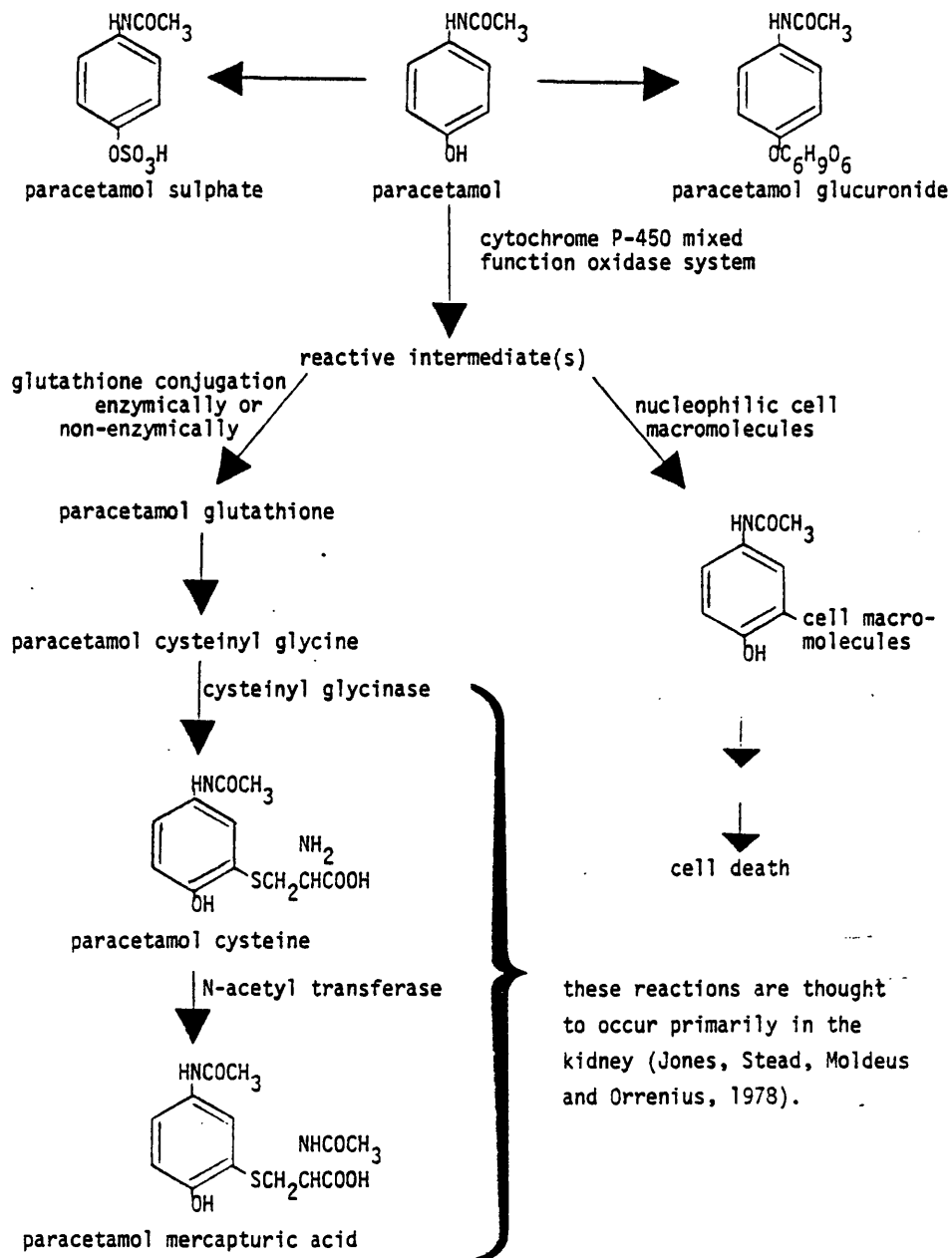
The major urinary metabolites of paracetamol are the glucuronic acid and sulphate conjugates which combine with paracetamol at the 4 position forming the non-toxic glucuronide and ethereal sulphate derivatives. In addition paracetamol is oxidised by the cytochrome P-450-dependent drug-metabolising enzyme(s) to a reactive intermediate. This intermediate is preferentially conjugated with hepatic glutathione and after further metabolism is excreted in the urine as paracetamol cysteine and paracetamol mercapturic acid. The mercapturate conjugate of paracetamol was first identified by Mitchell and Jollow in 1974 after conjugates of paracetamol containing divalent sulphur were found in urine. Hepatic glutathione however is limited and once it is depleted covalent binding to hepatocytes may occur.

The present understanding of the relationship between the metabolism of paracetamol and its hepatotoxicity is summarised in the scheme overleaf (Fig 1.1).

#### 1.4.1.3 Paracetamol poisoning

Paracetamol in normal therapeutic doses is one of the safest mild analgesics. Serious toxicity with paracetamol was first recognised in 1966 when fatal hepatic necrosis after overdosage was reported (Davidson and Eastham, 1966 and Thomson and Prescott, 1966). It was already known that paracetamol could produce centrilobular hepatic

Fig. 1.1 Pathways of paracetamol metabolism (modified from Mitchell et al, 1974)



necrosis in animals (Eder, 1964, Boyd and Bereczky, 1966). Paracetamol, as a result of its free availability is now commonly taken in overdose for suicide purposes and there is a steady increase in the number of paracetamol-poisoned patients admitted to poisoning treatment centres throughout Britain.

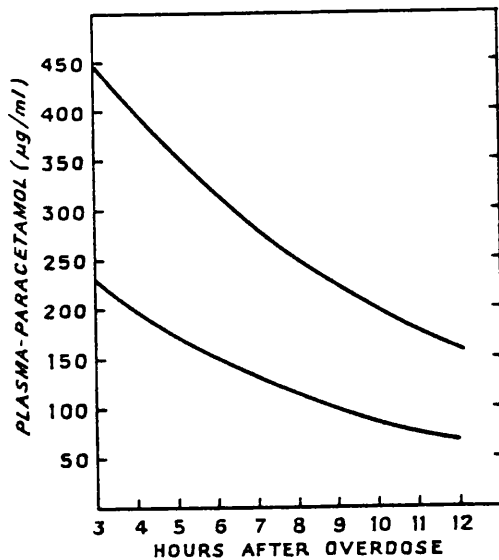
Current rheumatological textbooks recommend doses of paracetamol of up to 6g per day when used in the treatment of pain in rheumatoid arthritis. Fernandez and Fernandez-Brito (1977) reported a case in which 5.85g of paracetamol taken as a single dose caused liver damage. Another case report described the development of liver fibrosis which was attributed to ingestion of 2.7g paracetamol per day for one week followed by a week at a lower dose level (Olsson, 1978). An earlier case history reported chronic aggressive hepatitis in a female patient who ingested 2.925g of paracetamol per day for a year (Johnson and Tolman, 1977). One case of moderately severe liver necrosis after 4g per day for approximately one year was reported by Bonkowsky, Mudge and McMurtry (1978).

Notwithstanding these reports the dose regimen quoted in Martindale (Extra Pharmacopea) is 0.5 - 1g every 3 - 4 hours with a maximum of 4g per day. A one-day survey conducted at the Royal National Hospital for Rheumatic Diseases in Bath showed that some patients ingested as much as 8g paracetamol per day (Dr A St.J. Dixon personal communication). The possibility is thus raised that some patients with painful diseases such as rheumatoid arthritis may be unknowingly creating the potential for toxicity by longterm use of large doses of paracetamol.

In the first few days after acute paracetamol overdose there are few symptoms. In the first twenty four hours nausea, vomiting, anorexia and abdominal pain occur which may last for up to a week. Liver injury is usually evident on the second day with elevation of serum bilirubin, transaminase and lactate dehydrogenase concentrations and prolonged prothrombin time. In serious cases, encephalopathy, coma and death may ensue. Transient azotemia often occurs and in some patients renal failure dominates. A recent report suggests that

acute renal failure may occur in the absence of liver failure and may become evident one week after ingestion compared with two to four days for the liver damage (Cobden et al, 1982). The centrilobular necrosis which is seen on liver biopsy is reversible over the following months in non-fatal cases (Woodbury and Fingl, 1975).

Prescott, Sutherland, Park, Smith and Proudfoot (1976) suggested that the earliest evidence of liver damage is prolongation of the plasma half-time of paracetamol. They showed that a paracetamol half-time greater than four hours indicates the occurrence of hepatic necrosis and above twelve hours indicates that hepatic coma may be expected. The admission plasma paracetamol concentration has also been used to estimate the severity of intoxication and liver damage is likely to be severe above the upper line, mild between the lines and clinically insignificant below the lower line.



Prescott et al, 1976

#### 1.4.1.4 Paracetamol poisoning and specific therapy

The basis of the specific therapy employed in paracetamol poisoning stems from work by Mitchell, Jollow, Potter, Gillette and Brodie (1973) who demonstrated that glutathione had a crucial role in the metabolism of paracetamol. They found that pretreatment with diethyl maleate depleted hepatic glutathione and potentiated paracetamol-induced hepatic necrosis while pretreatment with cysteine (a precursor of glutathione) prevented the hepatotoxicity. Paracetamol caused a dose-dependent depletion of hepatic glutathione which was enhanced by treatments that potentiated hepatic damage and which was prevented by treatments that protected against the liver necrosis. Thus the treatment of paracetamol poisoning is directed towards removal of the active metabolite (which is responsible for the hepatotoxicity) by either the provision of sulphhydryl groups or by administration of compounds which may act as glutathione precursors. Compounds such as cysteamine, methionine and N-acetyl cysteine which contain thiol groups should be given within 12 hours of paracetamol ingestion because they may precipitate hepatic coma if given after this time. These antidotes were first found to be effective by Prescott and co-workers (Prescott, Swainson, Forrest, Newton, Wright and Mathew, 1974; Prescott, Sutherland, Park, Smith and Proudfoot, 1976; Prescott, 1977); glutathione itself is thought to be poor in penetrating cell membranes (see Black, 1980). Although Strolin-Benedetti, Malnoe, Schneider, Lam, Krebor and Smith (1975) found that glutathione given intravenously can protect mice against the hepatotoxicity of a large dose of paracetamol, Gazzard, Hughes, Portmann, Dordoni and Williams (1974) found intravenous glutathione to have no protective effect in rats.

## 1.4.2 Aspirin (acetylsalicylic acid)

### 1.4.2.1 Absorption, Distribution, Kinetics and Metabolism

Acetylsalicylic acid is absorbed from the stomach and from the small intestine. As it is an anionic drug which is unionized at low pH and has a  $pK_a$  of 3.5, the acidic environment of the stomach (about pH 1.4) would be expected to favour absorption. In fact most of an oral dose of aspirin is probably absorbed from the upper small intestine which has a vastly greater surface area than does the stomach. There is little difference between rates of absorption of unbuffered aspirin, buffered aspirin and aspirin solution (although aspirin absorption appears to be greatly affected by the fasting state absorption being much slower after a heavy meal, Davison, 1971). When non-dissociated acetylsalicylic acid molecules in the gastric lumen enter mucosal cells, the higher pH renders acetyl salicylate more ionized and less lipid soluble thus favouring accumulation in epithelial cells. High acetyl salicylic acid concentrations in superficial cells has been advanced as an explanation for the mucosal cell loss and also gastric damage due to aspirin (Wood, 1963).

Aspirin taken by mouth is mainly absorbed unchanged but some salicylic acid enters the portal circulation. Aspirin has a plasma half life of about fifteen minutes due to its rapid conversion to salicylic acid by hydrolases in liver and plasma. At clinical concentrations some 50 - 90% of salicylic acid is bound to plasma proteins, mainly albumin (Reynolds and Cluff, 1960) while aspirin is bound to only a very limited extent.



Stafford (1963) suggested however that in some pathological states plasma proteins may be quantitatively and qualitatively altered and this may result in higher concentrations of salicylate in the plasma; hypoalbuminaemia may occur in rheumatoid arthritis (Reynolds and Cluff, 1960).

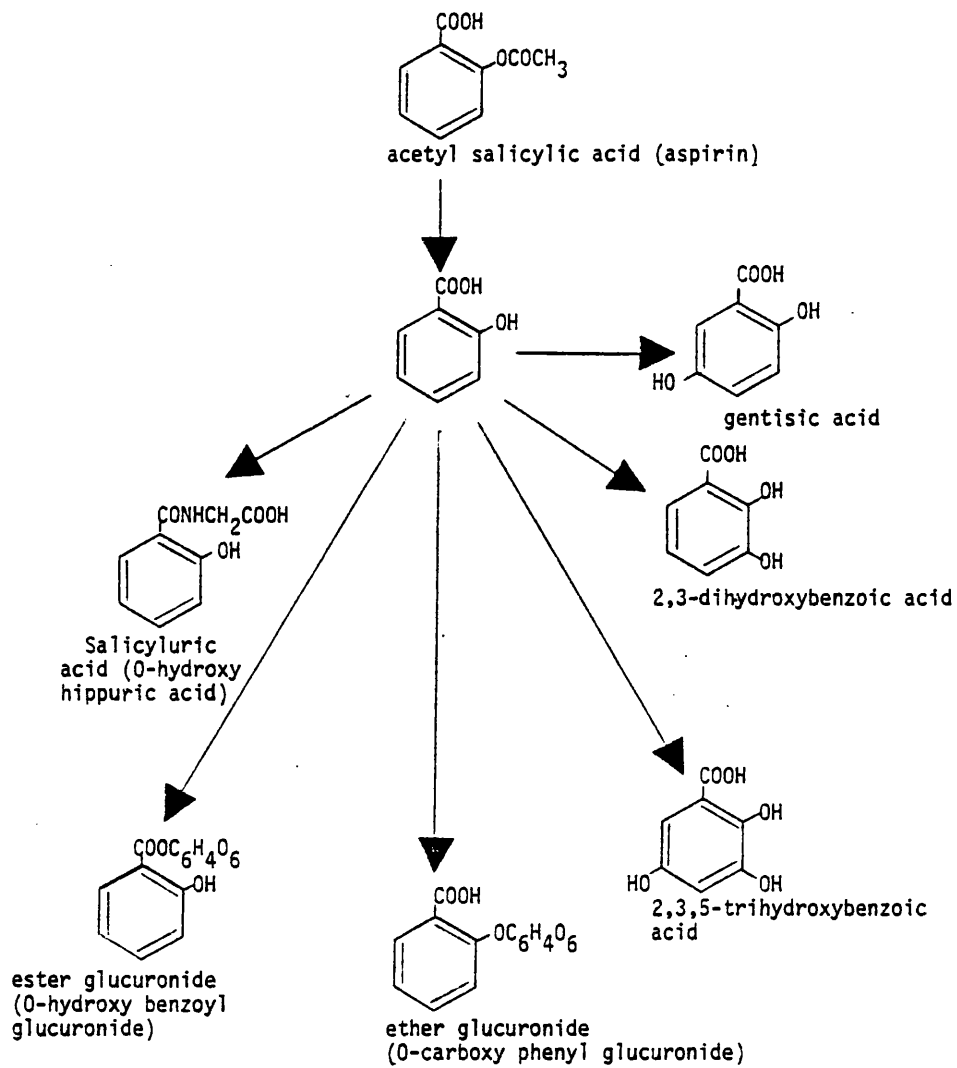
The usual therapeutic dose of aspirin is 600mg orally every 6 hours this gives rise to peak plasma free salicylate concentration of less than 60 $\mu$ g/ml. In arthritic conditions for which up to 10g per day is indicated, peak plasma levels may reach 200 - 300 $\mu$ g/ml (Davison, 1971) and these concentrations are often associated with symptoms of toxicity such as tinnitus, anaemia and gastric disturbances. In low doses elimination of salicylate follows first order kinetics but at higher therapeutic doses and in overdose saturation of the elimination process occurs due to the limited capacity of two major elimination pathways (salicylic acid and salicyl phenolic glucuronide). The plasma half life of salicylic acid is 4 - 6 hours in low doses. After high doses the time for plasma concentration to fall by half may be prolonged to over 15 hours (Levy, Tsuchiya and Amsel, 1972)

Salicylate biotransformation takes place mainly in the microsomal system and mitochondria of the liver. The three main metabolites of salicylate are salicylic acid (the glycine conjugate), the ether or phenolic glucuronide and the ester or acyl glucuronide. About 5% is oxidised to gentisic acid (2,5-dihydroxy benzoic acid) and to 2,3-dihydroxy benzoic acid and 2,3,5-dihydroxy benzoic acid. These are all found in urine but only a small proportion of these hydroxylated metabolites (1% of total salicylate) has been found in plasma (Woodbury and Fingl, 1975).

The main metabolic pathways of aspirin are outlined in Fig.

1.2. Excretion of salicylic acid and its metabolic products is mainly by the kidneys, only traces are found in sweat, bile and faeces. Urinary pH can profoundly alter the proportion excreted as free salicylate. The rate of urinary excretion of free salicylate is also influenced by the glomerular

Fig. 1.2 Metabolism of aspirin



filtration rate, the rate of proximal tubular secretion of salicylate and the rate of urine flow. Competition for the tubular transport system may also occur with anionic substances such as probenacid, p-aminobenzoic acid, p-aminohippuric acid and acetazolamide (Milne, 1963).

Dose dependent elimination of salicylic acid was first noted by Levy (1965). He found that after a 0.25g dose of aspirin overall elimination of salicylate and its metabolites followed first-order kinetics but with doses of 1g or more of aspirin administered to four subjects the formation of salicylic acid, which accounts for 80 - 90% of the total metabolites of salicylic acid, tended towards zero-order kinetics. Levy, Tsuchiya and Amsel (1972) suggested that the liver was limited in its ability to form these conjugates from urinary excretion rate data in man.

#### 1.4.2.2 Aspirin poisoning and therapy

Toxicity associated with chronic ingestion of acetyl salicylic acid described in Section 1.2.3 includes gastrointestinal bleeding and kidney and liver damage. Occult bleeding from the gut occurs in most people taking aspirin regularly due to painless gastric haemorrhage. Aspirin is probably also a cause of major gastrointestinal haemorrhage although this view was questioned recently when it was found by Langman (1977) that some patients who presented with bleeding were taking analgesics other than aspirin. Aspirin also causes renal irritation shown by increased urinary excretion of renal tubular cells and studies by Prescott (1965) showed that in therapeutic doses aspirin produced much larger amounts of renal tubular cells in urine than did paracetamol or phenacetin. Prescott and Ansari (1969) found that in rats a delayed rise in tubular cell excretion was accompanied by proximal tubular necrosis. Like phenacetin and paracetamol it is now thought that aspirin may be converted in the body to a highly reactive alkylating agent which binds covalently to tubular cell proteins causing necrosis. (Mitchell, McMurtry, Statham and Nelson (1977)). Again the formation of the reactive intermediate is via

cytochrome P450. More recently aspirin has been implicated as causing hepatotoxicity (Prescott, 1980) but this was usually minor compared with other manifestations of salicylate poisoning. Aspirin is rapidly metabolised to salicylic acid and Levy and Yaffe (1968) have found that some individuals may be slow metabolisers of salicylate so that accumulation to toxic levels may occur with a dose regimen which would normally be safe. Some pathological conditions (for example Gilberts disease) are manifest as a deficiency in the ability to conjugate with glucuronic acid, and give rise to high levels of salicylate in the body.

The effects of overdose of aspirin include tinnitus, deafness, nausea, vomiting, respiratory alkalosis, and metabolic acidosis. Doses of 500mg/kg salicylate or more are potentially lethal while doses in excess of 300mg/kg may result in prolonged and severe effects (Temple, 1981).

Treatment of salicylate poisoning is directed towards minimizing absorption, correcting acid-base disturbances, hastening elimination of absorbed salicylate and preventing complications. Forced alkaline diuresis has been used successfully in salicylate poisoning but is often complicated by hypokalaemia. Lawson, Proudfoot, Brown et al (1969) advocated a forced 'cocktail' diuresis which included a supplement of potassium chloride in addition to sodium bicarbonate, laevulose and saline. More recently Balali-Mood, Critchley, Proudfoot and Prescott (1982) reported that sodium bicarbonate given alone was more effective than either forced alkaline diuresis or forced diuresis alone in decreasing plasma salicylate concentrations and in increasing renal clearance and excretion of salicylate.

### 1.5 Scope of the Project and Background to the Study

Aspirin and paracetamol are commonly used drugs both of which have the potential for toxicity during chronic treatment and after overdosage.

Both drugs are extensively metabolised and it is possible to identify the major metabolites from each and hence follow their metabolism in situations which may be of clinical significance. The following studies were therefore undertaken:

- a) Metabolism in normal volunteers (aspirin and paracetamol)
- b) Metabolism in disease states (rheumatoid patients - aspirin and paracetamol; thyroid patients - paracetamol only)
- c) Metabolism of paracetamol in the presence of other drugs (salicylamide in man; phenobarbitone, phenylbutazone, rifampicin, L-ascorbic acid, salicylamide and  $\alpha$ -tocopherol in mice and guinea pigs).
- d) Metabolism of aspirin and paracetamol after overdose in man.
- e) Metabolism of paracetamol after specific therapy for high doses of paracetamol in rabbits.
- f) Chronic dosing with paracetamol in mice and guinea pigs.

A one-day survey of patients in the Royal National Hospital for Rheumatic Diseases (RNHRD) in Bath revealed that paracetamol is at least as popular as aspirin as the basic prescribed and self-administered drug for control of pain in rheumatoid arthritis. It also showed that concurrent treatment with combinations of paracetamol with other drugs which could affect the metabolism of paracetamol (for example inducing and metabolic competing agents) is common. Patients were found to be taking paracetamol with one or more other drug such as aspirin, immunosuppressives (e.g. azathioprine), penicillamine, gold, steroids, phenylbutazone and indomethacin (Dixon, Personal communication, 1978). This apparently is usual throughout Great Britain (see Editorial, BMJ, 8 March 1980). Access to patients at the RNHRD has enabled the study of possible alterations in metabolism which might be attributable to the disease state, to effects of chronic administration of aspirin and paracetamol, or to drug-drug interactions. In order to study the effects of chronic administration of paracetamol on its metabolism mice and guinea pigs were treated chronically with paracetamol and the urinary metabolic profile was assessed.

Salicylate and paracetamol poisoning remain a major clinical problem involving accidental ingestion in children and intentional overdose in adults. Consumption of paracetamol in Great Britain increased in the mid 1960s because it had fewer adverse effects at the recommended doses than aspirin and this increased therapeutic use of paracetamol was accompanied by an increase in the number of patients taking paracetamol for deliberate self-poisoning (Koch - Weser, 1976). Poisoned patients in the Bath area are mainly treated in the Clinical Pharmacology Unit (CPU) at the Royal United Hospital and this Unit has provided an excellent facility to study the metabolism of aspirin and paracetamol in such cases. A survey of patients admitted to the CPU over the period June 1979 - January 1981 was carried out to determine the drugs taken most frequently in overdose and it was found that the third and fourth most popular drugs taken in overdose admitted to the CPU are salicylates (accounting for 16.43% of total admissions) and paracetamol (accounting for 11.80%).

The availability of the CPU has enabled samples to be taken from patients after accidental or deliberate self-poisoning with aspirin or paracetamol so that the metabolism of these two drugs after high doses and also the effects of specific and non-specific antidotes may be ascertained. In addition, a control study from a wide age-range of human volunteers has allowed the establishment of a control metabolic profile after therapeutic doses of aspirin and paracetamol.

Inducing agents may affect the toxicity of paracetamol by increasing the formation of the reactive intermediate (Mitchell et al, 1973). As there is such a large number of environmental agents known to induce drug-metabolising enzymes as well as the possibility of induction by the practice of polypharmacy the effects of three agents (phenobarbitone, rifampicin (an antibiotic) and phenylbutazone (commonly used in rheumatoid arthritis) on the metabolism of paracetamol have been studied in animals.

In addition, effects of agents which may compete with paracetamol for available conjugating substrates have been examined in animals and man. Agents which conjugate with sulphate such as salicylamide or L-ascorbic acid may decrease sulphate availability to the extent that a higher proportion of a given dose of paracetamol may be oxidised to the reactive metabolite.

Antipyrine metabolism was found by Crooks, Hedley, Macnee and Stevenson (1973) to be increased in hyperthyroid patients and to be subnormal in hypothyroid patients. It is possible that accelerated metabolism in hyperthyroidism may enhance the toxicity of paracetamol by amplifying the rate of formation of the potentially hepatotoxic intermediate of paracetamol. The metabolism of paracetamol was therefore studied in a small group of patients with thyroid disease.

Finally after identification of two of the minor metabolites of paracetamol found in urine as 2- and 3-hydroxy paracetamol, it has been possible to show that considerable amounts of these metabolites and the cysteine and mercapturic acid metabolites of paracetamol in urine arise from acid-labile pre-aromatic unaromatized derivatives. These results give rise to the hypothesis that the pathways of O- and S-substitution are linked and arise as a consequence of nucleophilic attack on an epoxide ring. Thus it is proposed that 4-hydroxy-2,3-dihydroacetanilide 2,3-oxide is a likely intermediate of paracetamol metabolism and may be a hepatotoxic metabolite of the drug.

## Chapter 2

### Materials and Methods

#### 2.1 Compounds

- a) Analytes
- b) Solvents
- c) Other chemicals
- d) Drugs

#### a) Analytes

- i) acetanilide, (N-phenyl acetamide),  $\text{CH}_3 \text{CONH C}_6\text{H}_5$ ,  
MP = 114-115°C, MW = 135.17, (BDH Chemicals Ltd).
- ii) o-anisic acid, (o-methoxy benzoic acid),  $\text{O-CH}_3\text{OC}_6\text{H}_4\text{CO}_2\text{H}$ , MP = 98-100°C, MW = 152.15, (Aldrich Chemical Co. Ltd.).
- iii) aspirin, (acetyl salicylic acid),  $2\text{-(CH}_3\text{CO}_2\text{) C}_6\text{H}_4\text{CO}_2\text{H}$ , MP = 138-140°C, MW = 180.2, (Evans Medical Ltd).
- iv) gentisic acid, (2,5-dihydroxybenzoic acid), sodium salt,  $(\text{OH})_2\text{C}_6\text{H}_3\text{COONa}$ , MP = 205°C, MW = 176.1, (Sigma chemical Co.)
- v) 2-hydroxy paracetamol, (2,4-dihydroxy N-phenyl acetamide),  $2,4\text{-(OH)}_2\text{CH}_3\text{CONHC}_6\text{H}_4$ , MW = 261, (Stirling Winthrop R&D\*).
- vi) paracetamol B.P., (4-acetamidophenol),  $\text{CH}_3 \text{CONH C}_6\text{H}_4\text{OH}$ , MP = 169-172°C, MW = 151, (obtained from the Pharmacy, Royal United Hospital, Bath).
- vii) paracetamol cysteine, (3-[(1-acetamido-4-hydroxy phenyl)thio]alanine),  $1, 3, 4\text{-NH COCH}_3 \text{C}_6\text{H}_3 \text{S CH}_2\text{CH (NH}_2\text{) COOH (OH)}$ , MP = 192-193°C, MW = 270, (Stirling Winthrop R&D\*).
- viii) paracetamol glucuronide, (4-hydroxy N-phenyl acetamide 4-glucuronide),  $p\text{-CH}_3 \text{CONH C}_6\text{H}_4 \text{OC}_6\text{H}_9\text{O}_6$ , MW = 327, (Stirling Winthrop R&D\*).
- ix) paracetamol meta sulphate, (3,4-dihydroxy 3-sulphate N-phenyl acetamide),  $3,4\text{-(OH)}_2\text{CH}_3\text{CONHC}_6\text{H}_4\text{-OSO}_3\text{H}$ , MW = 261, (Stirling Winthrop R&D\*).



- x) paracetamol mercapturic acid, (1-acetamido-4-hydroxy phenyl mercapturic acid),  $1, 3, 4\text{-HNCOCH}_3 \text{C}_6\text{H}_3 \text{SCH}_2 \text{CH}(\text{NHCOCH}_3)\text{COOH}(\text{OH})$ , MW = 312, (Stirling Winthrop R&D\*).
- xi) paracetamol sulphate, (4-hydroxy N-phenyl acetamide-4-sulphate),  $p\text{-CH}_3\text{CONHC}_6\text{H}_4\text{OSO}_3\text{H}$ , MW = 230, (Stirling Winthrop R&D\*).
- xii) salicylic acid, (2-hydroxy benzoic acid),  $\text{HO C}_6\text{H}_4 \text{COOH}$ , MP = 157-162°C MW = 138.13, (BDH Chemicals Ltd).
- xiii) salicyluric acid, (O-hydroxy hippuric acid),  $\text{HO C}_6\text{H}_4\text{CONHCH}_2\text{CO}_2\text{H}$ , MP = 167-169°C, MW = 195.2, (Sigma Chemical Co.)

\*These compounds were kindly donated by Dr R S Andrews of Stirling Winthrop R&D.

#### b) Solvents

HPLC grade solvents were used throughout without further purification and all were obtained from Fisons.

- i) acetonitrile, (methyl cyanide),  $\text{CH}_3\text{CN}$ , MP = -48°C, MW = 41.05.
- ii) isopropanol,  $(\text{CH}_3)_2 \text{CHOH}$ , MP = -89.5°C, MW = 60.1.
- iii) methanol,  $\text{CH}_3\text{OH}$ , MP = -98°C, MW = 32.04.

#### c) Other Chemicals

- i) acetic acid (glacial),  $\text{CH}_3\text{COOH}$ , MW = 60.05, (BDH Chemicals Ltd).
- ii) formic acid, (98%),  $\text{HCOOH}$ , MW = 46.03, (BDH Chemicals Ltd).
- iii) ketodase ( $\beta$ -glucuronidase) buffered in acetate to pH 5.0, each ml contained 5,000 Fishman Units, (General Diagnostics).
- iv) perchloric acid (60%),  $\text{HClO}_4$ , MW = 100.46, (Fisons).
- v) potassium dihydrogen orthophosphate,  $\text{KH}_2\text{PO}_4$ , MP = 252.6°C, MW = 136.09, (BDH Chemicals Ltd).
- vi) sodium lauryl sulphate,  $n\text{-CH}_3(\text{CH}_2)_{10}\text{OSO}_3\text{Na}$ , MW = 288.38, (BDH Chemicals Ltd).

- vii) sulfatase, Type H-2 (from *Helix pomatia*) also containing  $\beta$ -glucuronidase, (Sigma Chemical Co.).
- viii) tetrabutyl ammonium phosphate (TBAP, ion pairing agent) was the gift of Dr C Riley, University of Bath.

All compounds used were of analytical grade purity or better where possible.

d) Drugs

- i) L-ascorbic acid (vitamin C),  $\text{COC(OH)C(OH)OCH}_2\text{CH}_2\text{(OH)}$ , MP = 190-192°C, MW = 176.1, (Sigma Chemical Co.)
- ii) soluble aspirin B.P. tablets, each tablet contained 300mg, used for human studies, (obtained from the Pharmacy, Royal United Hospital, Bath).
- iii) L-methionine B.P. (obtained from the Pharmacy, Royal United Hospital, Bath).
- iv) paracetamol B.P. tablets, each tablet contained 500mg, used for human studies, (obtained from the Pharmacy, Royal United Hospital, Bath).
- v) phenobarbitone sodium (5-ethyl 5-phenyl barbituric acid),  $\text{C}_6\text{H}_5\text{C}_2\text{H}_5\text{C(CO)NHOCNCONa}$  MP = 174-178°C, MW = 284.2, (BDH Chemicals Ltd).
- vi) phenylbutazone tablets, BP, (4-butyl-1, 2-diphenyl-pyrazolidine-3, 5-dione), MP = 105°C, MW = 308.4, each tablet contained 100mg phenylbutazone, (obtained from the dispensary, School of Pharmacy, University of Bath).
- vii) rifampicin capsules, BP, (5,6,9,17,19,21 - hexahydroxy-23-methoxy-2,4,12,16,18,20,22 - heptamethyl 8-[N-(4-methyl-1-piperazinyl) formimidoyl]-2,7-(epoxypentadeca [1,11,13] trienimino) naphtho [2,1-6] furan - 1,11(2H)-dione 21-acetate),  $\text{C}_{43}\text{H}_{58}\text{N}_4\text{O}_{12}$ , MW = 822.96, each capsule contained 150mg rifampicin (Ciba).
- viii) salicylamide B.P.,  $\text{C}_6\text{H}_4\text{(OH)CONH}_2$ , mp = 140°C, MW = 137.14, was the gift of A Davis, Beecham Pharmaceuticals.

- ix) DL- $\alpha$ -tocopherol acetate, (vitamin E), 5,7,8-trimethyl tocol,  $C_{29}H_{50}O_2$ , MP = 2.5-3.5°C, MW = 430.69, was the gift of Dr R Ehsanullah of Roche Products Limited.

Preparation of salicylamide solution:- 25ml 20% NaOH was made up using NaOH B.P. and sterile water. 1g salicylamide powder was weighed into each of 12 disposable plastic beakers and sterile water was added and slurried. 1.45ml 20% NaOH was added in drops with constant stirring to each beaker making sure that the pH at no time exceeded 10.5. This resulted in an equimolar solution of salicylamide with sodium hydroxide. Each beaker was then made up to a final volume of 200ml which included 5ml concentrated water of aniseed B.P.

## 2.2 Animals

The experimental animals used were male CFP mice weighing between 10 and 45g; male Dunkin Hartley guinea pigs weighing from 280-600g and female New Zealand white rabbits weighing from 2.8 to 4.6kg.

Guinea pigs and rabbits were housed in individual metabolism cages (Associated Crates Limited) and mice were placed in groups of four in metabolism cages (North Kent Plastic) which allowed the separate collection of urine and faeces. During each experiment water was allowed ad libitum but (except for the chronic studies) food was restricted for 24 hours while urine was being collected. The normal diet of the animals was FDI for rabbits and guinea pigs and CRM for mice. They were maintained on a constant light-dark cycle (14h light - 05.00-19.00 and 10h darkness - 19.00-05.00). All experiments were carried out in the animal house which was maintained at an ambient temperature of 24°C.

### 2.3 Human volunteers and patients

Human volunteers who were aged from 13 to 80 were healthy and ambulatory and gave their informed consent. The younger volunteers were hospital and laboratory workers or their children while the older volunteers (aged over 60 years) were contacted through local general practitioners. Studies of drug overdose were carried out on self-poisoned patients who had been admitted to the Clinical Pharmacology Unit, at the Royal United Hospital, Bath. In-patients in the Royal National Hospital for Rheumatic Diseases, Bath and out-patients with thyroid disorders who were attending the Medical Out Patients Department at the Royal United Hospital, Bath were also studied. A record was made of all drugs currently or regularly taken by volunteers and patients

### 2.4 Administration of compounds to animals and humans

#### a) Animal Studies

Guinea pigs, mice and rabbits received paracetamol in various doses either dissolved in propylene glycol (guinea pigs only) or as a saturated suspension in 0.9% saline (guinea pigs, mice and rabbits). Intraperitoneal injections were made unless otherwise stated.

Phenobarbitone was made up as a stock solution of 100mg/ml or 10mg/ml in saline for guinea pigs and mice respectively. Both species were pretreated with 100mg/kg phenobarbitone i.p. for 4 days and 7 days before administration of 150mg/kg paracetamol i.p. at the start of the last day. Phenylbutazone tablets were ground up using a pestle and mortar and then suspensions of 100mg/ml and 10mg/ml were made in 0.9% saline. Guinea pigs and mice were pretreated with 100mg/kg phenylbutazone i.p. for 4 days followed by 100mg/kg paracetamol i.p. on the final day.

Rifampicin was given to the same two species at a dose of 80mg/kg i.p. and was made up as a 40mg/ml solution in N-saline adjusted with 50 $\mu$ l 2N NaOH to achieve solution. Guinea pigs were treated with this solution but it was diluted to 8mg/ml for mouse studies. After 5 day pretreatment with rifampicin, paracetamol 150mg/kg i.p. was administered on the 5th day. After the administration of paracetamol, animals were housed in metabolic cages as previously described and 24 hour urine was collected.

L-ascorbic acid and salicylamide were both made up as 10mg/ml and 100mg/ml solutions in N-saline which were administered to mice and guinea-pigs respectively.  $\alpha$ -tocopherol was obtained in ampoules of 200mg/ml and this solution was used as such for guinea pig studies but was diluted to 10mg/ml for mouse work. These three metabolic competing agents were administered i.p. concurrently with 100mg/kg paracetamol i.p. and animals placed in metabolic cages while 24 hour urine was collected.

Paracetamol was administered orally to rabbits as a solution of 100mg/ml or 200mg/ml N-saline. L-methionine was made up as a standard solution of 200mg/ml in N-saline and was administered by mouth just prior to paracetamol. After being dosed with paracetamol rabbits were housed in metabolic cages for the collection of 24 hour urine. Blood (approximately 3ml) was taken every 45 minutes from an ear vein after the application of xylene to the ear, to dilate the blood vessels of the pinna. Blood samples were collected up until 315 minutes in each experiment.

In each experiment, urine was collected from metabolic cages in measuring cylinders, the volume was noted and an aliquot was taken for analysis.

## b) Human Studies

Each subject took paracetamol, 1g, (2 x 0.5g tablets) or soluble aspirin BP, 600mg (2 x 300mg tablets in 50ml of water) after fasting overnight and emptying the bladder. Salicylamide was administered as its sodium salt as described above. Each subject collected his/her own urine samples (0-8h and 8-24h collection periods) in acidified urine bottles.

### 2.5 Storage of biological samples

All samples were maintained at  $-20^{\circ}\text{C}$  until required for analysis. Urine was collected in 21 plastic bottles which contained 10ml, 1 N HCl so that the final pH of the urine was approximately 2 and aliquots of approximately 20ml urine were taken. Serum samples were stored in unheparinized tubes.

### 2.6 Treatment of urine and serum

#### a) Preparation of urine for HPLC

For animal and human studies, 1ml urine was added to 1ml of the internal standard solution (100 $\mu\text{g}/\text{ml}$  acetanilide in distilled water for paracetamol studies, 500 $\mu\text{g}/\text{ml}$  o-anisic acid in acetonitrile for aspirin studies) and made up to 10ml with distilled water. For human overdose studies and repeated studies in animals, dilution of urine was often necessary but the same concentrations of the internal standards were used throughout. Urine solutions were analysed by HPLC after pre-injection filtration (filters by Millipore (UK) Ltd, London).

**b) Preparation of serum for HPLC**

Serum was obtained by centrifuging clotted blood at 1310.4g for 15 minutes. The procedure described in the following flow diagram was then carried out. There is no dilution factor because the internal standard (acetanilide) was treated identically to paracetamol and its metabolites.

1ml serum + 1ml  
(100 $\mu$ g/ml) acetanilide +  
1ml 15% perchloric acid  
were whirlimixed and  
centrifuged at 1783.6g  
for 10 minutes to  
precipitate proteins.



2ml of supernatant were  
removed and added to 1ml  
KH<sub>2</sub>PO<sub>4</sub> to  
precipitate the  
perchlorate. This was  
whirlimixed and  
centrifuged at 1783.6g  
for 10 minutes.

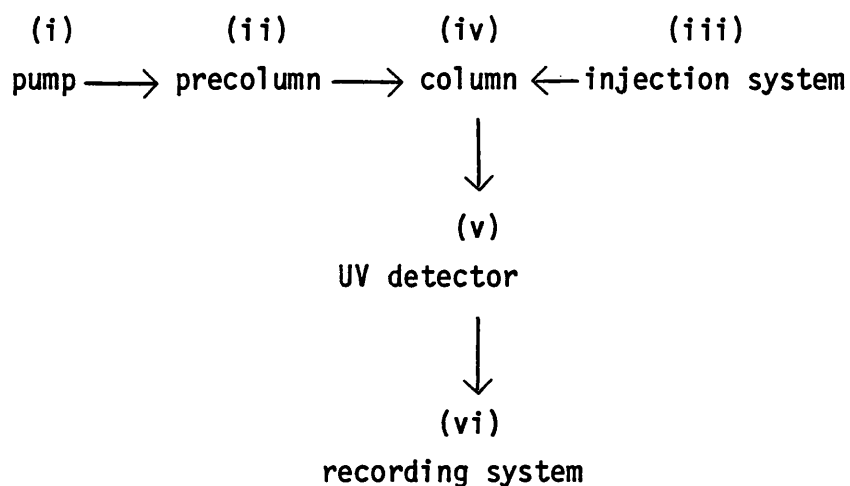


The supernatant was  
respun at 2329.6g for 15  
minutes and the  
supernatant analysed by  
HPLC after pre-injection  
filtration.

c) Calibration curves for parent drug and metabolites were constructed in urine and plasma as described above with the exception that no acid was added to the standard solutions made up in blank urine. 1ml blank urine was used in calibration curves made up in urine. See section 2.8.b.

## 2.7 High performance liquid chromatography

### a) Generalised Scheme



- i) Laboratory Data Control (LDC) Constametric III Metering Pump (Jones Chromatography, Llanbradach) which is a constant flow pump providing a pulsationless flow using dual reciprocating pistons.
- ii) precolumn (5cm long, 5mm internal diameter,  $\frac{1}{4}$  inch external diameter) containing Hypersil ODS.
- iii) Rheodyne valve, model 7125 which was a syringe-loading sample injection valve fitted with a  $20\mu\text{l}$  loop. An interchangeable Supraevaglass micromatic syringe was used fitted with a luer 33# needle.
- iv) chromatography column consisted of a 24cm long, 5mm internal diameter,  $\frac{1}{4}$  inch external diameter stainless steel tube packed with octadecyl silica (APEX, Jones Chromatography, Llanbradach). The column end fitting comprised a low dead-volume Swagelok connection with a  $\frac{1}{16}$  inch outlet which was connected to the detector by



microbore ptfe tubing (internal diameter 0.15mm). Low dead volume  $1/16$  inch stainless steel tubing (internal diameter 0.15mm) was used to connect the injection valve to the top of the column. All other connections were made of stainless steel tubing and standard Swagelok fittings (HETP Ltd).

- v) detector used was an LDC model Spectromonitor III spectrophotometer (Jones Chromatography, Llandradach) providing wavelength coverage in the uv spectrum from 190-350nm. This provided an output signal of 10mv which corresponded to full scale for recording absorbance.
- vi) chromatographs were produced on a JJ model CR 550 potentiometric chart recorder (JJ Lloyd Instruments Ltd).

All equipment was operated at ambient temperature throughout.

#### b) Degassing mobile phases

This was achieved by refluxing for 10 minutes, by sparging with helium for 5 minutes or ultrasonication for 10 minutes. The helium method was the most successful.

#### c) Internal standards

Internal standards were chosen which did not interfere chromatographically with analytes. Stock solutions were 100 $\mu$ g/ml acetanilide in distilled water and 500 $\mu$ g/ml o-anisic acid in acetonitrile for the assay for paracetamol and its metabolites and aspirin and its metabolites respectively.

d) Resolution of peaks

Resolution ( $R_s$ ) is a measure of how well two analytes are separated and

$$R_s = \frac{\text{differences in retention times (min)}}{\text{mean peak (base) width (min)}}$$

$R_s < 0.08$  partial resolution, some overlap

$R_s < 1.00$  normal criterion, small overlap

$R_s < 2.00$  excellent separation, no overlap unless peak tail.

In this system it was ensured that  $R_s > 0.08$  for all peaks.

e) Experimental conditionsi) Analysis of paracetamol and its metabolites

(Modified from the method of Adriaenssens and Prescott, 1978).

Mobile phase: 97.5% 0.08M  $\text{KH}_2\text{PO}_4$  (10.89g/l), 2.5% isopropanol, 0.075% TBAP (0.5M, 150 $\mu$ l/200ml) and 0.1% (v/v) formic acid (200 $\mu$ l/200ml); pH = 3.0.

flow: 1.5ml/min.

UV wavelength: 254 nm.

Retention times, capacity ratios and values of  $\lambda$  max

Analyte	Retention Time	Capacity Ratio $K^1$	$\lambda$ max (nm)
Paracetamol glucuronide	3 min 20s	0.86	242
Paracetamol cysteine	4 min 50s	1.47	200, 242, 294
Paracetamol	6 min 43s	3.15	242
Paracetamol sulphate	13 min 58s	7.82	240
Paracetamol mercapturate	16 min 30s	9.21	241, 296
Acetanilide (internal standard)	23 min 35s	13.59	273

ii) Analysis of 2-OH paracetamol and 3-OH paracetamol

Mobile phase: 97.5% 50mM sodium lauryl sulphate, 2.5% isopropanol, 0.1% formic acid, pH = 3.0.

flow: 0.5ml/min until 2-OH and 3-OH paracetamol eluted and then flow was increased to 3.0ml/min to elute the internal standard (acetanilide).

UV wavelength: 254nm.

2-OH paracetamol and 3-OH paracetamol eluted with retention times of 7 min 30s and 7 min 55s respectively. Acetanilide was eluted at 15 min. The standard solution of 3-OH paracetamol was obtained by incubating paracetamol metasulphate with sulfatase at pH 5 overnight.

iii) Analysis of aspirin and its metabolites

Mobile Phase: 35% methanol, 64% distilled water, 1% glacial acetic acid; pH = 3.0.

flow: 1.0 ml/min

UV wavelength: 250 nm

Retention times, capacity ratios and values of  $\lambda$  max

Analyte	Retention Time	Capacity Ratio $K^1$	$\lambda$ max (nm)
Salicylic acid	7 min 35s	1.85	237, 297
O-anisic acid (internal Standard)	10 min 6s	2.83	292, 235
Acetyl salicylic acid	11 min 17s	3.45	224, 295
Salicylic acid	17 min 20s	5.71	230, 295

f) Determination of salicyl glucuronides

As standards were not available, the salicyl glucuronides were quantified using a specific enzyme hydrolase ( $\beta$ -glucuronidase). Urine was adjusted to pH5 with dilute acetic acid. 0.5ml Ketodase was added ( $\beta$ -glucuronidase containing 5,000 Fishman Units per ml) and whirlmixed for 30s, stoppered and incubated overnight at 37°C. Analysis for salicylic acid was then carried out as above. The amount of salicyl glucuronides present was taken to be the difference in salicylic acid measured before and after  $\beta$ -glucuronidase treatment.

## 2.8 Estimation of paracetamol and aspirin by HPLC - standard curves

### a) Linearity of Response

Calibration curves were constructed by determining the peak height ratios of four to seven standard solutions containing a range of solute concentrations and a constant concentration of the relevant internal standard. Each solution was injected onto the HPLC in duplicate, the mean was taken and fitted to the following equation using linear regression.

$$y = mx + c$$

where  $y$  is the peak height ratio,  $x$  is the concentration of solute and  $m$  and  $c$  are regression coefficients. The constancy of the calibration curve was checked throughout the period of the experiments and no change was found.

### b) Standard curves for paracetamol and aspirin and their metabolites

Calibration curves were constructed for paracetamol and its metabolites in plasma and urine and for aspirin and two of its metabolites in urine. Figs 2.1, 2.2 and 2.3 show graphically the calibration curves for paracetamol in urine in the range 1-40 $\mu$ g/ml, paracetamol in plasma in the range 0.25-10 $\mu$ g/ml and salicylic acid in urine in the range 25-150 $\mu$ g/ml. The correlation coefficient  $r$ , the slope  $m$  and the intercept  $c$  are given on each graph. Calibration curves for metabolites of paracetamol and aspirin are not shown graphically but values for  $r$ ,  $m$  and  $c$  are given below.

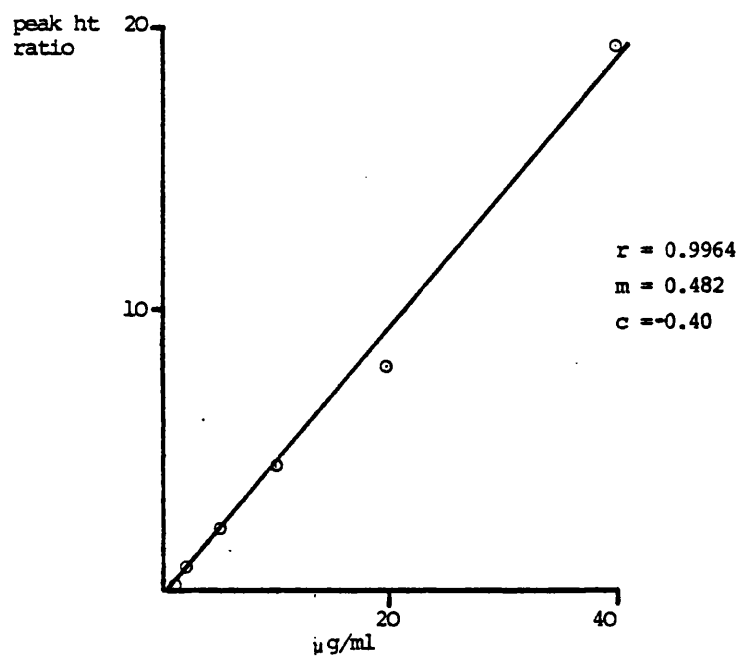
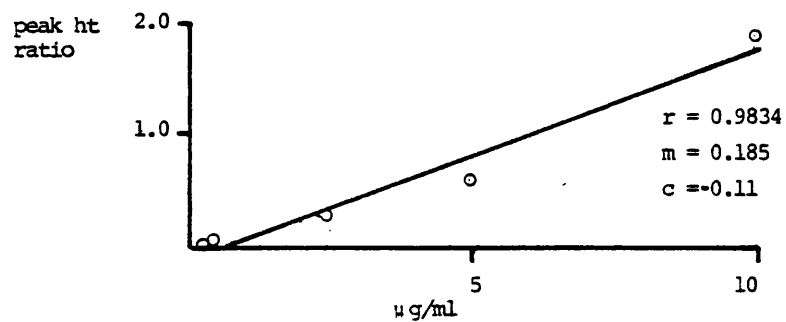
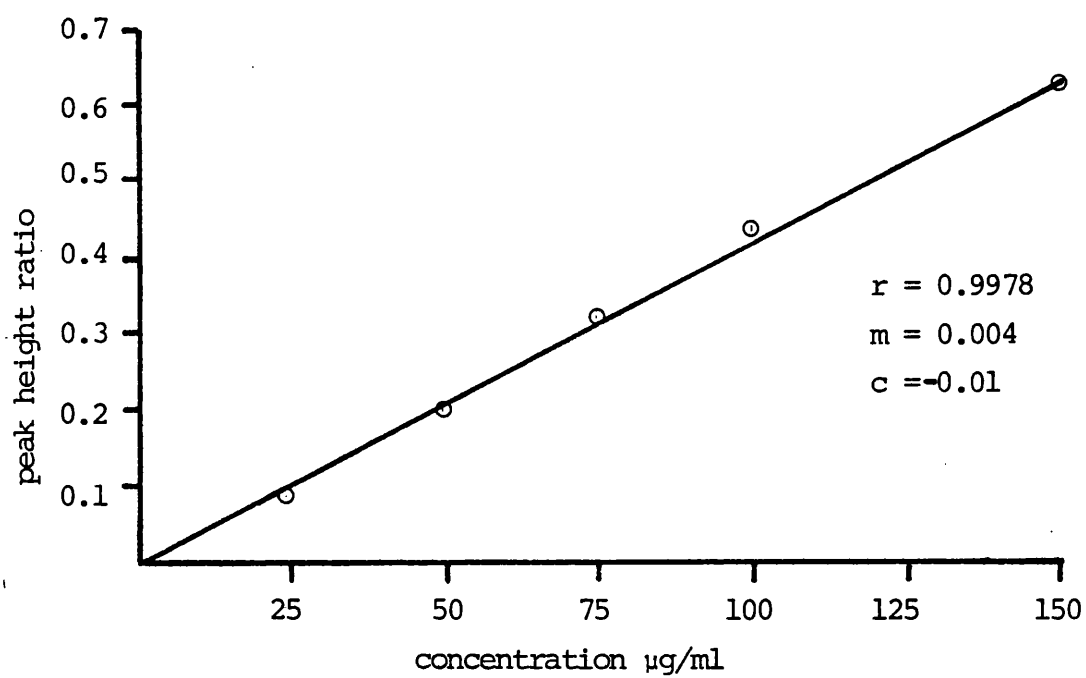
Fig. 2.1 Calibration curve for paracetamol in urineFig. 2.2 Calibration curve for paracetamol in plasma

Fig.2.3 Calibration curve for salicylic acid in urine

i) Paracetamol metabolites in urine

	r	m	c	n	Range ( $\mu\text{g/ml}$ )
paracetamol	0.9964	0.482	-0.40	6	1-40
paracetamol glucuronide	0.9996	0.086	0.37	7	10-880
paracetamol sulphate	0.9858	0.020	0.18	6	10-100
paracetamol cysteine	0.9966	0.407	-0.68	6	2.5-40
paracetamol mercapturic acid	0.9989	0.075	0.01	5	2.5-40

ii) Minor metabolites of paracetamol in urine

	r	m	c	n	Range ( $\mu\text{g/ml}$ )
2-hydroxy paracetamol	0.9989	0.184	0.218	5	2.5-4.0
3-hydroxy paracetamol	0.9982	0.111	-0.069	5	3.2-38.6

iii) Paracetamol metabolites in plasma

	r	m	c	n	Range ( $\mu\text{g/ml}$ )
paracetamol	0.9834	0.185	-0.11	5	0.25-10
paracetamol glucuronide	1.0000	0.032	0.03	3	10-50
paracetamol sulphate	1.0000	0.009	-0.02	3	10-50
paracetamol cysteine	0.9993	0.110	0.12	3	10-50
paracetamol mercapturic acid	0.9954	0.026	-0.09	3	10-50



iv) Aspirin metabolites in urine

	r	m	c	n	Range (µg/ml)
acetylsalicylic acid	0.9677	0.009	-0.14	4	25-100
salicyluric acid	0.9970	0.036	-0.23	5	25-150
salicylic acid	0.9978	0.004	-0.01	5	25-150

c) Concentration determination

Peak height ratios of parent drug and metabolites were determined in serum and urine samples and concentrations were calculated using the equation

$$x_i = \frac{y_i - c}{m}$$

Where  $x_i$  is the unknown concentration to be determined,  $y_i$  is the observed peak height ratio and  $c$  and  $m$  are regression coefficients calculated from the calibration curves.

2.9 Statistical Evaluation and Treatment of Results

Results were expressed as means and standard errors of the mean (SEM). The significance of the difference between means was taken as  $p < 0.05$  using Student's two-tailed t-test. Regression and correlation coefficients were calculated using the statistical package Genstat.

In all of the studies in which concentrations of paracetamol and its metabolites or aspirin and its metabolites were measured in urine or plasma, the weight of each metabolite recovered was converted to the weight of paracetamol or aspirin from which it had originated as

described below\*. Since there was considerable variation in total urinary recovery of paracetamol and aspirin and their metabolites between individuals in each collection period, direct comparisons of each treatment with control values yielded little information. Therefore, throughout this work results have been expressed either as percentage dose excreted or as percentage recovery of paracetamol or aspirin and their metabolites for studies in man and animals.

Percentage dose of paracetamol and aspirin excreted has been expressed in terms of the administered dose of paracetamol or aspirin as follows:

$$\% \text{ dose of paracetamol excreted: } \frac{P + PG^* + PS^* + PC^* + PM^* \text{ (mg)}}{1,000\text{mg}} \times 100$$

$$\% \text{ dose of aspirin excreted: } \frac{ASA^* + GA^* + SA^* + SUA^* + SG \text{ (mg)}}{460.2\text{mg}^*} \times 100$$

The percentage recovery in each urine collection period was also determined. For paracetamol, for example, the total weight of P + PG + PS + PC + PM was calculated and the fractional contribution of paracetamol and of individual metabolites was expressed as a percentage of this total i.e.

$$\% \text{ recovery of paracetamol} = \frac{\text{paracetamol or metabolite}^* \text{ (mg)}}{PG + PG^* + PS^* + PC^* + PM \text{ (mg)}} \times 100$$

correspondingly for aspirin

$$\% \text{ recovery of aspirin} = \frac{SA \text{ or } SUA^* \text{ or } SG \text{ (mg)}}{ASA^* + GA^* + SA^* + SUA^* + SG^* \text{ (mg)}} \times 100$$

These procedures were adopted because they express the metabolic disposition of paracetamol or aspirin in terms of their principal biotransformation products.

\*Corrected for molecular weight with respect to paracetamol or to salicylic acid. Correction factors are given in Appendix 1.

### Chapter 3

#### Paracetamol Results in Man

##### 3.1 Control Study

###### Introduction

In order to study the effects of different clinical situations and treatments on the metabolism of paracetamol, it was necessary to examine the urinary metabolic profile of paracetamol in a control group of normal subjects.

Drug metabolism is known to vary with age (Crooks, O'Malley and Stevenson, 1976). Thus it was important to structure the control group so that a wide age range was studied and 8 - 10 normal volunteers per decade of age were collected where possible. Many previous studies of drug metabolism with age have only compared young and old age groups and have measured drug half life. The present study a) measures metabolites and b) takes a spectrum of age. It thus constitutes one of the first detailed examinations of drug metabolism with age.

###### Procedures

Human volunteers under 60yrs of age were hospital and laboratory workers or their children; those aged over 60 years were contacted through local general practitioners. Each volunteer was asked to record smoking habits and was questioned about other drugs taken. Paracetamol, 1g (or 500mg for those under 15yrs) was taken by each subject (23 males, 26 females) early in the morning after emptying the bladder and fasting overnight. All urine passed in the subsequent 8 hours (0 - 8hour collection) and in the period 8 - 24 hours after dosing (8 - 24hour collection) was collected in separate acidified polypropylene urine bottles. Urine was analysed by HPLC for the four major metabolites of paracetamol (that is, paracetamol glucuronide (PG), paracetamol sulphate (PS), paracetamol cysteine (PC) and paracetamol mercapturic acid (PM)) and for paracetamol excreted unchanged (P).

## Results

In this and subsequent studies in which paracetamol and its metabolites were quantified in urine, the amount of each metabolite was converted to the weight of paracetamol from which it had originated (conversion factors used appear in Appendix 1). The total recovery of paracetamol in each collection period was calculated from the sum of the metabolites, thus converted, and of unchanged paracetamol. The recovery of individual metabolites or of unchanged paracetamol was then expressed as a percentage of the total.

Table 3.1.1 shows the percentage of the dose which was recovered in each collection period.

Table 3.1.1 Percentage of paracetamol and its metabolites recovered in urine following administration of 1.0g or 0.5g by mouth to healthy volunteers in a single dose (means  $\pm$  SEM)

Collection period	Percentage dose excreted	
	Mean	SEM
0 - 8h	49.21	3.31
8 - 24h	27.37	2.45
0 - 24h	76.59	4.53

No correlation was found between the percentage dose excreted and the age of the volunteers in either collection period or over the whole 24 hour period. Quantities of paracetamol and its metabolites expressed in mg (converted to parent paracetamol as described above) and the percentage recovery of each metabolite in each urine collection period for each volunteer are given in Appendix 2 and 3 respectively.

Table 3.1.2 shows means of the percentage total recovered amounts of paracetamol excreted unchanged and each of its four metabolites in the collection periods.

**Table 3.1.2 Recovery of paracetamol and its metabolites as a percentage of the total in the three collection periods following administration of 1.0g or 0.5g by mouth to normal volunteers in a single dose.**

Collection period	n	P		PG		PS		PC		PM		PC + PM	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0 - 8h	49	5.28 ± 0.72		52.08 ± 1.44	35.14 ± 1.45	3.41 ± 0.30	4.00 ± 0.34	7.41 ± 0.48					
8 - 24h	49	4.60 ± 0.57		56.38 ± 2.06	27.33 ± 1.74	6.03 ± 0.76	5.71 ± 0.47	11.74 ± 0.95					
0 - 24h	49	4.82 ± 0.59		53.46 ± 1.35	32.79 ± 1.16	4.28 ± 0.38	4.72 ± 0.31	9.01 ± 0.49					

The amount recovered as paracetamol sulphate was significantly lower in the second (8 - 24h) urine collection period than the first (0 - 8h) period ( $p < 0.002$ ). The decrease in the recovery of paracetamol sulphate in the second collection was accompanied by a significant increase in the percentage recovered as paracetamol cysteine plus paracetamol mercapturic acid ( $p < 0.001$ ). Recoveries of PM and PC individually were greater in the second than in the first collection period ( $p < 0.01$ ). These results are plotted in Fig. 3.1.1.

Table 3.1.3 shows the excretion of paracetamol and its metabolites in each urine collection period. Results are presented for convenience in cohorts of ten years of age and are illustrated in Figs. 3.1.2, 3.1.3 and 3.1.4.

Fig. 3.1.1.  
Percentage recovery of paracetamol and its metabolites in urine following administration of 1.0g or 0.5g by mouth to healthy volunteers (means  $\pm$  S.E.M.)

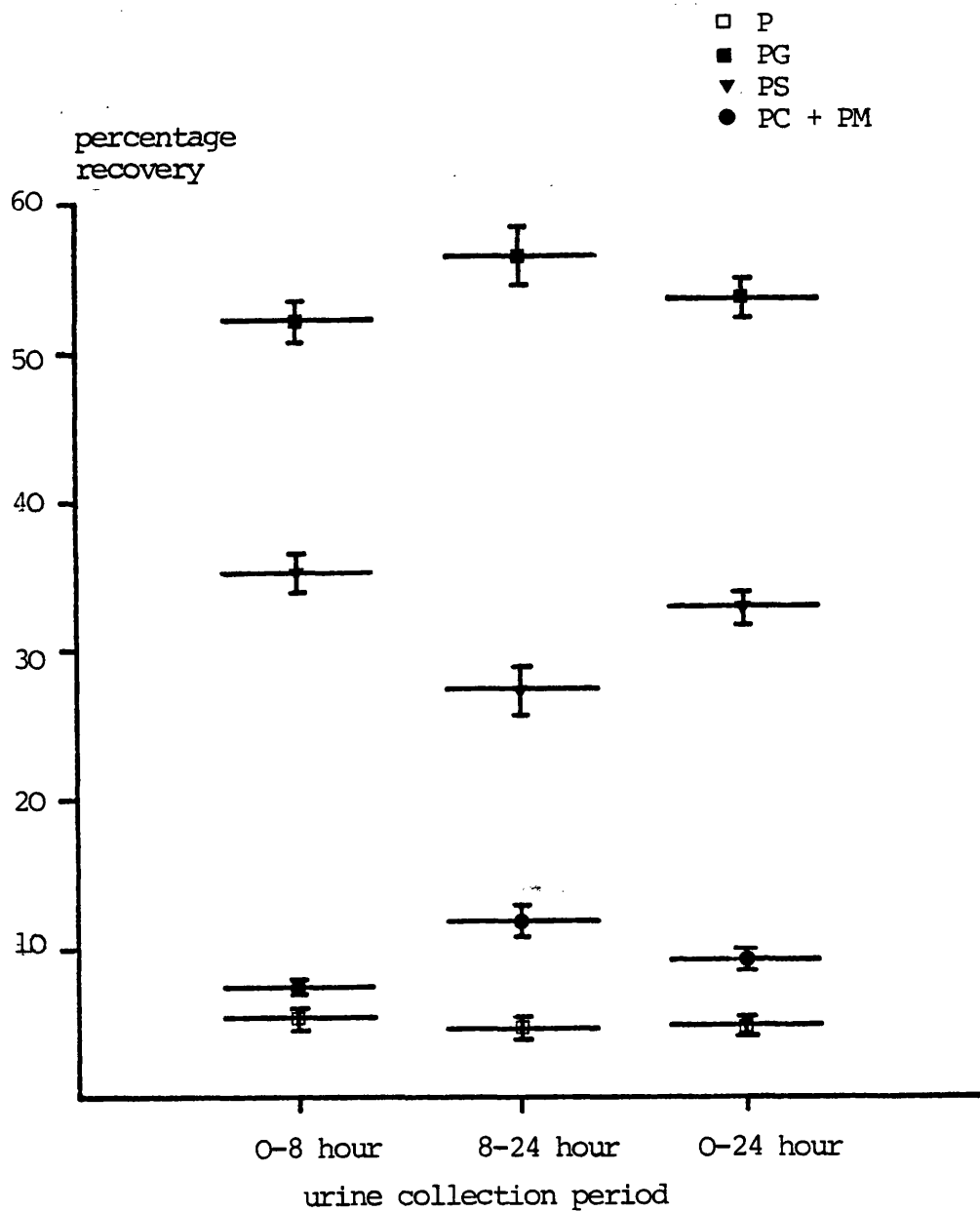




Table 3.1.3 Means  $\pm$  SEM of the percentage recovery of paracetamol and its metabolites in each ten year age group after administration of 1.0g or 0.5g by mouth to healthy volunteers.

0-8h age	n	P		PG		PS		PC		PM		PC+PM	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
11-20	2	5.03	1.31	45.59	8.19	35.08	6.46	7.82	0.84	4.62	0.34	12.44	1.18
21-30	8	4.25	1.16	49.77	5.55	37.14	4.28	5.53	0.66	3.31	1.16	8.83	1.20
31-40	9	8.11	2.82	51.39	3.76	32.56	4.28	2.89	0.39	5.03	0.81	7.93	1.03
41-50	10	5.53	1.09	54.83	2.97	33.16	3.37	3.07	0.46	3.41	0.82	6.48	1.02
51-60	8	6.34	1.93	54.22	1.05	32.61	2.31	3.20	0.82	3.60	0.73	6.80	1.46
61-70	6	2.72	1.17	53.96	2.82	36.90	2.48	2.20	0.36	4.20	0.70	6.42	0.68
71-80	6	3.24	1.24	49.03	4.41	41.31	4.35	1.92	0.52	4.50	1.00	6.42	1.23
8-24h age	n	P		PG		PS		PC		PM		PC+PM	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
11-20	2	3.80	1.88	56.82	7.52	17.46	1.24	13.15	5.44	8.75	5.20	21.90	10.64
21-30	8	2.63	1.07	44.54	4.45	39.02	3.52	7.87	1.82	5.93	1.57	13.80	2.84
31-40	9	6.23	1.92	57.20	5.53	26.64	4.11	4.64	1.33	6.38	1.15	11.03	1.52
41-50	10	8.16	1.88	58.40	3.03	23.06	3.32	5.59	1.64	5.29	0.90	10.88	1.80
51-60	8	3.09	0.64	58.65	3.30	28.72	2.64	4.76	1.49	4.50	1.12	9.37	1.92
61-70	6	4.17	1.19	60.22	8.71	21.87	6.04	8.32	3.49	5.41	0.96	13.73	3.38
71-80	6	3.08	0.78	59.72	5.60	28.36	5.22	3.42	1.02	5.42	0.83	8.83	1.51
0-24h age	n	P		PG		PS		PC		PM		PC+PM	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
11-20	2	6.31	0.15	48.10	8.58	31.46	5.56	8.80	1.41	5.32	1.24	14.13	2.87
21-30	8	3.56	1.12	48.20	4.83	37.41	2.94	6.30	0.95	4.52	0.99	10.83	1.29
30-41	9	7.50	2.34	51.07	3.39	32.92	3.16	3.96	0.80	5.51	0.76	9.47	0.97
41-50	10	6.02	1.18	55.97	2.80	30.04	3.14	3.89	0.72	4.44	0.69	8.32	1.00
51-60	8	4.84	1.14	56.17	2.08	29.90	2.34	3.77	0.93	4.07	0.78	7.84	1.44
61-70	6	3.10	1.01	56.91	2.15	32.04	1.00	2.94	1.03	4.50	0.76	7.94	0.98
71-80	6	3.05	0.76	54.58	3.68	34.42	3.39	2.78	0.75	5.18	0.76	7.96	1.24

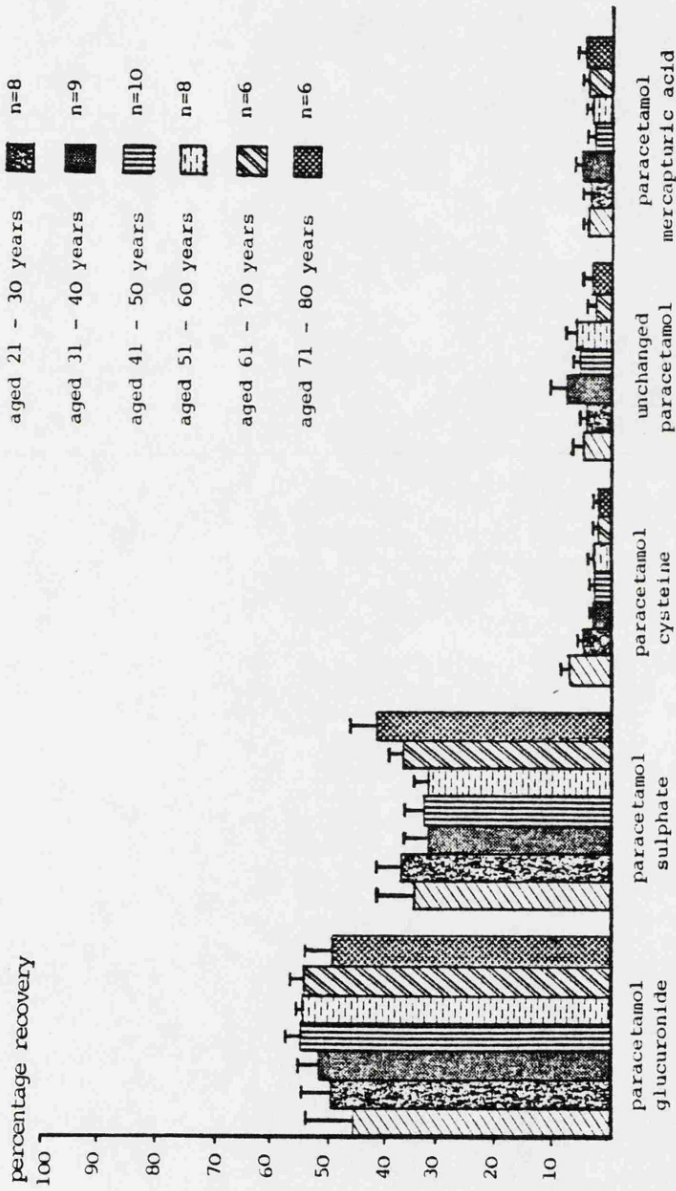


Fig. 3.1.2 Percentage recovery of paracetamol and its metabolites in 0 - 8 hour urine (means  $\pm$  S.E.M.)

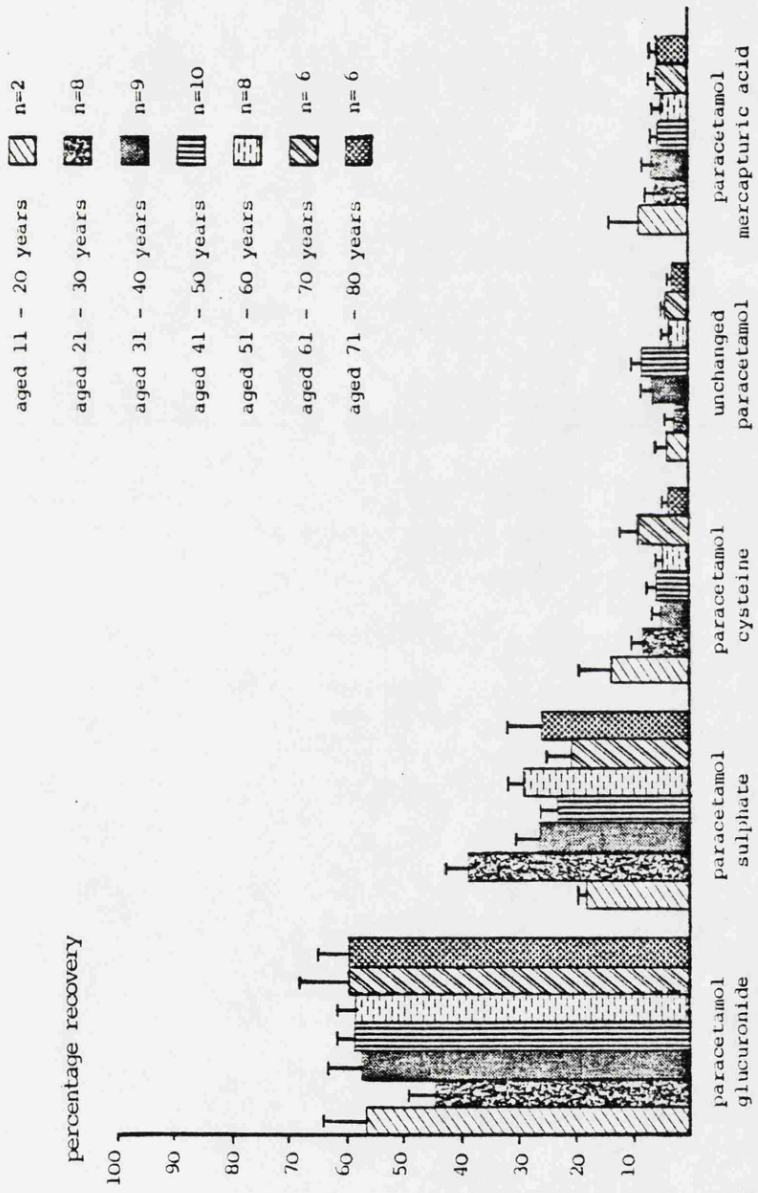


Fig. 3.1.3 Percentage recovery of paracetamol and its metabolites in 8 - 24 hour urine (means  $\pm$  S.E.M.)

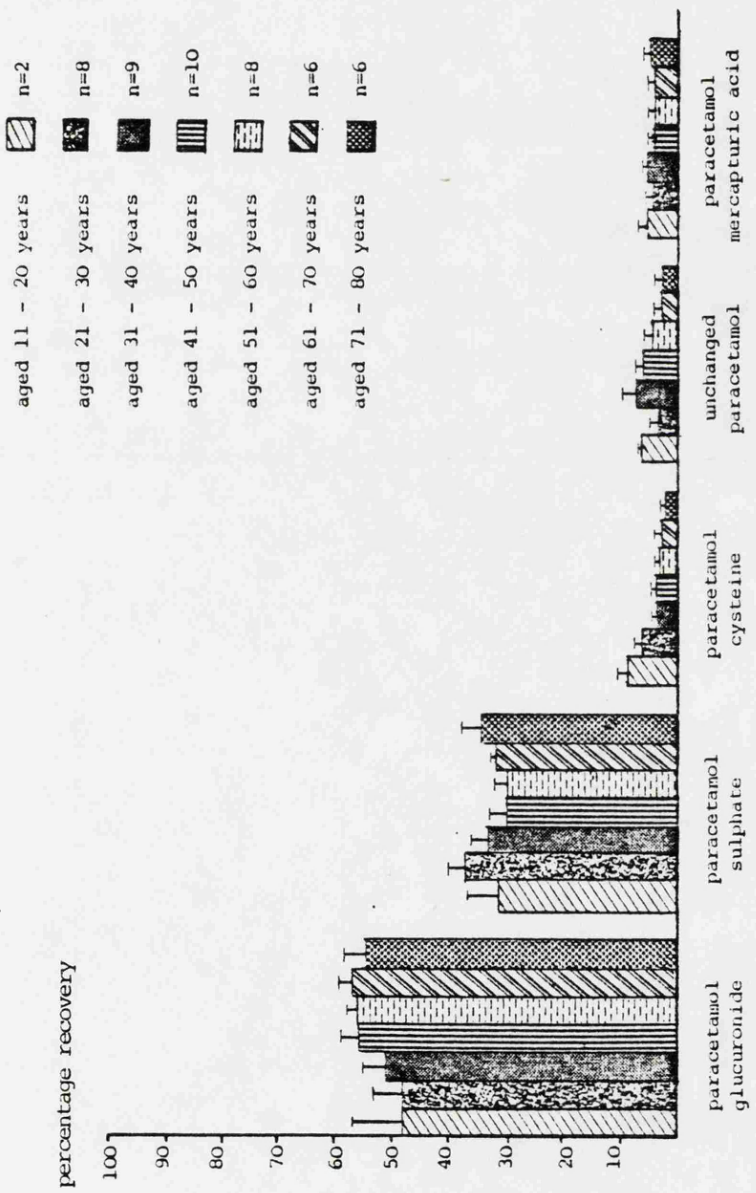


Fig. 3.1.4 Percentage recovery of paracetamol and its metabolites in 0 - 24 hour urine (means  $\pm$  S.E.M.)

Inspection of Figs. 3.1.2, 3.1.3 and 3.1.4 does not reveal an obvious relation of individual metabolites with age. Therefore the ratio of Phase II to Phase I metabolites was calculated, that is, the ratio of conjugated to oxidised plus conjugated metabolites or  $(PG + PS)/(PC + PM)$ . Fig. 3.1.5. shows that there was a clear relationship between this ratio and age. However it can be seen that the scatter increases with age. Statistically in order to calculate a mathematical relationship, it is important that this scatter is approximately the same at all ages. Therefore the natural logarithm of the ratio was calculated in order to minimize scatter in the higher age groups.  $\log_e$  ratio for each urine collection period is plotted against age in Figs. 3.1.6, 3.1.7 and 3.1.8. It can be seen that the scatter is now similar at all ages and that the ratios in 0 - 8h and 0 - 24h urine collections are significantly related to age while ratios in the 8 - 24h period do not reach significance. For the whole 24 hour period the following regression equation was obtained:

$$\log_e [(PG + PS)/(PC + PM)] = 1.902 + 0.0093 (\text{age})$$

The regression coefficient 0.0093 is more than  $2\frac{1}{2}$  times its standard error of  $\pm 0.0034$  and thus is highly significantly different from zero ( $p < 0.01$ ). It is apparent from the equation that the ratio  $(PG + PS)/(PC + PM)$  increases by just under 1% per year of age. The line for this equation is given in Fig. 3.1.8 as a full line. A slightly closer relationship is given when natural logarithms of both the ratio and age are taken, giving

$$\log_e [(PG + PS)/(PC + PM)] = 0.746 + 0.423 \log_e (\text{age}).$$

The regression coefficient 0.423 is now more than 3 times its standard error of  $\pm 0.134$  and is significantly different from zero ( $p < 0.01$ ). This equation is shown as the dashed line in Fig 3.1.8. It can be seen that it accommodates the low values of  $\log_e [(PG + PS)/(PC + PM)]$  in the two children and also brings out the fact that at older ages the trend seems to be diminishing.

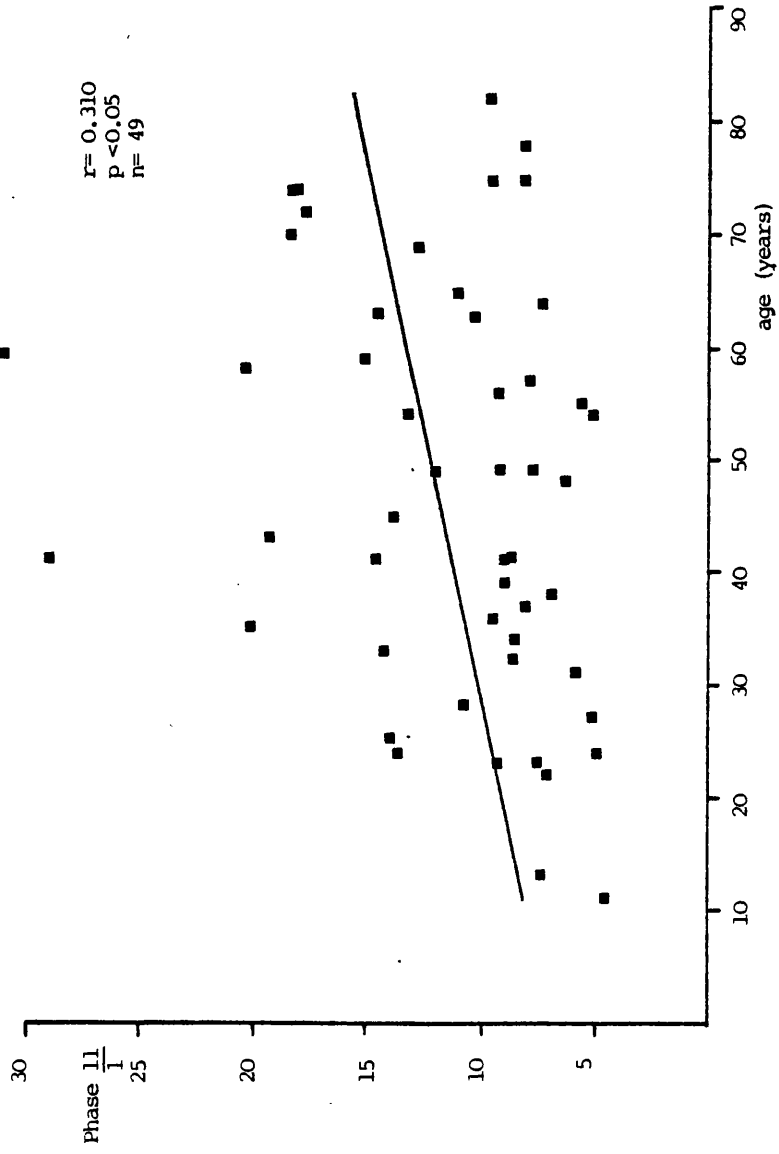


Fig. 3.1.5. Graph of Phase II metabolites of paracetamol against age in Phase I urine collected over 24 hours from normal volunteers

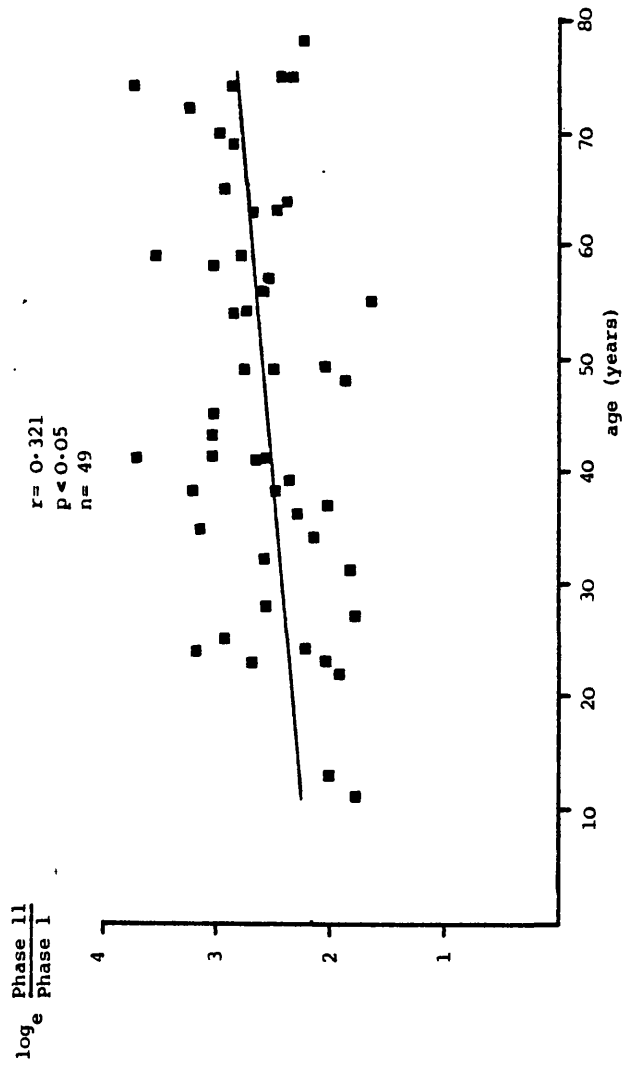


Fig. 3.1.1.6.  $\log_e$  Phase II metabolites of paracetamol plotted against Phase I age in 0 - 8 hour urine of healthy volunteers

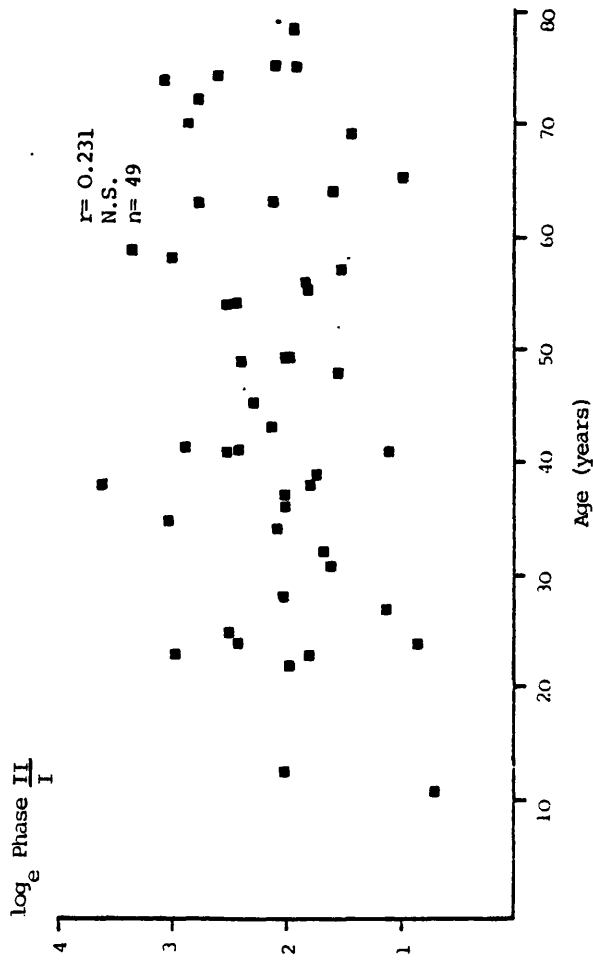


Fig. 3.1.7.  $\log_e$  Phase II metabolites of paracetamol plotted against age in 8 - 24 hour urine of healthy volunteers



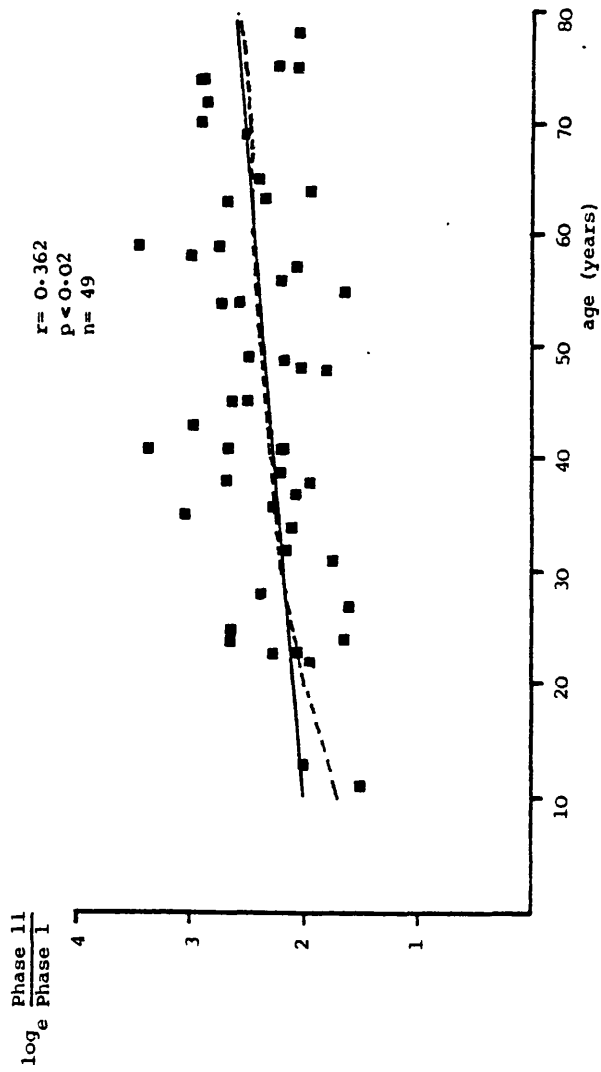


Fig. 3.1.8.  $\log_e \frac{\text{Phase II}}{\text{Phase I}}$  metabolites of paracetamol plotted against age in 0 - 24 hour urine of healthy volunteers

In order to examine the relationship of  $\log_e [(PG + PS)/(PC + PM)]$  to  $\log_e$  age in more detail, the recovery of the individual metabolites over the 0 - 24h collection period was considered separately. The natural logarithm of the amount of paracetamol glucuronide excreted was positively correlated with age ( $r = +0.356$ ,  $p < 0.05$ ) and the relationship may be expressed by the equation:

$$\log_e PG\% = 0.152 \times \log_e \text{ age} - 1.225$$

The regression coefficient 0.152 has a standard error of  $\pm 0.058$  and is significantly different from zero ( $p < 0.02$ ). In the 0 - 24 hour urine collection percentage recovery of paracetamol cysteine plus mercapturic acid was negatively correlated with age ( $r = -0.374$ ,  $p < 0.01$ ) and the relationship is expressed by the equation:

$$\log_e (PC + PM)\% = -0.355 \times \log_e \text{ age} + 3.43.$$

The regression coefficient (-0.355) has a standard error of  $\pm 0.121$  and is significantly different from zero ( $p < 0.01$ ). Recovery of paracetamol cysteine was negatively correlated with age ( $r = -0.486$ ,  $p < 0.01$ ) in the 0 - 24 hour urine collection and the relationship may be expressed by the equation:

$$\log_e PC\% = -0.715 \times \log_e \text{ age} + 0.663$$

The regression coefficient (-0.715) has a standard error of  $\pm 0.188$  and is significantly different from zero ( $p < 0.001$ ). Correlations between paracetamol mercapturic acid and paracetamol sulphate and  $\log_e$  of age were poor.

When the individual metabolites were examined in relation to age in the 0 - 8 hour and 8 - 24 hour collection periods, percentage recoveries of (PC + PM) and PC decreased significantly with age in the first 8 hours of collection ( $p < 0.02$  and  $p < 0.001$  respectively). In the 8 - 24 hour collection period (PC + PM) and PC decreased with age at a similar rate to

that of the first 8 hour period but the scatter was so much greater that statistical significance was not attained. Percentage recovery of PG was not related to age in the 0 - 8 hour period but increased with age in the 8 - 24 urine collection period ( $p < 0.05$ ).

In summary, the decrease in percentage recoveries of (PC + PM) and PC with age in the first 8 hours and the increase with age of the percentage recovery of PG in the 8 - 24 hour period served to increase the ratio of Phase II in the 0 - 8 hour period. Figs. 3.1.9

#### Phase I

3.1.10 and 3.1.11 show percentage recovery of paracetamol glucuronide plotted against age. For clarity, percentage recoveries of metabolites are plotted against age without transformation so that the scatter of results can be seen. Fig. 3.1.12 shows the percentage recovery of paracetamol cysteine plus paracetamol mercapturic acid plotted against age and Fig. 3.1.13 shows the relation of paracetamol cysteine alone with age. Finally all the analyses were repeated excluding the ten volunteers under the age of 30. This is because, as has been already described, the trend with age is in general less marked in the older volunteers and its overall significance may have been disproportionately affected by values found in the younger volunteers.

In volunteers aged over 30 years the following findings were made: the percentage recovery of paracetamol glucuronide increased with age but not now to a statistically significant extent, the percentage recovery of paracetamol sulphate was unrelated to age; the percentage recovery of paracetamol cysteine decreased with age but not to a statistically significant extent and the percentage recovery of paracetamol mercapturic acid was unrelated to age. The ratio Phase II/Phase I metabolites still increased with age but not to a statistically significant extent.

Fig. 3.1.9.

Percentage recovery of paracetamol glucuronide from healthy Volunteers plotted against age in years

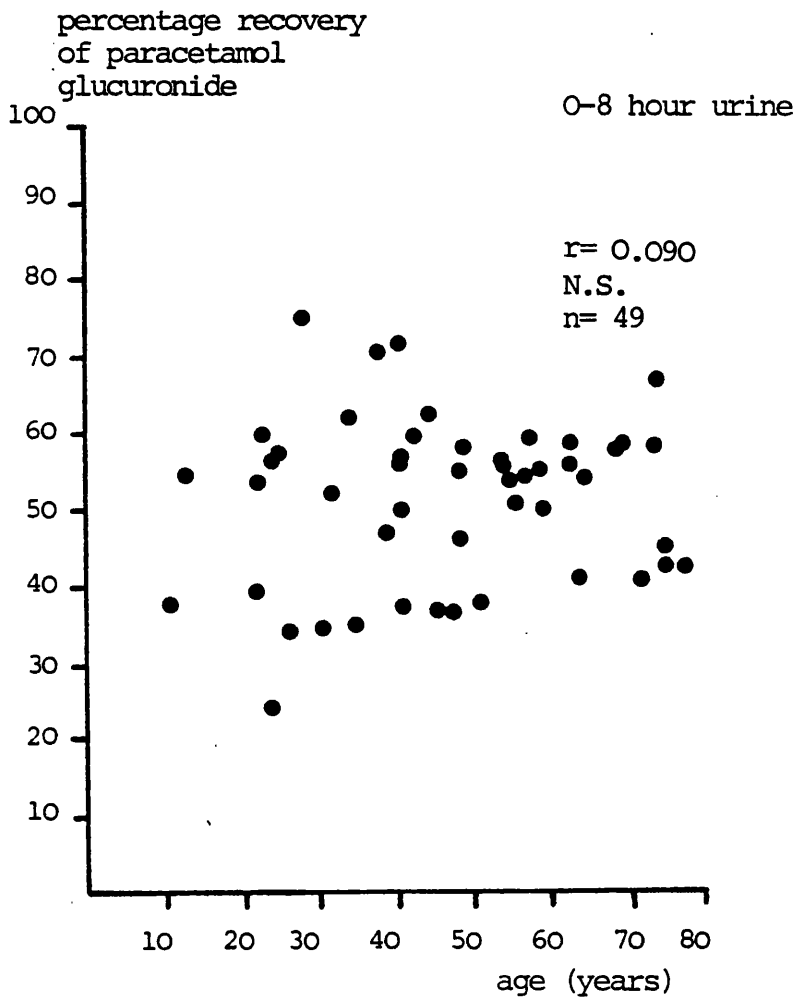


Fig. 3.1.10.  
Percentage recovery of paracetamol glucuronide from healthy  
volunteers plotted against age in years

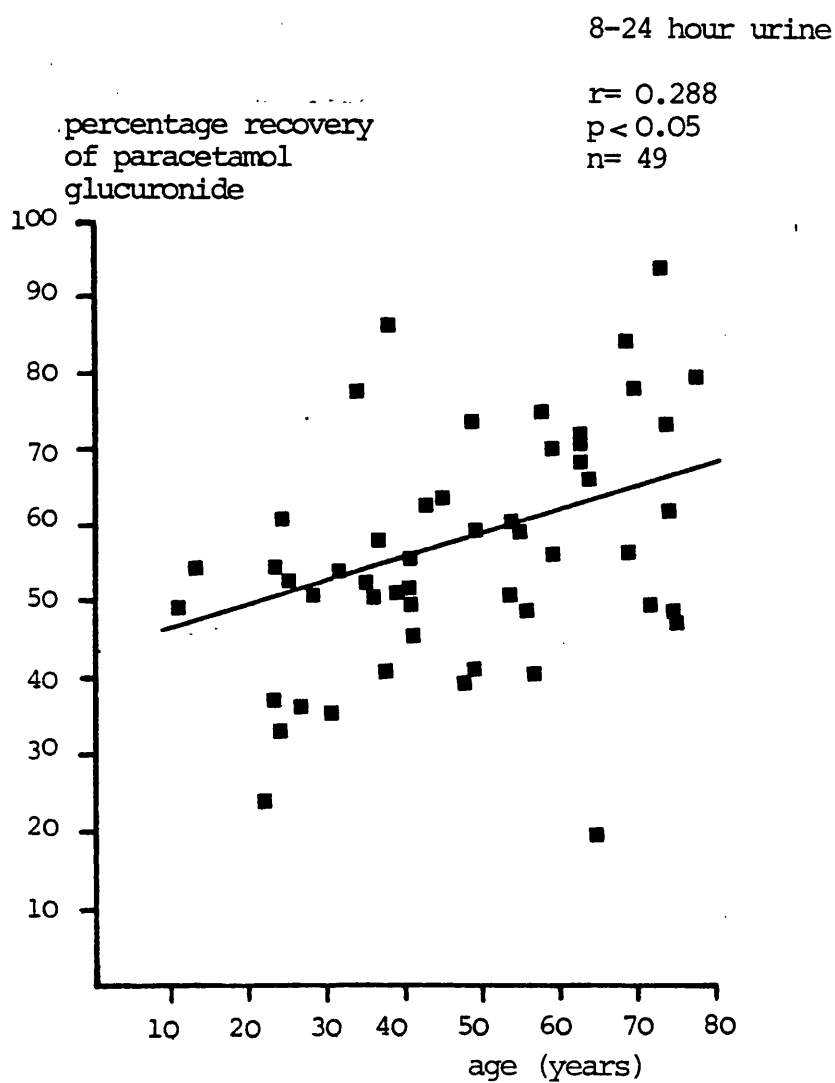


Fig. 3.1.11.  
Percentage recovery of paracetamol glucuronide from healthy  
volunteers plotted against age in years

0-24 hour urine

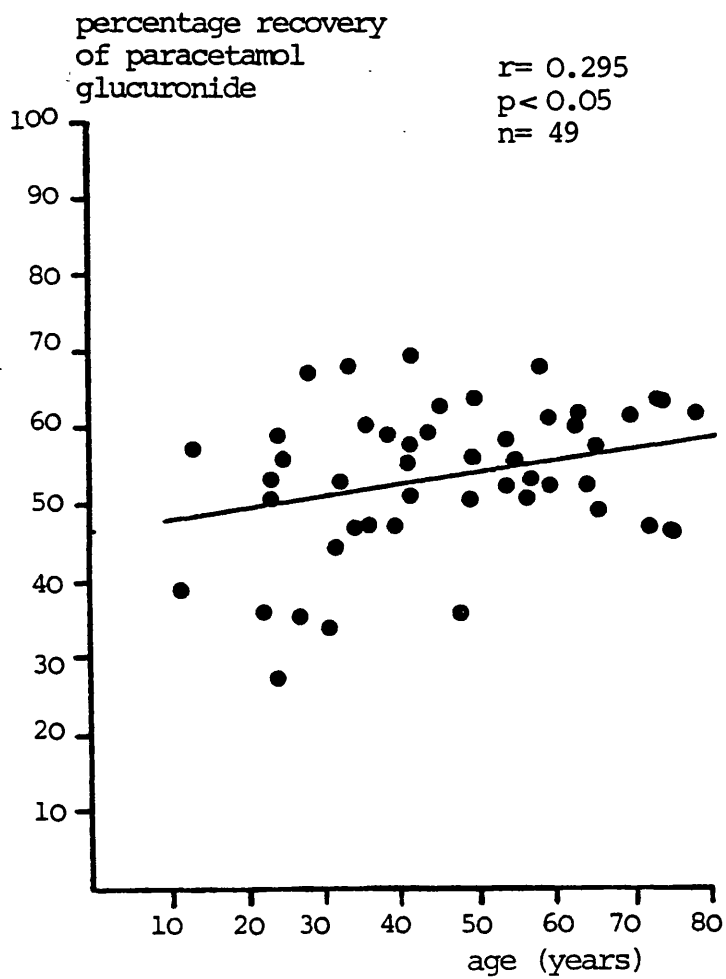


Fig. 3.1.12 Percentage recovery of paracetamol cysteine plus mercapturic acid from healthy volunteers against age in years

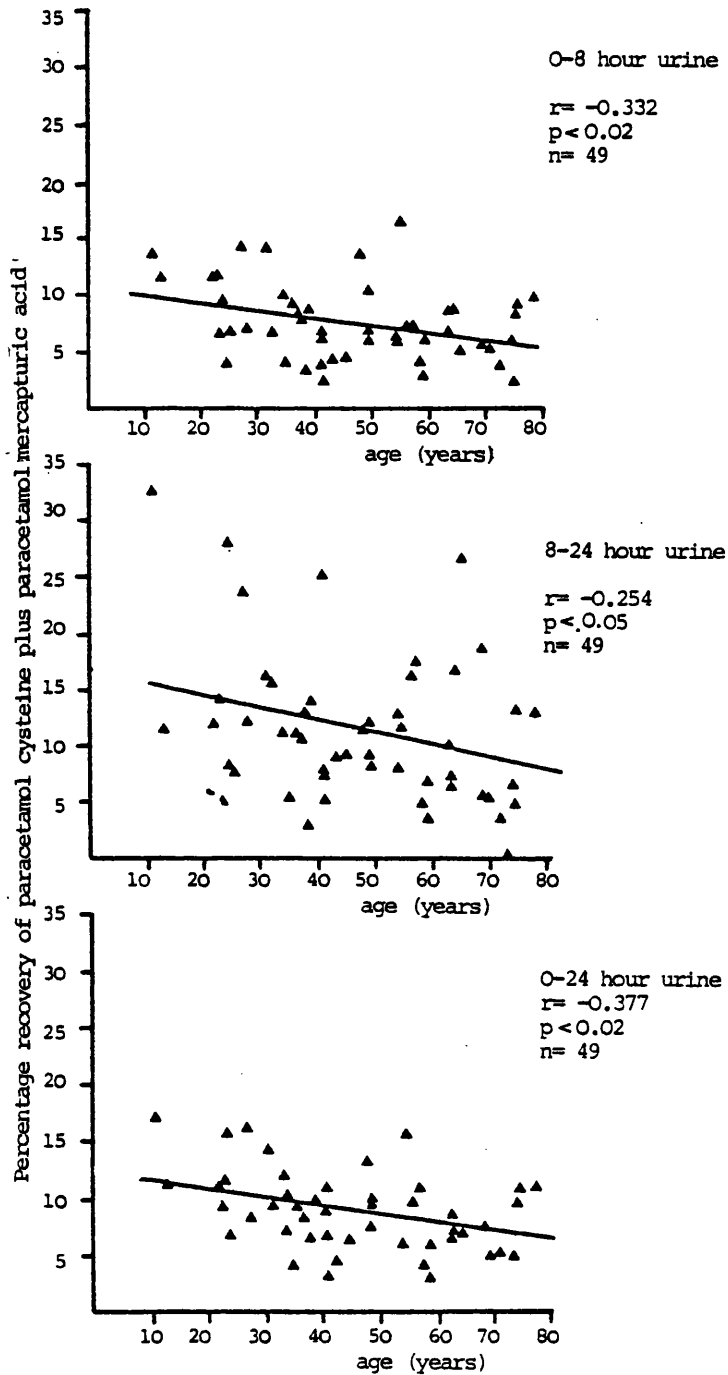
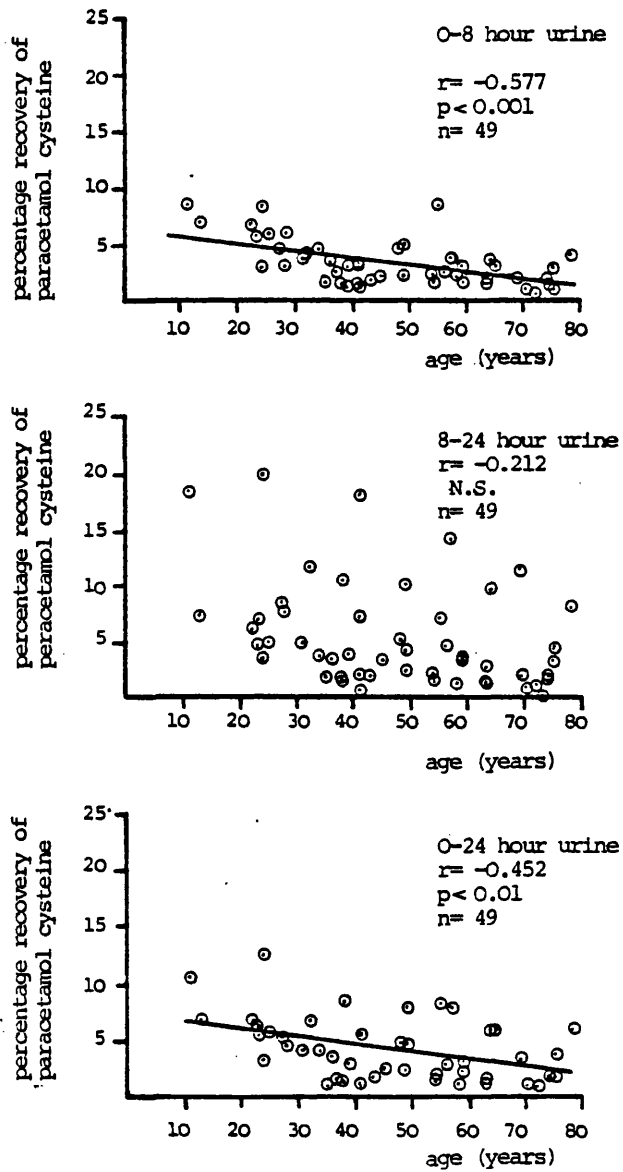


Fig. 3.1.13.  
Percentage recovery of paracetamol cysteine from healthy  
volunteers against age in years





Thus the changes in metabolic pattern with age are slight and only reach statistical significance when results from the under 30s are included.

There were also significant correlations between recoveries of individual metabolites. PG was significantly negatively correlated with PS ( $p < 0.001$ ), PC ( $p < 0.01$ ), PM ( $p < 0.05$ ) and PC + PM ( $p < 0.001$ ) in both urine collection periods and over the whole 24 hours. PS was negatively correlated with unchanged paracetamol in each period ( $p < 0.05$ ).

These results are plotted in Figs. 3.1.14, 3.1.15, 3.1.16, 3.1.17, 3.1.18, 3.1.19 and 3.1.20.

In order to compare the metabolism of paracetamol in normal volunteers after a therapeutic 1g dose with the metabolism of paracetamol under conditions such as disease or overdose two further ratios were calculated: namely  $(PG + PS)/(PC + PM)$  and  $PG/PS$ . The ratios  $PG/PS$  and  $(PG + PS)/(PC + PM)$  were chosen for the following reasons: because the amount of paracetamol ingested has to be fairly large before glucuronic acid is depleted or glucuronyl transferase activity is saturated the ratio  $PG/PS$  reflects the recovery of paracetamol sulphate i.e. if  $PG/PS$  increases this suggests a decrease in recovery of paracetamol sulphate and vice versa. The ratio  $(PG + PS)/(PC + PM)$  increases when either paracetamol sulphate recovery increases (which would be obvious from the  $PG/PS$  ratio) or when  $(PC + PM)$  falls. The ratio  $(PG + PS)/(PC + PM)$  decreases either when paracetamol sulphate recovery decreases (which shows up in the  $PG/PS$  ratio) or when  $(PC + PM)$  recoveries increase. Therefore the two ratios summarise the metabolic patterns of paracetamol sulphate and paracetamol cysteine plus mercapturic acid. Mean values of this ratio in each collection period are given in Table 3.1.4.

Fig. 3.1.14 Percentage recovery of paracetamol glucuronide and paracetamol sulphate after administration of paracetamol 1g by mouth to healthy volunteers

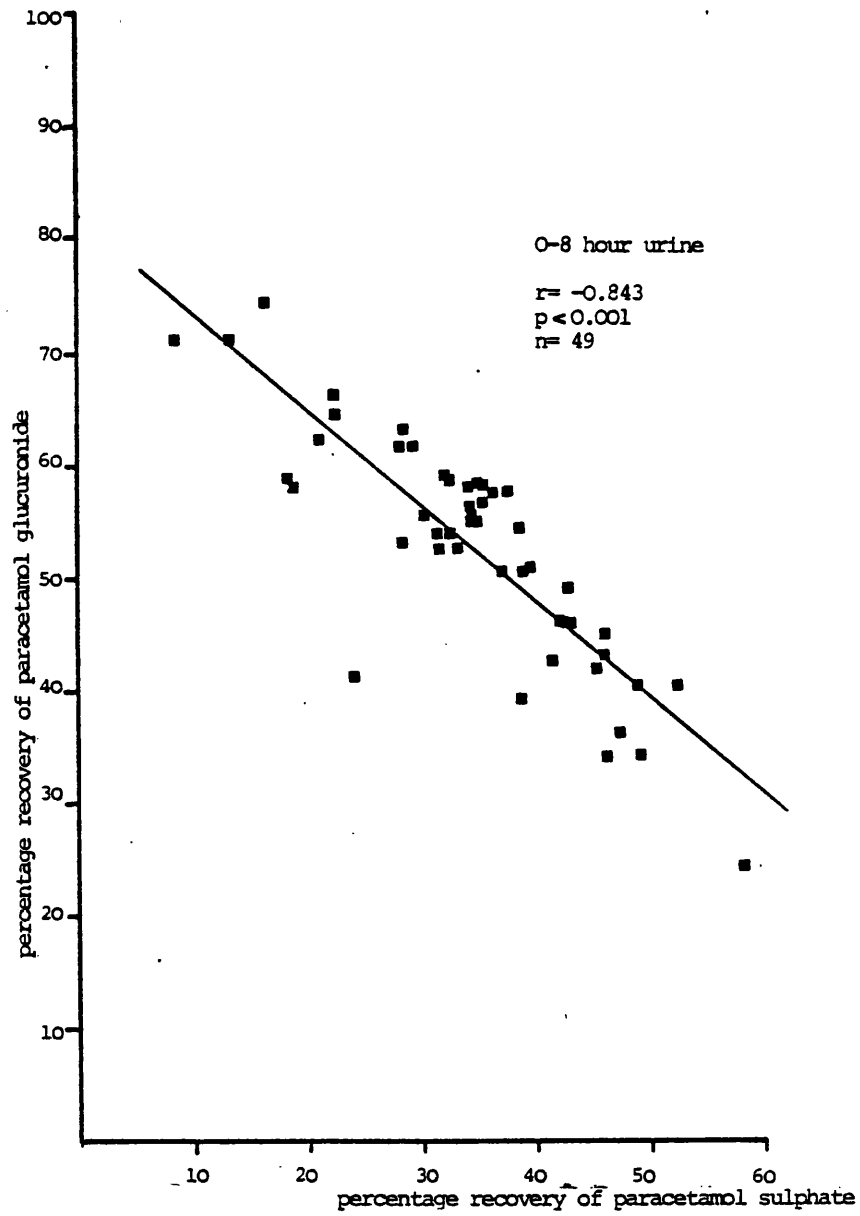


Fig. 3.1.15 Percentage recovery of paracetamol glucuronide and paracetamol sulphate after administration of paracetamol 1g by mouth to healthy volunteers

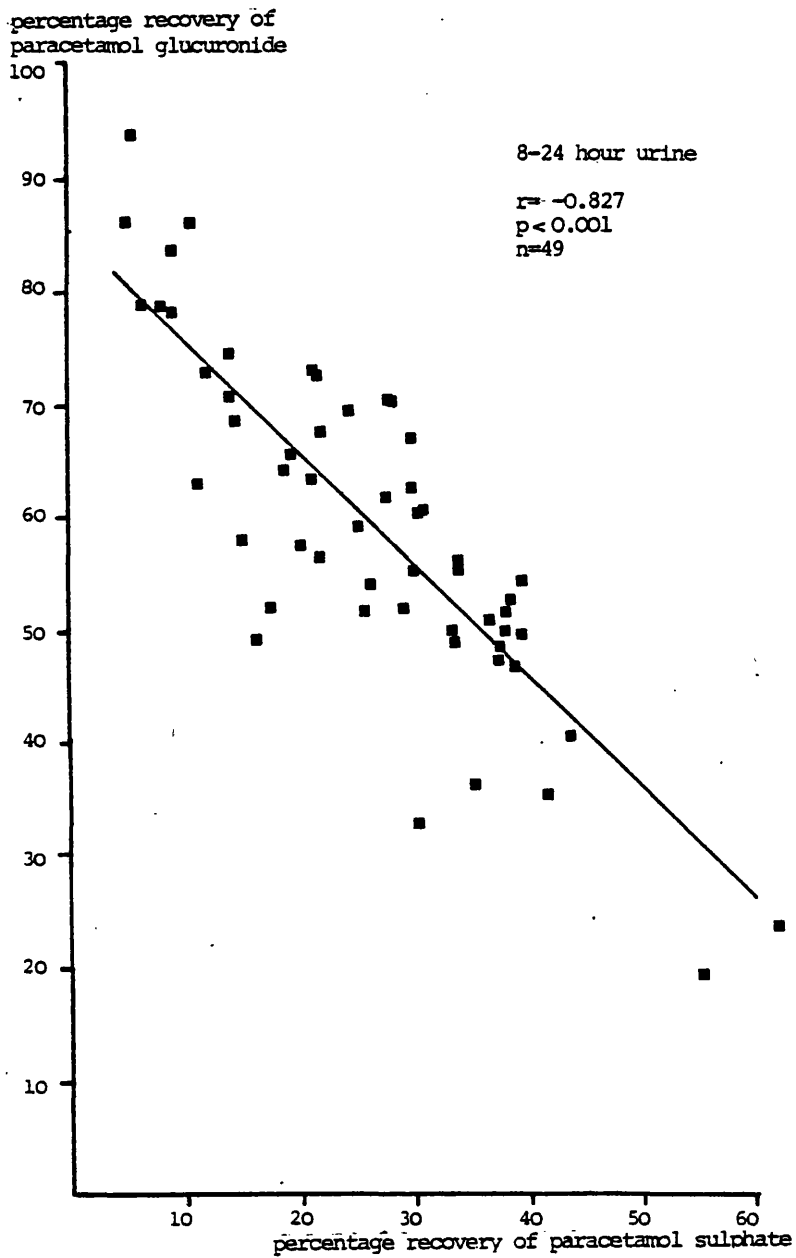


Fig. 3.1.16.  
Percentage recovery of paracetamol glucuronide and paracetamol sulphate after administration of paracetamol 1.0g by mouth to healthy volunteers

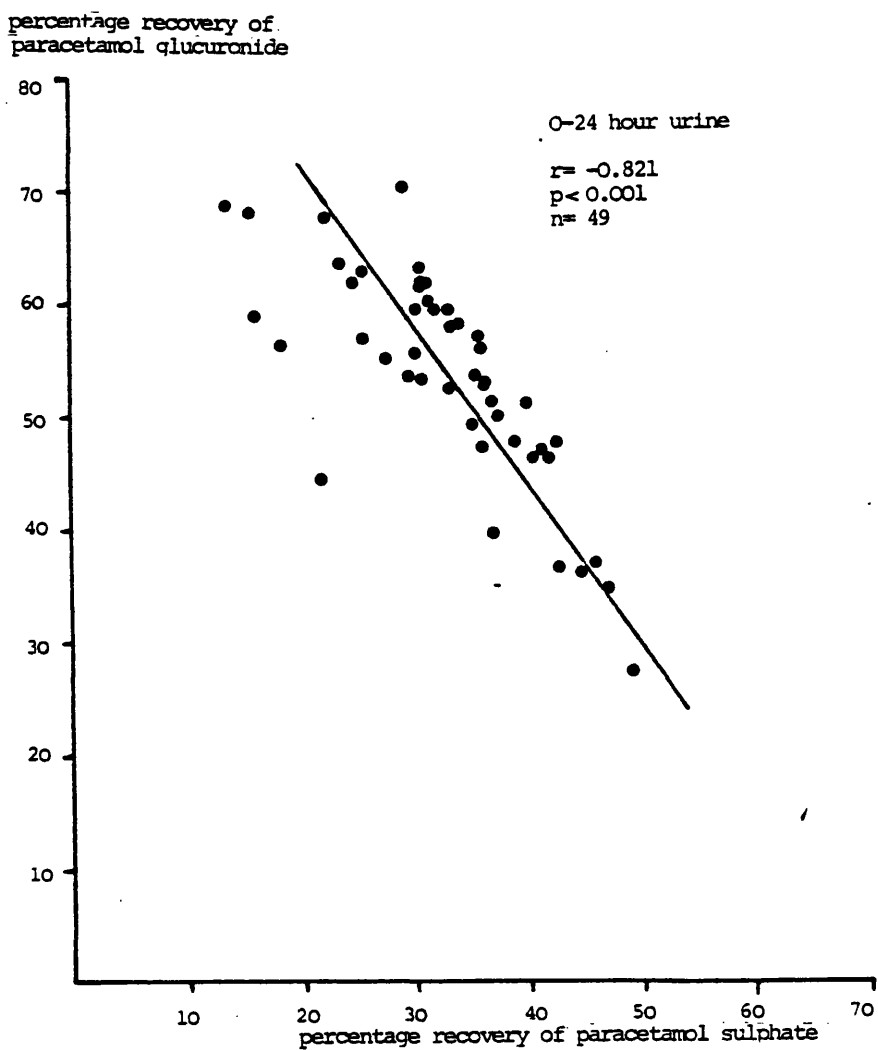


Fig. 3.1.17. Percentage recovery as paracetamol cysteine plotted against percentage recovery as paracetamol glucuronide

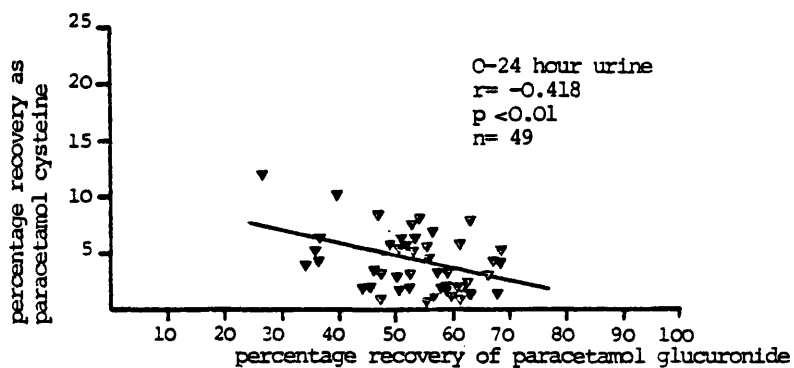
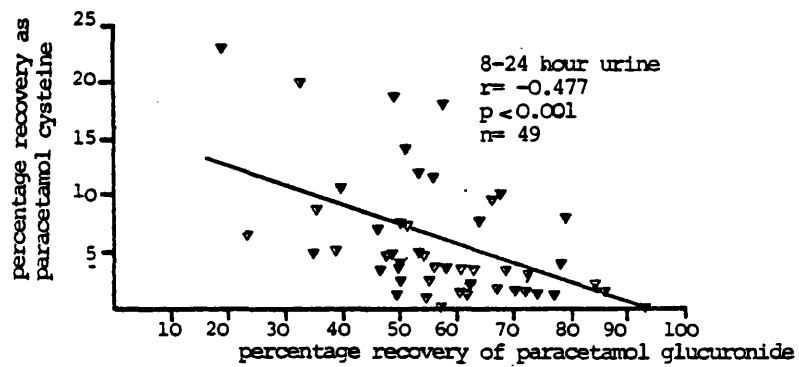
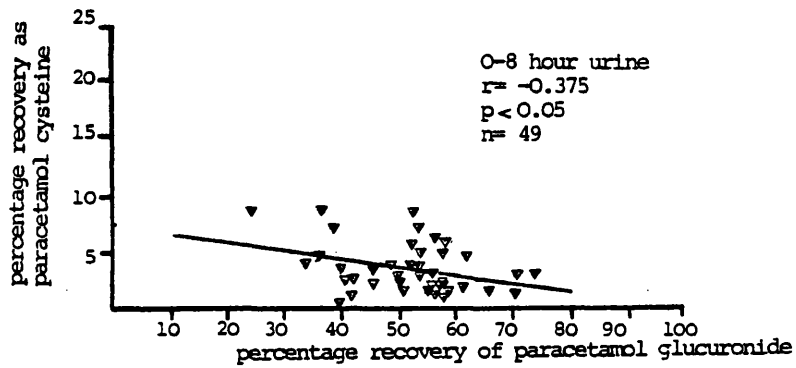


Fig. 3.1.18. Percentage recovery as paracetamol mercapturic acid plotted against percentage recovery of paracetamol glucuronide

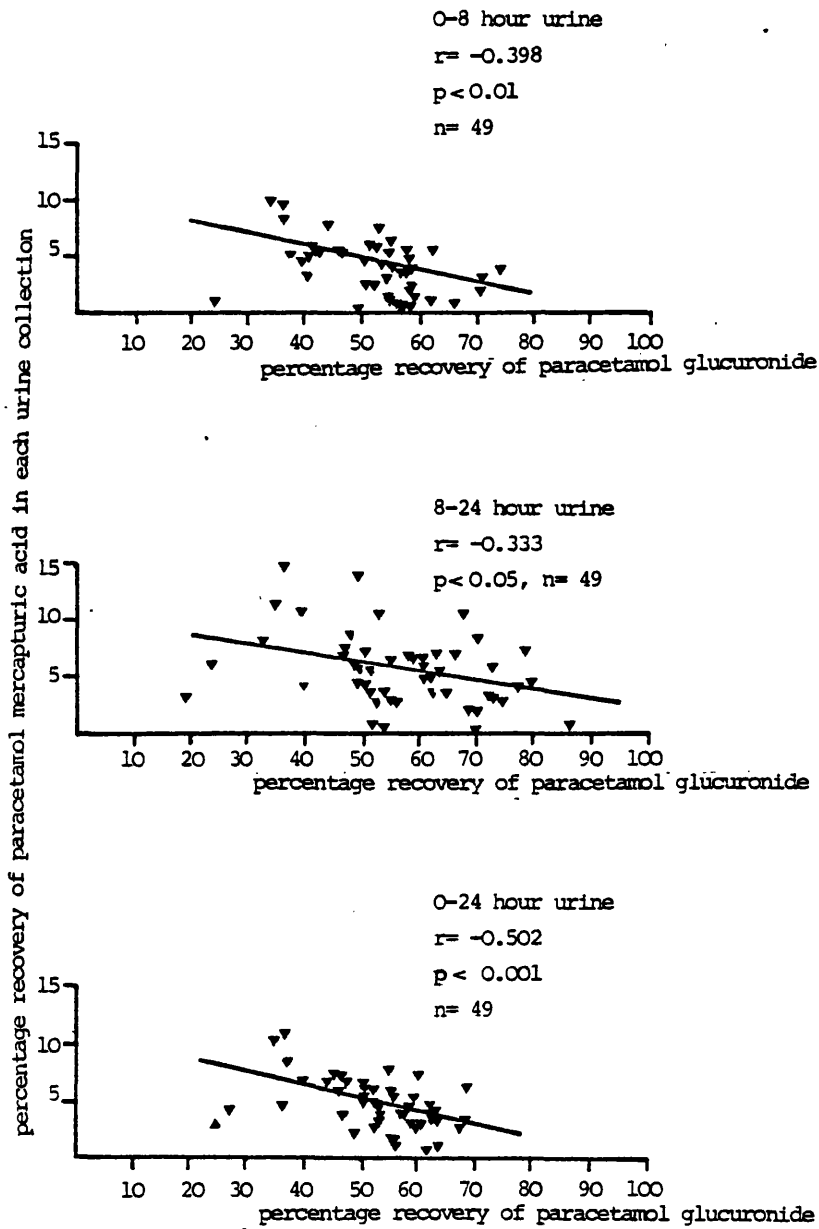


Fig. 3.1.19.  
Percentage recovery as paracetamol cysteine plus paracetamol mercapturic acid plotted against percentage recovery as paracetamol glucuronide

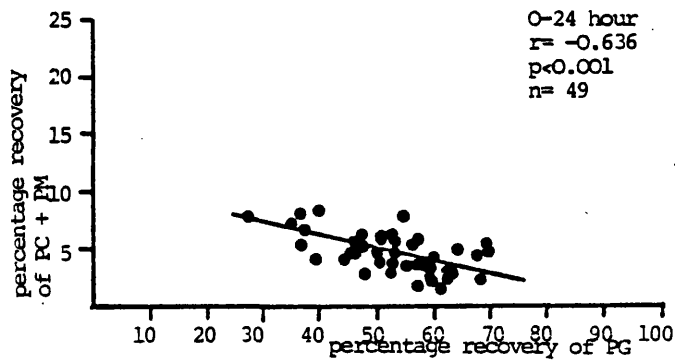
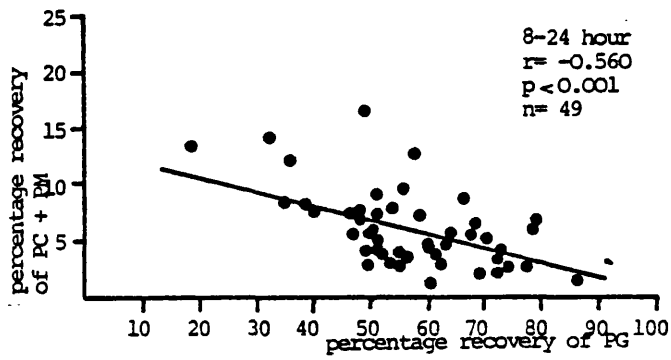
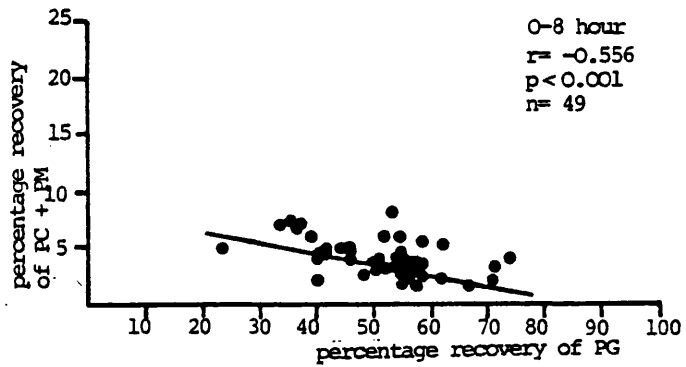


Fig. 3.1.20 Percentage recovery of paracetamol sulphate plotted against percentage recovery of unchanged paracetamol

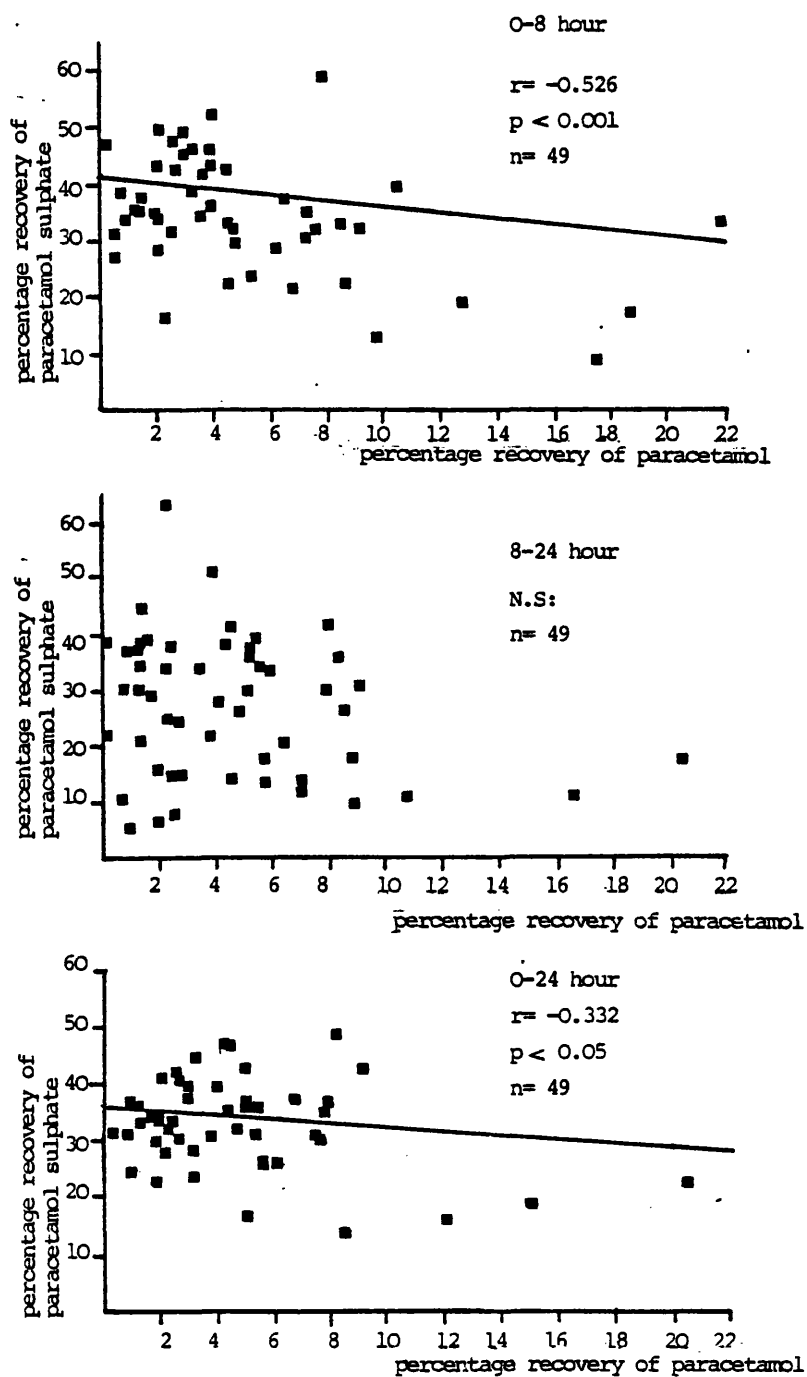




Table 3.1.4 PG/PS and (PG+PS)/(PC+PM) ratios in each collection

	(PG+PS)/(PC+PM)	PG/PS
0 - 8h	14.84 ± 1.14	1.80 ± 0.19
8 - 24h	10.09 ± 0.97	3.64 ± 0.72
0 - 24h	11.29 ± 0.82	1.83 ± 0.13

Table 3.1.4 shows that the ratio (PG + PS)/(PC + PM) was significantly lower in the 8 - 24h period than the 0 - 8h period ( $p < 0.01$ ) reflecting the increased recovery of paracetamol cysteine and mercapturic acid in the second collection period which has already been described. The ratio PG/PS was significantly higher in the 8 - 24h collection compared with the 0 - 8h interval ( $p < 0.02$ ) reflecting the decrease in recovery of paracetamol sulphate in the second collection period.

In order to determine the values of these ratios after a higher dose of paracetamol, a study was carried out in 4 normal volunteers each of whom took paracetamol 2.5g orally after fasting overnight and collected urine two hourly for ten hours. Individual details and recovery of paracetamol and its metabolites in mg and expressed as a percentage are given in Appendix 4.2. Percentage recoveries of each metabolite and of unchanged paracetamol are given in Table 3.1.5 for each period.

Table 3.1.5

Percentage recoveries of unchanged paracetamol and its metabolites after administration of paracetamol 2.5g to 4 healthy volunteers by mouth.

Time (h)	n	P	PG	PS	PC	PM	PC + PM
2	4	7.81±1.49	60.77±4.98	25.91±3.68	4.29±0.81	1.21±0.12	5.50±0.78
4	4	4.36±1.07	73.54±3.15	17.00±1.36	2.88±0.96	2.22±0.32	5.10±0.78
6	4	2.68±0.47	67.98±3.46	22.78±3.32	3.33±0.42	3.21±0.52	6.55±0.38
8	3	1.58±0.13	70.53±4.30	20.54±3.67	3.77±0.41	3.58±0.57	7.35±0.88
10	4	1.04±0.20	71.19±3.63	20.23±2.68	3.71±0.29	3.84±0.53	7.56±0.33

Percentage recoveries of unchanged paracetamol decreased significantly when 8 and 10 hour values were compared with values at 2 hours ( $p < 0.05$ ) but percentage recoveries of the metabolites did not change significantly with time.

The ratios  $(PG + PS)/(PC + PM)$  and  $PG/PS$  were calculated for each sample for each individual and means and standard errors of the values are given in Table 3.1.6.

Table 3.1.6.

(PG + PS)/(PC + PM) and PG/PS ratios with time in 4 healthy volunteers after paracetamol 2.5g by mouth

Time (h)	(PG + PS)/(PC + PM)	PG/PS
2	16.79 ± 2.46	2.66 ± 0.76
4	20.54 ± 4.49	4.39 ± 0.65
6	14.92 ± 1.21	3.19 ± 0.48
8	12.86 ± 1.91	3.70 ± 0.75
10	12.18 ± 0.57	3.78 ± 0.66

(PG + PS)/(PC + PM) values after paracetamol 2.5g were significantly higher at 2, 4 and 6 hours after ingestion than in 8 - 24h urine and at 10h compared with 0 - 8h urine after paracetamol 1.0g ( $p < 0.05$ ) (See Table 3.1.4) but at later times the ratios were not significantly different from values calculated for the 1g dose. These initial high values are probably due to increased conjugation of paracetamol with glucuronic acid and sulphate at the higher dose. However, (PG + PS)/(PC + PM) and PG/PS values given in Table 3.1.6 were not significantly different during the whole ten hours. PG/PS values from 4h onwards after 2.5g paracetamol were significantly higher than 0 - 8h and 0 - 24hour values but not 8 - 24h values after 1g paracetamol ( $p < 0.05$ ) (See Table 3.1.6). This suggests that sulphate depletion occurred with the 2.5g dose but not to any greater extent than that seen after the 1g dose in the 8 - 24h urine collection period.

## 3.2 Neonatal Metabolism of Paracetamol

### Introduction

The writer is unaware of any previous studies of paracetamol metabolism in the neonate. Because of ethical difficulties in giving paracetamol to neonates, in this study the metabolism of paracetamol has been studied in neonatal urine after administration of paracetamol to nursing mothers for analgesic purposes so that infants ingested paracetamol from breast milk. It is likely that unchanged paracetamol but not its more polar metabolites can cross from mother to infant via breast milk and paracetamol but not its metabolites was found in breast milk by Notarianni (1981) (personal communication) and it was shown by Berlin, Yaffe and Ragni (1980) that paracetamol did indeed appear in breast milk. Although these authors could not detect paracetamol or its metabolites in neonatal urine, in the present study both parent drug and metabolites were found in nursing infants' urine. The metabolites of paracetamol and unchanged drug were therefore quantified in neonatal urine after maternal ingestion of paracetamol in order to assess the metabolic capacity of the infant as described in Chapter 2.

### Procedures and Results

Urine was obtained from 6 neonates (mean age  $\pm$  SEM = 3.3  $\pm$  0.92 days) after mothers had taken 1-3g paracetamol up to eight hours before breast feeding.

Times at which mothers ingested paracetamol, times of breast feeding and neonatal urine collection, maternal dose and recovery of paracetamol in neonatal urine are given in Table 3.2.1. Larger recoveries of paracetamol were obtained from neonates whose mothers took 2 or more grams of paracetamol. Recoveries in the urine of the twins (R.M.) were different probably due to different urine volumes collected and milk volume ingested.

**Table 3.2.1. Individual values of dosage of paracetamol ingested, times of feeding and urine collections and total recovery of paracetamol and its metabolites in urine of 6 neonates**

Neonatal Initials	Time at which mothers ingested paracetamol	Dose at time stated	Time of breast feeding	Time of neonatal urine collection	Neonatal urine volumes (ml)	Total recovery of paracetamol $\mu$ g corrected for molecular weight
L.D.	10.20	1g	14.45	15.00 - 18.00	2.8	85.19
F.C.	11.30	1g	14.45	18.00	1.4	59.81
R.M.	10.40	1g	13.00	13.00 - 15.30	0.56	24.85
R.M.	10.40	1g	13.00	13.00 - 14.00	10.5	486.58
D.C.	06.00 10.35	1g 1g	14.45	17.30	12.5	22.24
M.C.	07.15 10.00 14.45	1g 1g 1g	14.15	18.00	11.5	921.92

Table 3.2.2 shows means  $\pm$  SEM of concentrations and percentage recovery of paracetamol and its metabolites in urine of these 6 neonates compared with mean percentage recoveries in the control study (Section 3.1).

Table 3.2.2. Means  $\pm$  S.E.M. of total amount and percentage recoveries of paracetamol and its metabolites excreted by neonates compared with percentage recoveries in the control study

	P	PG	PS	PC + PM
Neonates				
Total amount ( $\mu$ g)	120.02 $\pm$ 67.32	177.13 $\pm$ 64.44	68.71 $\pm$ 23.87	84.24 $\pm$ 26.78
Range (n=6)	2.91-432.40	17.71-348.52	2.35-150.06	0-158.04
Percentage recovery	24.85 $\pm$ 6.65	54.14 $\pm$ 5.96	9.85 $\pm$ 1.92	11.13 $\pm$ 3.19
Range (n=6)	9.53-46.90	32.82-71.27	4.98-18.25	0-20.47
Control Study percentage recovery in 24 hours after paracetamol 1g	4.82 $\pm$ 0.59	53.46 $\pm$ 1.35	32.79 $\pm$ 1.16	9.01 $\pm$ 0.49

Variations in amount excreted in neonatal urine are introduced by differences in milk intake by the infants and in the length of time between the mother's ingestion of paracetamol and breast feeding. Nevertheless, it is apparent that the neonates excreted a significantly larger amount of unchanged paracetamol ( $p < 0.05$ ) and significantly lesser amounts of paracetamol sulphate ( $p < 0.001$ ) compared with normal volunteers. Thus even if some metabolites were ingested by the neonates in breast milk, the amount of unchanged paracetamol recovered in neonatal urine was still greater than in the control study.

Individual recoveries in the neonates expressed in  $\mu$ g and as percentages are given in Appendix 5.1 and 5.2 respectively.

### 3.3 Paracetamol Metabolism in Patients with Rheumatoid Arthritis

#### Introduction

The metabolism of paracetamol was investigated in 22 in-patients aged between 36 and 78 years in the Royal National Hospital for Rheumatic Diseases in Bath. All twenty two were diagnosed by consultant rheumatologists as having rheumatoid arthritis using the criteria of the American Rheumatism Association (see Appendix 6). All 22 took paracetamol for analgesic purposes during their stay but were also taking other drugs as detailed in Appendix 7. There do not appear to have been any studies previously carried out on paracetamol metabolism in patients with rheumatoid arthritis. The metabolism of paracetamol could potentially be altered by the disease state itself, by concurrent therapy with other drugs or by the large amounts of paracetamol taken by patients with this disease.

#### Procedures

Urine was collected from 0-8h and 8-24h in separate acidified urine bottles after paracetamol (1g) was taken orally in the early morning. Urine was assayed for paracetamol and its major metabolites by HPLC as described in Section 2.7.e(i). Several patients took extra 1g doses of paracetamol during the 24 hours of the study and details of dosages obtained from hospital drug records are given in Appendix 7.

#### Results

Appendix 8 gives individual values of the total quantities of paracetamol and its metabolites recovered and Appendix 9 shows individual values of the percentage recoveries of paracetamol and its metabolites in each collection period for the 22 patients.

Table 3.3 shows the mean percentage recoveries of paracetamol and its metabolites in the 0-8h, 8-24h and 0-24h urine collection periods in those patients. Results over the whole 24 hour collection period are compared with control 0-24 hour values from normal volunteers after a 1g dose in Fig. 3.3. Significantly greater amounts of the glucuronide ( $p < 0.001$ ), lesser amounts of sulphate ( $p < 0.001$ ) and lesser amounts of unchanged paracetamol ( $p < 0.005$ ) were recovered from the rheumatoid patients than from the controls. Percentage recoveries of the cysteine and mercapturic acid metabolites of paracetamol were not significantly different from control values.

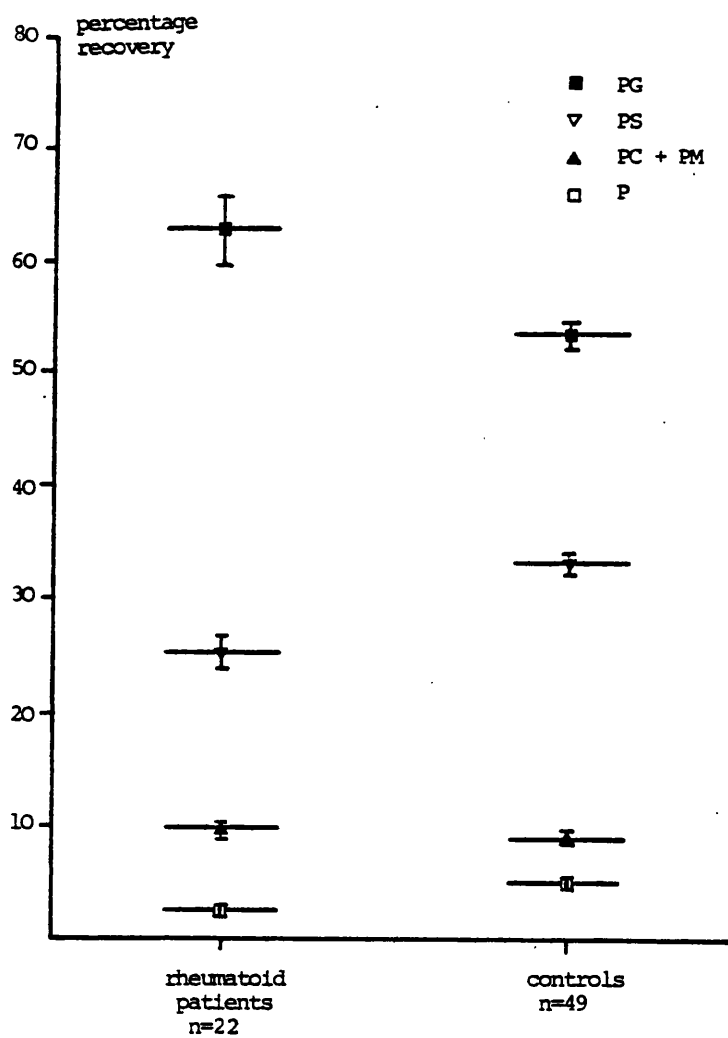
Table 3.3 Means  $\pm$  S.E.M. of percentage recovery of paracetamol and its metabolites in 22 rheumatoid patients

	P	PG	PS	PC + PM
0-8h	2.75 $\pm$ 0.42	60.74 $\pm$ 2.50	27.53 $\pm$ 2.41	8.83 $\pm$ 0.99
8-24h	2.48 $\pm$ 0.45	64.78 $\pm$ 1.68	23.20 $\pm$ 1.51	9.53 $\pm$ 0.78
0-24h	2.43 $\pm$ 0.37	68.13 $\pm$ 1.88	25.00 $\pm$ 1.52	9.38 $\pm$ 0.78



Fig. 3.3.

Percentage recovery of paracetamol and its metabolites in urine collected for 24 hours comparing patients with rheumatoid arthritis after varying amounts of paracetamol taken by mouth with normal volunteers after paracetamol 1.0g taken by mouth showing means  $\pm$  S.E.M.



### 3.4 Metabolism of Paracetamol in Patients with Thyroid Disease

#### Introduction

When thyroid function is altered, a series of physiological effects occur which are likely to affect drug absorption, metabolism or excretion (Shenfield, 1981). Forfar, Pottage, Toft, Irvine, Clements and Prescott (1980) reported that in thyrotoxic patients paracetamol absorption was faster; there was also an apparent increase in total body clearance and shorter plasma half-life of paracetamol than when these patients subsequently became euthyroid. On the basis of antipyrine half-life, Shenfield (1981) demonstrated acceleration of hepatic metabolism in hyperthyroid patients and reduced metabolism in hypothyroid patients. Induction or inhibition of the hepatic mixed function oxidase system may have implications for the toxicity of paracetamol which may be increased if the rate of formation of the reactive intermediate is enhanced or the activity of glutathione transferase diminished. Therefore the effects of thyroid disease on paracetamol metabolism were investigated in 7 hyperthyroid patients.

#### Procedures

Patients whose serum thyroxine levels were in excess of 135nmol/l (Bold and Wilding, 1975) were admitted to the study. The patients took paracetamol 1.0g by mouth with water in a single dose. Urine was collected over 0-8h and 8-24h periods and concentrations of paracetamol and its metabolites were measured by HPLC.

#### Results

Details of these 7 patients appear in Table 3.4.1.

Table 3.4.1. Clinical details of 7 hyperthyroid patients

Patient	Sex	Age (years)	Weight (kg)	FTI (Units)	Thyroxine (nmol l <sup>-1</sup> )
A.M.	M	61	57.5	268	>300
I.H.	F	74	not measured	194	194
A.A.	F	53	62.0	233	281
H.R.	M	75	70.0	280	230
E.C.	F	70	57.6	199	183
D.S.	F	48	54.0	>300	275
G.G.	F	74	63.5	176	146

Table 3.4.2 Means  $\pm$  S.E.M. of percentage recovery of paracetamol and its metabolites in the 7 hyperthyroid patients

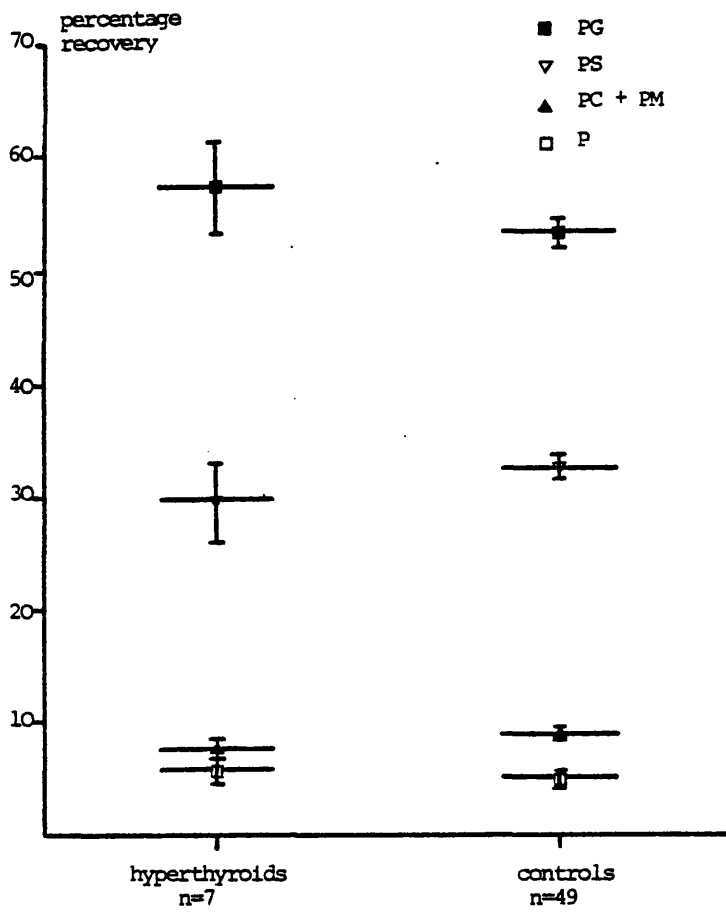
	P	PG	PS	PC	PM	PC + PM
0-8h	5.38 $\pm$ 1.42	55.86 $\pm$ 4.20	31.79 $\pm$ 3.67	2.94 $\pm$ 0.33	4.04 $\pm$ 0.75	6.97 $\pm$ 0.74
8-24h	7.32 $\pm$ 1.73	58.74 $\pm$ 4.64	24.65 $\pm$ 3.46	3.98 $\pm$ 0.68	5.30 $\pm$ 1.68	9.29 $\pm$ 2.17
0-24h	5.66 $\pm$ 1.10	57.30 $\pm$ 4.15	29.55 $\pm$ 3.48	3.20 $\pm$ 0.34	4.28 $\pm$ 0.84	7.49 $\pm$ 0.88

Table 3.4.2. gives the percentage recoveries of paracetamol and its metabolites in 7 hyperthyroid patients. In Section 3.1 (the control study) it was shown that in normal volunteers percentage recoveries of paracetamol sulphate and paracetamol cysteine plus paracetamol mercapturic acid were significantly different in 0-8 hour and 8-24 hour urine collections. In the hyperthyroid patients however the percentage recoveries of paracetamol and its metabolites were not significantly different in the two collection periods ( $p > 0.05$ ). When values from these hyperthyroid patients were compared with values from normal volunteers in the control study (see Fig. 3.4), no significant differences in the percentage recoveries of paracetamol and its metabolites were found in either urine collection or over the whole 24 hours.

An attempt was made to correlate the percentage recoveries of paracetamol and its metabolites excreted in urine with individual FTI and thyroxine levels but no relationship was found.

Appendix 10 gives total amounts of unchanged paracetamol and its metabolites in mg for each individual. Appendix 11 gives the same values corrected for percentage recovery.

Fig. 3.4.  
Percentage recovery of paracetamol and its metabolites in urine collected for 24 hours comparing patients with serum thyroxine concentrations greater than 135nmol/l with normal volunteers after paracetamol 1.0g taken by mouth showing means  $\pm$  S.E.M.



### 3.5 Effect of Salicylamide on Paracetamol Metabolism in Man

#### Introduction

Salicylamide is a mild analgetic and antipyretic which is now rarely used in the United Kingdom. The drug is however of interest in that it is largely conjugated with endogenous glucuronic acid and sulphate (Levy and Yamada, 1971). Salicylamide thus constitutes a potentially useful model of a drug which may compete with paracetamol for sulphate and glucuronic acid. Any effects observed which can be attributable to the competing effects of this drug may also apply to other drugs taken concurrently with paracetamol which also conjugate with sulphate and glucuronic acid.

The work was undertaken to explore the possibility that an agent which competes with paracetamol for metabolic substances may decrease amounts of paracetamol excreted as its glucuronide and sulphate conjugates thus making available increased amounts of unchanged paracetamol for oxidation by the mixed function oxidase system and increasing its potential for hepatotoxicity.

#### Procedures

Two experiments were carried out:

##### Experiment 1:

Six normal volunteers took paracetamol 1.0g by mouth with 100ml H<sub>2</sub>O after an overnight fast. Venous blood samples were taken immediately before ingestion of paracetamol and thereafter at 45 minute intervals for 5.25h. Urine was collected for 0-8 hours and 8-24 hours after dosing in separate containers.

### Experiment 2:

Seven days later, Experiment 1 was repeated in the same six volunteers but in addition salicylamide 1g was taken twice by mouth half an hour before and 2 hours after the 1g oral dose of paracetamol. Salicylamide was given each time as its sodium salt in a final volume of 200ml. Preparation of the salicylamide solution is described in Section 2.1.d.

Urine and serum samples were analyzed by HPLC as described in Section 2.7 e i. Total amounts of each urinary and serum metabolite were corrected for molecular weight. Urinary results are presented as percentage recovery.

The serum half life of paracetamol was calculated for each volunteer in each experiment, in addition the area under the serum concentration time curves (AUC) for paracetamol and its glucuronide and sulphate conjugates was calculated by the trapezoidal rule described by Natari (1975) up to 315 minutes after administration of paracetamol.

Results

Table 3.5.1 gives details of the volunteers studied in experiments 1 and 2.

Table 3.5.1 Details of volunteers studied in Experiments 1 and 2

Volunteer	Age (years)	Sex	Weight (kg)	Cigarette usage	Alcohol consumption	Tea & Coffee ingestion per day
B	22	M	79	Non smoker	3-4 pints beer per day	3½ pints tea
Pa	21	M	76	15 cigarettes per day	3-4 pints beer per day	3 pints tea
S	26	M	92	20 cigarettes per day	3 pints beer per day	2½ pints coffee
Ha	25	F	58	Ex smoker	2 gins per week	2 pints coffee /tea
Pe	42	M	82	Non smoker	2 glasses wine per day	2 pints coffee /tea
He	22	F	44	Non smoker	1 pint beer per day	2 pints coffee /tea



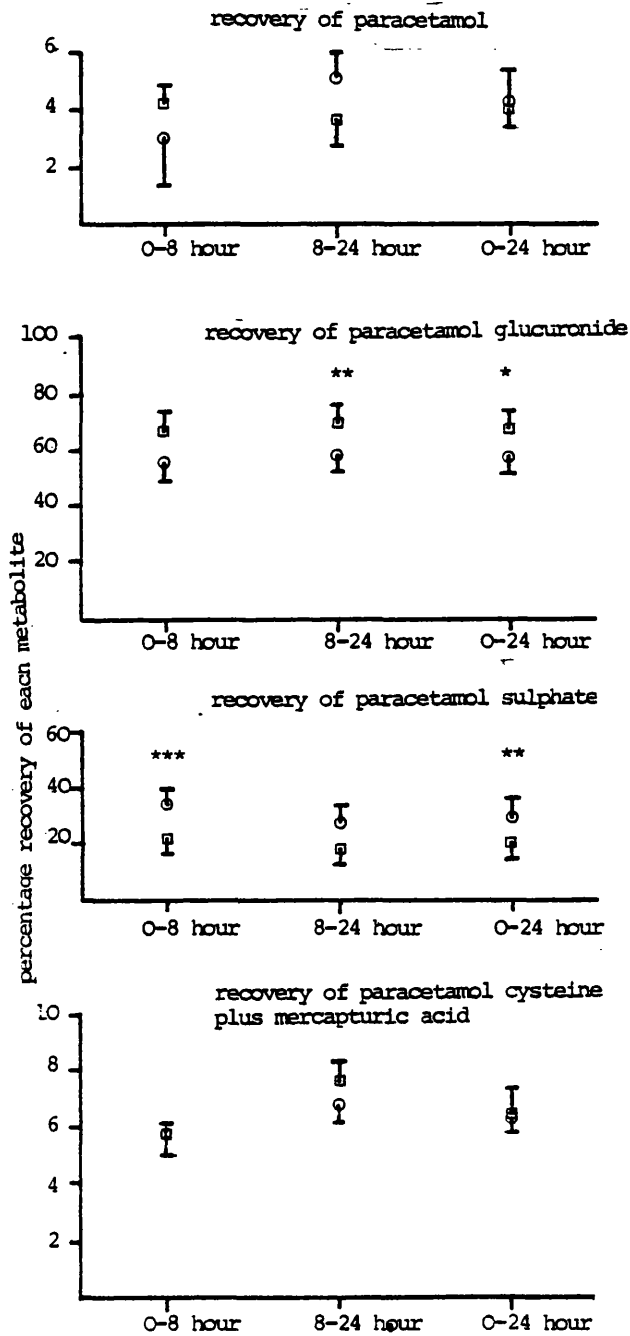
Table 3.5.2. gives the mean percentage recovery of paracetamol and its metabolites in urine for both experiments.

Table 3.5.2. Mean  $\pm$  S.E.M. percentage recovery of the major metabolites of paracetamol and unchanged paracetamol in 6 healthy volunteers following paracetamol 1.0g taken alone (Expt. 1) and following paracetamol 1.0g taken with salicylamide 2.0g (Expt. 2) (Details of drug administration appear in the text).

	P	PG	PS	PC	PM	PC + PM
<u>0-8h</u>						
Expt.1	3.32 $\pm$ 0.60	56.52 $\pm$ 6.21	34.76 $\pm$ 5.49	1.82 $\pm$ 0.26	3.57 $\pm$ 0.60	5.88 $\pm$ 0.38
Expt.2	4.28 $\pm$ 0.56	67.53 $\pm$ 5.70	22.57 $\pm$ 5.39	2.28 $\pm$ 0.22	3.33 $\pm$ 0.63	5.86 $\pm$ 0.79
<u>8-24h</u>						
Expt.1	5.16 $\pm$ 0.78	59.34 $\pm$ 5.47	28.57 $\pm$ 5.44	2.84 $\pm$ 0.26	3.73 $\pm$ 0.32	6.77 $\pm$ 0.59
Expt.2	3.68 $\pm$ 0.85	70.32 $\pm$ 6.76	18.79 $\pm$ 6.61	3.22 $\pm$ 0.26	4.00 $\pm$ 0.76	7.70 $\pm$ 0.76
<u>0-24h</u>						
Expt.1	4.26 $\pm$ 1.08	58.92 $\pm$ 6.10	30.80 $\pm$ 5.71	2.27 $\pm$ 0.17	3.74 $\pm$ 0.34	6.30 $\pm$ 0.42
Expt.2	3.98 $\pm$ 0.50	68.43 $\pm$ 5.90	21.26 $\pm$ 5.58	2.70 $\pm$ 0.22	3.62 $\pm$ 0.62	6.50 $\pm$ 0.83

Table 3.5.2 shows that the percentage recovery of paracetamol sulphate was considerably lower when salicylamide was taken (Expt. 2) than in the control study (Expt. 1) in the 0-8 hour urine collection ( $p < 0.005$ ). In 8-24 hour urine there was no significant difference between Experiments 1 and 2. In the second collection period, paracetamol glucuronide excretion increased after salicylamide ( $p < 0.02$ ). Over the whole 24 hour period, paracetamol sulphate was less ( $p < 0.02$ ) when salicylamide and paracetamol were taken together compared with paracetamol given alone. There were no significant changes in the excretion of the other metabolites in the 24 hour urine. Percentage recoveries of paracetamol and its metabolites in each urine collection are shown in Fig. 3.5.1.

Fig. 3.5.1 Percentage recovery of paracetamol and its metabolites in 0 - 8 hour, 8 - 24 hour and 0 - 24 hour urine (means  $\pm$  S.E.M.)



Key: Experiment 1 1g paracetamol only ○  
 Experiment 2 1g paracetamol + 2g salicylamide □  
 \*  $p < 0.05$  \*\*  $p < 0.02$  \*\*\*  $p < 0.005$

Concentrations of paracetamol, paracetamol sulphate and paracetamol glucuronide in serum measured in both experiments appear in Tables 3.5.3. and 3.5.4. and are shown graphically in Figs 3.5.2, 3.5.3 and 3.5.4. It can be seen that when paracetamol was taken with salicylamide, serum concentrations of paracetamol were significantly greater ( $p < 0.05$ ) from 135 to 360 minutes after dosing than when paracetamol was given alone. From Table 3.5.4. and Fig. 3.5.3 it can be seen that serum concentrations of paracetamol sulphate were significantly lower when salicylamide was given with paracetamol in Experiment 2 from 45 minutes to 180 minutes ( $p < 0.05$ ) than corresponding times in Experiment 1 when paracetamol was given alone. Paracetamol glucuronide concentrations were significantly higher after salicylamide (Experiment 2) only at the 225 minute measurement as shown in Table 3.5.4 and Fig 3.5.4..

The half life of paracetamol in serum was calculated for each volunteer in both experiments and values compared using the paired 't' test (Table 3.5.5.). The half-life of paracetamol was found to be significantly longer after salicylamide than when paracetamol was taken alone ( $p < 0.025$ ). The area under the serum paracetamol concentration time curve (AUC) was found to be significantly greater after salicylamide than for paracetamol taken alone ( $p < 0.01$ ). AUC for paracetamol glucuronide was not altered but the AUC for paracetamol sulphate was significantly decreased by salicylamide administration ( $p < 0.01$ ). Values for paracetamol half life and AUCs for paracetamol, paracetamol glucuronide and paracetamol sulphate calculated from 0 min to 315 mins are given in Table 3.5.5.

**Table 3.5.3**      Concentrations of paracetamol in serum ( $\mu\text{g/ml}$ ) in the control study (Experiment 1) and after salicylamide (Experiment 2) (Means  $\pm$  SEM)

Time	Experiment 1 Paracetamol ( $\mu\text{g/ml}$ )	Experiment 2 Paracetamol ( $\mu\text{g/ml}$ )
-30 mins	-	1g SAM
0 mins	1g paracetamol	1g paracetamol
0 mins	0.16 $\pm$ 0.12 n=6	0.10 $\pm$ 0.07 n=6
45 mins	3.44 $\pm$ 0.17 n=6	4.02 $\pm$ 0.53 n=6
90 mins	3.24 $\pm$ 0.34 n=6	3.52 $\pm$ 0.35 n=6
120 mins	-	1g SAM
135 mins	2.87 $\pm$ 0.32 n=6	3.61 $\pm$ 0.33 n=6
180 mins	1.92 $\pm$ 0.36 n=6	3.14 $\pm$ 0.30 n=6
225 mins	1.51 $\pm$ 0.31 n=5	2.28 $\pm$ 0.20 n=6
270 mins	1.14 $\pm$ 0.21 n=6	1.87 $\pm$ 0.21 n=5
315 mins	0.86 $\pm$ 0.21 n=6	1.40 $\pm$ 0.18 n=6

SAM - salicylamide

Table 3.5.4. Concentrations of paracetamol glucuronide and paracetamol sulphate in serum ( $\mu\text{g}/\text{ml}$ , corrected for molecular weight\*) in the control study (Experiment 1) and after salicylamide (Experiment 2)

Time	Experiment 1		Experiment 2			
	PG( $\mu\text{g}/\text{ml}$ )	PS( $\mu\text{g}/\text{ml}$ )	PG( $\mu\text{g}/\text{ml}$ )	PS( $\mu\text{g}/\text{ml}$ )		
-30 mins	-		1g SAM			
0 mins	n	1g paracetamol	n	1g paracetamol		
0 mins	6	0.37 $\pm$ 0.14	0.71 $\pm$ 0.71	6	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
45 mins	6	1.18 $\pm$ 0.26	1.09 $\pm$ 0.16	6	1.08 $\pm$ 0.18	0.16 $\pm$ 0.16
90 mins	6	2.50 $\pm$ 0.49	1.41 $\pm$ 0.21	6	3.48 $\pm$ 1.34	0.16 $\pm$ 0.16
120 mins			1g SAM			
135 mins	6	3.18 $\pm$ 0.69	1.25 $\pm$ 0.20	6	3.03 $\pm$ 0.54	0.16 $\pm$ 0.16
180 mins	6	2.86 $\pm$ 0.56	0.94 $\pm$ 0.34	6	3.03 $\pm$ 0.51	0.31 $\pm$ 0.20
225 mins	5	2.64 $\pm$ 0.56	0.56 $\pm$ 0.23	6	2.96 $\pm$ 0.64	0.00 $\pm$ 0.00
270 mins	6	2.07 $\pm$ 0.30	0.47 $\pm$ 0.32	5	2.73 $\pm$ 0.41	0.00 $\pm$ 0.00
315 mins	6	1.61 $\pm$ 0.30	0.31 $\pm$ 0.20	6	2.19 $\pm$ 0.52	0.00 $\pm$ 0.00

\*Corrected for molecular weight as described in Appendix 1.

SAM - salicylamide

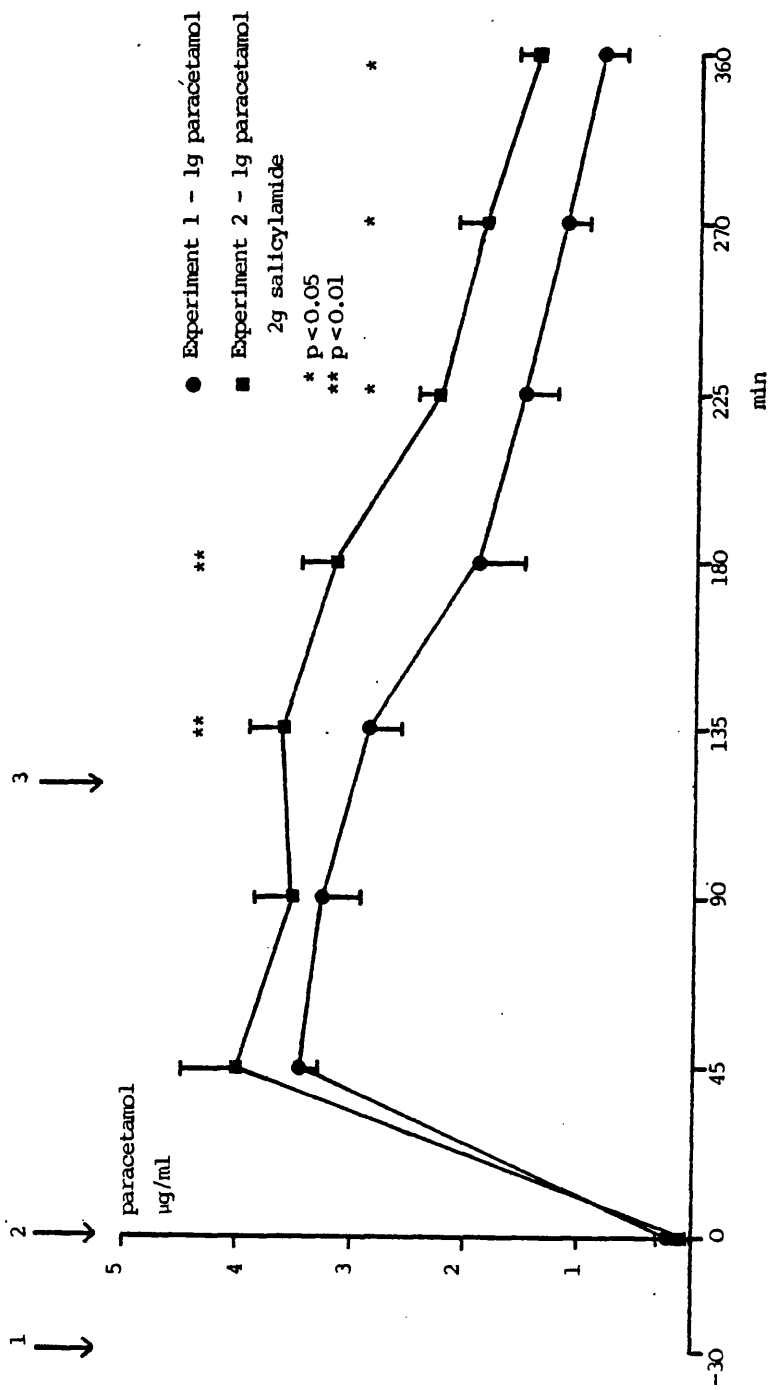


Fig. 3.5.2 Effect of salicylamide on paracetamol metabolism showing concentrations of paracetamol in serum against time (means  $\pm$  S.E.M.)  
 1. 1g paracetamol (Experiment 1 only)  
 2. 1g paracetamol + 2g salicylamide (Experiment 2 only)  
 3. 1g salicylamide (Experiment 3 only)

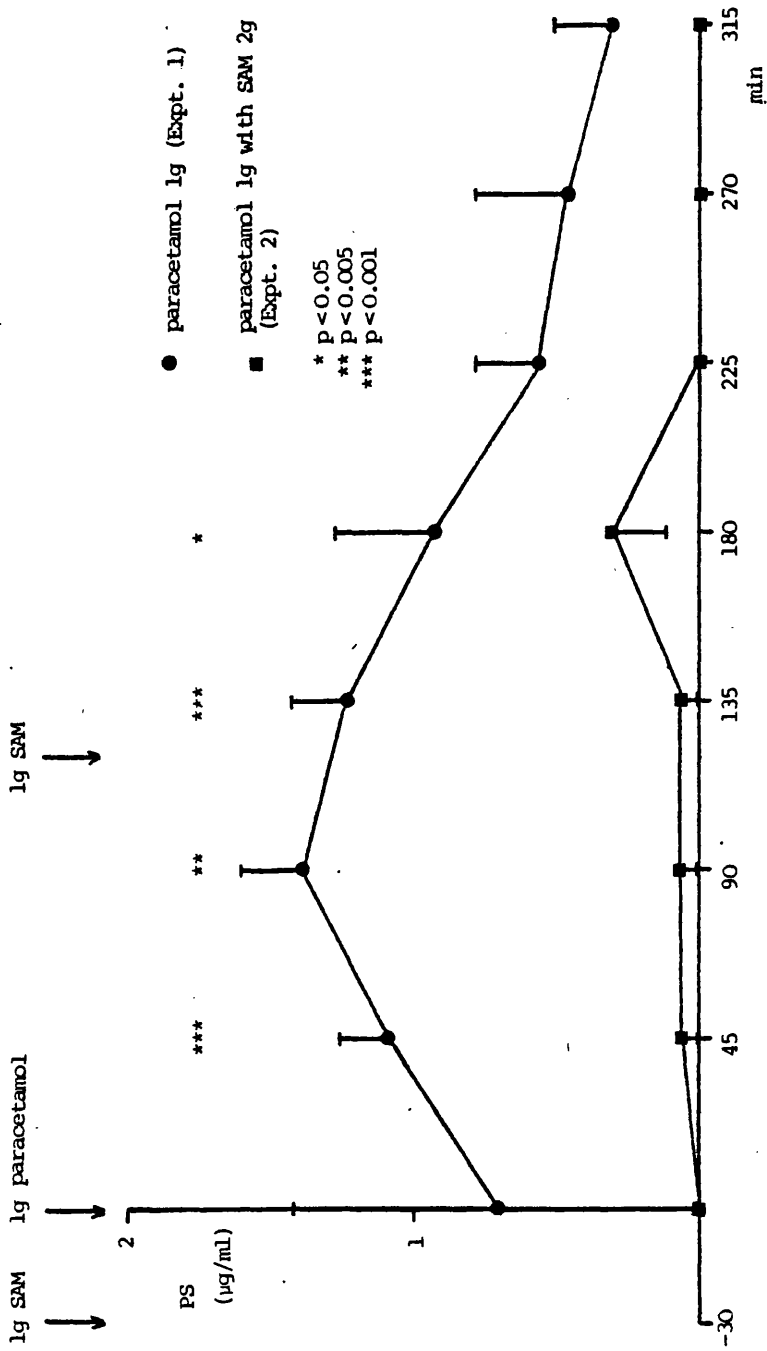


Fig. 3.5.3. Effect of salicylamide on serum levels of paracetamol sulphate corrected for molecular weight (means  $\pm$  S.E.M.)

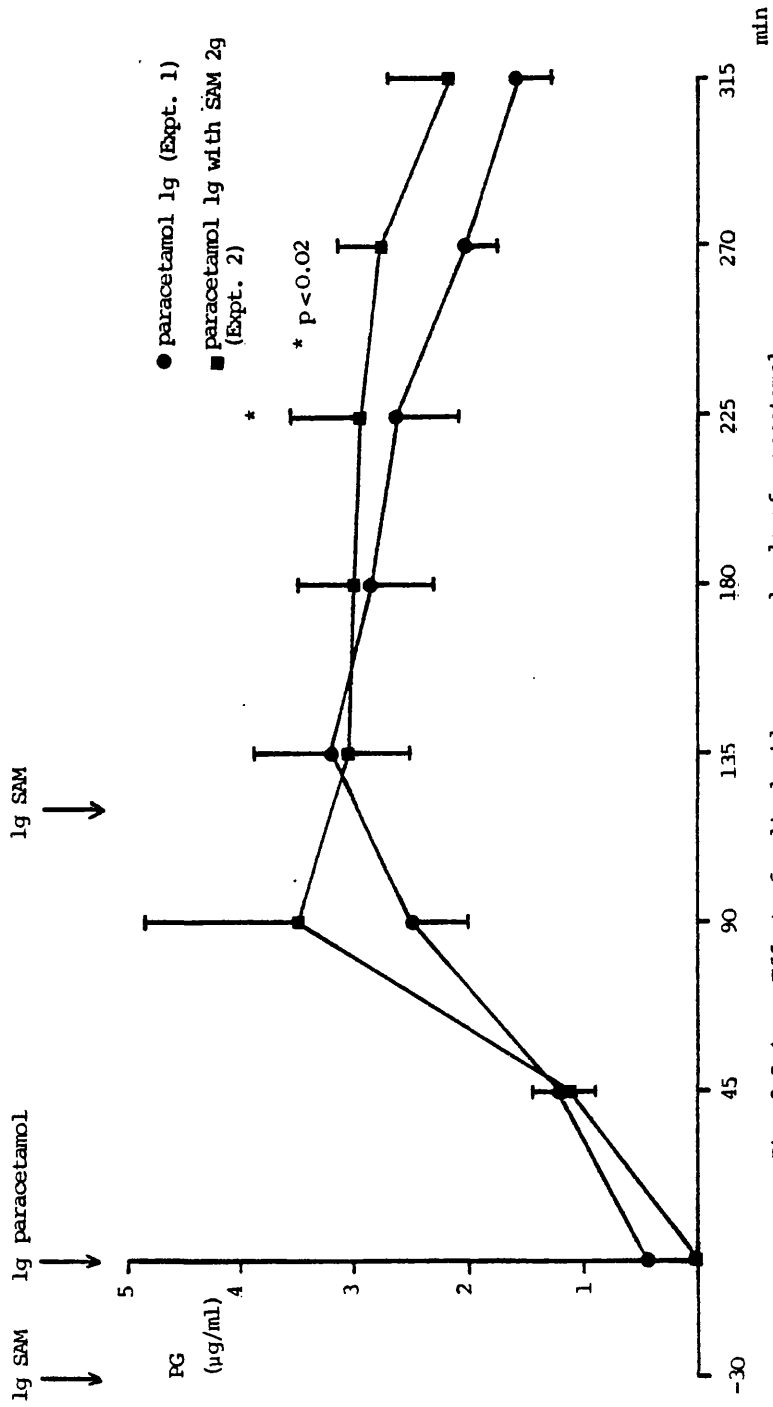


Fig. 3.5.4. Effect of salicylamide on serum levels of paracetamol glucuronide corrected for molecular weight (means  $\pm$  S.E.M.)



Table 3.5.5. Half life of paracetamol and AUC for paracetamol, paracetamol glucuronide and paracetamol sulphate for each volunteer in Experiment 1 and Experiment 2

Volunteer	Half life of paracetamol (min)		AUC paracetamol ug min/ml		AUC paracetamol glucuronide ug min/ml		AUC paracetamol sulphate ug (min)	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
He	165.0	165.0	913.06	1149.64	703.84	1049.64	210.56	0.00
Ha	90.0	107.9	748.99	906.35	1261.62	1189.10	170.14	0.00
B	86.6	147.4	595.62	949.24	345.35	381.63	602.40	133.95
Pa	86.6	144.4	502.76	795.01	675.02	554.78	125.96	0.00
Pe	115.5	162.7	634.02	852.90	460.66	639.43	400.44	0.00
S	115.5	133.5	541.97	627.72	636.88	581.94	191.29	0.00
Mean	109.9	143.5	656.07	880.14	680.56	732.75	283.46	22.32
S.E.M.	12.4	8.6	62.00	70.66	129.21	128.46	74.58	22.32

When the data from the volunteers in Experiment 1 are compared with those in the control study (Section 3.1) who took paracetamol 1.0g no significant differences were found in the percentage urinary recoveries of unchanged paracetamol paracetamol glucuronide and paracetamol sulphate. However, the six volunteers in Experiment 1 excreted a significantly lower amount of paracetamol cysteine ( $p < 0.001$ ) over the 0-8h, 8-24h and 0-24h collection periods and significantly less paracetamol mercapturate in the 8-24h ( $p < 0.002$ ) and 0-24h ( $p < 0.05$ ) urine collections than did subjects in the control study (Section 3.1). Percentage recovery of (PC + PM) was significantly lower in the 6 volunteers in Experiment 1 in 0-8h ( $p < 0.02$ ), 8-24h ( $p < 0.001$ ) and 0-24h urine ( $p < 0.01$ ) than in the control study.

Appendix 12 gives individual values of paracetamol and its metabolites in mg excreted in urine for Experiment 1 and 2.

Appendix 13 gives individual values of percentage recovery of paracetamol and its metabolites in urine for both experiments.

Appendix 14 gives serum concentrations of paracetamol, paracetamol glucuronide and paracetamol sulphate for both experiments.

### 3.6 Patients with Paracetamol Overdose

#### Introduction

Overdose is the most important and common circumstance in which paracetamol toxicity is found. The metabolic mechanism of the toxicity of paracetamol has already been described (Section 1.4.1.2); a certain proportion of a therapeutic dose of paracetamol is believed to be converted to a reactive intermediate which combines with glutathione before excretion. In overdose however, there is insufficient glutathione to cope with the increased amounts of active metabolite formed and liver damage may occur as a result of alkylation of hepatic macromolecules by the toxic intermediate. The normal treatment for paracetamol poisoning, as discussed in Section 1.4.1.4, is L-methionine by mouth or N-acetyl cysteine intravenously. These antidotes should be given within 10 hours of ingestion of the overdose. After this time covalent binding of paracetamol to liver macromolecules has probably occurred and giving amino acids to a damaged liver may provoke hepatic encephalopathy. The metabolic profile of paracetamol was therefore studied after high doses of the analgesic had been ingested by patients who attempted suicide, in order to determine the metabolism of paracetamol in overdose and the effects that these antidotes had on paracetamol metabolites.

#### Procedures

Patients were managed as follows:

after gastric lavage had been performed in the Casualty Department of the Royal United Hospital, Bath, a urine sample was collected where possible. Methionine 2.5g was then administered through the washout tube. Blood was taken for measurement of paracetamol concentration. Patients were then transferred to the Clinical

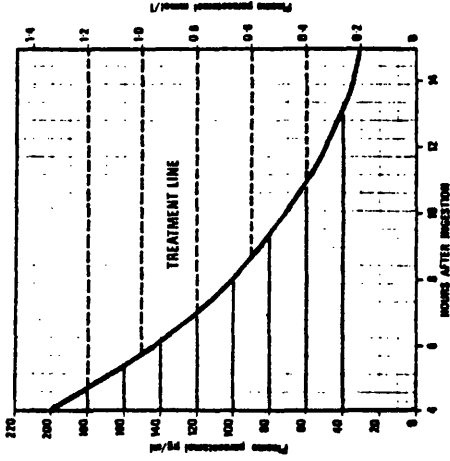
Pharmacology Unit. Subsequent management depended on the result of the plasma paracetamol concentration estimation in relation to the recommended treatment schedule issued by Duncan, Flockhart, and Co. manufacturers of Parvolex (see overleaf). Patients in whom plasma paracetamol concentrations indicated treatment with N-acetyl cysteine received this drug. Those patients in whom plasma paracetamol concentrations indicated that treatment with N-acetyl cysteine was not necessary received L-methionine by mouth. Urine samples were collected at approximately 2 hourly intervals in acidified urine containers and were assayed for paracetamol glucuronide, paracetamol sulphate, paracetamol cysteine, paracetamol mercapturic acid and unchanged paracetamol as previously described.

### Results

Results are presented separately for each individual because the number of tablets ingested varied widely between patients. Tables 3.6.1 - 3.6.10 show results from 10 overdosed patients. Since doses ingested were usually unknown or unreliably reported, records are given of total amounts of paracetamol and its metabolites recovered and expressed in mg normalized to the molecular weight of paracetamol for each urine sample. Times at which patients reported they ingested paracetamol and total intake are recorded where this information is available. The ratios of  $(PG)/(PS)$  and  $(PG + PS)/(PC + PM)$  are also calculated for each urine sample. It can be seen that for many patients, as the treatment progresses the ratios of  $(PG)/(PS)$  and  $(PG + PS)/(PC + PM)$  decrease. Two graphs are plotted, the first showing these two ratios for the patient described in Table 3.5.1 and the second showing the these ratios of the patient described in Table 3.5.10. for purposes of illustration (Figs. 3.6.1 and 3.6.2).

Both these patients are compared with normal volunteers described in Section 3.1 who had ingested paracetamol 2.5g and collected urine 2 hourly for ten hours. Values of  $(PG)/(PS)$  and  $(PG + PS)/(PC + PM)$  ratios for these normals are given in Table 3.1.6.

None of the ten overdose patients had any evidence of liver damage



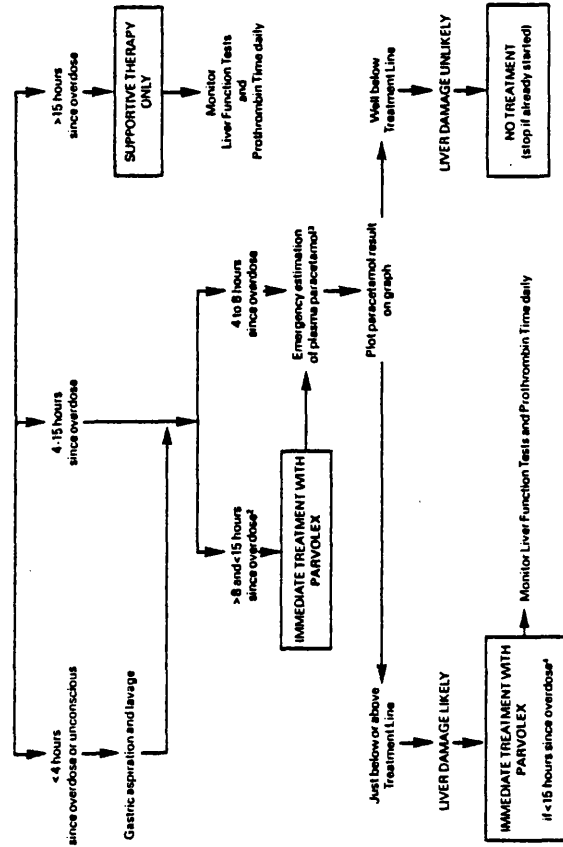
Plasma paracetamol concentrations in relation to time after overdose as a guide to prognosis. Patients at risk are indicated in patients with values just below or above the treatment line. Adapted from Prescott, L. F. Health Bull. (1978) 4, 204

(i) Plasma paracetamol must be measured using a method specific for unchanged paracetamol (see Stewart, et al., Ann. Clin. Biochem., 1979, 16, 89).

(ii) Regimen for Intravenous Parvolex:  
 50mg/kg in 200ml 5% dextrose intravenously over 15 minutes.  
 100mg/kg in 200ml 5% dextrose intravenously at 18 hrs.  
 100mg/kg in 200ml 5% dextrose intravenously at 18 hrs.  
 (Total 300mg/kg in 20 hrs.) See dosage chart.

(iii) Supportive therapy:  
 Fluid and electrolyte replacement, cautious administration of opiate for persistent nausea and vomiting. Conventional treatment for impending or established hepatic failure if prothrombin time ratio exceeds 2.5-3.0 on the second or third day after overdose.

# The Management of Paracetamol Poisoning in Adults<sup>1</sup>



**NOTES**

1. If the reasonably certain that < 7.5g has been taken, liver damage is unlikely and no further treatment is necessary.
2. If the time of ingestion is not known, 8 g in the unconscious patient, treatment with Parvolex should be started immediately.
3. Results of plasma paracetamol estimation cannot be interpreted < 4 hours after ingestion of the overdose.
4. Parvolex is very effective when given up to 8 hours after overdose. The results of plasma paracetamol estimation are unreliable after 10 hours and increasingly between 12 and 15 hours. It is ineffective after 15 hours and may then be associated with harmful effects.

Table 3.6.1

P1, Female, 49 yr.

Time	Event	P	PG	PS	PC+PM	PG/PS	$\frac{PG+PS}{PC+PM}$
16.00	Admission	150.67	408.97	24.93	15.34	16.40	28.28
16.20	Methionine 2.5g p.o.						
17.00	NAC, 9g i.v.						
17.15	NAC, 3g i.v.						
19.15		1544.62	3939.70	307.75	304.49	12.80	13.95
21.20	NAC, 6g i.v.						
22.30		1027.05	5752.31	1827.39	696.04	3.19	10.89
03.00		332.50	2311.96	710.61	197.94	3.25	15.27
05.30		56.35	1442.93	446.11	167.96	3.23	11.25
07.00		4.18	97.68	31.05	19.71	3.14	6.53
08.30		6.02	131.25	41.54	35.49	3.16	4.87
Total		3121.39	14084.80	3389.38	1421.63		

Total recovery of paracetamol in urine = 22017.20mg

In this patient the ratios of (PG)/(PS) and (PG+PS)/(PC+PM) were initially large suggesting both sulphate and glutathione availability were compromised. However, after treatment both ratios decrease with time.

Table 3.6.2

P2, Male, 49 yr.

Time	Event	P	PG	PS	PC+PM	PG/PS	$\frac{PG+PS}{PC+PM}$
13.00	ingestion of 100 tablets of paracetamol 500mg						
18.40	Methionine 2.5g p.o.						
21.00		1946.70	4152.92	0.00*	298.45	$\infty$	13.91
21.30	NAc 10.5g i.v.						
21.45	NAc 3.5g i.v.						
23.30		212.07	936.39	20.63	229.17	45.39	4.18
01.00		176.15	833.48	17.06	200.88	48.85	4.23
02.00	NAc 7.0g i.v.						
02.15		126.00	1317.11	66.12	160.88	19.92	8.60
11.50		6.63	721.55	95.07	60.75	7.59	13.44
15.30		9.10	284.18	39.95	36.29	7.11	8.93
17.30		1.66	40.15	6.66	9.07	6.03	5.16
22.00		2.87	83.49	18.13	-11.86	4.60	8.57
07.45		4.57	112.40	13.68	18.66	8.22	6.76
Total		2309.60	8481.67	277.30	1026.01		

Total recovery of paracetamol in urine = 12094.58mg.

This table shows that initially no paracetamol sulphate was excreted but after treatment with N-acetyl cysteine and L-methionine paracetamol sulphate recovery in urine was increased.

\* paracetamol sulphate may have been hydrolysed by bacteria or acid

Table 3.6.3

P3, Male, 31 yr.

Time	Event	P	PG	PS	PC+PM	PG/PS	<u>PG+PS</u>
							PC+PM
06.00-18.00 ingestion of 100 tablets of paracetamol 500mg							
05.00	Admission						
06.00	Methionine 2.5g p.o.						
07.00		459.42	6071.39	385.14	502.37	15.76	12.85
10.00	Methionine 2.5g p.o.						
14.00	Methionine 2.5g p.o.						
15.45		830.25	14492.71	1355.46	1383.29	10.69	11.46
18.00	Methionine 2.5g p.o.						
19.30		571.88	3872.43	702.33	1011.17	5.51	4.52
24.00	Methionine 2.5g p.o.						
06.30		248.12	1971.01	747.84	662.29	2.64	4.10
15.00		189.75	738.61	438.16	415.14	1.68	2.83
02.20		21.48	224.46	115.72	89.62	1.94	3.80
06.00		39.52	403.87	185.70	126.35	2.17	4.67
13.10		18.12	203.65	112.38	93.28	1.81	3.39



Table 3.6.3 Continued

P3, Male, 31 yr.

Time	Event	P	PG	PS	PC+PM	PG/PS	$\frac{PG+PS}{PC+PM}$
16.15		5.79	75.79	32.55	28.37	2.33	3.82
19.00		9.68	55.54	66.96	43.31	0.83	2.83
01.30		9.76	52.86	54.03	44.39	0.98	2.41
22.00		6.66	24.72	22.62	19.55	1.09	2.42
11.00		10.92	6.05	48.44	11.79	0.12	14.62
11.15		9.21	18.76	40.17	27.02	0.47	2.18
16.30		12.84	43.30	56.84	39.92	0.76	3.35
21.00		4.58	2.59	19.73	8.68	0.13	2.57
22.00		6.60	2.47	14.99	12.54	0.16	1.39
07.30		13.75	122.82	34.70	26.11	3.54	6.03
11.00		17.84	6.14	49.96	28.94	0.12	1.94
16.30		7.32	2.11	17.17	9.26	0.12	2.08
20.00		10.29	36.07	41.26	17.10	0.87	4.52
03.00		10.71	0.00	23.75	19.40	0.00	1.22
06.45		5.80	0.00	19.84	0.00	0.00	0.00
09.00		13.04	0.00	51.26	17.60	0.00	2.91
Total		2533.33	28427.35	4637.00	4627.49		

Total urinary recovery of paracetamol in urine = 40225.17mg.

Although the ratios (PG)/(PS) and (PG+PS)/(PC+PM) were only slightly above normal before treatment this might be because glucuronic acid, sulphate and glutathione were depleted. Treatment with L-methionine seemed to decrease both ratios.

Table 3.6.4

P4, Female, 28 yr

Time	Event	P	PG	PS	PC+PM	PG/PS	<u>PG+PS</u> PC+PM
16.00	Methionine 2.5g p.o.						
16.20		8.07	29.08	26.05	5.43	1.12	10.15
19.50		113.28	609.40	104.63	134.58	5.82	5.30
20.00	Methionine 2.5g p.o.						
21.40		60.86	246.30	40.00	112.14	6.16	2.55
24.00	Methionine 2.5g p.o.						
00.40		28.57	193.13	36.30	102.73	5.32	2.23
04.00	Methionine 2.5g p.o.						
06.00		31.84	185.44	35.71	119.01	5.19	1.86
09.40		4.07	37.73	11.97	26.60	3.20	1.86
13.50		5.10	47.62	6.78	44.75	7.02	1.21
17.20		0.46	0.00	2.5	3.08	0.00	0.81
Total		252.25	1248.70	263.75	548.32		

Total recovery of paracetamol in urine = 2313.02mg.

Treatment with L-methionine resulted in a decrease in (PG+PS)/(PC+PM) with time but had no effect on (PG)/(PS)

Table 3.6.5

P5, Female, 38 yr

Time	Event	P	PG	PS	PC+PM	PG+PS	
						PG/PS	PC+PM
22.00	ingestion of 70 tablets of paracetamol 500mg						
11.00	gastric washout followed by full NAc treatment (300mg/kg over 20 hours)						
18.15		377.76	4905.10	702.18	928.96	6.98	6.04
07.00		64.50	599.71	485.97	266.74	1.23	4.07
10.00		64.32	5.99	68.94	33.33	0.09	2.25
15.00		8.06	7.10	13.58	12.51	0.52	1.65
17.00		0.90	1.70	1.68	4.72	1.01	0.72
22.30		1.46	1.82	1.02	3.89	1.78	0.73
11.00		1.94	5.76	3.50	6.06	1.64	1.53
13.30		3.70	3.42	0.41	3.26	8.34	1.17
16.00		2.00	2.48	0.07	3.31	33.43	0.77
17.00		1.81	4.42	2.39	7.03	1.85	0.97
Total		526.45	5537.50	1279.74	1269.81		

Total recovery of paracetamol in urine = 8613.50mg.

N-acetyl cysteine decreased (PG)/(PS) and (PG+PS)/(PC+PM) with time.

The first urine collection analysis suggests that sulphate but not glutathione was depleted by this dose of paracetamol. After treatment with N-acetyl cysteine, (PG)/(PS) and (PG+PS)/(PC+PM) decreased with time.

Table 3.6.6.

P6, Male, 40 yr.

Time	Event	P	PG	PS	PC+PM	PG+PS	
						PG/PS	PC+PM
22.00	Ingestion of 10 tablets of paracetamol 500mg						
00.15		8.86	83.22	32.06	2.37	2.60	48.44
	Methionine						
	2.5g p.o.						
03.00		8.92	105.59	68.88	3.31	1.53	52.71
04.15		2.73	50.94	23.73	5.47	2.15	13.65
09.30		3.18	39.58	11.14	1.96	3.55	25.88
12.00		3.82	37.40	10.56	2.39	3.54	20.07
23.00		10.16	42.86	8.97	5.40	4.78	9.60
Total		37.67	359.59	155.34	20.90		

Total recovery of paracetamol in urine = 573.50mg.

Table 3.6.7

P7, Female, 19 yr

Time	Event	P	PG	PS	PC+PM	PG/PS	<u>PG+PS</u> PG+PM
17.30	Ingestion of 30 tablets of Distalgesic						
23.00	Methionine 2.5g p.o.						
03.00	Methionine 2.5g p.o.						
07.00	Methionine 2.5g p.o.						
10.00		34.74	1574.37	36.98	117.79	42.57	13.68
11.00	Methionine 2.5g p.o.						
01.30		18.95	108.78	7.86	10.73	13.84	10.86
07.55		4.36	41.95	21.82	9.09	1.92	7.01
22.30		4.94	18.73	1.89	2.48	9.91	8.31
Total		62.99	1743.83	68.55	140.09		

Total recovery of paracetamol in urine = 2015.46mg.

Although both ratios were decreased after treatment with L-methionine, (PG)/(PS) rose again in the last urine collection suggesting that further treatment with L-methionine might have been beneficial.

There was little evidence that sulphate depletion had occurred in this patient despite the low recoveries of the glutathione conjugates of paracetamol initially. As (PG+PS)/(PC+PM) decreased after treatment with L-methionine, this drug may have made more glutathione available for conjugation.

Table 3.6.8

P8, Female, 29 yr

Time	Event	P	PG	PS	PC+PM	PG/PS	<u>PG+PS</u> PC+PM
23.30	Ingested 20 tablets of paracetamol 500g and then vomited						
00.30	Methionine 2.5g p.o.						
01.00	Methionine 2.5g p.o.						
03.00		102.68	798.18	304.44	60.78	2.62	18.14
04.00		52.41	469.64	168.10	34.52	2.79	18.47
05.00	Methionine 2.5g p.o.						
06.00		40.98	747.93	233.93	76.97	3.20	12.76
08.18		31.22	656.18	222.00	71.59	2.96	12.27
09.00	Methionine 2.5g p.o.						
10.00		22.02	468.77	155.34	66.04	3.02	9.45
11.10		11.32	227.96	73.35	41.26	3.11	7.30
13.00		8.49	242.38	77.36	43.64	3.13	7.33
Total		269.12	3611.04	1234.52	394.81		

Total recovery of paracetamol in urine = 5509.49 mg.

Methionine treatment appeared only to affect (PG+PS)/(PC+PM) in this patient.

Table 3.6.9

P9, Female, 28 yr

Time	Event	P	PG	PS	PC+PM	PG/PS	<u>PG+PS</u> PC+PM
12.45		71.41	890.98	52.48	110.29	16.98	8.55
	Methionine 2.5g p.o.						
21.45		143.85	3444.21	172.20	570.89	20.00	6.33
	Methionine 2.5g p.o.						
05.45		30.80	409.42	66.12	53.15	6.19	8.95
	Methionine 2.5g p.o.						
20.45		9.73	116.39	68.76	35.95	1.69	5.15
	Methionine 2.5g p.o.						
07.00		16.41	93.86	109.06	44.49	0.86	4.56
Total		272.20	4954.86	468.62	814.77		

Total recovery of paracetamol in urine = 6510.45mg.

In this patient treatment with L-methionine appeared to increase recoveries of both sulphate and glutathionine conjugates of paracetamol

Table 3.6.10

P10, Female, 17 yr

Time	Event	P	PG	PS	PC+PM	PG+PS	
						PG/PS	PC+PM
10.50	Ingestion of 48 tablets of paracetamol 500mg						
12.30	Gastric washout + methionine 2.5g p.o.						
12.45	Methionine 2.5g p.o.						
13.00		264.15	2134.05	175.94	57.37	12.13	40.26
14.30	Methionine 2.5g p.o.						
17.00		142.17	2787.94	216.15	111.74	12.90	26.88
19.00		32.30	1185.08	118.63	58.38	9.99	23.33
20.00		16.47	446.53	46.85	28.94	9.53	17.05
21.00		1126.72	8204.07	593.34	665.43	13.83	13.22
21.30		16.72	494.77	41.01	59.06	12.06	9.07
08.15		8.42	724.40	84.13	87.46	8.61	9.24
09.15		67.04	1721.87	381.12	282.74	8.49	6.81
11.15		1.17	53.35	8.28	6.83	6.44	9.02
Total		1675.16	16113.41	1378.26	1088.87		

Total recovery of paracetamol in urine = 20255.70mg.

L-methionine resulted in a decrease in (PG+PS)/(PC+PM) but had little effect on (PG)/(PS) suggesting that if further treatment with L-methionine had been given elimination of paracetamol might have been hastened



Fig. 3.6.1 Values of G/S and  $\frac{G+S}{C+M}$  in urine for the patient shown in Table 3.6.1 compared with normals who ingested paracetamol 2.5g (as described in Section 3.1) All values are corrected for molecular weight

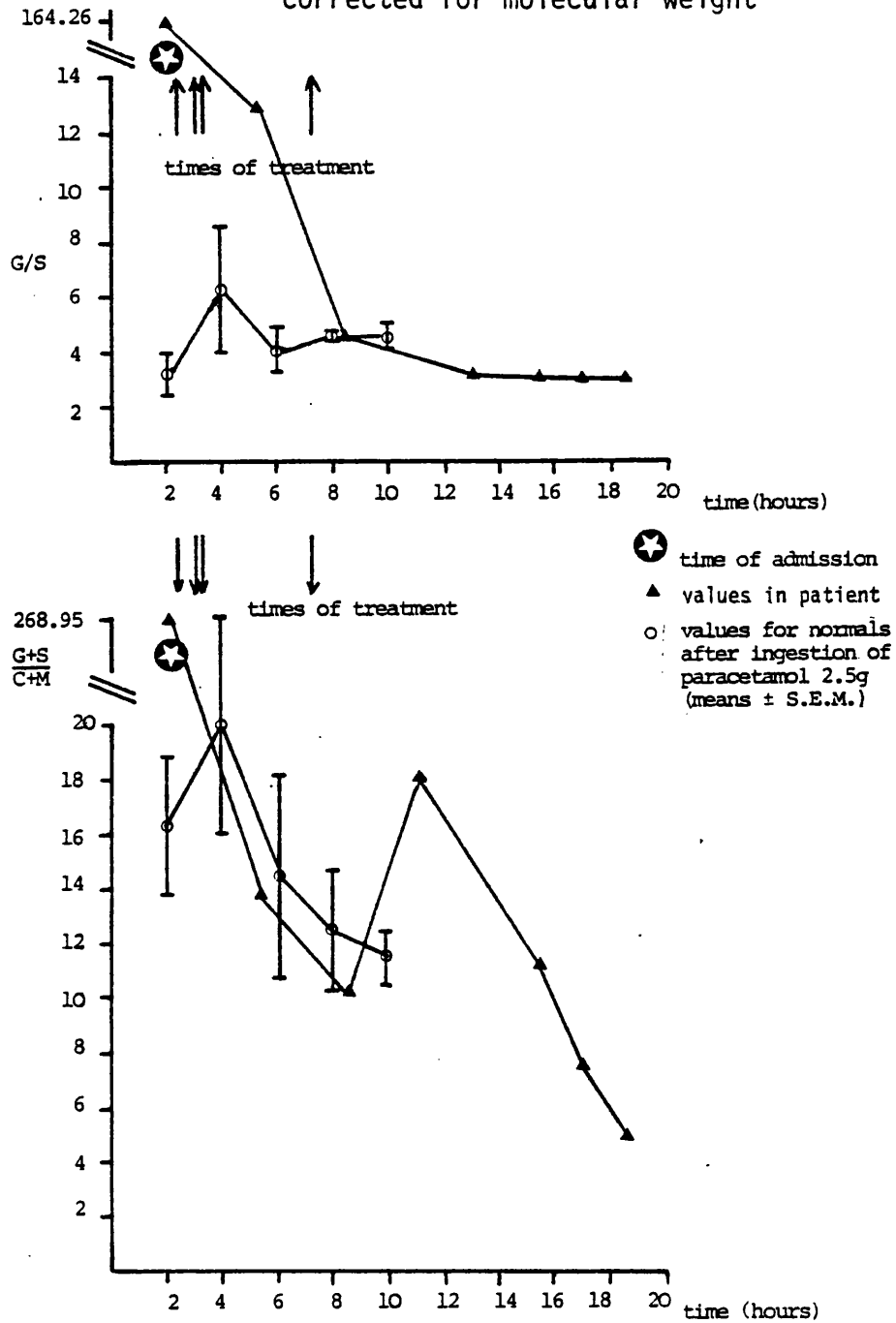
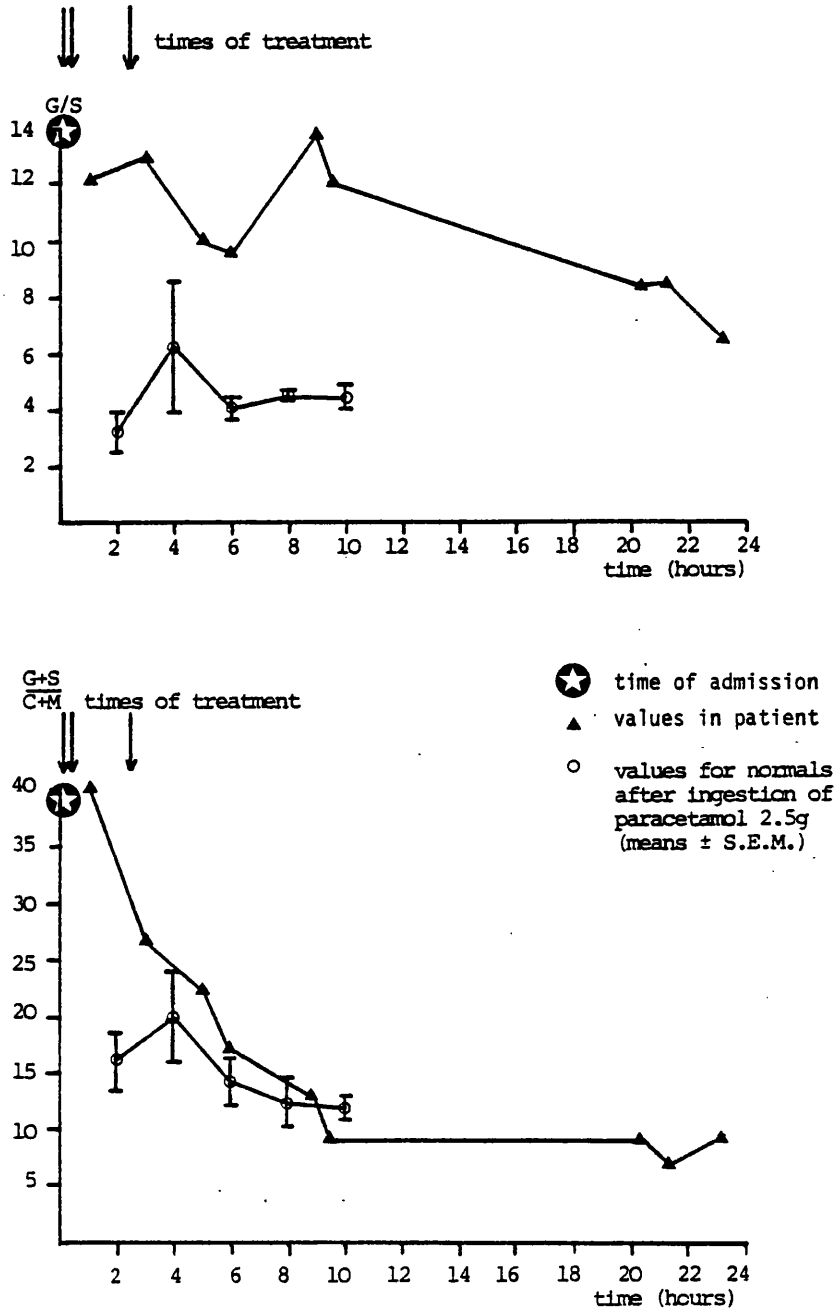


Fig. 3.6.2 Values of  $\frac{G/S}{C+M}$  and  $\frac{G+S}{C+M}$  in urine for the patient shown in Table 3.6.10 compared with normals after paracetamol 2.5g (as described in Section 3.1) All values are corrected for molecular weight



### 3.7 A possibly toxic intermediate metabolite of paracetamol

#### Introduction

After it has been taken in overdose, the toxicity of paracetamol may be manifest as fulminant hepatic failure. Recently renal tubular necrosis has been shown to follow paracetamol overdose - sometimes in the absence of liver damage (Cobden et al, 1982). Both these effects are thought to be due to covalent binding of a reactive metabolite of paracetamol to microsomal protein (Mitchell et al, 1977). This reactive metabolite is thought to arise as a result of oxidation by the cytochrome P-450 mixed function oxidase system and in therapeutic doses is safely detoxicated by conjugating with glutathione; the products of this reaction appear in urine as paracetamol cysteine and paracetamol mercapturic acid. However glutathione availability is limited so that after high doses of paracetamol sufficient of the reactive metabolite is left unconjugated to bind to liver and possibly kidney macromolecules to cause cell death.

The identity of the toxic intermediate has been debated for many years. N-hydroxy paracetamol was initially considered to be a likely candidate and was thought to act by spontaneous dehydration to N-acetyl benzoquinonimine which is known to covalently bind to protein and to react with glutathione to form a paracetamol-glutathione conjugate (Hinson, Nelson and Mitchell, 1977). However, toxicity mediated by N-hydroxy paracetamol was refuted by Healey, Calder, Young et al, (1978) and by Gemborys, Gribble and Mudge (1978) because of its half-life (15 minutes at physiological pH) indicating that it was not dehydrated as rapidly as was previously postulated, and because of its limited toxicity. The N-acetyl benzoquinonimine has not been rejected as a possible toxic metabolite although its mechanism of formation is not known.

During the analysis of urine from overdose patients, several additional drug-related peaks were noted which were not present in blank urine and it was thought possible that these peaks might represent metabolites of paracetamol which arose from the toxic pathway. Therefore these metabolites were examined with a view of increasing information of the metabolism of paracetamol.

### Procedures

Paracetamol 1.5g was taken orally by normal volunteers and all urine passed in the first 8 hours was collected in unacidified urine bottles. Aliquots were treated with  $\beta$ -glucuronidase and sulphatase and incubated at pH5 at 37°C overnight. As well as the known metabolites of paracetamol (paracetamol glucuronide, paracetamol sulphate, paracetamol cysteine and paracetamol mercapturic acid) the urine was examined for the presence of 2-OH and 3-OH paracetamol by HPLC as described in Section 2.7.e.ii. Peaks corresponding to the retention times of 2- and 3-OH paracetamol were collected by preparative HPLC and sent for mass spectrometric analysis on a Vg Micromass 258 mass spectrometer.

Substituted benzenes have been reported to form acid-labile pre-aromatic metabolites (Knight and Young, 1958). In order to determine whether the oxidation products of paracetamol metabolism arose from such species, urine was further treated with HCl at pH 0.3 at 37°C for 20 hours and was then re-assayed for O-substituted and S-substituted metabolites. Standard samples of O- and S-substituted metabolites were incubated under identical conditions to assess any effect of this treatment on the results.

## Results

Standards of the two O-substituted metabolites were verified as authentic by mass spectrometry. Examination of the urinary metabolic profile of paracetamol by HPLC after treatment with  $\beta$ -glucuronidase and sulphatase revealed the presence of two peaks which had identical retention times to the 2-OH and 3-OH paracetamol standards (7 min 20s and 7 min 55s respectively). Preparative HPLC permitted the collection of samples of 2-OH and 3-OH paracetamol present in urine after ingestion of paracetamol and these were subjected to direct insertion mass spectrometry. 3-OH paracetamol was shown to be a urinary metabolite of paracetamol but 2-OH paracetamol was present in too low a concentration to permit mass spectrometric analysis.

After enzymic hydrolysis followed by acid treatment for 20 hours (jointly performed by Dr L Notarianni and myself) there was found to be a decrease in the concentrations of the oxidation products of paracetamol compared with concentrations before acid treatment. When standard samples of O- and S- substituted metabolites were incubated for 20 hours under acid conditions (again performed jointly) breakdown of these metabolites was found to be considerable. The breakdown followed a first order degradation process with the following rate constants: 2- and 3-hydroxy paracetamol-  $-0.031 \text{ hr}^{-1}$ , 3- 5-cysteiny1-paracetamol-  $-0.055 \text{ hr}^{-1}$  breakdown therefore had to be considered in calculating the amounts of oxidised metabolites present.

Table 3.7 shows the mean concentrations of 3-OH paracetamol and S-substituted metabolites of paracetamol after enzymic hydrolysis alone and after enzymic hydrolysis followed by acid treatment. Results are also shown in Fig. 3.7.1. Decomposition of these metabolites as shown in Fig. 3.7.2. was allowed for when assessing the concentrations of these metabolites in urine before acid treatment by applying the above rate constants.

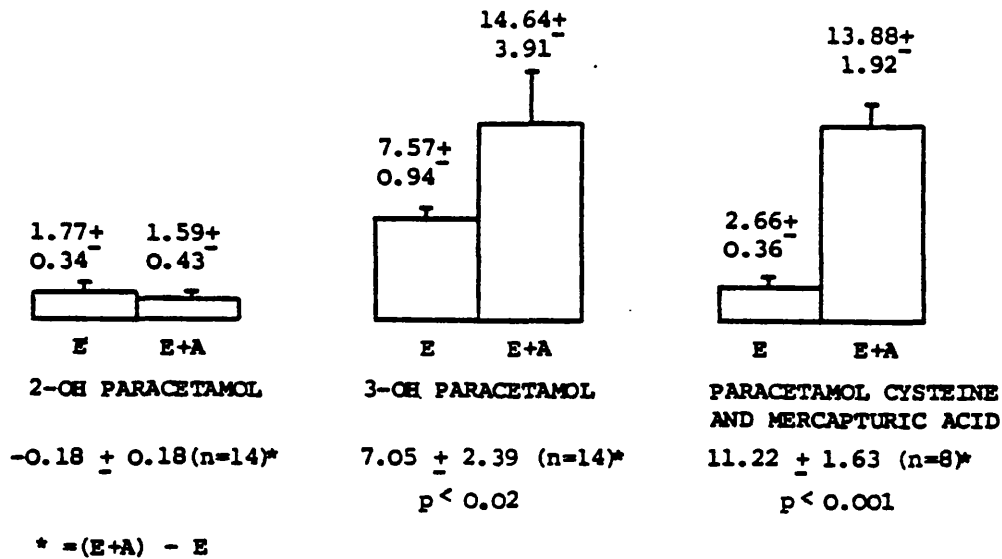
Table 3.7. Concentrations of 3-OH paracetamol and S-substituted metabolites in human urine after paracetamol 1.5g administered to normal volunteers

Conditions	Concentration ( $\mu\text{g/ml}$ )	
	3-hydroxy paracetamol	S-metabolites
Urine after enzymic hydrolysis (sulphatase and $\beta$ -glucuronidase)	7.55 $\pm$ 0.94*	2.66 $\pm$ 0.35*
Urine after enzymic hydrolysis and incubated at pH 0.3 for 20 hours	14.64 $\pm$ 3.91	13.88 $\pm$ 1.92
	n = 14, p<0.02	n = 8, p<0.001

Results shown above indicate that acid-labile species present in urine were converted to 3-hydroxy paracetamol and paracetamol cysteine plus paracetamol mercapturic acid by incubation at pH 0.3 for 20 hours. Concentrations of 2-OH paracetamol did not change significantly after the 20 hour acid treatment. In order to follow the time course of the formation and decomposition of O-substituted and S-substituted metabolites in urine, one urine sample was incubated at pH 0.3 at 37<sup>o</sup>C (following enzymic hydrolysis). Concentrations of O- and S-substituted metabolites were measured by Dr L Notarianni at intervals for 20 hours. This time course is shown in Fig. 3.7.3. It can be seen that in this urine sample, concentrations of 2-OH paracetamol also rose initially as a result of the treatments but by 20 hours, concentrations had fallen again due to breakdown.

The mass spectrum of a standard of 3-OH paracetamol and a mass spectrum of 3-OH paracetamol urine after preparative HPLC following oral ingestion of paracetamol is shown in Fig. 3.7.4.

Fig 3.7.1.



Mean ± SEM concentrations (µg/ml) of O- and S- substituted metabolites of paracetamol in human urine before (E) and after incubation at pH 0.3 and 37°C for 20 hr (E+A). E represents values for urines pretreated with β-glucuronidase and sulphatase and corrected for degradation as described in the text. Student's *t*-test for paired data was used to assess the statistical significance of the results.

Fig. 3.7.2. Decomposition of 2-hydroxy paracetamol, 3-hydroxy paracetamol, 3-S- cysteinyl paracetamol and 3-S-(N-acetyl cystenyl)paracetamol with time.

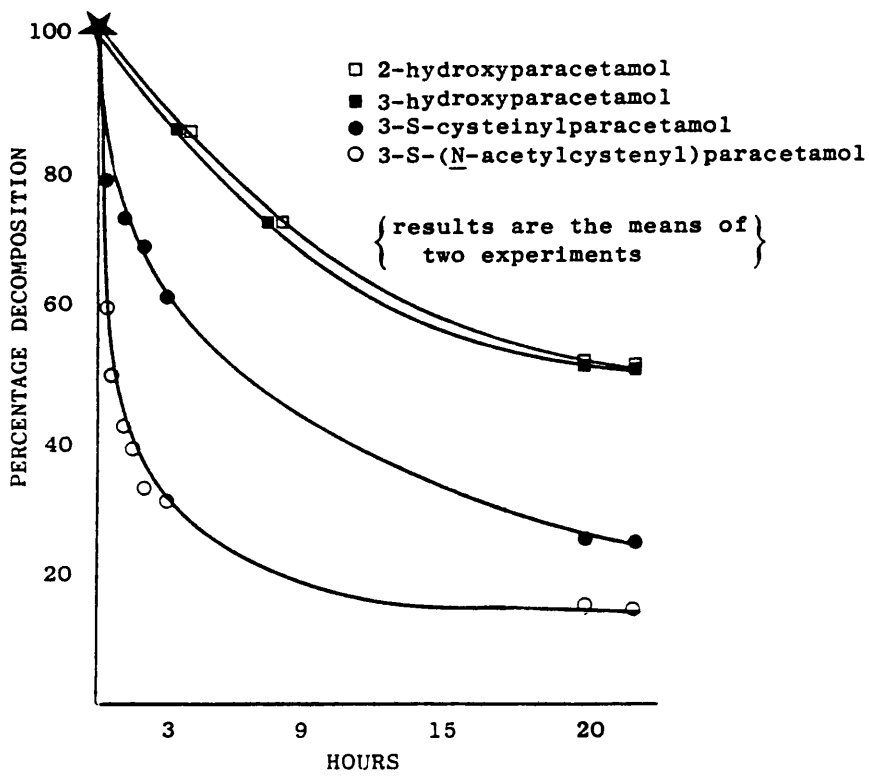
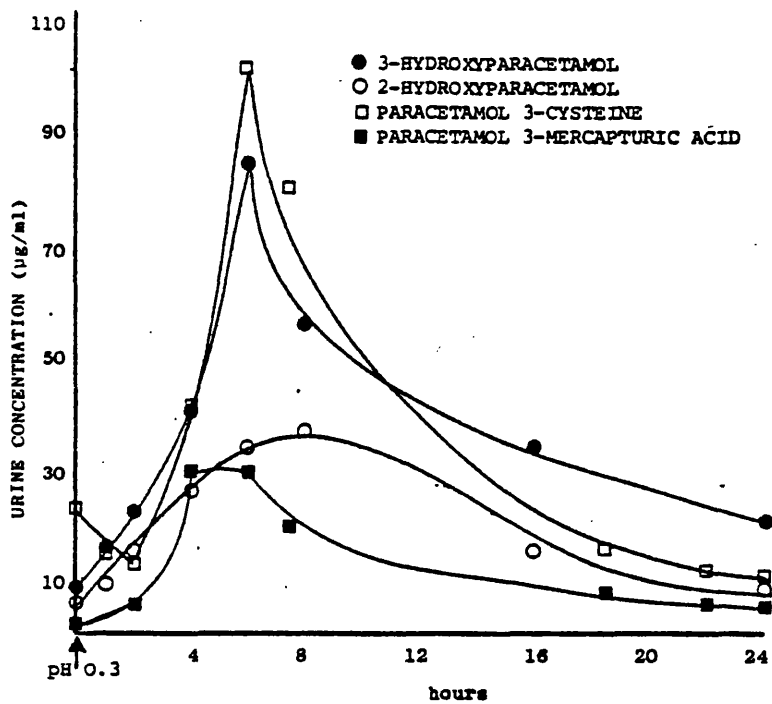




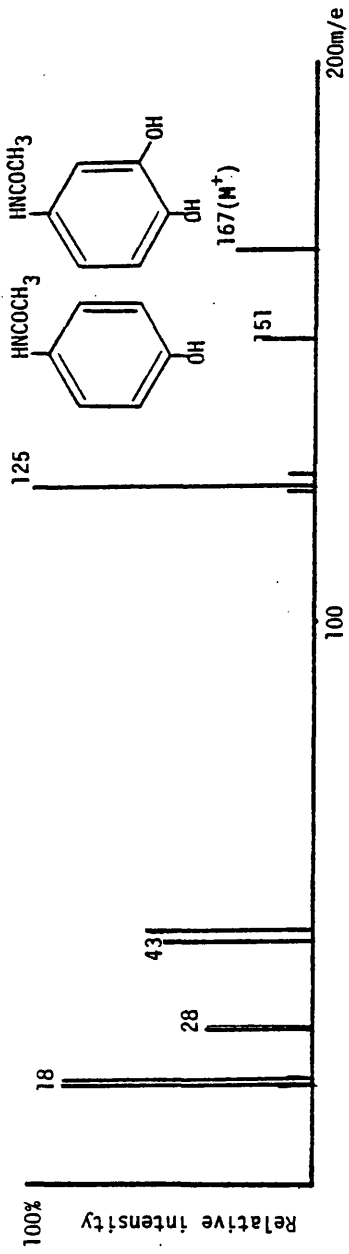
Fig. 3.7.3. Time course of concentrations of O and S- substituted metabolites of paracetamol in urine incubated at pH 0.3 at 37°C after enzymic hydrolysis.



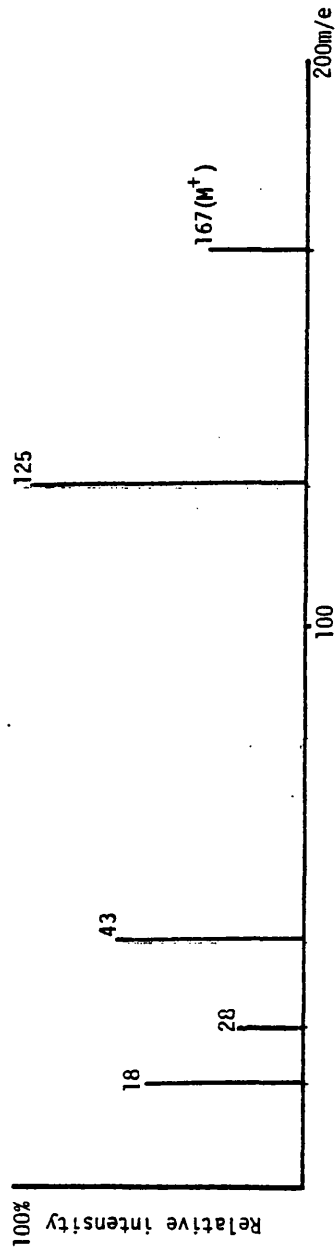
CONCENTRATIONS OF PARACETAMOL METABOLITES IN URINE FOLLOWING INCUBATION AT pH 0.3 and 37°C WITH RESPECT TO TIME

Fig. 3.7.4

Mass Spectrum of a pure standard of 3-OH paracetamol collected by preparative HPLC.



Mass Spectrum of 3-OH paracetamol in human urine collected by preparative HPLC after administration of paracetamol by mouth.



## Chapter 4

### Discussion of Paracetamol Results in man

#### 4.1 Control Study

In this study the urinary metabolic profile of paracetamol after a therapeutic 1g or 500mg (children) dose administered to normal volunteers was established using high performance liquid chromatography. Previous findings by Cummings, King and Martin (1967) showing that the major metabolites of paracetamol are paracetamol-4-glucuronide and paracetamol-4-sulphate were confirmed and in agreement with the work of Potter, Thorgeirsson, Jollow and Mitchell (1974) it was found that these metabolites made up some 80% of the administered dose. A smaller percentage of paracetamol appears in the urine as its 3-S-cysteiny1 and 3-S-(N-acetylcysteiny1) derivatives and these metabolites arise presumably from conjugation of the P-450 oxidation product of paracetamol with glutathione. These metabolites were first described by Jagenburg and Toczko, (1960).

The present results showed that in every volunteer but one, more of the glucuronide conjugate than the sulphate conjugate was recovered ( $53.46 \pm 1.35\%$  and  $32.79 \pm 1.16\%$  in 24 hour urine respectively). It was found that the percentage recoveries of paracetamol glucuronide and paracetamol sulphate in this population were negatively correlated (although absolute amounts of these metabolites were found to be positively correlated largely as a result of large variations in the total amounts of these two metabolites excreted). The negative correlation between paracetamol glucuronide and paracetamol sulphate implies that these two pathways were in competition.

Recoveries of paracetamol sulphate were significantly lower in the second urinary collection period (8 - 24 hours) than in 0 - 8 hour urine ( $p < 0.002$ ). This suggests that even after a therapeutic dose of paracetamol, sulphate normally available for conjugation is being noticeably depleted. Bray, Humphries, Thorpe, White and Wood (1952) suggested that because of the limited capacity of the total sulphate pool sulphate availability may readily be exhausted. This suggests that with increasing doses of paracetamol sulphate conjugation may become a less utilized pathway due to decreased availability of sulphate. Figs. 3.1.4, 3.1.5 and 3.1.6 suggest that paracetamol glucuronidation and sulphonation are competing pathways but in the present study there was no apparent increase in paracetamol glucuronide recovered in 8 - 24 hours to compensate for the decreased excretion of paracetamol sulphate. However, there was a significant increase ( $p < 0.05$ ) in the percentage recoveries of the glutathione conjugates of paracetamol (i.e. paracetamol cysteine and mercapturic acid). Since these two metabolites arise via the oxidation pathway of paracetamol this suggests that sulphate depletion was sufficient to result in increased production of the reactive intermediate of paracetamol. If glutathione availability is compromised sufficiently hepatotoxicity may result. The implication exists that co-administration of compounds which also utilize sulphate for conjugation may also increase the risk of toxicity. The relationship between depletion of hepatic glutathione, arylation of liver macromolecules and hepatic damage was first described by Mitchell, Jollow, Potter, Gillette and Brodie (1973) in mice. Glutathione has been demonstrated to act as a nucleophile with a number of electrophilic substances and more recently has been found also to have a role as a detoxicant acting as a reducing agent or as a cofactor. It has been suggested that high intracellular concentrations of glutathione may be essential for the prevention of cytotoxicity as a result of drug metabolism (Orrenius and Jones, 1978).

In the past, studies on the effects of age on drug metabolism have largely been confined to measurement of plasma half life. It has been found by several workers that the half lives of drugs such as antipyrine (O'Malley, Crooks, Duke and Stevenson, 1971), aminopyrine (Jori, Di Salle and Quadri, 1972), phenylbutazone (O'Malley et al, 1971), warfarin, (Hewick, Moreland, Shepherd and Stevenson, 1975), phenytoin, (Hayes, Langman and Short, 1975) and diazepam (Klotz, Avant, Hoyumpa, Schenker and Wilkinson, 1975) were increased in the elderly (over 60 years of age) compared with younger people (aged under 50 years).

The half life of paracetamol was found to be longer in people over 73 years old than people under the age of 27 years by Triggs, Nation, Long and Ashley (1975) and by Briant, Liddle, Dorrington and Williams (1975) who compared 20 - 40 year olds with people over the age of 65 years. In the present study in 0 - 8 hour urine, paracetamol cysteine plus mercapturic acid recovery decreased significantly with age ( $p < 0.01$ ) due to a significant decrease in the percentage recovery of paracetamol cysteine. These results suggest that with increasing age either the percentage of a given dose of paracetamol oxidised to the reactive intermediate decreases or that further metabolism of this intermediate to yield the products of conjugation with glutathione in urine diminishes with age. Kato and Takanaka (1968) demonstrated that in old rats the liver weight to body weight ratio and liver cytochrome P-450 content was lower and liver microsomal preparations from old rats had a lower activity in metabolizing a number of substrates resulting in an increased sensitivity to a number of agents. Lauterbur, Vaishrav, Stillwell and Mitchell (1980) showed that in male rats the rate of glutathione turnover decreased with increasing age. Rumack and Peterson (1978) demonstrated that children are less susceptible to the toxic effects of paracetamol than adults. Results from this study tend to endorse this as the higher percentage recoveries of cysteine and mercapturic acid metabolites in the younger age groups suggest an increased

ability to conjugate with glutathione and perhaps an increased capacity for detoxifying the reactive metabolite.

Results from this study also showed that following the decreased recoveries with increasing age of the S-substituted metabolites of paracetamol in the 0 - 8 hour urine collections there was a significant ( $p < 0.05$ ) increase in the percentage recovery of paracetamol glucuronide with increasing age in 8 - 24 hour urine. This suggests that, rather than a decreasing ability to conjugate with glutathione with increasing age, the decrease in the oxidation products of paracetamol arises as a result of a smaller proportion of paracetamol undergoing cytochrome P-450 oxidation with age. This conjugation of paracetamol with glutathione is greatest in children and with increasing age the mixed function oxidase system may become impaired.

It is of interest that other workers have found the half life of paracetamol to be increased in older people (Crooks, O'Malley and Stevenson, 1976). The present study showed that in 24 hour urine from the over 30s the proportion of unchanged paracetamol increased with age although individual percentage recoveries of individual metabolites was found not to change with age. This is consistent with a general decrease in drug metabolizing ability with age resulting in increased proportions of unchanged drug recovered in urine. It should be added that catechol derivatives of paracetamol which arise from P-450 oxidation were not measured in this study (see Section 3.7) and alterations in recoveries of these metabolites with age might give an indication of whether oxidation or glutathione conjugation decreased with increasing age.

The effects of these changes in metabolism are unlikely to be of clinical significance as the changes are small (less than 1% increased or decreased percentage recovery of metabolite per year of age). Although the percentage recovery of paracetamol increased with age in the over 30s, there were marked differences between

individuals in urinary recoveries of each metabolite and parent drug. Other workers have reported a wide intersubject variation in the percentage recoveries of the glucuronide and sulphate metabolites of paracetamol (Caldwell, Davies and Smith, 1980). This appears to be the first study in vivo to show that recoveries of urinary metabolites and parent drug change with age.

Although there was a significant correlation between age and  $(PG+PS)/(PC+PM)$ , in all subjects when the results for female volunteers alone were examined no such correlation existed. These findings could represent a clinically unimportant sex difference in paracetamol metabolism but may also be explained by the fact that female volunteers showed a much higher scatter in this ratio than the males. Sex differences in drug metabolism reported in the literature are scarce but include the finding that men are slower metabolisers of antipyrine (O'Malley, Crooks, Duke and Stevenson, 1971).

Because of their relationships with age, it follows that percentage recoveries of paracetamol glucuronide are negatively correlated with recoveries of paracetamol plus mercapturic acid in each urine collection period.

Values of  $(PG+PS)/(PC+PM)$  and  $(PG)/(PS)$  were calculated for each individual in the age study and the means were calculated. When they were compared with values in the study in which 4 volunteers took 2.5g paracetamol, it was found that  $(PG)/(PS)$  ratios in the latter study did not alter significantly over a 10 hour period. This will be discussed later in relation to the metabolism of paracetamol after overdosage. Likewise  $(PG+PS)/(PC+PM)$  did not change significantly over the first 10 hours after ingestion. The slight decrease observed is probably due to a decrease in sulphate recovered with time and not to increased cysteine plus mercapturic acid recoveries.

#### 4.2 Paracetamol metabolism in neonates

The metabolism of paracetamol was studied in six nursing infants after their mothers were given from 1 to 3g of paracetamol. The parent drug and its four metabolites were detectable in the urine of all six babies. This finding contrasts with that of Berlin, Yaffe and Ragni (1980) who also used an HPLC method of analysis and could detect neither paracetamol nor its metabolites in the urine of nursing infants. When paracetamol recoveries of each metabolite and unchanged paracetamol in neonates were examined in the present study it was found that the infants excreted substantially higher amounts of unchanged paracetamol but lower amounts of paracetamol sulphate than had subjects in the control study. Despite this, unlike the control study, there was no increase seen in the products of cytochrome P-450 oxidation suggesting that there may either be lower concentrations or lower activities of the mixed function oxidase system or glutathione transferase in neonates than in older subjects. As percentage recoveries of the cysteine and mercapturic acid metabolites of paracetamol in neonates were similar to values in the control study, there is no evidence to suggest that neonates may have different sensitivities to the hepatotoxic effects of paracetamol than do adults. Results from mice described by Hart and Timbrell (1979) showed that levels of cytochrome P-450 and hepatic glutathione concentrations in neonatal mice was less than in adult mice. Therefore, in human neonates either the formation of the reactive metabolite of paracetamol or its detoxication may be less developed than in adults.

Mancini, Sonaware and Yaffe (1980) reported that young mice and rats were less vulnerable to paracetamol toxicity compared with adult animals by determining LD<sub>50</sub> values for paracetamol at different ages. Pulkkinen (1963) and Percy and Yaffe (1964) described a significant ability of rats



and mice to form sulphate esters in contrast to their other conjugating systems such as the formation of glutathione conjugates, glycine conjugates and glucuronide conjugation (Testa and Jenner, 1976). The present results suggest that a species difference may exist in the deficiency of neonatal man to conjugate with sulphate although its other drug metabolising enzymes appear to be well developed by this age.

#### 4.3 Metabolism of paracetamol in patients with rheumatoid arthritis

The metabolic profile of paracetamol has been studied in patients with rheumatoid arthritis. Glynn (1972) described classical chronic arthritis as being a two phase disease in which the first phase possibly results from a systemic infection by an organism which has a tendency to settle in the joints. Here it excites an inflammatory reaction probably as a result of a local immune response. This phase may last as long as a year but after that a second phase may follow which results, in most instances, from development of autoimmunisation to some antigen or antigens produced by the initial inflammation. Whatever the cause, rheumatoid arthritis is a chronic crippling disease that requires analgesic drugs. In the present study, patients with rheumatoid arthritis defined by a physician according to the criteria of the American Rheumatism Association (see Appendix 6) took paracetamol at their normal doses.

There are more variables to be taken into account in this study than in the control study because these patients may differ in their ability to metabolise paracetamol as a consequence of their disease state, the high doses of paracetamol taken and concurrent therapy with other drugs.

It was found that these patients showed higher percentage recoveries of paracetamol glucuronide and lower percentage recoveries of paracetamol sulphate and of unchanged paracetamol than normal volunteers after a 1g dose of paracetamol in the control study. Percentage recoveries of the glutathione conjugates of paracetamol were not significantly different from those found in normal volunteers. As many rheumatoid patients took more than 1g of paracetamol in the 24 hours of the study, these patients were compared with the four normal volunteers in the control study who took paracetamol 2.5g. The rheumatoid patients showed a significantly higher percentage recovery of paracetamol sulphate than those controls who took 2.5g paracetamol ( $p < 0.05$ ) suggesting that less sulphate depletion had occurred in the rheumatoid patients. This may however, be a result of the different dosing regimens between controls and rheumatoid patients namely that the controls took paracetamol 2.5g at the beginning of the study while the rheumatoid patients took 1g doses throughout the day. Thus, depletion of endogenous sulphate may have occurred early after dosing but the rheumatoid patients may have been able to replete their sulphate pools by normal dietary intake of precursors of sulphate.

It is known that serum sulphydryl (SH) concentrations are low in patients with rheumatoid arthritis but after treatment with D-penicillamine, aurothiomalate, fenclofenac and benoxaprofen serum SH concentrations rise to normal in those patients showing clinical improvement (Hall, Blake and Bacon, 1981). Low serum SH concentrations probably reflect reduced glutathione availability and suggest that a deficiency in the intracellular detoxication mechanism may exist in patients with rheumatoid arthritis (Munthe, Kass and Jellum, 1981). As paracetamol is thought to be detoxified by conjugation of its active metabolite with glutathione (Mitchell et al, 1973) a decrease in glutathione concentration might result in

increased susceptibility to paracetamol toxicity. In the present study at least ten of the patients with rheumatoid arthritis were being treated with drugs containing thio groups so that their glutathione concentrations may not have been compromised.

As there was no indication in the rheumatoid patients of increased utilization of the toxic pathway of paracetamol metabolism despite their large total daily consumption of paracetamol there seem to be no grounds for believing that rheumatoid arthritis patients run a special risk of hepatotoxicity from paracetamol taken in the doses used in this study.

#### 4.4. Metabolism of paracetamol in patients with thyroid disease

Thyrotoxicosis appeared to have no appreciable effect on the metabolism of a 1g dose of paracetamol when recoveries of each metabolite and unchanged paracetamol were compared with recoveries in normal volunteers in the control study. Crooks, Hedley, Macnee and Stevenson, (1973) studied the activity of liver microsomal enzymes in female thyrotoxic and hypothyroid patients by examining antipyrine and  $^{35}\text{S}$ -methimazole half-life. Half-lives of both drugs were increased in patients with untreated hypothyroid disease while they were decreased in patients with thyrotoxicosis. These workers suggested that changes in drug metabolising activity occurred in abnormal thyroid states and that this also resulted in increased clearance in hyperthyroid patients. When the percentage dose of paracetamol recovered in 24 hours was compared in normal volunteers and hyperthyroid patients no statistically significant difference was found. However the scatter between individuals was quite large so any differences between the groups were probably masked by the interindividual differences. It does at least appear that recoveries of the glutathione conjugates of paracetamol were not affected by thyrotoxicosis nor was the percentage recovery of unchanged paracetamol diminished (which might have resulted from an accelerated metabolism).

#### 4.5. The effect of salicylamide on paracetamol metabolism in man

Salicylamide was administered to 6 volunteers concurrently with a therapeutic dose of paracetamol in order to determine how a metabolic competing agent might affect the metabolism of paracetamol. When salicylamide was present, paracetamol serum concentrations were significantly higher than when paracetamol was given alone presumably because clearance of paracetamol was reduced. Serum and urinary concentrations of paracetamol sulphate were lower after salicylamide suggesting that depletion of endogenous sulphate had occurred as both paracetamol and salicylamide conjugate with this anion. This led to a significant lengthening of the half-life of paracetamol and the area under the serum paracetamol concentration time curve was significantly higher after salicylamide. The AUC for paracetamol sulphate was significantly lower after salicylamide but because the AUC for paracetamol glucuronide was not significantly increased it appears that glucuronidation does not compensate totally for the increased unchanged paracetamol. Furthermore the AUC for unchanged paracetamol was increased. This may be because endogenous glucuronic acid is also compromised by salicylamide with which it also conjugates. The urinary excretion pattern however gave no suggestion of glucuronic acid depletion as unchanged paracetamol was not increased after salicylamide and paracetamol glucuronide recovery was increased compared with control values.

Levy and Yamada (1971) also administered paracetamol to healthy human subjects in the presence and absence of salicylamide and found that the excretion rate of paracetamol sulphate and glucuronide decreased after salicylamide. This may have been a result of competition for secretion at kidney tubules between the conjugates of salicylamide and paracetamol. However, as these workers collected urine samples every 15 - 30 minutes, they were able to monitor small changes in the rate of excretion of each metabolite.

It is interesting that in the present study there was no evidence for increased production of the toxic metabolite because concentrations of urinary cysteine and mercapturic acid were not increased by salicylamide treatment. This suggests that depletion of sulphate available for conjugation does not necessarily lead to a potentially toxic situation.

#### 4.6. Paracetamol metabolism after overdose

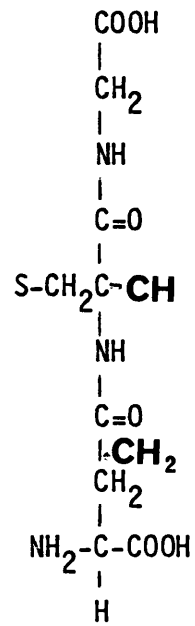
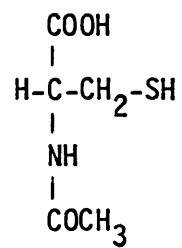
Since paracetamol is freely available it has become one of the more popular drugs for deliberate self poisoning. A knowledge of its metabolism was an aid in the original derivation of a regimen for treating paracetamol overdose. After high doses paracetamol is associated with hepatotoxicity and the rationale behind the treatment of paracetamol poisoning is to provide a suitable substrate to conjugate with the toxic metabolite of paracetamol. Glutathione itself prevents in vitro covalent binding to microsomal protein but glutathione administered to man is not thought to cross cell membranes readily (Prescott, Newton, Swainson et al, 1974).

However glutathione administered intravenously to mice was found to be taken up by the liver and to protect against the hepatotoxic effects of a large dose of paracetamol (Strolin-Benedetti et al, 1975). It was found by Mitchell et al, (1973) that glutathione precursors such as cysteine and cysteamine could prevent experimental paracetamol-induced hepatic necrosis. Prescott et al (1974) reported that intravenous cysteamine was successful in treating patients with massive paracetamol overdoses; however this treatment had unpleasant side effects. In consequence more acceptable alternatives were investigated and oral methionine was found to effect moderate protection against paracetamol- induced hepatotoxicity (Crome, Vale, Volans et al, 1976). N-acetyl cysteine was administered orally by Peterson and Rumack (1977) and by intravenous infusion by Prescott et al, (1977) and both found this

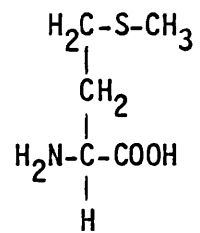
drug to be very effective without significant side effects. The structures of these compounds in relation to glutathione which conjugates with paracetamol in the 3-position are shown below.

The success of sulphur containing amino acids has been attributed either to direct conjugation with the reactive intermediate of paracetamol via the sulphur atom or to repletion of glutathione stores.

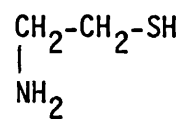
glutathione

N-acetyl cysteine

L-methionine



cysteamine



In order to examine whether these compounds indeed act by combining either directly or indirectly with the reactive metabolite of paracetamol, the metabolic profile of 10 patients admitted to the casualty department of the Royal United Hospital, Bath suffering from paracetamol poisoning was examined. The patients were treated with either L-methionine or N-acetyl cysteine as dictated by blood concentrations of paracetamol and time after ingestion of the analgesic as described earlier. The latter parameter is often difficult to determine as information given by these patients is often inaccurate (Schnaps, Halken, Davy and Tirash, 1980). Values of the ratios  $(PG)/(PS)$   $(PG+PS)/(PC+PM)$  were calculated for each urine collection obtained from the individual patients.

Before treatment values of these ratios (where obtained) in overdose patients were very much higher than the mean values in normal volunteers for whom  $PG/PS$  ranged from 0.24 - 4.23 and  $(PG+PS)/(PC+PM)$  ranged from 4.88 - 30.53 in 24 hour urine suggesting that sulphate and glutathione depletion had occurred. In the seven cases P.3, P.4, P.6, P.7, P.8, P.9 and P.10 to whom L-methionine alone was given, the ratio of  $PG/PS$  decreased with time in three (P.3, P.7 and P.9) and the ratio of  $(PG+PS)/(PC+PM)$  decreased with time in all seven. In the other 3 cases to whom N-acetyl cysteine was administered alone or in combination with L-methionine, (P.1, P.2 and P.5) ratios of  $(PG)/(PS)$  and  $(PG+PS)/(PC+PM)$  decreased with time in all three cases. This suggests that both agents serve to increase the urinary products of glutathione conjugation with the toxic metabolite of paracetamol. The results further suggest that N-acetyl cysteine and methionine increase the availability of sulphate for conjugation with paracetamol. As sulphate conjugation accounts for some 33% of the excreted material after therapeutic 1g doses of paracetamol, low concentrations of endogenous sulphate will lead to a slower rate of decay of parent drug. Balanowska and Gessner (1980) described sulphation as a high affinity, low capacity pathway which is readily saturable. Sulphate depletion may result in a relatively

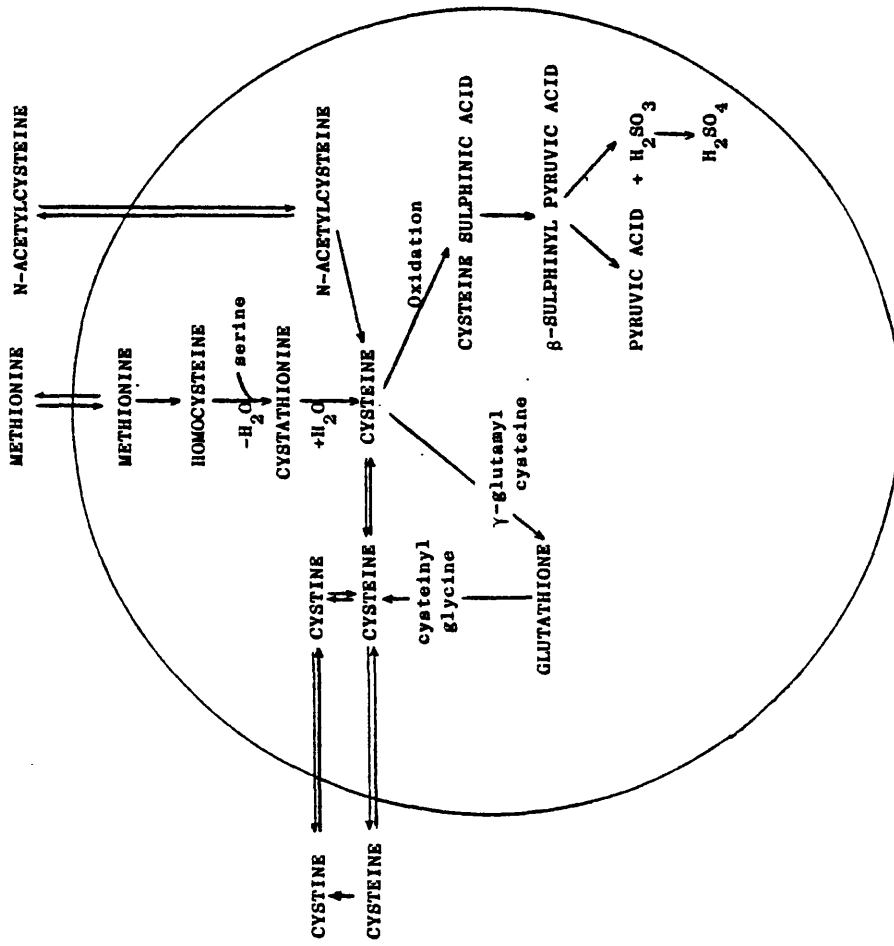


greater proportion of paracetamol undergoing oxidation to the active metabolite causing an even greater demand on glutathione. N-acetyl cysteine and L-methionine, by increasing the availability of sulphate will therefore aid paracetamol elimination and hence reduce the amounts of oxidised intermediate formed. Possible routes of metabolism which L-methionine and N-acetyl cysteine may undergo to produce glutathione or cysteine to conjugate with the reactive metabolite and produce sulphate are shown in Fig. 4.1.

It might be argued that with time, depletion of sulphate and glutathione might be replenished by intermediary metabolism resulting in decreased ratios. However by this time toxicity might well have occurred. Comparison of values of the ratios  $(PG)/(PS)$  and  $(PG+PS)/(PC+PM)$  after a 1g dose with values from the four normal individuals who took paracetamol 2.5g (Section 3.1) showed that with no treatment the ratios did not alter significantly over the ten hour period. Therefore the decrease in each ratio which occurred after treatment in the overdose patients may be attributed to the effects of N-acetyl cysteine and L-methionine.

It is important to distinguish between increases and decreases in production of the cysteine and mercapturic acid metabolites of paracetamol as indicators of increased production of the reactive metabolite in judging the potential of a given clinical situation for toxicity. After therapeutic doses a rise in the recovery of cysteine and mercapturic acid metabolites suggests an increased production of the reactive metabolite and that there are adequate resources of glutathione to detoxify it. After paracetamol overdose it must be assumed that glutathione availability is compromised so that now a decrease in cysteine and mercapturic acid metabolites of paracetamol may represent the inherently more dangerous situation that already sufficient concentrations of the hepatotoxic metabolite may be present to arylate liver macromolecules.

Fig. 4.1. INTER-RELATIONSHIP AND POSSIBLE FATE OF AMINO ACIDS ADMINISTERED IN CASES OF PARACETAMOL POISONING



Prescott (1981) found intravenous N-acetyl cysteine to be more successful than intravenous L-methionine in treating severe paracetamol poisoning and also found that cysteamine produced unpleasant side effects. Vale, Meredith and Goulding (1981) found oral methionine to be just as effective as intravenous N-acetyl cysteine but found that incidence and severity of liver damage increased if treatment was delayed beyond 10 hours (both methionine and N-acetyl cysteine should be given within eight hours for most effective protection against liver damage). These authors suggested that if patients were vomiting, N-acetyl cysteine could be given intravenously but otherwise methionine should be used as it is inexpensive, reaches the liver directly if given orally and the total treatment is completed within 12 hours (compared with 20 hours for intravenous N-acetyl cysteine).

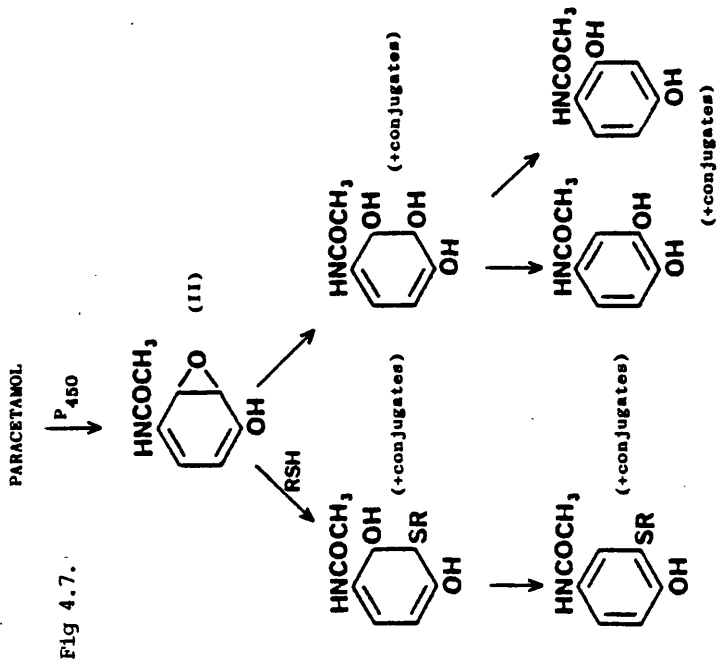
Tredger, Smith, Davis and Williams (1980, 1981), found in vitro that N-acetyl cysteine decreased the covalent binding of paracetamol to mouse hepatic microsomes but methionine showed no such effect. N-acetyl cysteine also decreased the concentrations of NADPH-cytochrome c reductase. Thus N-acetyl cysteine's protective effect against paracetamol-induced hepatotoxicity may be exerted by processes affecting the binding of a reactive paracetamol metabolite and to a lesser degree the formation of that metabolite. These authors concluded that methionine's effectiveness in vivo is due to mechanisms other than those involving interactions with the mixed function oxidase system or its products. They suggested that as methionine was metabolised in vivo to homocysteine, cysteine and glutathione, these too might be responsible for its effects.

Galinsky and Levy (1979) found that oral administration of N-acetyl cysteine to rats caused an increased formation of paracetamol sulphate and concluded that this may be partly responsible for its protective effect. Galinsky, Slattery and Levy (1979) found that administration of sodium sulphate to rats (as a bolus dose i.p.

followed by an intravenous infusion over 2 hours) caused increased paracetamol sulphate formation and enhanced the elimination of paracetamol. When it was first shown by Prescott, Newton, Swainson, Wright, Forrest and Mathews (1974) that cysteamine was a successful scavenger of the reactive metabolite, McLean (1974) suggested that methionine or cysteine (as glutathione precursors) could be added to paracetamol tablets. If it is found clinically in man that administration of sodium sulphate has a protective effect, it may be feasible to include sodium sulphate or another source of sulphate in paracetamol - containing pharmaceutical products as suggested by Galinsky et al, (1979).

#### 4.7. A possible toxic intermediate metabolite of paracetamol

Knight and Young (1958) found that for various substituted benzenes e.g. naphthalene, conjugation of an epoxide (a product of hepatic oxidation) with glutathione initially resulted in the production of acid-labile derivatives which they termed pre-mercapturates. These compounds are relatively stable in urine at pH5 but undergo rapid dehydration in strong acid to give the corresponding aromatic metabolites. Results presented above indicate the presence of similar unaromatised metabolites of paracetamol and greatly increased amounts of S-substituted metabolites ( $p < 0.001$ ) and 3-hydroxy paracetamol ( $p < 0.02$ ) were found after incubation at pH0.3. These results are however an underestimate of concentrations of acid-labile metabolites released by acid treatment because no account was taken of the continuing conversion of acid-labile metabolites to final compounds during acid treatment. These acid-labile species which constitute a large proportion of the oxidised metabolites of paracetamol may be considered to be hydroxy dihydrobenzenes of the type shown in Fig. 4.7. Recovery of unaromatised and aromatised 2-hydroxy paracetamol was consistently and substantially less than that of 3-hydroxy paracetamol probably because of steric hindrance due to the position of the N-acetyl group.



Proposed oxidation of paracetamol.

Although, largely as a result of in vitro work the hepatotoxic metabolite of paracetamol is believed to be N-acetyl benzoquinonimine (Hinson, Pohl and Gillette, 1979), it may be proposed as an alternative that toxicity may be due to the 2,3- or 3,4-oxides of paracetamol, either of which could give rise to 3-hydroxy paracetamol. Recent work by Hinson, Pohl, Monks, Gillette and Guengerich (1980) suggests that the S-substituted metabolites which are believed to derive from the toxic intermediate, are formed by a pathway distinct from that which gives rise to 3-hydroxy paracetamol. The present results suggest that pathways of O- and S-substitution are linked. As considerable amounts of acid-labile forms of both groups of the ultimate oxidation products are found in urine, this suggests that these arise from nucleophilic attack on an epoxide ring (which may occur at either the 2- or 3-position) to produce, initially, a hydroxydihydrobenzene (Fig. 4.7.1). As both 2-OH paracetamol and 3-OH paracetamol were found in urine, it is proposed that the pathways of O- and S-substitution originate from 4-hydroxy-2,3-dihydroacetanilide-2,3-oxide (II in Fig. 4.7.1). Epoxides have been shown to be intermediates in the metabolic formation of phenols and transhydrodiols (see Garner, 1976) and the fast ring opening and closing of arene oxides via carbonium ions is thought to be responsible for their activity with even weakly nucleophilic sites on cell macromolecules (Jerina and Daly, 1974). In addition, epoxides are believed to be mediators in the tissue necrosis induced by a number of aromatic compounds, for example bromobenzene (Brodie, Reid, Cho, Sipes, Krishna and Gillette (1971). It is therefore proposed that 4-hydroxy-2,3-dihydroacetanilide-2,3-oxide is likely to be an hepatotoxic metabolite of paracetamol in man.

## Chapter 5

### Paracetamol Metabolism in Animals - Results

#### General Introduction

Davis and his colleagues first noted in 1974 that certain species including hamsters and mice are susceptible to paracetamol-induced liver necrosis (Davis, Potter, Jollow and Mitchell, 1974). Other species such as rats, rabbits and guinea pigs were found to be relatively resistant to the hepatotoxic effects of paracetamol. It will be apparent that man may be described as a susceptible species.

A variety of factors are believed to alter paracetamol metabolism and some of those which might be relevant to man were investigated in animal species. The effects of inducing agents and agents which compete for metabolic substrates were investigated because these may promote the metabolism of paracetamol by the potentially toxic oxidation pathway as indicated by an increase in cysteine and mercapturic acid excretion. Repeated dosing studies were also performed to see if there were any indications of toxicity. Because paracetamol serum half lives were not measured in overdosed human patients, experiments were carried out in rabbits to determine whether the effects that L-methionine showed in human overdosed patients (increasing the recovery of paracetamol as its sulphate and glutathione conjugates) could be reproduced in rabbits and whether they resulted in a decrease in the area under the serum concentration time curve of paracetamol and/or a shorter plasma elimination half-life.

Four studies were carried out:

- 1) Effect of enzyme inducing agents on paracetamol metabolism.
- 2) Paracetamol metabolism following single and repeated doses.
- 3) Effect on paracetamol metabolism of agents which compete for metabolic substrates.
- 4) Effect of L-methionine on paracetamol metabolism.

In these experiments one susceptible species (the mouse) and one non-susceptible species (the guinea pig) were chosen for study. These species were also studied so that the pattern of metabolites associated with toxicity could be examined.

### 5.1 Effect of inducing agents on the metabolism of paracetamol in guinea pigs and mice

#### Introduction

The effects of phenobarbitone, phenylbutazone and rifampicin on the metabolism of paracetamol were studied in guinea pigs and mice. The enzyme inducing effects of phenobarbitone, phenylbutazone and rifampicin after repeated dosing for several days are well known (Gelboin, 1971; Burns, 1971; Goldberg, 1980). Walker, Kelleher, Dixon and Losowsky, (1973) showed that phenobarbitone-pretreated mice showed a greater degree of liver necrosis than those treated with paracetamol alone. Jollow, Thorgeirsson, Potter, Hashimoto and Mitchell, 1974 showed that pretreatment with inducing agents known to potentiate hepatotoxicity increased the formation of paracetamol mercapturic acid. They also showed that the fraction of paracetamol converted to the mercapturic acid conjugate was highest in mice and hamsters (the most susceptible species) and lowest in species such as the rat which were less susceptible to paracetamol's hepatotoxic effects. Wright and Prescott (1973) suggested that a 'potentially induced' group of paracetamol-poisoned patients developed more severe hepatic necrosis than 'non-induced' patients. However Prescott, Critchley, Balali-Mood and Pentland (1981) failed to find an increase in the cysteine and mercapturic acid conjugates of paracetamol in patients with microsomal enzyme induction although they did excrete less unchanged drug and paracetamol sulphate and correspondingly more paracetamol glucuronide than did healthy volunteers.



This study was therefore carried out to confirm that pretreatment with phenobarbitone, phenylbutazone and rifampicin resulted in an increase in the oxidation pathway in these 2 species, suggesting a predisposition towards toxicity. These effects could then be compared with results obtained from repeated dosing studies and after the simultaneous administration with paracetamol and metabolic competing agents.

### Procedures

24 male mice and 4 male guinea pigs were pretreated with either:

- 1) phenobarbitone 100mg/kg i.p. per day for four days
- or 2) phenobarbitone 100mg/kg i.p. per day for seven days
- or 3) phenylbutazone 100mg/kg i.p. per day for four days
- or 4) rifampicin 80mg/kg i.p. per day for five days.

Paracetamol 150mg/kg was given intraperitoneally on the morning of the final day using standard solutions of 75mg/ml in 0.9% saline for guinea pigs and 15mg/ml in 0.9% saline for mice. Guinea pigs and mice were housed in metabolic cages for the collection of urine for 24 hours after dosing with paracetamol. Urine was then analyzed by HPLC for paracetamol and its metabolites. Details of the standard solutions of inducing agents are given in Table 5.1.1 and 5.1.2 for guinea pigs and mice respectively. Cytochrome P<sub>450</sub> concentrations were measured but are not presented because of marked variation in the results obtained presumably because of a methodological error. However the doses given are conventionally accepted as causing induction.

## Results

Percentage recoveries of unchanged paracetamol and its metabolites in 24 hour urine are given in Tables 5.1.1. and 5.1.2. and individual quantities in mg and percentage recoveries of paracetamol and its metabolites are given in Appendix 15 for guinea pigs and Appendix 16 for mice.

It can be seen from Table 5.1.1. that paracetamol cysteine and mercapturic acid recoveries were increased significantly by all three inducing agents in guinea pigs ( $p < 0.05$ ) but recoveries of the other metabolites of paracetamol were not altered. Control paracetamol glucuronide concentrations were low because one guinea pig excreted approximately 0% as the glucuronide conjugate. These results are plotted in Figs. 5.1.1., 5.1.2. and 5.1.3.

Table 5.1.1.1. Means  $\pm$  S.E.M. of percentage recovery of unchanged paracetamol and its metabolites in 24 hour urine after pretreatment of guinea pigs with an inducing agent or saline

Treatment	n	% recovery						
		PG	PS	PC	P	PM	PC + PM	
paracetamol 150mg/kg in saline	4	65.15 $\pm$ 21.76	5.17 $\pm$ 2.21	0.00 $\pm$ 0.00	29.68 $\pm$ 19.60	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	
paracetamol 150mg/kg after 7 days pretreatment with phenobarbitone sodium 100mg/kg/day in saline	3	94.35 $\pm$ 0.95	2.78 $\pm$ 0.88	0.68 $\pm$ 0.41	1.61 $\pm$ 0.26	0.63 $\pm$ 0.06	1.30 $\pm$ 0.47	
paracetamol 150mg/kg after 4 days pretreatment with phenylbutazone 100mg/kg/day in saline	4	94.91 $\pm$ 0.65	2.32 $\pm$ 0.20	0.30 $\pm$ 0.10	2.06 $\pm$ 0.57	0.41 $\pm$ 0.25	0.72 $\pm$ 0.19	
paracetamol 150mg/kg after 5 days pretreatment with rifampicin 80mg/kg/day in saline	7	90.06 $\pm$ 1.72	5.65 $\pm$ 0.88	0.15 $\pm$ 0.10	3.58 $\pm$ 1.33	0.56 $\pm$ 0.16	0.71 $\pm$ 0.2	

Table 5.1.2. Means  $\pm$  S.E.M. of percentage recovery of paracetamol and its metabolites in 24 hour urine after pretreatment of mice with an inducing agent of vehicle

Treatment	n	% recovery						
		PG	PS	PC	P	PM	PC + PM	
paracetamol 150mg/kg in saline	6	63.90 $\pm$ 3.74	8.42 $\pm$ 2.37	14.64 $\pm$ 1.57	11.74 $\pm$ 3.73	1.30 $\pm$ 0.83	15.94 $\pm$ 1.2	
paracetamol 150mg/kg after 4 days pretreatment with phenobarbitone sodium 100mg/kg/day in saline	6	53.34 $\pm$ 2.78	3.43 $\pm$ 1.78	24.70 $\pm$ 1.66	7.29 $\pm$ 1.14	11.04 $\pm$ 1.17	35.74 $\pm$ 2.4	
paracetamol 150mg/kg after 7 days pretreatment with phenobarbitone sodium 100mg/kg	6	49.59 $\pm$ 1.41	3.70 $\pm$ 0.56	34.31 $\pm$ 1.23	3.57 $\pm$ 0.36	8.82 $\pm$ 0.43	43.14 $\pm$ 1.4	
paracetamol 150mg/kg after 4 days pretreatment with phenylbutazone 100mg/kg/day in saline	6	65.20 $\pm$ 0.53	6.44 $\pm$ 0.42	15.98 $\pm$ 0.60	8.30 $\pm$ 0.34	4.08 $\pm$ 0.62	20.06 $\pm$ 0.9	
paracetamol 150mg/kg after 5 days pretreatment with rifampicin 80mg/kg/day in saline	5	58.12 $\pm$ 2.20	5.19 $\pm$ 1.51	30.30 $\pm$ 1.10	2.20 $\pm$ 0.92	4.19 $\pm$ 1.77	34.49 $\pm$ 2.7	

Fig. 5.1.1 Effect of phenobarbitone pretreatment on paracetamol metabolism in guinea pigs (means  $\pm$  S.E.M.)

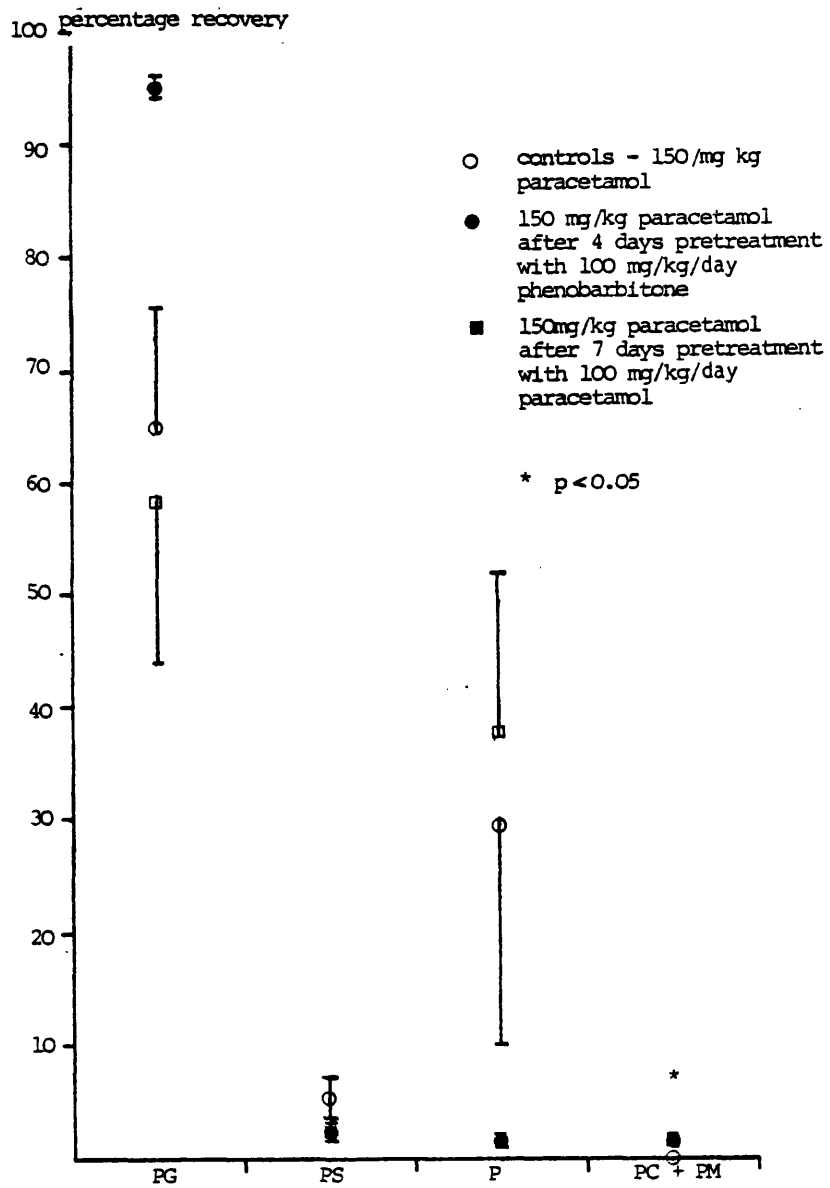


Fig. 5.1.2 Effect of phenylbutazone pretreatment on paracetamol metabolism in guinea pigs (means  $\pm$  S.E.M.)

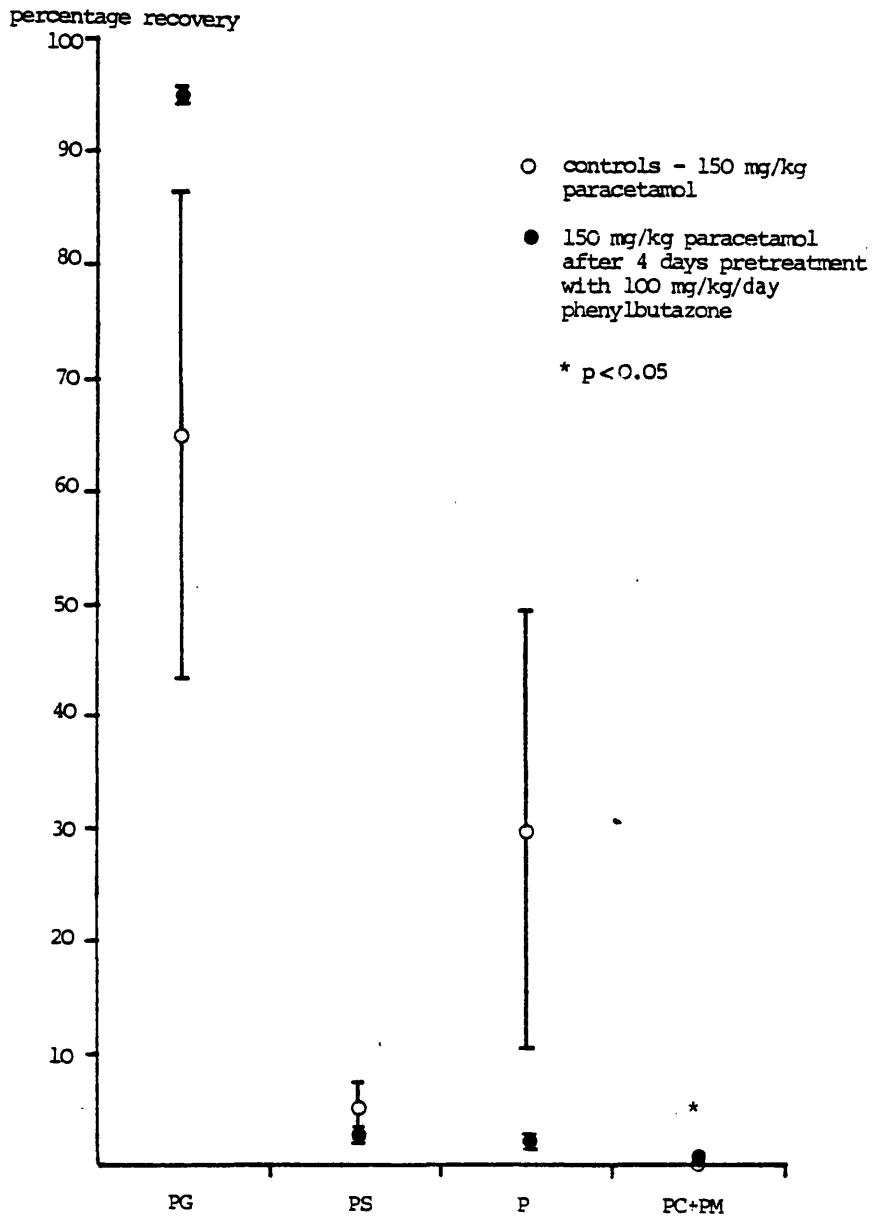
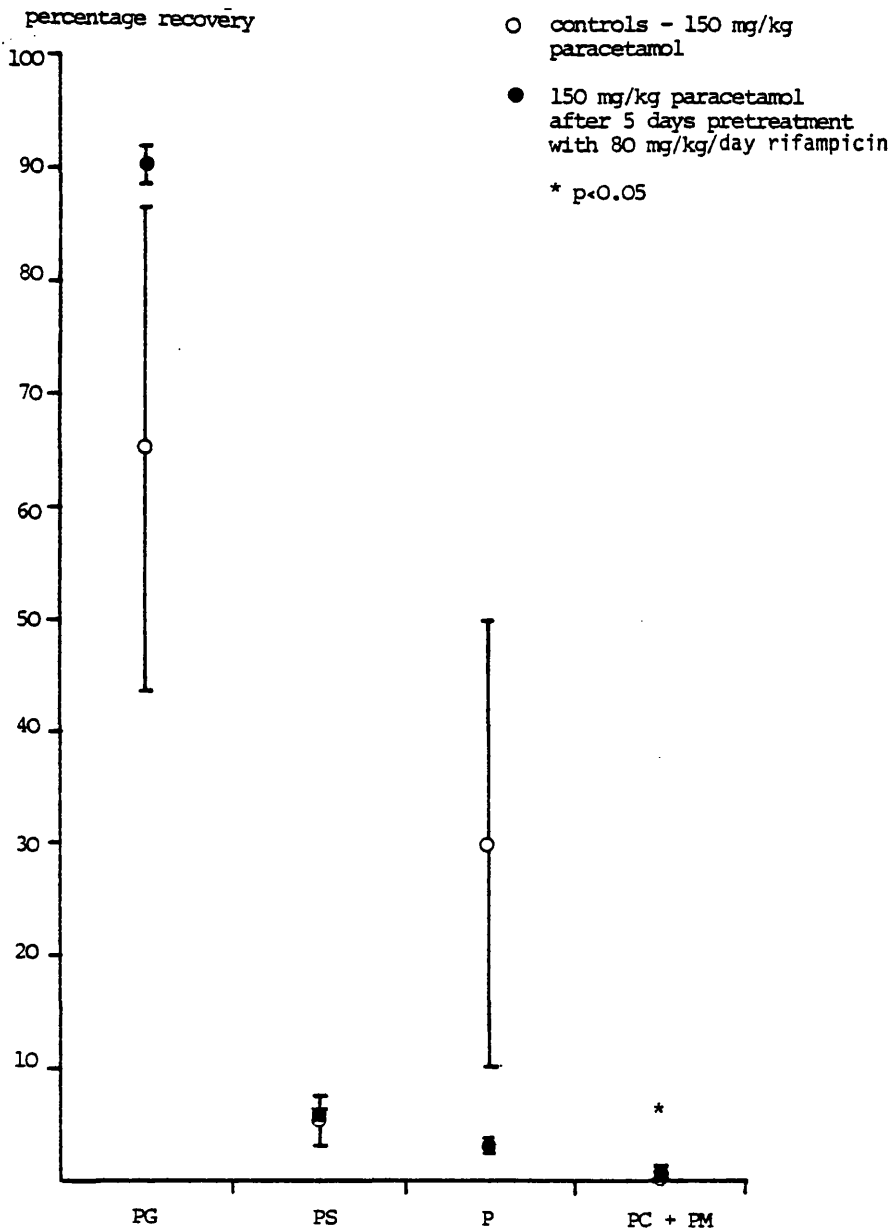


Fig. 5.1.3.  
Effect of rifampicin pretreatment on paracetamol metabolism  
in guinea pigs (means  $\pm$  S.E.M.)



Examination of the effects of these 3 agents on paracetamol metabolism in mice (Table 5.1.2.) revealed that 4-day and 7-day phenobarbitone pretreatment significantly reduced paracetamol glucuronide recovery ( $p < 0.05$ ) and significantly increased the percentage recoveries of paracetamol cysteine and mercapturic acid compared with controls ( $p < 0.001$ ). Phenylbutazone significantly increased paracetamol mercapturic acid recoveries ( $p < 0.025$ ) but not paracetamol cysteine and rifampicin increased the percentage of paracetamol cysteine plus paracetamol mercapturate recovered ( $p < 0.001$ ) while also decreasing the recovery of unchanged paracetamol ( $p < 0.05$ ) when compared with controls. None of the other metabolites of paracetamol was significantly increased or decreased by these 3 agents in mice. These results are plotted in Fig. 5.1.4, 5.1.5 and 5.1.6.



Fig. 5.1.4.  
Effect of phenobarbitone pretreatment on paracetamol metabolism  
in mice (means  $\pm$  S.E.M.)

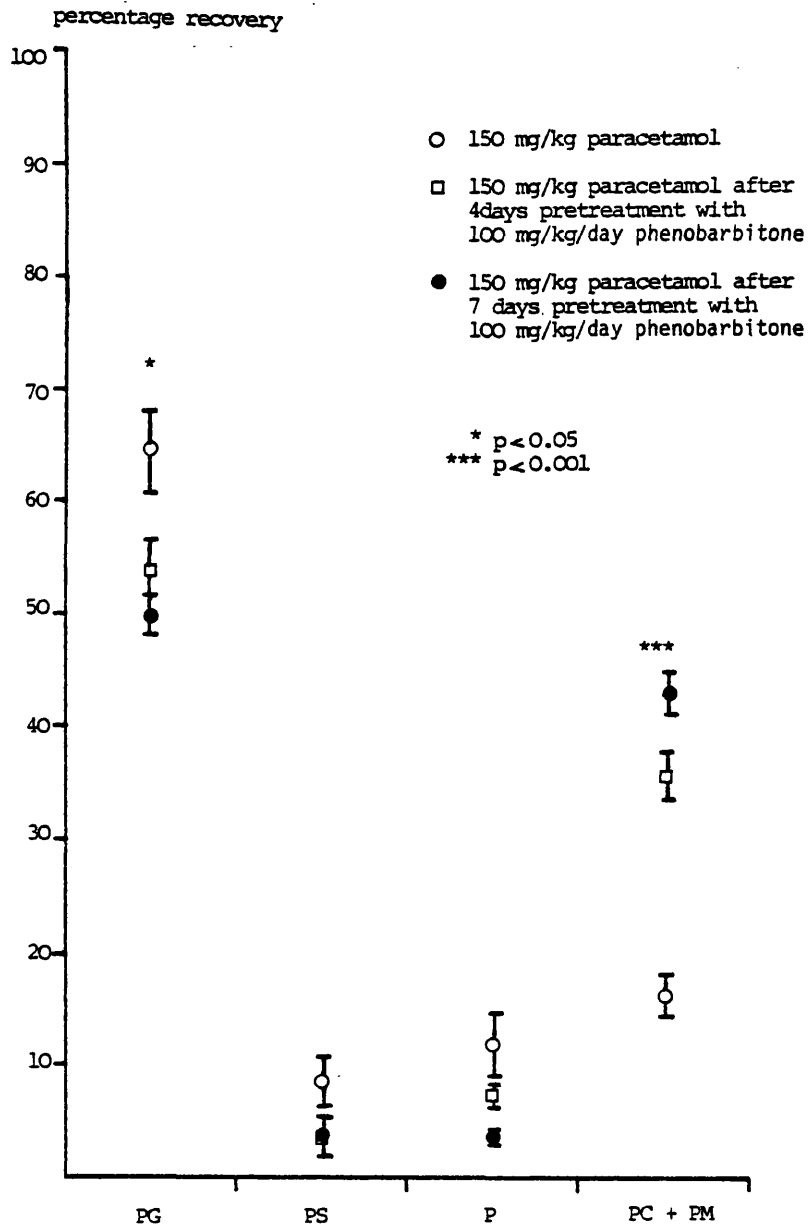


Fig. 5.1.5 Effect of phenylbutazone pretreatment on paracetamol metabolism in mice (means  $\pm$  S.E.M.)

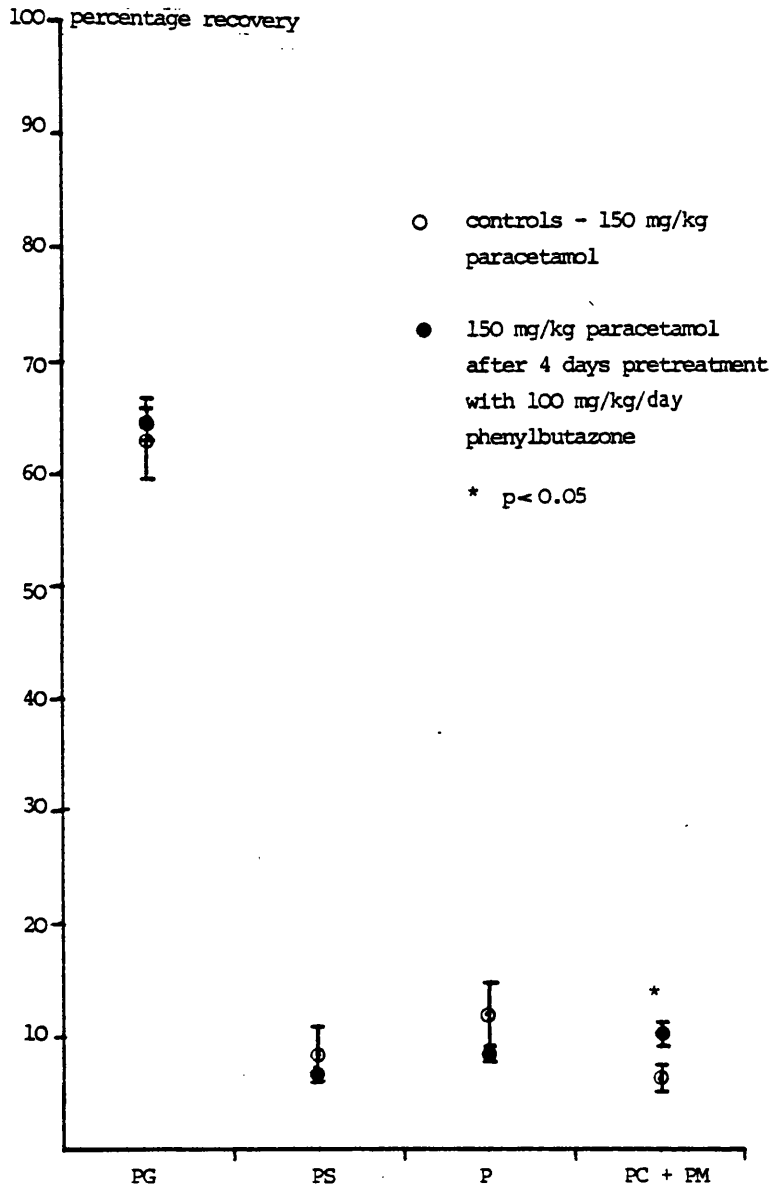
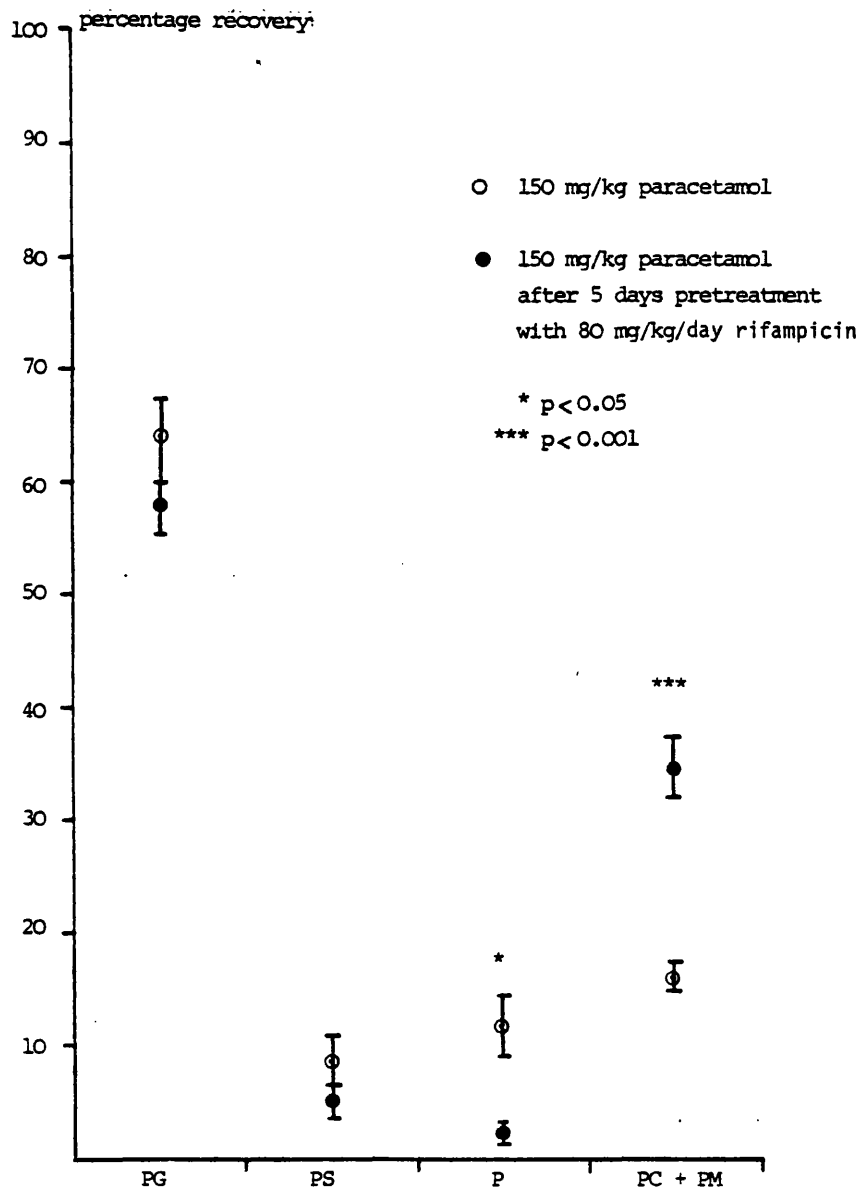


Fig. 5.1.6.  
Effect of rifampicin pretreatment on paracetamol metabolism  
in mice (means  $\pm$  S.E.M.)



## 5.2 Paracetamol metabolism in animals following single and repeated doses

### Introduction

The effects of a single dose of paracetamol 100mg/kg and of paracetamol 100mg/kg twice daily for 9 days (guinea pigs) or 11 days (mice) on the urinary metabolites of paracetamol were investigated.

### Procedures

#### a) Single doses

- i) 4 male guinea pigs were given paracetamol 100mg/kg i.p. in a single dose using a standard solution of 100mg/ml in 0.9% saline. The guinea pigs were housed in individual metabolic cages (Associated Crates Limited) and maintained in a constant light (5am-7pm) - dark (7pm-5am) cycle. Urine from the guinea pigs was collected for 24 hours after dosing and was analyzed by HPLC for paracetamol, paracetamol glucuronide, paracetamol sulphate, paracetamol cysteine and paracetamol mercapturic acid as described in Section 2.7.e.
- ii) 24 male mice were given paracetamol 100mg/kg i.p. in a single dose using a standard solution of 10mg/ml in 0.9% saline. The mice were housed in metabolic cages (North Kent Plastic) and maintained in a constant light (5am-7pm) - dark (7pm-5am) cycle. Urine from the mice was collected for 24 hours after dosing and was assayed for paracetamol and its metabolites by HPLC.

#### b) Repeated doses

- i) 4 male guinea pigs were given paracetamol 100mg/kg i.p. morning and evening for 9 days using a standard solution of 100mg/ml in 0.9% saline. The guinea pigs were housed in individual metabolic cages (Associated Crates Limited) and maintained in a constant light

(5am-7pm) - dark (7pm-5am) cycle. Urine from the guinea pigs was collected in separate containers for the periods 0-24h, 24-72h, 72-120h, 120-168h and 168-216h after dosing and was analyzed by HPLC for paracetamol and its metabolites.

- ii) 24 male mice were given paracetamol 100mg/kg i.p. morning and evening for 11 days using a standard solution of 10mg/ml in 0.9% saline. The mice were housed in metabolic cages (North Kent Plastic) and maintained in a constant light (5am-7pm) - dark (7pm-5am) cycle. Urine from the mice was collected in separate containers for the periods 0-24h, 24-72h, 72-120h, 120-168h, 168-216h and 216-264h urine after dosing and was analyzed by HPLC for paracetamol and its metabolites.

## Results

Values obtained for each metabolite of paracetamol in urine were converted to the weight of paracetamol using correction factors given in Appendix 1 and then expressed as percentage recovery of the total recovery of parent drug and metabolites in each urine collection period.

Results after single doses of paracetamol are given in Table 5.2.1. Individual results are given in Appendix 17 and Appendix 18. Mean results for repeated doses of paracetamol in guinea pigs and mice are given in Table 5.2.2 and 5.2.3 and individual results are given in Appendix 19 and Appendix 20 for guinea pigs and mice respectively.

Table 5.2.1. Means  $\pm$  S.E.M. of percentage recoveries of unchanged paracetamol and its metabolites in guinea pigs and mice in urine collected for 24 hours after a single dose of paracetamol 100mg/kg i.p.

Species	n	P	PG	PS	PC	PM	PC + PM
guinea pigs	4	51.76 $\pm$ 15.74	46.00 $\pm$ 15.24	0.28 $\pm$ 0.28	0.38 $\pm$ 0.16	1.57 $\pm$ 0.82	1.96 $\pm$ 0.95
mice	6	30.31 $\pm$ 7.67	40.68 $\pm$ 8.10	2.98 $\pm$ 0.78	22.10 $\pm$ 1.29	3.93 $\pm$ 0.80	26.03 $\pm$ 1.53

Table 5.2.2. Means  $\pm$  S.E.M. of percentage recoveries of unchanged paracetamol and its metabolites in guinea pigs excreted in serial urine collection periods after paracetamol 100mg/kg i.p. twice daily for 9 days

Collection period	n	P	PG	PS	PC	PM	PC + PM
0-24h	4	62.13 $\pm$ 16.17	36.58 $\pm$ 15.95	not detectable	1.29 $\pm$ 0.78	0.00 $\pm$ 0.00	1.29 $\pm$ 0.78
24-72h	4	91.13 $\pm$ 3.57	4.72 $\pm$ 4.73	"	4.14 $\pm$ 1.84	0.00 $\pm$ 0.00	4.14 $\pm$ 1.84
72-120h	4	99.26 $\pm$ 0.74	0.00 $\pm$ 0.00	"	0.00 $\pm$ 0.00	0.74 $\pm$ 0.74	0.74 $\pm$ 0.74
120-168h	4	88.76 $\pm$ 4.27	6.04 $\pm$ 5.69	"	5.20 $\pm$ 2.14	0.00 $\pm$ 0.00	5.20 $\pm$ 2.14
168-216h	4	92.90 $\pm$ 1.98	2.26 $\pm$ 2.26	"	4.84 $\pm$ 0.84	0.00 $\pm$ 0.00	4.84 $\pm$ 0.84

**Table 5.2.3 Means  $\pm$  S.E.M. of percentage recoveries of unchanged paracetamol and its metabolites in mice excreted in serial urine collection periods after paracetamol 100mg/kg i.p.**

Collection period	n	P	PG	PS	PC	PM	PC + PM
0-24h	6	1.66 $\pm$ 1.22	66.51 $\pm$ 1.72	not detectable	28.64 $\pm$ 1.18	3.18 $\pm$ 0.76	31.82 $\pm$ 1.76
24-72h	6	53.01 $\pm$ 11.93	19.96 $\pm$ 11.06	"	23.30 $\pm$ 1.47	3.73 $\pm$ 0.40	27.03 $\pm$ 1.30
72-120h	6	64.50 $\pm$ 10.69	9.53 $\pm$ 9.53	"	21.96 $\pm$ 1.28	4.01 $\pm$ 1.18	25.96 $\pm$ 1.23
120-168h	6	69.23 $\pm$ 7.72	5.52 $\pm$ 5.52	"	21.29 $\pm$ 2.87	3.96 $\pm$ 1.04	25.24 $\pm$ 2.56
168-216h	6	38.99 $\pm$ 12.10	26.87 $\pm$ 12.11	"	30.39 $\pm$ 1.20	3.74 $\pm$ 0.86	34.13 $\pm$ 0.99
216-264h	6	46.39 $\pm$ 12.01	18.45 $\pm$ 11.67	"	32.78 $\pm$ 0.93	2.38 $\pm$ 0.73	35.16 $\pm$ 1.12

Percentage recoveries of paracetamol and its metabolites after repeated doses in guinea pigs and mice are shown in Figs. 5.2.1. and 5.2.2. respectively.

When the percentage recoveries of unchanged paracetamol and its metabolites for single and repeat dosing in guinea pigs were compared, the recoveries in the first 24 hours of the repeated dose study were not significantly different from recoveries in the single dose study. In 24-72h urine, percentage recovery of paracetamol glucuronide was significantly ( $p < 0.05$ ) lower than after the single dose; in 72-120h urine percentage recovery of paracetamol glucuronide was significantly lower ( $p < 0.025$ ) while unchanged paracetamol was significantly higher ( $p < 0.025$ ) than after the single dose; in 120-168h urine, percentage recoveries of paracetamol glucuronide were significantly higher ( $p < 0.05$ ) and of unchanged paracetamol were significantly lower ( $p < 0.05$ ) than after the single dose. When repeat dosing over the 9 day period was examined in guinea pigs using the paired 't' test, there were found to be no significant changes in the recoveries of urinary metabolites over the whole 9 days except for a significant increase in paracetamol cysteine percentage recovery in the last 2 days ( $p < 0.001$ ) compared with the first 24 hour period. There was a trend for paracetamol glucuronide recovery to decrease and unchanged paracetamol to increase over the whole period but this did not reach statistical significance.

When urinary recoveries after a single dose of paracetamol 100mg/kg were compared with repeated twice-daily dosing over 11 days in mice marked differences were found. In the first 24 hours of their repeated dosing regimen, mice excreted significantly higher amounts of paracetamol glucuronide ( $p < 0.02$ ) and paracetamol cysteine plus mercapturic acid ( $p < 0.05$ ) due to increased recoveries of paracetamol



Fig. 5.2.1 Effect of repeated dosing on paracetamol metabolism in guinea pig showing percentage recovery (means  $\pm$  S.E.M.) of paracetamol and its metabolites. Concentrations of paracetamol sulphate were below detection ( $<10\mu\text{g/ml}$ )

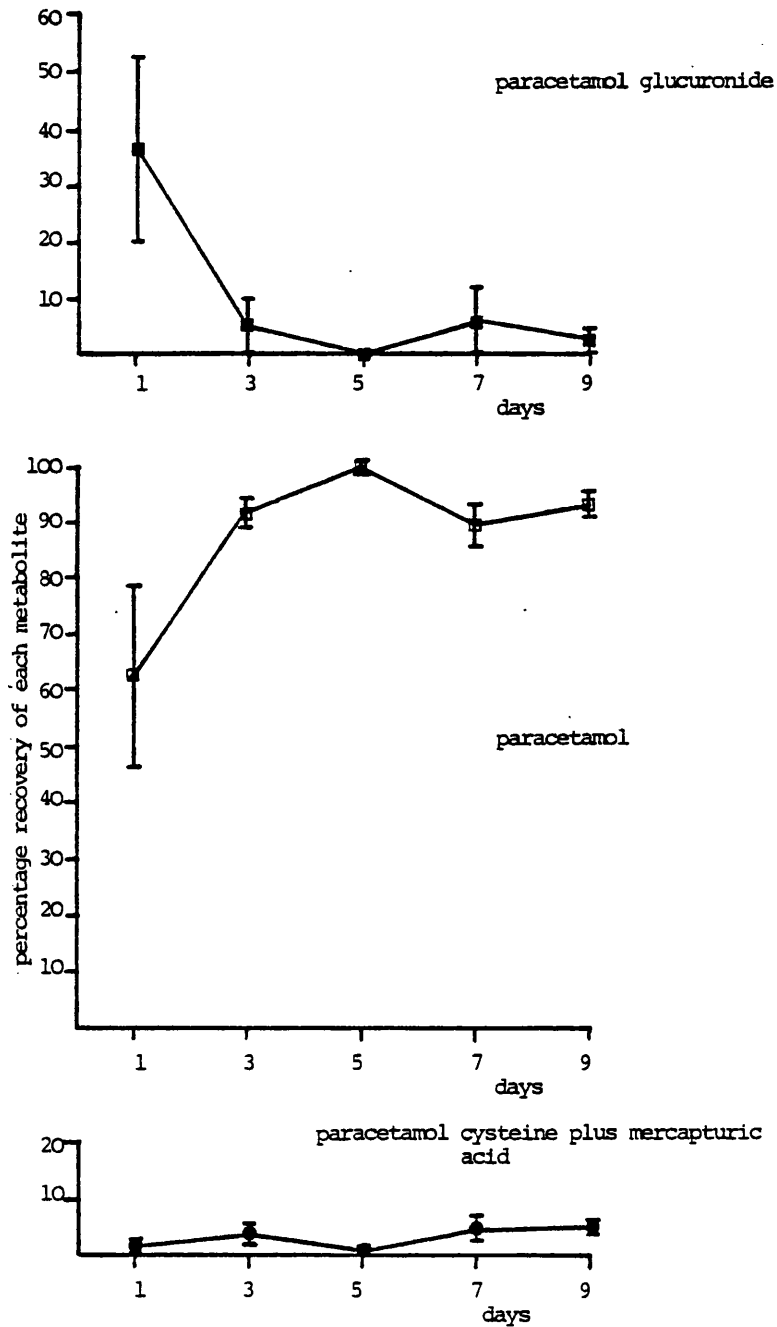
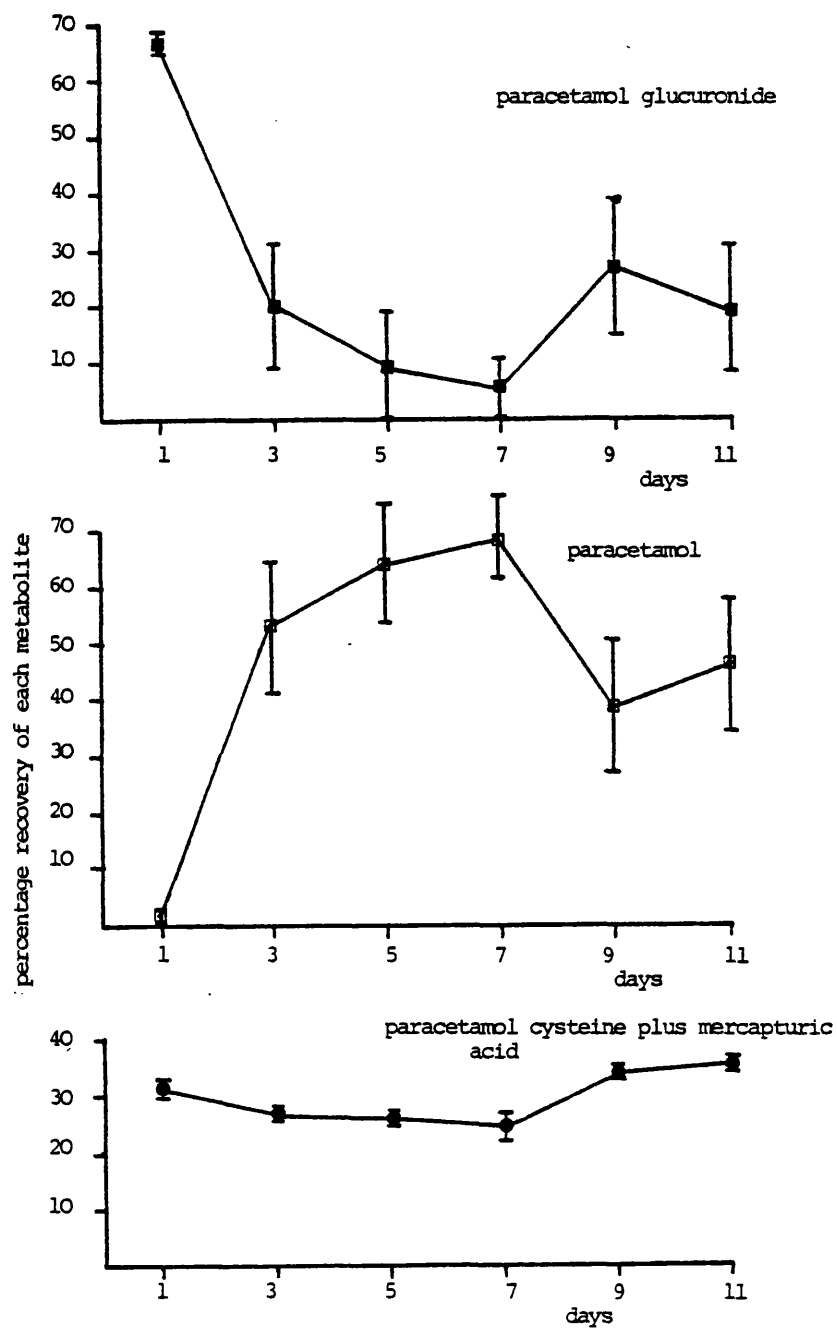


Fig. 5.2.2 Effect of repeated dosing on paracetamol metabolism in mice showing percentage recovery (means  $\pm$  S.E.M.) of paracetamol and its metabolites. Concentrations of paracetamol sulphate were below detection ( $<10\mu\text{g/ml}$ )



cysteine ( $p < 0.005$ ) and lower recoveries of unchanged paracetamol ( $p < 0.005$ ) and paracetamol sulphate ( $p < 0.005$ ) than mice after the single 100mg/kg dose. However, paracetamol glucuronide recovery decreased significantly with repeated dosing compared with the single dose recovery ( $p < 0.05$ ) in periods 72-120h and 120-168h although glucuronide excretion appeared to increase (though not to a significant extent) towards the end of the 11 day period compared with the single dose. The decrease in glucuronide excretion was accompanied by a significant increase in unchanged paracetamol in urine ( $p < 0.05$ ) in the same periods when compared with the single dose value. The amount of paracetamol sulphate excreted was always lower after repeated dosing than with the single dose ( $p < 0.005$ ). Recoveries of paracetamol cysteine plus mercapturic acid were significantly increased in the 168-216h and 216-264h collections ( $p < 0.005$ ) compared with the single dose values. These were due to significant increases in paracetamol cysteine recoveries ( $p < 0.001$ ) after repeated doses while mercapturate recoveries did not change.

Repeat dosing in mice was examined to compare the metabolic pattern seen in the first 24 hours with that seen in each subsequent 2 day period. There was a significant decrease in the recovery of paracetamol glucuronide ( $p < 0.05$ ) accompanied by a significant increase in paracetamol recovered unchanged ( $p < 0.025$ ) over the 11 days. No detectable paracetamol sulphate was excreted by any of the mice during the 0-11 day period in contrast to the finding with the single dose. Percentages of paracetamol cysteine plus mercapturic acid recovered were significantly decreased in the 72-120h period ( $p < 0.05$ ) but at later collection periods the trend was reversed and by the 216-264 hour period recoveries of these metabolites were significantly increased ( $p < 0.05$ ) compared with recoveries in the 0-24h period. When paracetamol cysteine recoveries alone were examined, they were found to be significantly lower from 24h-120h compared with the first 24 hours ( $p < 0.05$ ) but by the 216-264h period were significantly higher ( $p < 0.025$ ) than recoveries in 0-24 hour urine. Paracetamol mercapturate concentrations did not alter until they became significantly higher in the 216-264h period compared with 0-24 hour urine.

### 5.3 The Effect of Metabolic Competing Agents on Paracetamol Metabolism in Guinea Pigs and Mice

#### Introduction

In this experiment the effects of three drugs which could alter the metabolism of paracetamol by competing for metabolic substrates were studied. Salicylamide may compete with paracetamol for sulphate and glucuronic acid (Levy and Yamada, 1971). L-ascorbic acid and  $\alpha$ -tocopherol also conjugate with sulphate but also have other properties which appear to protect the liver from the toxicity of paracetamol (Raghuram, Krishnamurthi and Kalamegham, 1978; Walker, Kelleher, Dixon and Losowsky, 1974).

#### Procedures

4 male guinea pigs were given paracetamol 100mg/kg i.p. in a single dose using a standard solution of 100mg/ml in 0.9% saline. The competing agent was given immediately before this in a dose of 100mg/kg i.p. using a standard solution of 100mg/ml in 0.9% saline for L-ascorbic acid and salicylamide and a standard solution of 200mg/ml for  $\alpha$ -tocopherol. Guinea pigs were housed in individual metabolic cages (Associated Crates Limited) for 24 hours in a constant light (5am - 7pm) - dark (7pm-5am) cycle for urine collection. Urine was analyzed by HPLC for paracetamol and its metabolites.

24 male mice were given paracetamol 100mg/kg i.p. in a single dose using a standard solution of 10mg/kg in 0.9% saline for L-ascorbic acid and salicylamide and a standard solution of 10mg/ml in distilled water for  $\alpha$ -tocopherol. Mice were housed in metabolic cages (North Kent Plastic) for 24 hours in a constant light (5am - 7pm) - dark (7pm - 5am) cycle for urine collection. Urine was analyzed by HPLC for paracetamol and its metabolites.

## Results

Table 5.3.1. and 5.3.2. give the percentage recoveries of unchanged paracetamol and its metabolites and its four major metabolites after paracetamol 100mg/kg alone and after the same dose of paracetamol given with 100mg/kg of each competing agent to guinea pigs and mice. The results are plotted in Figs. 5.3.1, 5.3.2 and 5.3.3. for guinea pigs and Figs 5.3.4, 5.3.5 and 5.3.6 for mice. Individual results are given in Appendix 21 (guinea pigs) and Appendix 22 (mice).

The three competing agents appear to alter the metabolism of paracetamol in guinea pigs to a similar extent. All three caused a significant increase in the percentage recovery of paracetamol glucuronide ( $p < 0.05$ ) accompanied by a significant decrease in unchanged paracetamol ( $p < 0.05$ ). L-ascorbic acid and salicylamide did not change the metabolic profile of paracetamol significantly in mice but  $\alpha$ -tocopherol significantly increased the recoveries of paracetamol glucuronide and paracetamol cysteine and mercapturic acid ( $p < 0.05$ ) and decreased the recovery of unchanged paracetamol ( $p < 0.025$ ). None of the percentage recoveries of other metabolites of paracetamol in guinea pigs and mice was significantly changed.

**Table 5.3.1** Means  $\pm$  S.E.M. of percentage recoveries of paracetamol and its metabolites after administration of paracetamol concurrently with metabolic competing agents to guinea pigs

	n	P	PG	PS	PC + PM
paracetamol 100mg/kg	4	51.76 $\pm$ 15.74	46.00 $\pm$ 15.24	0.28 $\pm$ 0.28	1.96 $\pm$ 0.95
paracetamol 100mg/kg + salicylamide 100mg/kg	4	6.08 $\pm$ 0.47	92.50 $\pm$ 0.88	0.00 $\pm$ 0.00	1.42 $\pm$ 0.88
paracetamol 100mg/kg + L-ascorbic acid 100mg/kg	4	7.90 $\pm$ 1.47	90.43 $\pm$ 1.91	1.68 $\pm$ 1.04	0.00 $\pm$ 0.00
paracetamol 100mg/kg + $\alpha$ -tocopherol 100mg/kg	4	1.73 $\pm$ 0.56	95.42 $\pm$ 1.92	2.18 $\pm$ 1.73	0.66 $\pm$ 0.66

**Table 5.3.2. Means  $\pm$  S.E.M. of percentage recoveries of paracetamol and its metabolites after administration of paracetamol concurrently with metabolic competing agents to mice**

	n	P	PG	PS	PC + PM
paracetamol 100mg/kg	6	30.31 $\pm$ 7.67	40.68 $\pm$ 8.10	2.98 $\pm$ 0.78	26.03 $\pm$ 1.53
paracetamol 100mg/kg + salicylamide 100mg/kg	6	23.75 $\pm$ 4.07	42.87 $\pm$ 6.76	1.08 $\pm$ 0.42	32.29 $\pm$ 3.47
paracetamol 100mg/kg + L-ascorbic acid 100mg/kg	6	14.62 $\pm$ 2.48	50.60 $\pm$ 2.46	10.11 $\pm$ 0.91	24.67 $\pm$ 1.27
paracetamol 100mg/kg + $\alpha$ -tocopherol 100mg/kg	6	5.36 $\pm$ 0.71	62.18 $\pm$ 1.01	1.74 $\pm$ 0.66	30.43 $\pm$ 1.15

Fig. 5.3.1.  
Effect of salicylamide on paracetamol metabolism in guinea pigs  
(means  $\pm$  S.E.M.)

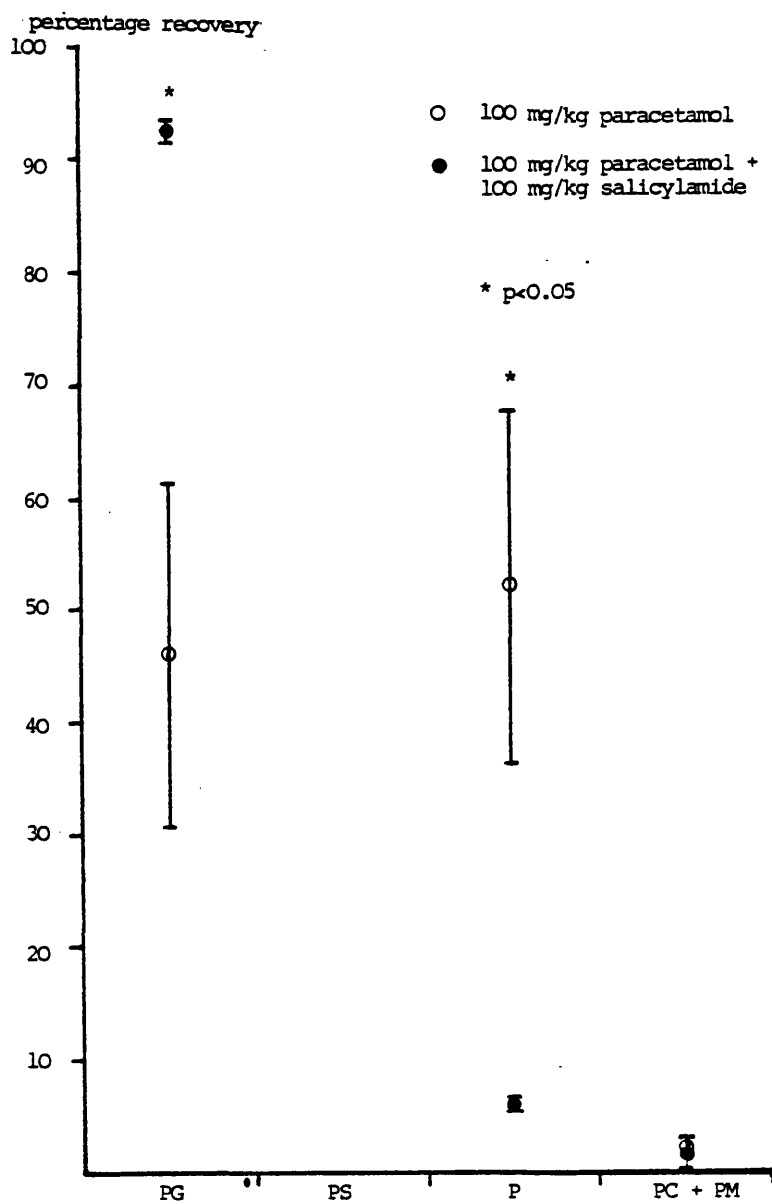




Fig. 5.3.2.  
Effect of L-ascorbic acid on paracetamol metabolism in guinea pigs (means  $\pm$  S.E.M.)

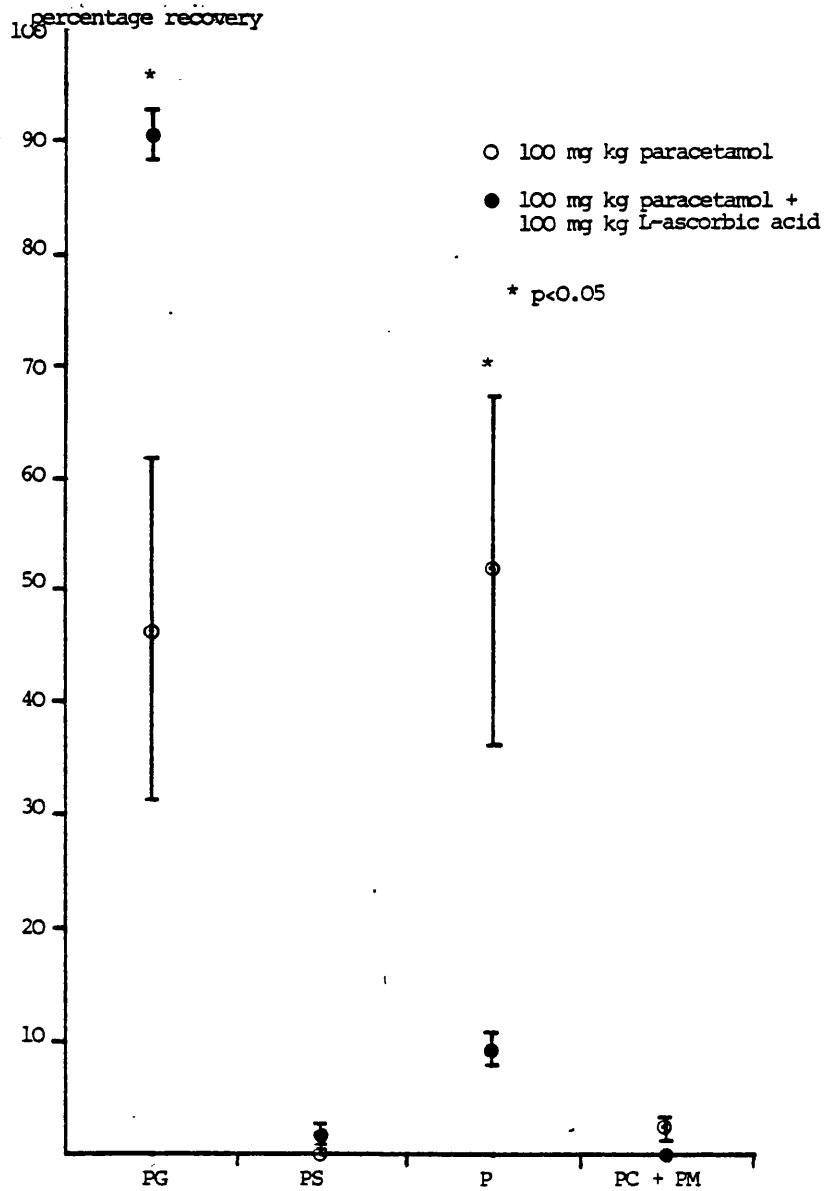


Fig. 5.3.3 Effect of  $\alpha$ -tocopherol on paracetamol metabolism in guinea pigs (means  $\pm$  S.E.M.)

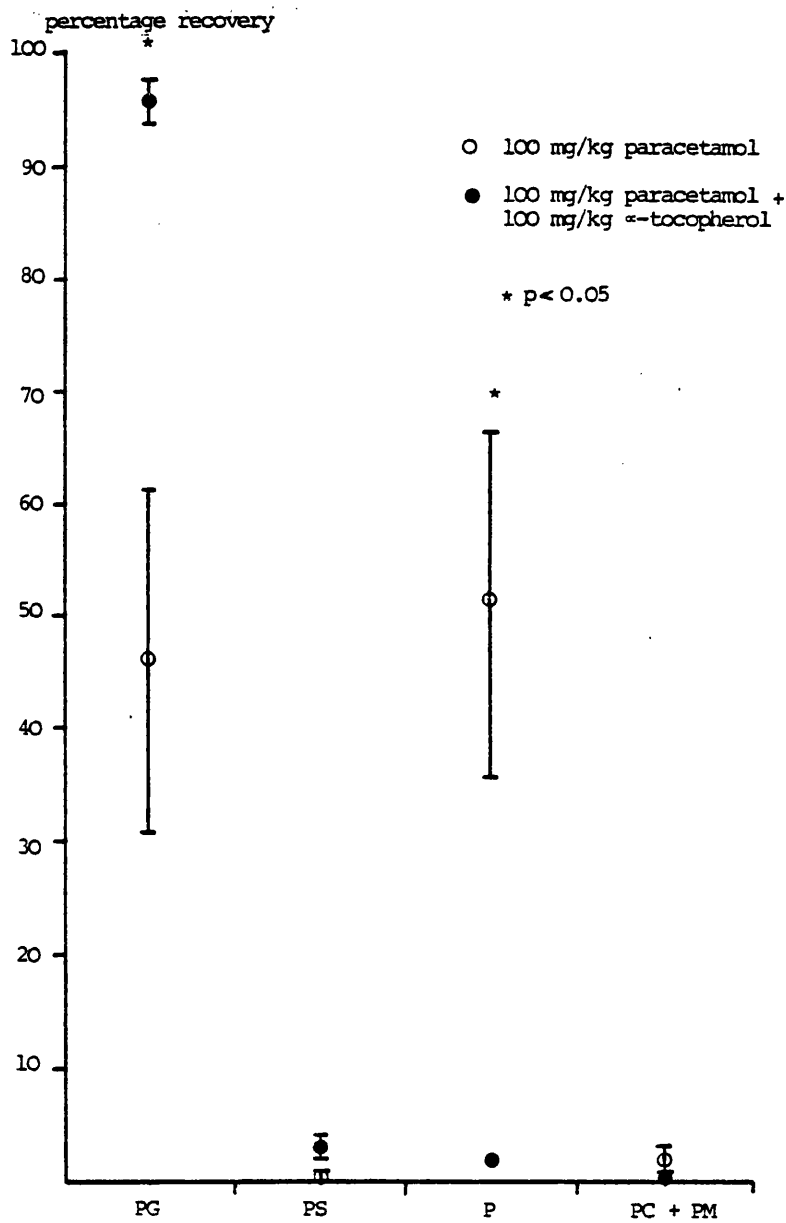


Fig. 5.3.4 Effect of salicylamide on paracetamol metabolism in mice (means  $\pm$  S.E.M.)

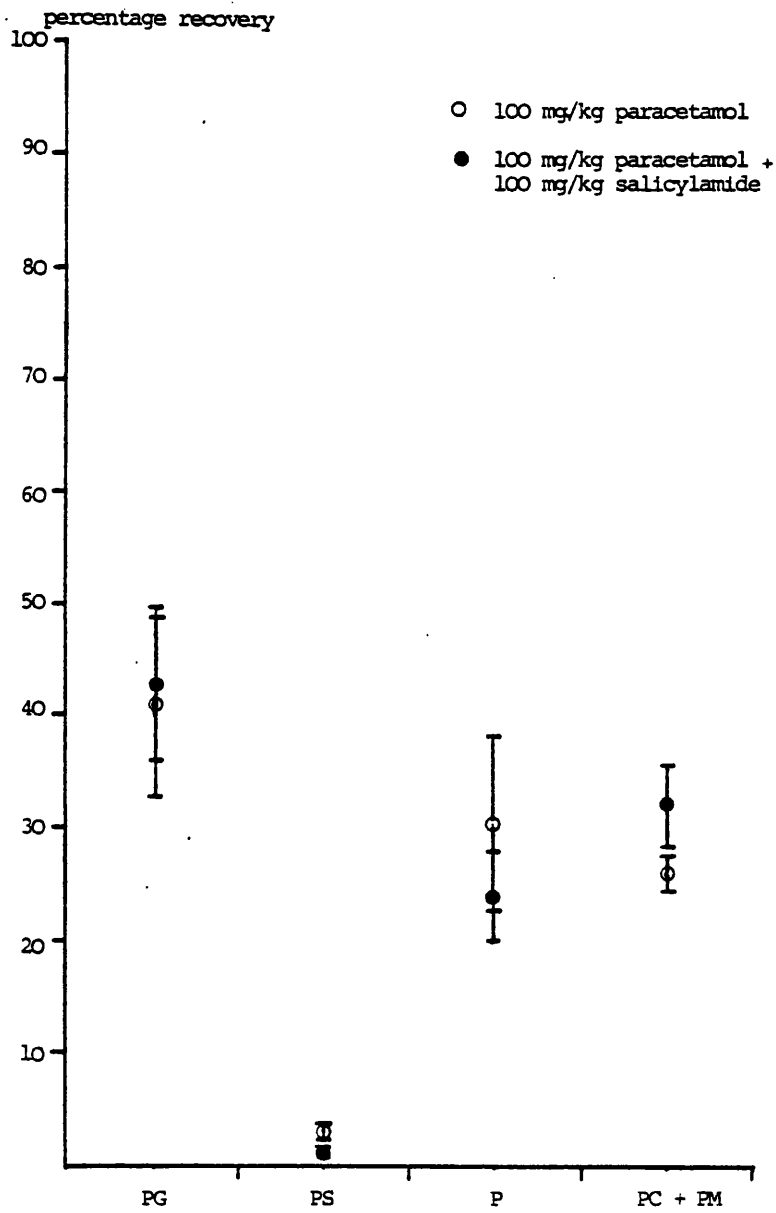


Fig. 5.3.5.  
Effect of L-ascorbic acid on paracetamol metabolism in mice  
(means  $\pm$  S.E.M.)

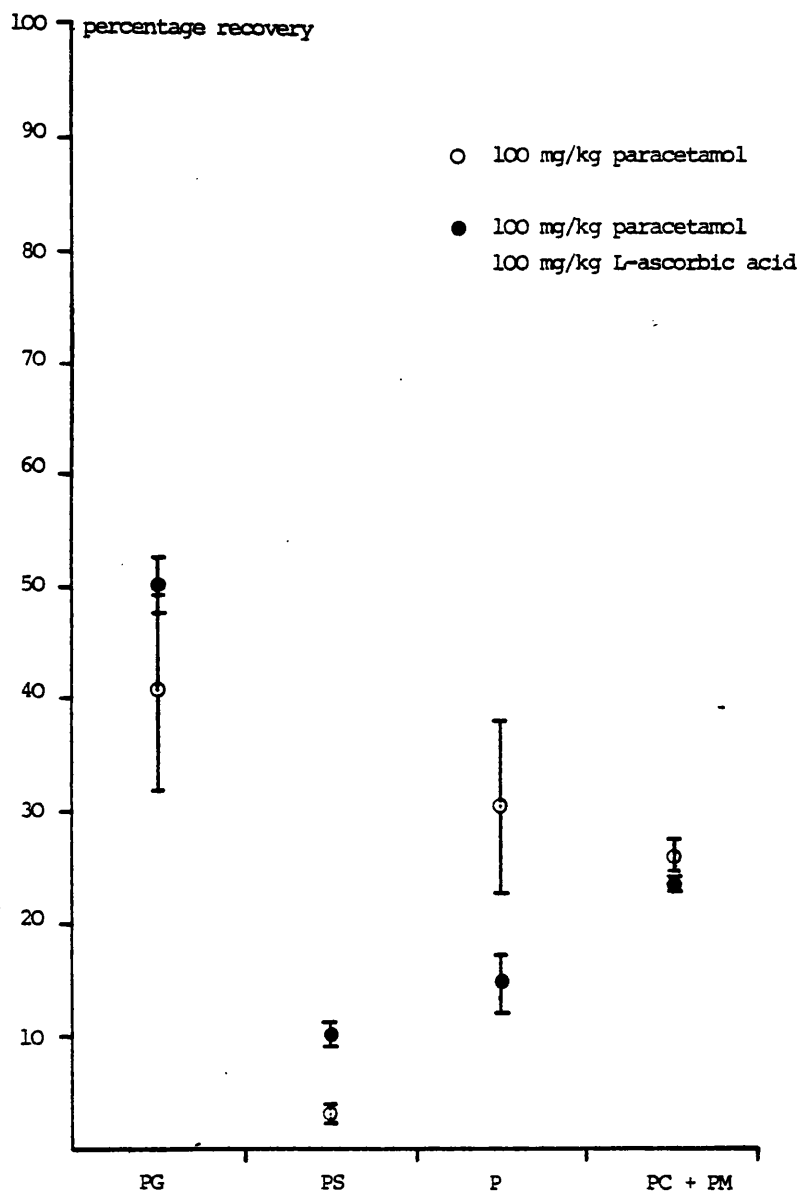
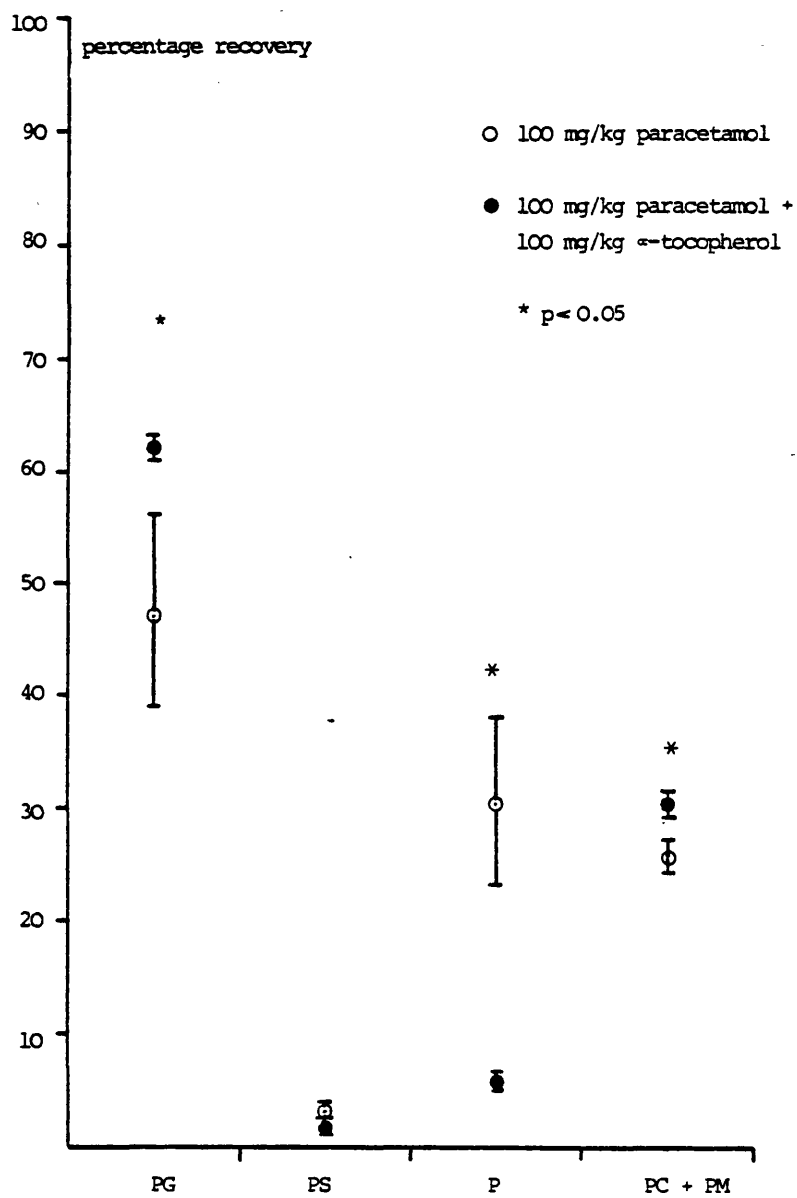


Fig. 5.3.6.  
Effect of  $\alpha$ -tocopherol on paracetamol metabolism in mice  
(means  $\pm$  S.E.M.)



#### 5.4 Metabolism of paracetamol in rabbits in the presence of L-methionine

##### Introduction

Results presented in Section 3.6 suggested that L-methionine administered to patients with paracetamol poisoning caused increased recoveries of the sulphate and cysteine plus mercapturic acid conjugates of paracetamol compared with recoveries of these metabolites before treatment. The experiments described below were carried out to examine the overdose situation in detail and were carried out in rabbits because of the relative ease of obtaining blood samples from this species compared with small laboratory animals. Although Davis et al, (1974) suggested that the rabbit is not a species susceptible to paracetamol-induced hepatotoxicity (8% showed some degree of centrilobular hepatic necrosis after paracetamol 750mg/kg), its urinary metabolic profile is similar to man.

##### Procedures

As no males were available, 3 female rabbits were used throughout the study, each acting as its own control. Table 5.4.1. details the doses of paracetamol and L-methionine given in Experiments 1, 2,, 3, 3 repeat and 4 which occurred in sequence. During each experiment blood samples (approximately 3ml from each rabbit) were taken every 45 minutes for 5.25 hours after dosing. Food and were allowed ad libitum and rabbits were housed in individual metabolic cages (Associated Crates Ltd) for 24 hours after dosing so that urine could be collected. Serum and urine samples were analyzed by HPLC as described in Section 2.7.b. At least fourteen days were allowed to elapse between experiments so that recovery of blood volume might be achieved. The area under the serum concentration time curve was calculated using the trapezoidal rule described by Natari (1975) from 0 - 315 minutes.

Table 5.4.1 Experimental regime carried out to determine the effects of L-methionine on metabolism and kinetics of paracetamol

Experiment	Time (days)	Dosing regimen (mg/kg)	Stock Solutions (mg/ml)	Comments
1	0	paracetamol 100mg/kg p.o.	100mg/ml saturated N-saline solutions	Study of the metabolism of paracetamol following a non-toxic dose of paracetamol (Davis et al, 1974)
2	19	paracetamol 400mg/kg p.o.	200mg/ml saturated N-saline solutions	Study of the metabolism of paracetamol following a potentially toxic dose of paracetamol but one unlikely to cause death Davis et al, (1974) found that paracetamol 750mg/kg caused 40% mortality in rabbits.
3	40	paracetamol 100mg/kg	100mg/ml saturated N-saline solution	These experiments were performed to ensure that no hepatotoxicity had occurred as result of expt. 2 as shown by absence of a change in metabolic pattern from experiment 1.
3 repeat	54	paracetamol 100mg/kg	100mg/ml saturated N-saline solution	Because it was found that paracetamol AUC was longer in Experiment 3 than in Experiment 1 Experiment 3 was repeated 14 days after the previous Experiment 3.
4	82	paracetamol 400mg/kg p.o. given just prior to L-methionine 400mg/kg p.o.	Both administered as 200mg/ml saturated N-saline solution	Study of the metabolism of a potentially toxic dose of paracetamol in the presence of L-methionine

## Results

Values of AUC for each animal during each experiment from 0 - 315 minutes after dosing are given in Table 5.4.2. When these figures were corrected for rabbit weight (shown in brackets), there was found to be a significant increase in AUC after paracetamol 400mg/kg (Experiment 2) compared with after paracetamol 100mg/kg in Experiments 1, 3 and 3 repeat ( $p < 0.02$ ). There was no significant difference between AUCs in Experiments 1, 3 and 3 repeat after doses of 100mg/kg ( $p > 0.05$ ). When methionine 400mg/kg was given with paracetamol 400mg/kg in Experiment 4, there was no significant difference between the AUCs calculated for this and Experiment 2 ( $p > 0.005$ ).



Table 5.4.2. Area under serum concentration time curves for paracetamol ( $\mu\text{g min ml}^{-1}$ ) with corrections for rabbit weight shown in brackets

		Rabbit A	Rabbit B	Rabbit C
Expt. 1	dose = paracetamol 100mg/kg	834.75 (0.184)	484.65 (0.171)	404.25 (0.113)
Expt. 2	dose = paracetamol 400mg/kg	9275.62 (2.027)	2992.70 (0.983)	4584.38 (1.280)
Expt. 3	dose = paracetamol 100mg/kg	861.75 (0.188)	586.35 (0.206)	368.55 (0.103)
Expt. 3 repeat	dose = paracetamol 100mg/kg	1028.70 (0.225)	567.60 (0.200)	642.15 (0.179)
Expt. 4	dose = paracetamol 400mg/kg	4489.65 (0.981)	2002.72 (0.705)	1800.90 (0.503)

Table 5.4.3 AUC ratios for Experiments 2 and 4 to Experiment 1

Rabbit	dose ratio	$\frac{\text{AUC Expt. 2}}{\text{AUC Expt. 1}}$	dose ratio	$\frac{\text{AUC Expt. 4}}{\text{AUC Expt. 1}}$
Rabbit A	4	11.0	4	5.3
Rabbit B	4	5.8	4	4.1
Rabbit C	4	11.3	4	4.4

Table 5.4.3 gives AUC ratios for Experiment 2 and 4 relative to Experiment 1 for each rabbit. It can be seen that after paracetamol 400mg/kg in Experiment 2 there is no indication of proportionality between AUC ratio and dose ratio compared with paracetamol 100mg/kg (Expt. 1). In Experiment 4 though when paracetamol 400mg/kg and L-methionine 400mg/kg were given in conjunction the data now suggest that first order kinetics are resumed although changes in two rabbits were more marked than in the third.

Serum concentrations of paracetamol against time are shown in Figs 5.4.1, 5.4.2, 5.4.3, 5.4.4, and 5.4.5. Figs 5.4.6, 5.4.7, 5.4.8, 5.4.9 and 5.4.10 show serum concentrations of paracetamol glucuronide and paracetamol sulphate in each experiment and levels of cysteine and mercapturic acid metabolites of paracetamol where these appeared in high enough concentrations. Concentrations of these four metabolites are (corrected for molecular weight) and expressed as percentage recovery and these values are also given in Table 5.4.4. It can be seen that while mercapturic acid concentrations in serum were low (and were only detectable in Experiments 1 and 2) compared with serum cysteine levels, in urine mercapturate levels showed a mean of  $8.3\% \pm 1.3\%$  compared with mean urinary cysteine levels of  $0.8\% \pm 0.3\%$  ( $n = 12$ , calculated from Experiments 1, 2, 3 repeat and 4 as shown in Table 5.4.4 and in Fig 5.4.11).

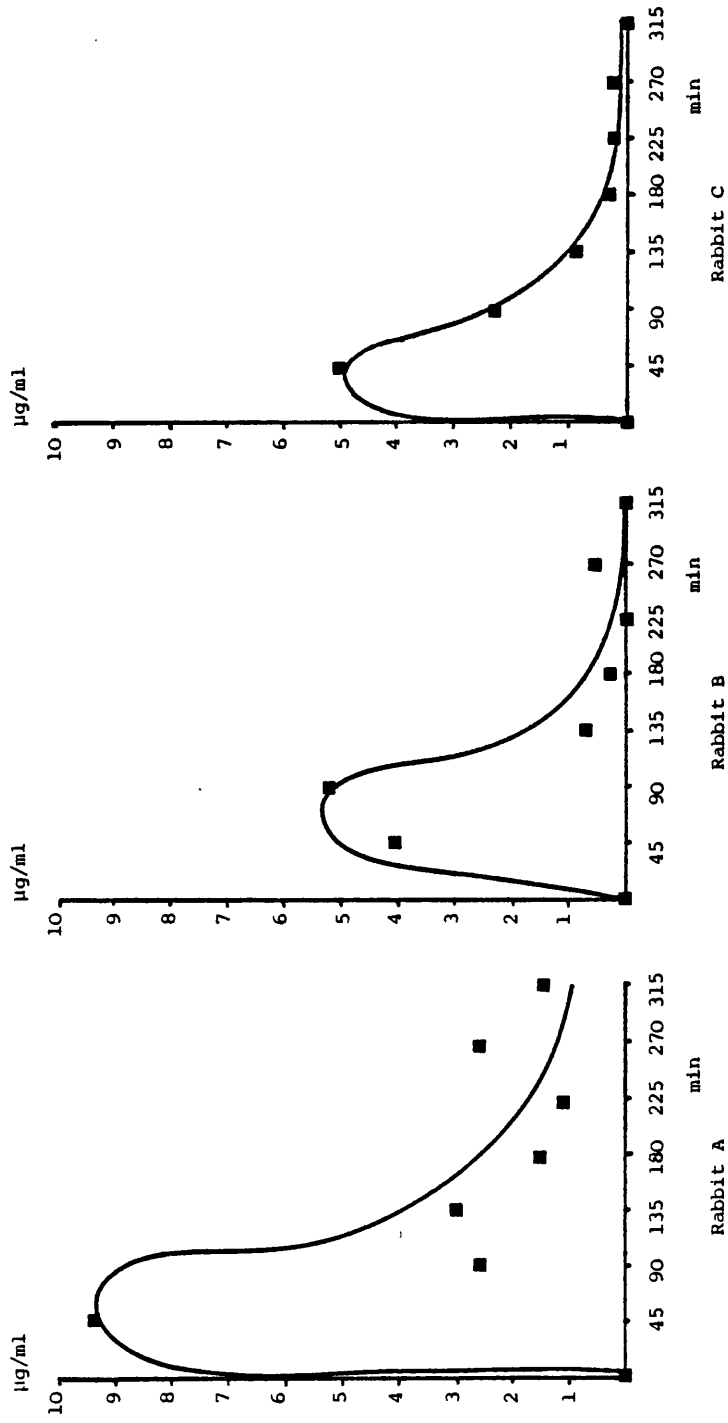


Fig. 5.4.1. Serum concentrations of unchanged paracetamol in rabbits after paracetamol 100mg/kg p.o. plotted against time (Experiment 1)

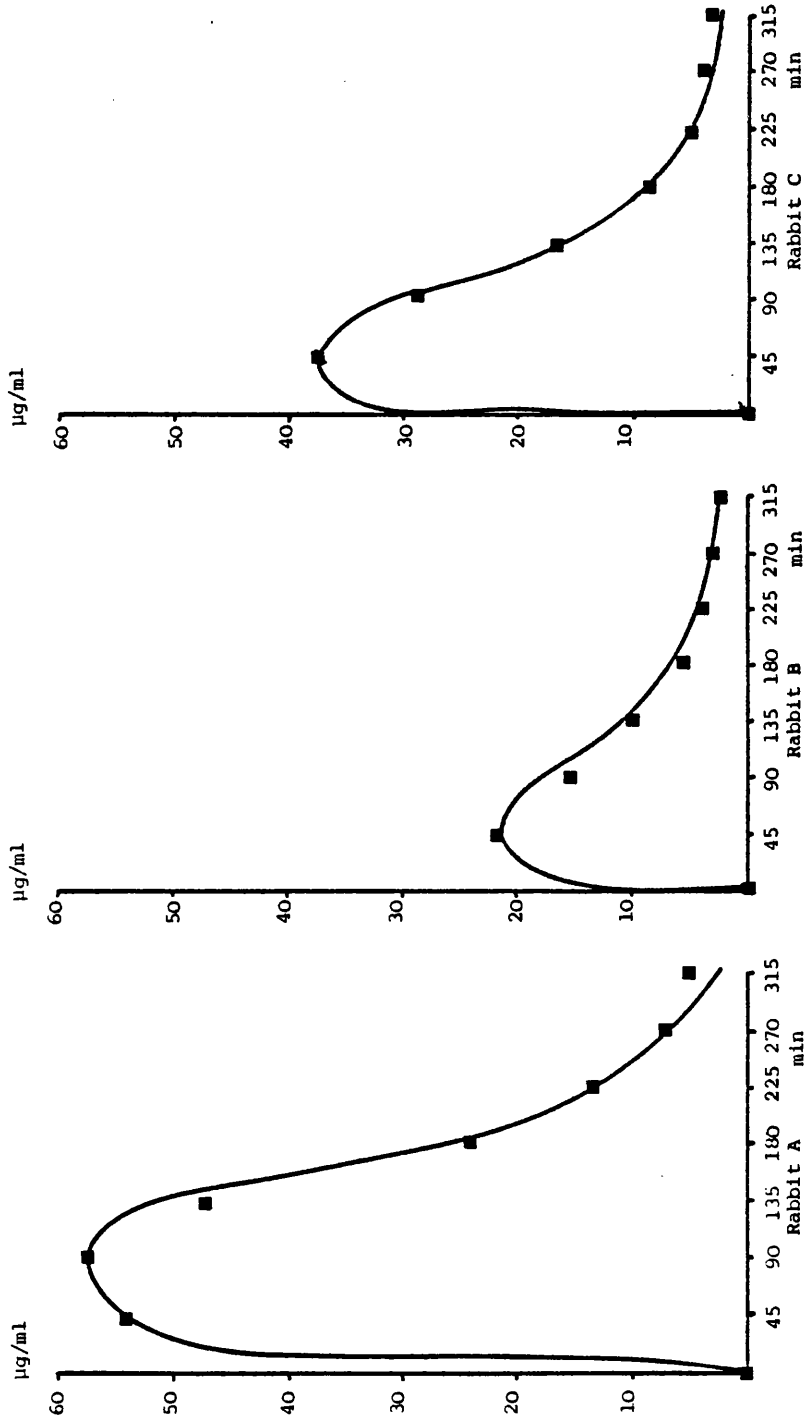


Fig. 5.4.2. Serum concentrations of unchanged paracetamol in rabbits after paracetamol 400mg/kg p.o. plotted against time (Experiment 2)

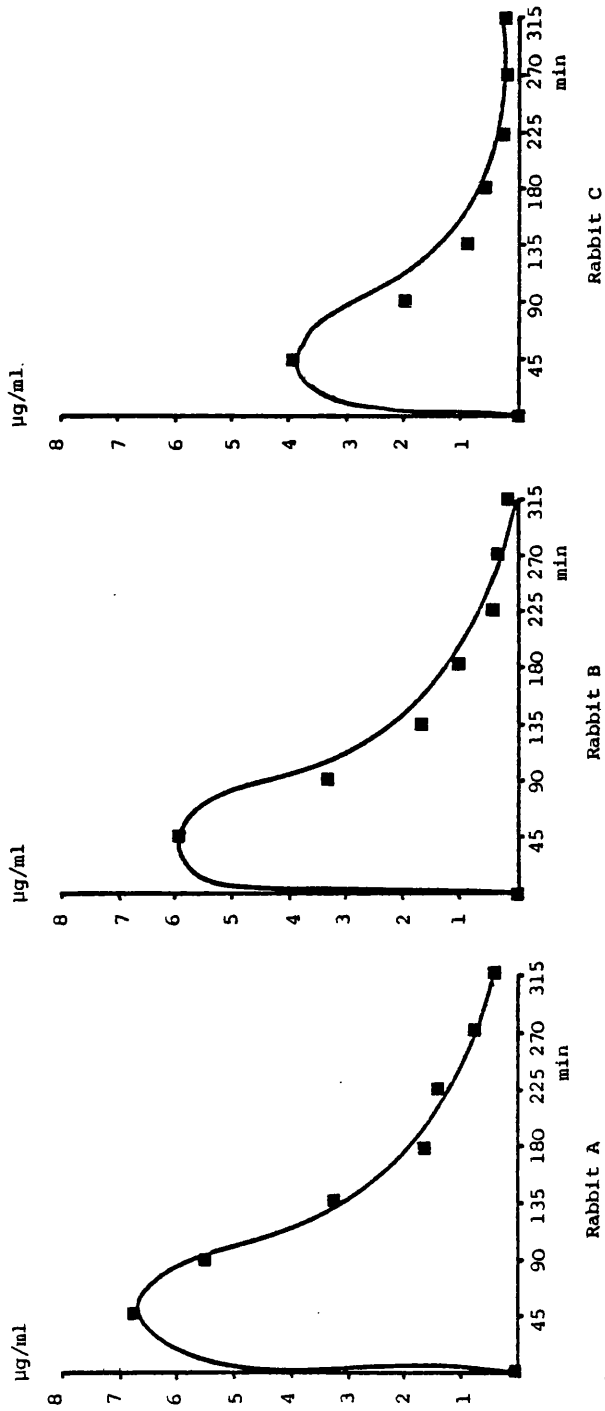


Fig. 5.4.3. Serum concentrations of unchanged paracetamol in rabbits after paracetamol 100mg/kg p.o. plotted against time (Experiment 3)

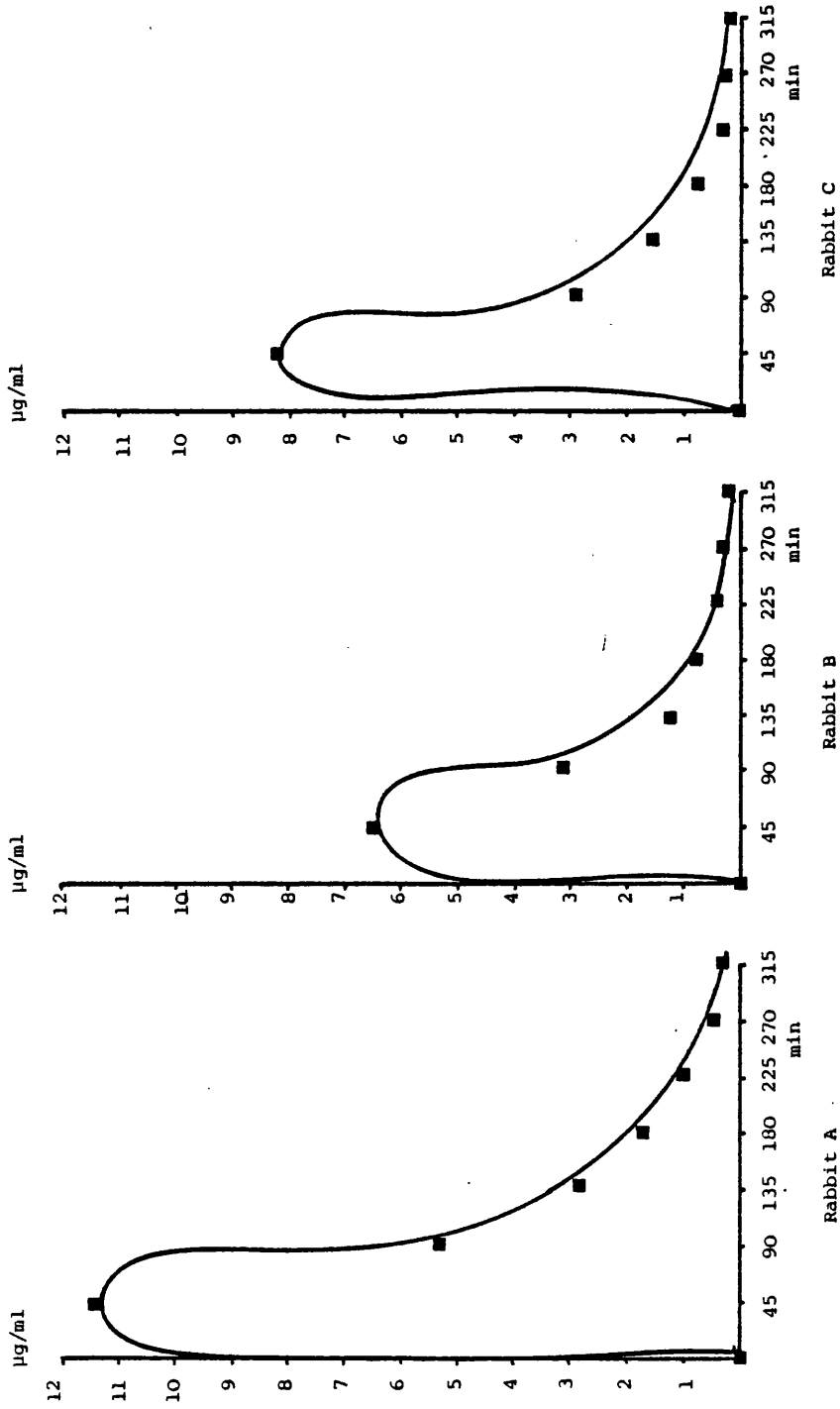


Fig. 5.4.4. Serum concentrations of unchanged paracetamol in rabbits after paracetamol 100mg/kg p.o. plotted against time (Experiment 3 repeat)

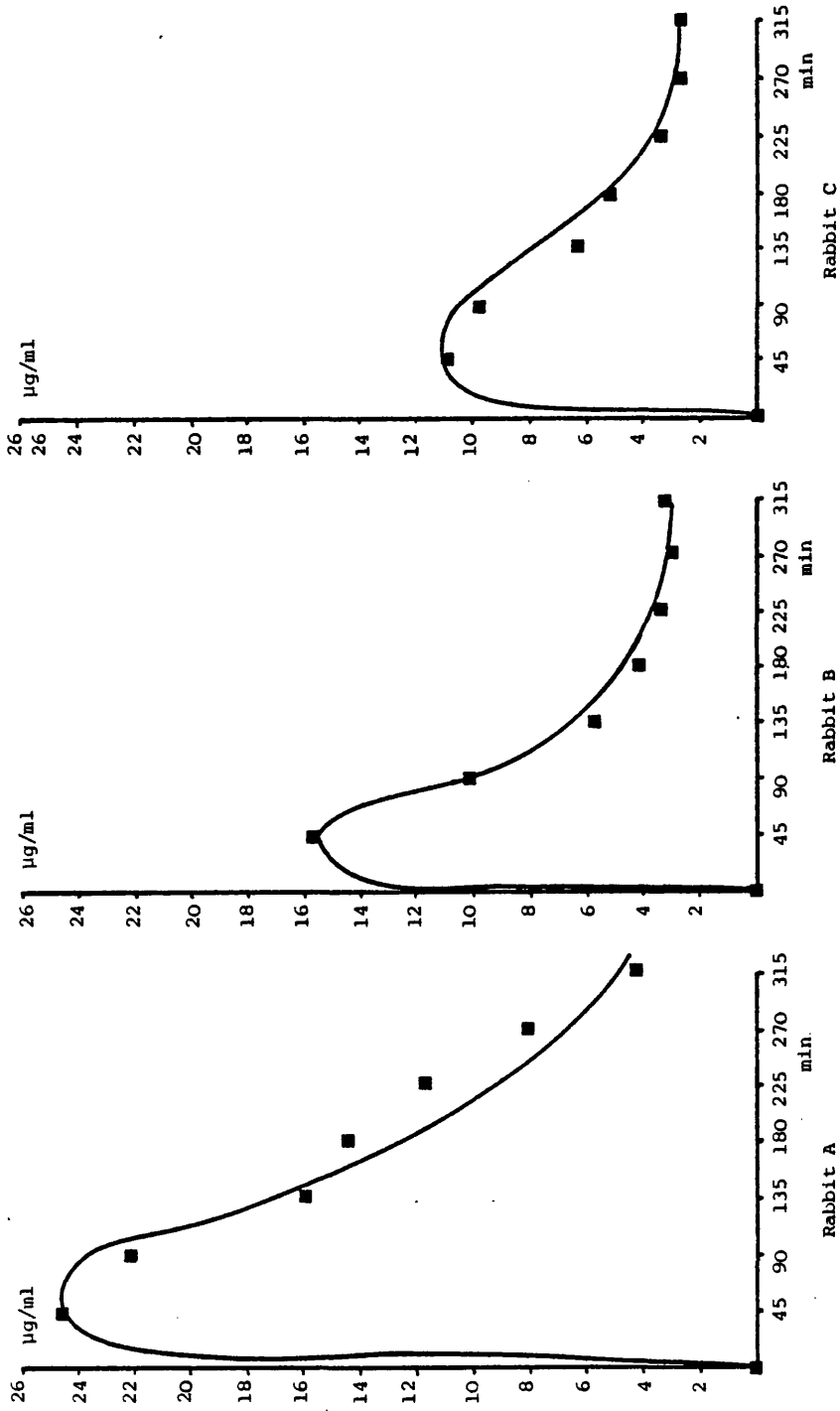


Fig. 5.4.5. Serum concentrations of unchanged paracetamol in rabbits after paracetamol 400mg/kg p.o. and L-methionine 400mg/kg p.o. plotted against time (Experiment 4)

Fig. 5.4.6 Serum concentrations of the glucuronide, sulphate, cysteine and mercapturate conjugates of paracetamol after 100mg/kg orally (Experiment 1)

Key: ■ paracetamol glucuronide  
 ▲ paracetamol cysteine  
 □ paracetamol mercapturic acid  
 △ paracetamol sulphate

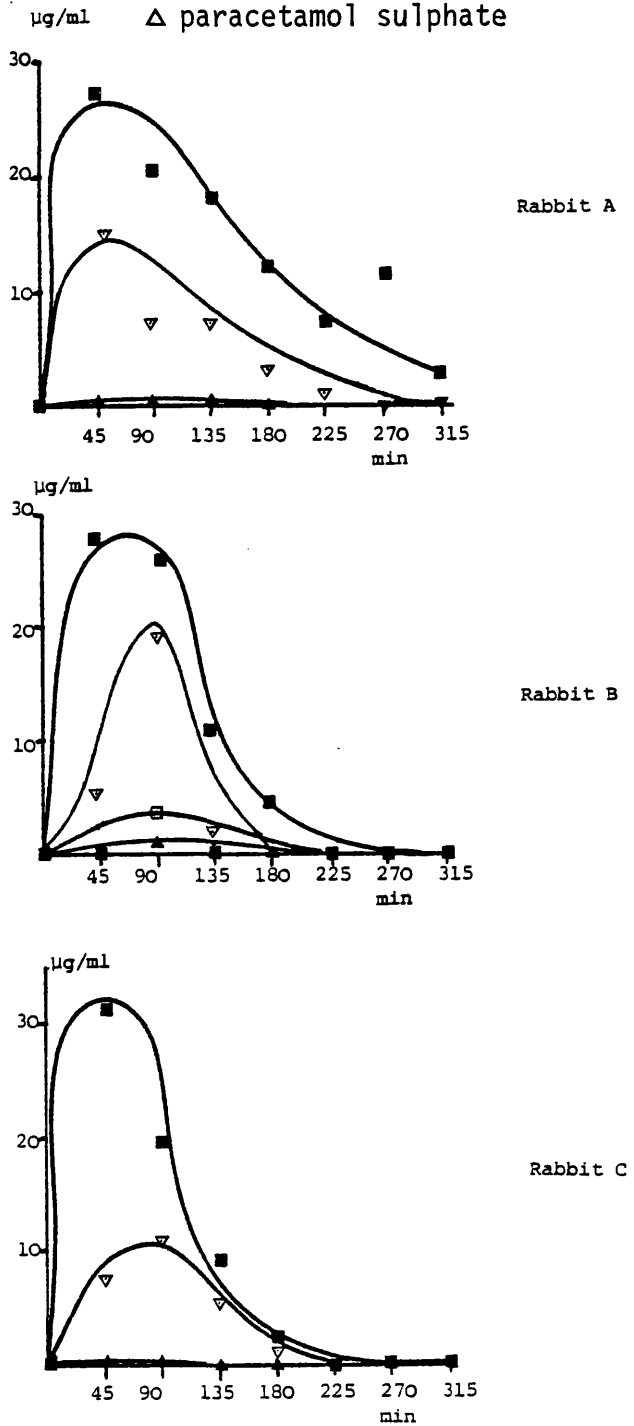




Fig. 5.4.7 Serum concentrations of the glucuronide and sulphate conjugates of paracetamol after 400mg/kg orally (Experiment 2)

Key: ■ paracetamol glucuronide  
 ▽ paracetamol sulphate

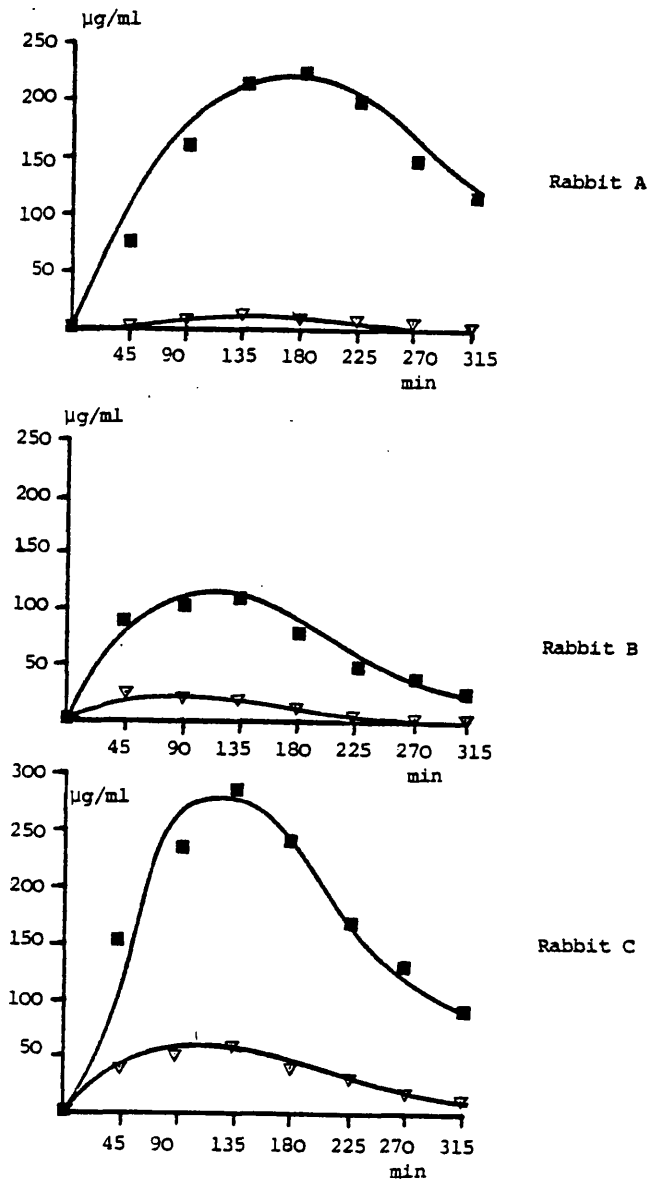


Fig. 5.4.8 Serum concentrations of the glucuronide, sulphate and cysteine conjugates of paracetamol after 100mg/kg paracetamol orally (Experiment 3)

Key: ■ paracetamol glucuronide  
▽ paracetamol sulphate  
▼ paracetamol cysteine

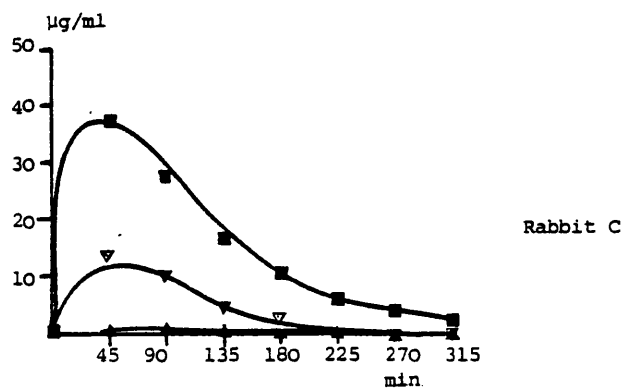
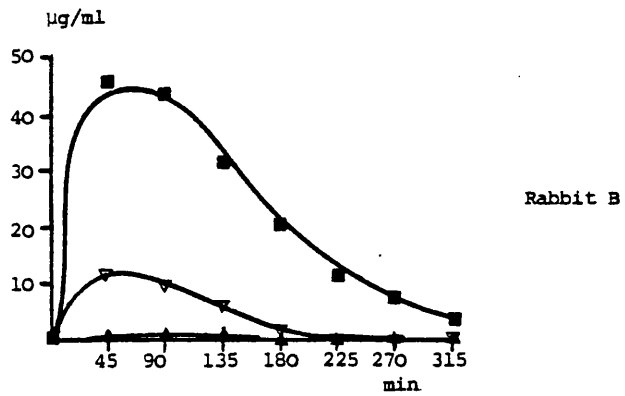
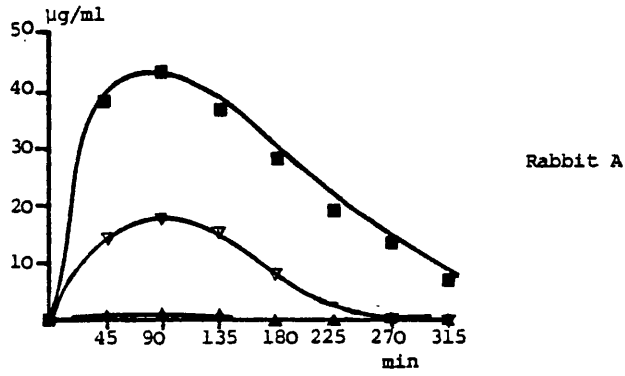
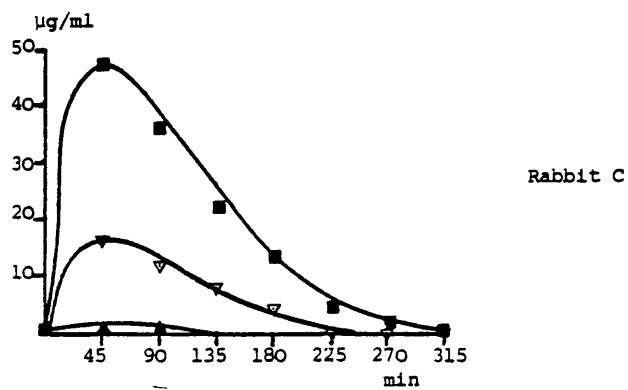
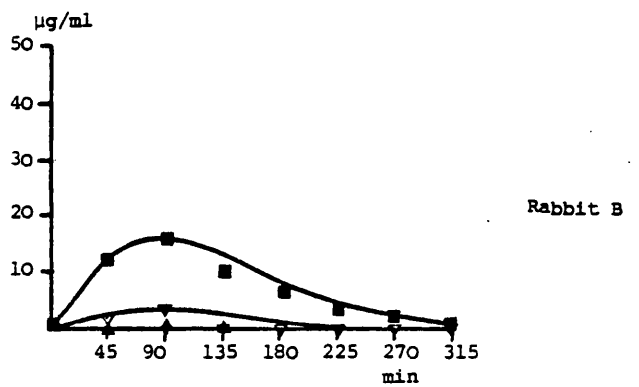
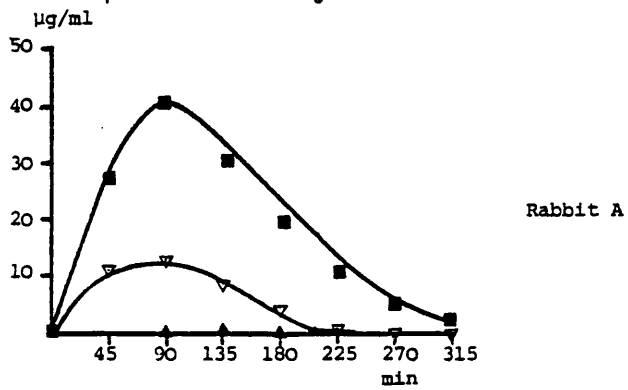


Fig. 5.4.9 Serum concentrations of the glucuronide, sulphate and cysteine conjugates of paracetamol after 100mg/kg orally (Experiment 3 repeat)

Key: ■ paracetamol glucuronide

▽ paracetamol sulphate

▼ paracetamol cysteine



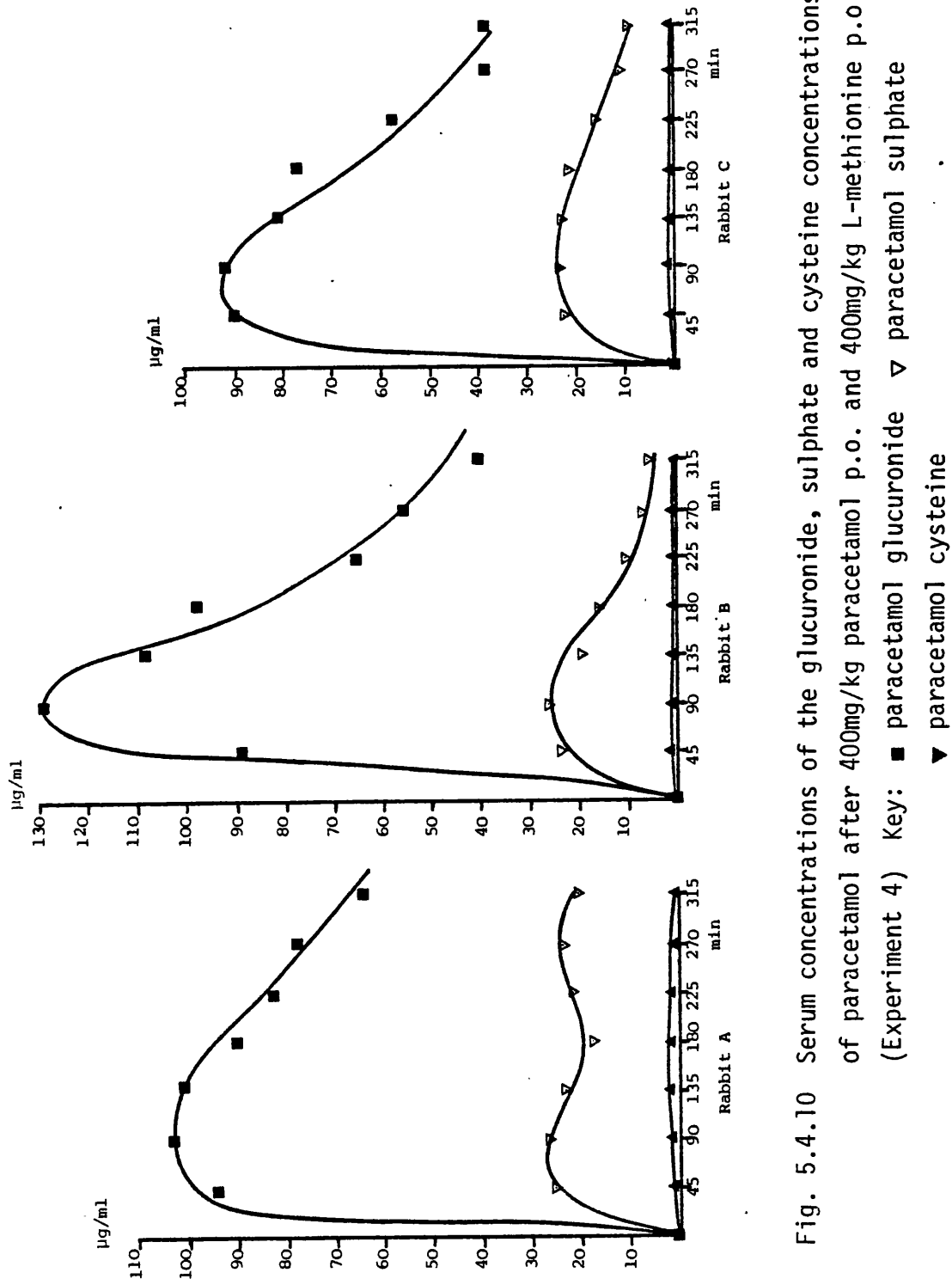


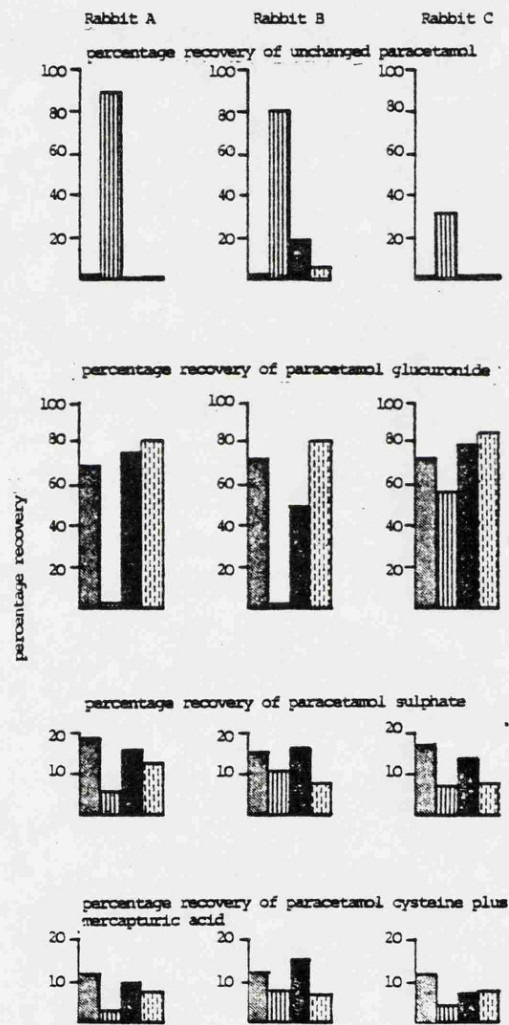
Fig. 5.4.10 Serum concentrations of the glucuronide, sulphate and cysteine concentrations of paracetamol after 400mg/kg paracetamol p.o. and 400mg/kg L-methionine p.o. (Experiment 4) Key: ■ paracetamol, ▽ paracetamol glucuronide, ▴ paracetamol cysteine

**Table 5.4.4** Percentage urinary recovery of paracetamol and its metabolites

	P	PG	PS	PC	PM	PC + PM
<u>Expt. 1</u>						
Rabbit A	1.46	68.44	18.42	0.21	11.46	11.67
Rabbit B	1.35	71.85	14.80	0.16	11.83	11.99
Rabbit C	1.02	71.39	16.95	0.24	10.40	10.64
<u>Expt. 2</u>						
Rabbit A	88.66	2.78	5.69	0.09	2.78	2.87
Rabbit B	80.86	0.70	10.58	0.11	7.74	7.85
Rabbit C	33.67	55.87	6.31	0.55	3.59	4.14
<u>Expt. 3 rpt</u>						
Rabbit A	0.10	74.51	15.63	0.42	9.34	9.76
Rabbit B	19.76	48.62	16.45	0.41	14.76	15.17
Rabbit C	0.30	78.71	14.07	0.93	5.99	6.92
<u>Expt. 4</u>						
Rabbit A	0.39	80.38	12.13	3.17	3.92	7.09
Rabbit B	4.59	81.27	7.43	1.20	5.51	6.71
Rabbit C	0.66	4.70	7.46	2.24	4.93	7.17

After the high dose of paracetamol (400mg/kg) in experiment 2 liver damage may well have occurred

Fig. 5.4.11 Urinary excretion of paracetamol and its metabolites in rabbits. Results are expressed as a percentage recovery for each of three rabbits in four experiments



Key: Experiment 1 dose of 100mg/kg paracetamol  
 Experiment 2 dose of 400mg/kg paracetamol  
 Experiment 3 repeat dose of 100mg/kg paracetamol  
 Experiment 4 dose of 400mg/kg paracetamol + 400mg/kg L-methionine

Urinary concentrations of paracetamol cysteine plus mercapturic acid were significantly decreased after paracetamol 400mg/kg (Experiment 2) compared with both the control 100mg/kg dose in Experiment 1 ( $p < 0.05$ ) and Experiment 3 repeat ( $p < 0.02$ ). Two out of 3 rabbits showed an increase in the percentage cysteine and mercapturic acid recovery when L-methionine was given just prior to paracetamol 400mg/kg (Experiment 4) compared with paracetamol 400mg/kg alone (Experiment 2). However, when compared with concentrations found in Experiment 1, cysteine and mercapturic acid concentrations after methionine were significantly lower ( $p < 0.02$ )

It can also be seen from Table 5.4.4 and Fig 5.4.11 that after the 400mg/kg dose of paracetamol administered in Experiment 2, the amount excreted as paracetamol glucuronide was markedly decreased, accompanied by an increase in paracetamol recovered unchanged (compared with values in Experiments 1 and 3 repeat) although this did not reach significance when a paired 't' test was used. However, peak serum concentration of paracetamol glucuronide in Experiment 2 showed the opposite, that is that peak serum glucuronide concentrations were higher than paracetamol concentrations (as shown in Table 5.4.5). In Experiment 4, Table 5.4.5 shows that peak serum paracetamol concentrations were lower than in Experiment 2 and paracetamol glucuronide peak levels were lower in 2 of the 3 rabbits.

**Table 5.4.5 Peak serum concentrations of paracetamol and paracetamol glucuronide in Experiments 2 and 4**

		Peak serum concentrations ( $\mu\text{g}/\text{ml}$ ) corrected for molecular weight)					
		paracetamol			paracetamol glucuronide		
		Rabbit A	Rabbit B	Rabbit C	Rabbit A	Rabbit B	Rabbit C
Expt. 2	paracetamol 400mg/kg	57.76	22.09	37.50	225.63	106.24	287.15
Expt. 4	paracetamol 400mg/kg+	24.75	15.91	11.15	103.80	128.85	91.70
	methionine 400mg/kg						



Table 5.4.6 gives values for the PG/PS ratio for each rabbit in each experiment in serum and urine. It can be seen that the PG/PS ratio in serum is higher over a longer period in Experiment 2 (dose of 400mg/kg) than in Experiment 1 (dose of 100mg/kg) (although this change does not reach significance) suggesting that sulphate depletion may have occurred. However, in urine in these two experiments the reverse occurred (because little paracetamol glucuronide was excreted, as has already been described). After L-methionine (Experiment 4) PG/PS ratios in serum remained similar to values in Experiment 1 because glucuronide concentrations were not as high as in Experiment 2.

In this series of experiments there was little to be gained from examination of (PG+PS)/(PC+PM) ratios as paracetamol glucuronide was present in by far the greatest amounts in both serum and urine except in Experiment 2 in which unchanged paracetamol was the dominant excretory product.

**Table 5.4.6** PG/PS ratio for each rabbit in urine (corrected for molecular weight)

Minutes	45	90	135	Serum 180	225	270	315	Urine
<u>Expt. 1</u>								
Rabbit A	2.55	3.87	3.39	5.29	9.87	∞	∞	3.71
Rabbit B	7.29	1.88	7.94	∞	∞	∞	∞	4.85
Rabbit C	5.84	2.56	2.35	3.15	∞	∞	∞	4.21
<u>Expt. 2</u>								
Rabbit A	16.33	19.32	21.42	29.30	26.01	27.51	38.26	0.49
Rabbit B	5.18	7.66	8.10	10.33	10.94	17.11	20.69	0.07
Rabbit C	5.29	6.52	6.54	8.07	7.31	9.90	9.28	8.85
<u>Expt. 3</u>								
Rabbit A	4.18	3.78	3.74	6.18	12.59	17.99	∞	∞
Rabbit B	5.44	6.35	6.97	13.44	∞	∞	∞	1.86
Rabbit C	3.79	3.57	4.29	4.54	∞	∞	∞	∞
<u>Expt. 3 rept</u>								
Rabbit A	3.57	4.41	4.88	6.49	15.10	∞	∞	4.77
Rabbit B	8.13	7.04	∞	∞	∞	∞	∞	2.96
Rabbit C	4.10	4.26	4.24	4.62	∞	∞	∞	5.59
<u>Expt. 4</u>								
Rabbit A	5.31	5.62	6.28	7.33	5.42	4.65	4.45	6.62
Rabbit B	5.25	6.97	7.89	8.49	8.53	9.01	10.61	10.93
Rabbit C	5.57	5.41	5.02	5.01	5.03	5.00	5.63	11.34

## Chapter 6

### Paracetamol Metabolism in Animals - Discussion

The capacity of paracetamol to cause hepatic necrosis was first noted in 1964 when Eder carried out toxicity studies in cats. Ten years later it was reported that serious hepatic necrosis also occurred in man after high doses of paracetamol (Davidson and Eastham, 1966 and Thomson and Prescott, 1966) and more recently it was reported that some species were susceptible to paracetamol-induced hepatic necrosis while other species seemed to be resistant to the hepatotoxic effects of paracetamol (David, Potter, Jollow and Mitchell, 1974). A table summarising the dose levels of paracetamol causing centrilobular liver necrosis and mortality of various species taken from Davis et al, 1974 is shown in Table 6.1. It is interesting that susceptibility to hepatic necrosis and mortality seem to be inversely related in this but death in the species most resistant to paracetamol hepatotoxicity may have been caused by glycogen depletion.

In the experiments carried out in the present animal studies, it was decided to examine the metabolism of paracetamol in the guinea pig (a non-susceptible species) and in the mouse (a susceptible species). The metabolism of paracetamol after pretreatment with three enzyme inducing agents, during repeated dosing and during the concurrent administration of three metabolic competing agents was studied.

The metabolism of paracetamol in the presence of L-methionine was also examined in rabbits. Rabbits might, (from Table 6.1) be described as a non-susceptible species but they show a similar urinary metabolic profile to man and in addition have a large enough blood volume to permit serial blood samples to be taken over 5.25 hours.

Table 6.1

Species	Dose	Percent showing some degree of centrilobular hepatic necrosis	Percent mortality
Hamsters	150 mg/kg	64	0
Mice	375 mg/kg	46	3
Rabbits	750 mg/kg	8	40
Rats	1500 mg/kg	6	69
Guinea Pigs	625 mg/kg	0	100

(from Davis et al., 1974)

### 6.1 Effects of three enzyme inducing agents on paracetamol metabolism in mice and guinea pigs

In the present study the effects of phenobarbitone, phenylbutazone and rifampicin on the metabolism of paracetamol in mice and guinea pigs were examined.

In guinea pigs control recoveries of paracetamol cysteine and mercapturic acid were zero but small amounts of these metabolites were recovered after pretreatment with each of the three inducing agents. The low recoveries of these metabolites may be due to a limited ability of the guinea pig either to oxidise paracetamol to its toxic metabolite or to detoxify the reactive intermediate to the cysteine and mercapturic acid metabolites via glutathione transferase. Davis et al (1974) found this species to be relatively resistant to the glutathione depletion, covalent binding and hepatic necrosis caused by paracetamol (despite causing a high percentage mortality probably as a result of glycogen depletion). These results suggest that guinea pigs form low amounts of the toxic intermediate metabolite even after induction of the P-450 system.

In mice all three inducing agents markedly increased the proportions of paracetamol cysteine plus mercapturic acid recoveries and decreased the proportion of paracetamol glucuronide recovered. Since concentrations of the glutathione conjugates were high even in control animals (nearly 16% of the percentage recovery) this route represents a major pathway even when compared with sulphate conjugation. Although sulphate conjugation remained low, it is possible that glucuronide conjugation (which tends to predominate over sulphate conjugation if there is a high circulating concentrations of paracetamol) would decrease as a result of the greater amounts being oxidised and then conjugated with glutathione

caused by the inducing agent. Alternatively the inducing agents may have caused glucuronic acid depletion. Balonowska and Gessner (1980) described glucuronyl transferase in rats as having a much higher  $K_m$  value for paracetamol than sulphotransferase. Thus sulphate becomes a predominant route of metabolism if low concentrations of paracetamol are present and the  $V_{max}$  for sulphation is lower than that for glucuronidation. However, these observations may be more applicable to man and rat in which species sulphate conjugation is a more prominent pathway.

Several studies have shown that paracetamol is activated metabolically by a cytochrome P-450-dependent drug metabolising enzyme located in the endoplasmic reticulum of the liver to an arylating agent which binds covalently probably to amine acids on liver proteins. Pretreatment with various inducers of drug metabolising activity were shown by Jollow, Thorgeirsson, Potter, Hashimoto and Mitchell, (1974) to increase the formation of the mercapturic acid metabolite of paracetamol and to potentiate hepatic necrosis in some species while inhibitors of drug metabolising enzymes inhibited the metabolism of paracetamol and protected against hepatic necrosis. This suggested that paracetamol-induced hepatic necrosis was mediated by a toxic metabolite of paracetamol (Mitchell, Jollow, Potter, Davis, Gillette and Brodie, 1973). Jollow, Mitchell, Potter, Davis, Gillette and Brodie, (1973) found the pretreatment with inducers or inhibitors of drug metabolism increased and decreased respectively the extent of covalent binding which in turn altered the severity of hepatic necrosis in some species. They found that peak concentrations of the binding of  $^3\text{H}$ -paracetamol preceded the development of recognisable necrosis by one to two hours. In hamsters, however, it was found that pretreatment with phenobarbitone markedly increased the excretion of paracetamol glucuronide and excretion of paracetamol mercapturate was unaffected while 3-methylcholanthrene pretreatment enhanced the excretion of the mercapturic acid metabolite (Jollow et al, 1974).

This suggests that in hamsters oxidation of paracetamol to its toxic metabolite may perhaps be mediated by cytochrome P-448 alone while in mice and rats oxidation to the reactive intermediate may involve both cytochrome P-448 and P-450.

Rifampicin has been described as an inducer of drug metabolism by several workers (e.g. Miguet, Mavier, Soussey and Dhumsaur, 1977, Ohnhaus and Park, 1979, Goldberg, 1980). Bolt and Remmer (1976) found that induction of NADPH-cytochrome c reductase occurred after pretreatment of rats with 20mg/kg rifampicin over 5 days. Recently Prescott, Critchley, Balali-Mood and Pentland (1981) examined the metabolism of paracetamol in man after pretreatment with rifampicin or anticonvulsants. They found that glucuronide conjugation of paracetamol was increased, that sulphate conjugation and unchanged paracetamol excretion were decreased but cysteine and mercapturic acid metabolites were largely unchanged. This implies that only a small induction of the potentially toxic pathway had occurred (if at all) so that treatment of man with rifampicin or anticonvulsants does not appear to increase the toxicity of paracetamol. The increase in glucuronidation suggests that these inducers may act in a similar fashion to phenobarbitone in hamsters and may even protect against paracetamol-induced liver disease.

Phenylbutazone caused a significant increase in the proportion recovered as the cysteine plus mercapturic acid metabolites of paracetamol by increasing the percentage recovery of mercapturate but not paracetamol cysteine. Phenylbutazone has been reported to cause the induction of hepatic microsomal enzymes in man and as phenylbutazone and paracetamol may be used concurrently in rheumatoid arthritis and gout, it may be important to determine the metabolism of paracetamol in man in the presence of phenylbutazone (or indeed any commonly used drug known to induce drug metabolising enzymes). Although results from Prescott et. al. (1973) suggest that inducing agents may not increase the toxicity of paracetamol in

man, it was reported by Critchley, Cregen, Balali-Mood, Pentland and Prescott (1982) that in some heavy drinkers, chronic consumption of alcohol may enhance the recovery of paracetamol as its cysteine and mercapturic acid metabolites. If the metabolism of paracetamol in man is similar to hamster metabolism rather than mouse metabolism of paracetamol then induction of paracetamol hepatotoxicity may be via a P-448-type mechanism rather than a phenobarbitone-type reaction. Thus the possibility is raised that phenobarbitone-type inducers might increase the proportion of paracetamol excreted as its glucuronide (a safe pathway) while inducers of P-448 might result in increased formation of the reactive metabolite of paracetamol and hence might lead to toxicity.

#### 6.2 The effect of repeated dosing of paracetamol in mice and guinea pigs

A study of the metabolism of paracetamol has been carried out in mice and guinea pigs to determine the urinary metabolic profile of paracetamol in these species after repeated dosing. When repeated and acute dosing studies were compared, there were found to be significant decreases in the percentage recoveries of paracetamol glucuronide and increase in the percentage recoveries of unchanged paracetamol in both species after repeated dosing suggesting that glucuronic acid available for conjugation may have become depleted. In mice, percentage recoveries of the cysteine and mercapturic acid metabolites firstly decreased and then, towards the end of the study increased again. Buttar, Chow and Downie (1977) found that in rats, paracetamol caused a dose-dependent depletion of hepatic glutathione with maximum depletion occurring 3 hours after acute dosing, returning to normal by 12 hours after low doses. However, before returning to normal, liver glutathione concentrations became significantly greater than control values suggesting a glutathione rebound action. They also found that when animals were treated for 7 days with paracetamol 1g/kg b.d. liver glutathione concentrations



were significantly higher after 7 days than in rats treated with a single dose of paracetamol (1g/kg). From the present results the increases in percentage recovery of paracetamol cysteine plus mercapturic acid with time may reflect a greater availability of glutathione for conjugation with the oxidised metabolite perhaps as a result of a homeostatic feedback mechanism while initially glutathione may not have been so freely available. Thus the decreased cysteine and mercapturic acid recoveries may have reflected a potentially toxic situation as glucuronide conjugation was also decreased. Further experiments could be carried out to determine hepatic glutathione concentrations during the whole period so that the true course of the initial depletion of glutathione and the possibility of an increased glutathione availability with time might be assessed.

Barker, Carle and Anvras (1977) described 3 cases of toxic hepatitis in man associated with the chronic ingestion of 5 - 8g paracetamol per day for a period of several weeks. They suggested that hepatotoxicity after chronic ingestion may be quite common especially in those patients with predisposing conditions. Chronic administration of paracetamol to patients with inflammatory joint disease might show a similar metabolic pattern to the repeat dosing in mice although there was no evidence of this in the study described in Section 3.3. Therefore the 'glutathione rebound action' of paracetamol seen after single high doses of paracetamol in rats (Buttar et al, 1977) might also occur after repeated dosing in mice and might also explain the lack of evidence for increased susceptibility to paracetamol toxicity in the rheumatoid patients.

### 6.3 Metabolism of paracetamol in the presence of competing agents

The effects of three metabolic competing agents on the metabolism of paracetamol in mice and guinea pigs were determined. Salicylamide has been reported to conjugate both with glucuronic acid and sulphate in man (Levy and Yamada, 1971), ascorbic acid conjugates with endogenous sulphate (Raghuram, Krishnamurthi and Kalamegham, 1978) and  $\alpha$ -tocopherol conjugates with glucuronic acid.

In addition ascorbic acid and  $\alpha$ -tocopherol have both been reported to act as antioxidants (Burns, 1975, Cohn, 1975).

In guinea pigs, all 3 agents were found to increase paracetamol glucuronide recovery and to decrease unchanged paracetamol recovery ( $p < 0.05$ ). In mice,  $\alpha$ -tocopherol increased paracetamol glucuronide recovery and increased paracetamol cysteine plus mercapturic acid recoveries ( $p < 0.05$ ) while reducing the recovery of unchanged paracetamol ( $p < 0.025$ ). Salicylamide and ascorbic acid did not significantly alter the percentage recoveries of the metabolites of unchanged paracetamol in mice. It thus appears that all 3 agents induce glucuronyl transferase activity in the guinea pig and in the mouse  $\alpha$ -tocopherol appears to act by inducing glucuronyl transferase activity and by increasing the products of oxidation of paracetamol either by inducing the formation of the reactive intermediate or by increasing the activity of glutathione transferase. Thus  $\alpha$ -tocopherol in vivo does not, under these experimental conditions, appear to be acting in its capacity as an antioxidant. Free D-glucuronic acid, formed from UDP-glucuronic acid by enzymic hydrolysis is a precursor in the biosynthesis of L-ascorbic acid (Lehninger, 1972). It is possible that the increase in paracetamol glucuronide seen after L-ascorbic acid administration to guinea pigs is related to this biochemical pathway. Perhaps normally a certain proportion of free glucuronic acid is sequestered for the formation of the small amount of L-ascorbic acid that guinea pigs produce (compared with other species). Thus administration of exogenous L-ascorbic acid may make glucuronic acid available for conjugation with paracetamol. Some workers have suggested that the dosage form of L-ascorbic acid may be important (Butterworth, Evans, Hargreaves and Pelling, 1982) and it is possible that intraperitoneal administration of L-ascorbic acid may not reach its site of action in mice. L-ascorbic acid has been shown to inhibit covalent binding

in vitro (Lake, Harris, Phillips and Gangolli, 1981) and these authors suggested that endogenous L-ascorbic acid formed in rodent and human liver and exogenously applied L-ascorbic acid may supplement endogenous protective mechanisms (such as reduced glutathione) by maintaining SH-activated enzyme systems in their reduced form. Alternatively, L-ascorbic acid might act to reduce the oxidised metabolite although the present results do not support this hypothesis. Following this the reactive intermediate may either proceed along other minor pathways of metabolism or may even form a complex with ascorbic acid itself. Alternatively the increase in glucuronidation may have been effected because of methionine's protective effect on the liver

#### 6.4 Metabolism of paracetamol in rabbits in the presence of L-methionine

The effects of L-methionine on paracetamol metabolism in rabbits was studied. Rabbits were not considered by Davis et al, (1974) to be a species susceptible to paracetamol-induced hepatic necrosis. AUCs calculated for the higher dose of paracetamol with and without L-methionine were not significantly different from each other but both were significantly higher than those calculated for the 100mg/kg doses of paracetamol ( $p < 0.05$ ).

Assuming complete absorption of drug from the gut an increase in the AUC may be considered to be due either to saturation of the drug elimination process or to saturation of certain metabolic processes. As peak serum concentrations of both paracetamol and paracetamol glucuronide were lower in general when methionine was given than

paracetamol 400mg/kg alone, L-methionine may have decreased the rate or extent of absorption of paracetamol. Methionine may also have increased the rate of elimination of paracetamol perhaps by inducing glucuronyl transferase activity as paracetamol glucuronide recovery in urine was higher after L-methionine than paracetamol 400mg/kg alone. The large AUC of paracetamol in Experiment 2 may be the result of glucuronic acid depletion or saturation of glucuronyl transferase activity. Alternatively hydrolysis of the glucuronide by bacteria could account for the low amounts of PG recovered.

It is suggested that in rabbits the enzyme catalyzing the transformation of paracetamol cysteine to paracetamol mercapturic acid (N-acetyl transferase) is located in the kidney as serum concentrations of paracetamol cysteine were higher than paracetamol mercapturic acid concentrations but in urine the reverse was true. Jones et al, (1978) considered that both of the metabolic reactions transforming paracetamol glutathione to paracetamol cysteine and mercapturic acid occur in the kidney.

After the high dose of paracetamol alone (Experiment 2), although peak glucuronide concentrations in serum were higher than peak serum concentrations of paracetamol, the opposite was true in urine. This suggests that paracetamol glucuronide was broken down by  $\beta$ -glucuronidase present perhaps in the kidney. It is however also feasible that paracetamol glucuronide was hydrolyzed exogenously as the metabolic cage did not allow for the complete separation of urine and faeces. It would be surprising if this were to be the explanation though as this effect would have been seen in the other experiments. There did not appear to be any effect on the total percentage of the administered dose of paracetamol excreted although it was difficult to assess as there was such a wide variation in the percentage dose excreted.

There is little evidence that L-methionine acts to increase the availability of sulphate or glutathione for conjugation with paracetamol in rabbits. Thus L-methionine does not appear to act similarly in man and rabbits. However recoveries of paracetamol sulphate and paracetamol cysteine plus mercapturic acid in urine appeared to be lower after 400mg/kg paracetamol alone than 100mg/kg paracetamol and similar effects have been noted in man (see Section 3.5).

L-methionine. It is apparent therefore that the rabbit is not an ideal model for the effects of L-methionine on paracetamol metabolism observed in man.

In conclusion from these experiments on paracetamol metabolism in animals, some reasons are suggested as to why some species are susceptible and why some are non-susceptible. The mouse (a susceptible species) metabolises a large proportion (approximately 25%) of a dose of paracetamol to the reactive metabolite and this is normally successfully scavenged by glutathione until some 45% is depleted. The guinea pig however does not appear to oxidise paracetamol to its toxic metabolite to any significant extent and this may be the reason why the formation of only small amounts of paracetamol cysteine and mercapturic acid are seen. This was first suggested by Jollow et al, (1974). It is interesting that although the rabbit is not a susceptible species (from Davis et al, 1974), percentage recoveries of paracetamol cysteine and mercapturic acid in urine of the same order of magnitude as those seen after a therapeutic 1g dose of paracetamol administered to man. Man is considered to be susceptible to paracetamol-induced hepatic necrosis and this discrepancy may perhaps be explained by the presence of pre-aromatic derivatives of paracetamol found in human urine. To fully categorize a species as being susceptible or non-susceptible to paracetamol hepatotoxicity, these pre-aromatic metabolites, plus the O-substituted and S-substituted metabolites, would need to be quantified.

## Chapter 7

### Aspirin Results in Man

#### 7.1 Control Study

##### Introduction

In order to examine the effects of rheumatoid arthritis and overdose on aspirin metabolism a study was undertaken to determine the metabolism of aspirin (acetylsalicylic acid, ASA) in normal volunteers after a therapeutic dose. The major urinary metabolites of aspirin were quantified. Although Caldwell, O'Gorman and Smith (1979) carried out an investigation into the glycine conjugation of salicylate in 85 healthy volunteers, these were aged between 20 and 31 years and no age related studies of salicylate metabolism appear to have been reported.

##### Procedures

As in the control paracetamol study, human volunteers were hospital and laboratory workers or their children. Those aged over 60 years were contacted through local general practitioners. Each gave an account of smoking habits and of other drugs being taken and details are given in Appendix 23. Aspirin 600mg was taken by each subject orally early in the morning after emptying the bladder and fasting overnight. All urine passed in the subsequent 8 hours (0 - 8 hour collection) and in the period 8 - 24h after dosing (8 - 24 hour collection ) was collected in separate acidified plastic urine bottles. Urine was analysed by HPLC as described in Section 2.7 for the major urinary metabolites of aspirin i.e. salicylic acid (SA), salicyluric acid (SUA) and the ester and ether glucuronides (SG). In some cases it was possible to quantify the gentisic acid metabolite (GA) but in general it was found to chromatogram with an endogenous urinary peak and could not be resolved. In any case less than 5% of a given dose of aspirin is oxidised to gentisic acid (Woodbury and Fingl, 1975), and this was confirmed in the present study when measurement of gentisic acid was possible.

In this and subsequent studies in which aspirin and its metabolites were quantified in urine corrections were made for molecular weight to express each as the equivalent of SA (using correction factors given in Appendix 1) so that the fractional contributions of each metabolite and aspirin excreted unchanged to total recovery could be compared. Salicylic acid was chosen as the standard for calculating recovery since this is the convention in the literature. A 600mg dose of aspirin is equivalent to 460.2mg salicylic acid. The salicyl glucuronides were measured as salicylic acid after treatment with  $\beta$ -glucuronidase, thus no correction factor was necessary in this case. There was considerable interindividual variation in total amounts excreted and results are expressed as percentage dose excreted or as percentage recovery of aspirin and its metabolites in each urine collection. When quantification of gentisic acid could be achieved values were included when calculating percentage dose excreted and percentage recoveries.

## Results

Table 7.1.1 gives the percentage dose of acetylsalicylic acid and its metabolites recovered in each collection period. Individual results (expressed both in mg and as percentage recovery) are given in Appendix 24 and Appendix 25 respectively. Forty one subjects were studied of which there were 18 males and 23 females.

Table 7.1.1 Means  $\pm$  SEM of percentage dose excreted of acetyl salicylic acid and its metabolites in 41 subjects

Collection period	Percentage dose excreted
0 - 8h	46.11 $\pm$ 3.51
8 - 24h	27.18 $\pm$ 2.57
0 - 24h	73.27 $\pm$ 3.64

When individual results were examined it was found that the percentage dose recovered was negatively correlated with age in 0 - 8 hour urine ( $r = -0.493$ ,  $p < 0.01$ ) but not in 8 - 24 hour urine or over the whole 24 hour period. These results are plotted in Fig. 7.1.1.

Urinary pH was measured in 25 volunteers and a positive correlation was found between percentage recovery of salicylic acid in 0 - 8 hour urine ( $r = 0.526$ ,  $p < 0.01$ ) but not in 8 - 24 hour urine. Acetylsalicylic acid reached quantifiable concentrations in only 11 volunteers (mean percentage recovery  $4.14 \pm 0.82$  in 24 hour urine) and gentisic acid was quantified in only 5 volunteers (mean percentage recovery  $1.65 \pm 0.60$  in 24 hour urine).

Table 7.1.2 gives percentage recoveries of salicylic acid, salicyluric acid and the salicyl glucuronides in each collection period in 41 volunteers and these results are plotted in Fig. 7.1.2

Table 7.1.2 Mean  $\pm$  SEM of percentage recovery of salicylic acid, salicyluric acid and salicyl glucuronides in 41 normal volunteers

Collection period	n	SA	SUA	SG
0 - 8h	41	$6.00 \pm 1.31$	$83.96 \pm 1.73$	$9.16 \pm 1.14$
8 - 24h	41	$2.60 \pm 0.85$	$88.79 \pm 1.76$	$5.79 \pm 1.42$
0 - 24h	41	$4.68 \pm 0.85$	$85.65 \pm 1.28$	$8.35 \pm 0.87$



Fig. 7.1.1.

Percentage dose excreted of acetyl salicylic acid and its metabolites from normal volunteers who took aspirin 600mg by mouth (means  $\pm$  S.E.M.)

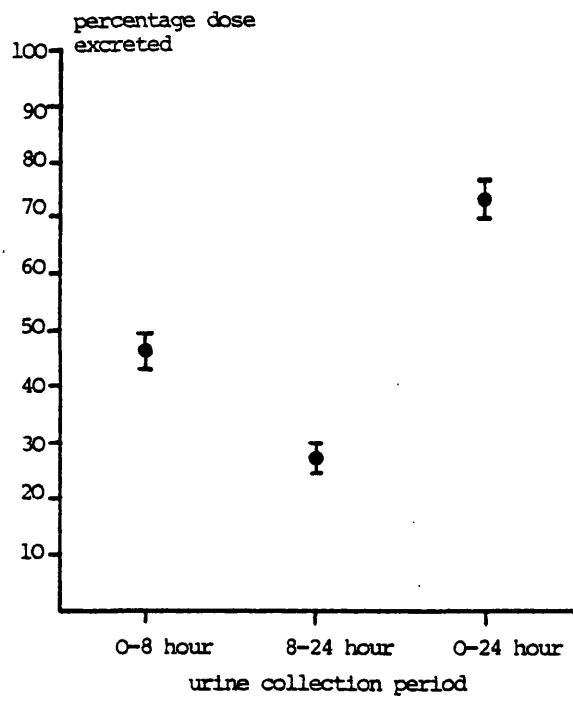


Fig. 7.1.2.

Percentage recoveries of the metabolites of acetyl salicylic acid in each urine collection period from normal volunteers who took aspirin 600mg by mouth (means  $\pm$  S.E.M.)

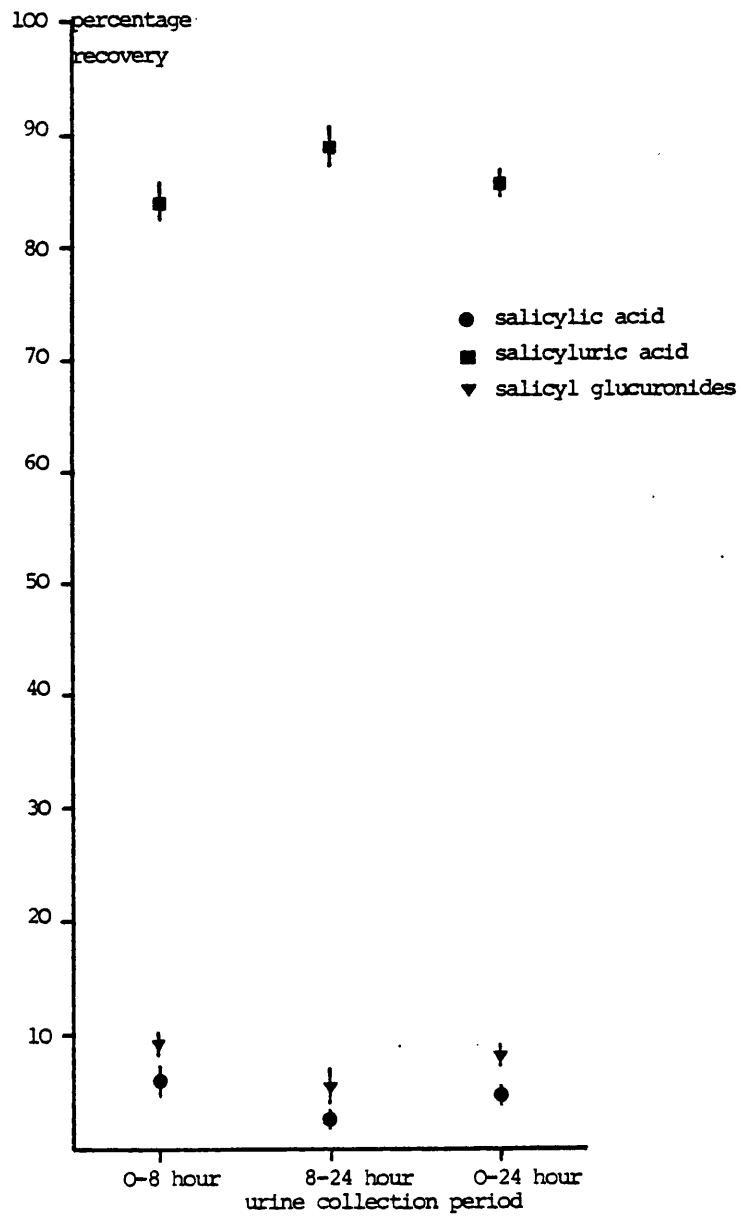


Table 7.1.2 shows that the major metabolite of acetylsalicylic acid was salicyluric acid which accounted for some 90% of the excreted metabolic products. Percentage recoveries of salicylic acid were significantly lower in 8 - 24 hour urine than 0 - 8 hour urine. Salicyluric acid concentrations and salicyl glucuronide concentrations were not significantly different in 0 - 8 hour and 8 - 24 hour periods.

Table 7.1.3 gives the percentage recoveries of the salicylic acid, salicylurate and glucuronic acid metabolites of acetyl salicylic acid with age presented in cohorts of ten years. These results are plotted in Fig 7.1.3, 7.1.4 and 7.1.5.

Table 7.1.3 Percentage recoveries of salicylic acid, salicylurate and glucuronide metabolites of acetyl salicylic acid (means  $\pm$  SEM) from normal volunteers who took acetyl salicylic acid 600mg by mouth

Age	n	SA	SUA	SG
<u>0 - 8h</u>				
11 - 20	4	6.24 $\pm$ 3.38	81.85 $\pm$ 5.11	10.77 $\pm$ 3.22
21 - 30	9	3.33 $\pm$ 1.92	84.86 $\pm$ 4.17	11.13 $\pm$ 1.74
31 - 40	7	11.17 $\pm$ 5.07	77.12 $\pm$ 5.00	9.74 $\pm$ 3.81
41 - 50	7	6.31 $\pm$ 2.19	81.18 $\pm$ 4.38	10.82 $\pm$ 2.57
51 - 60	5	2.53 $\pm$ 1.93	86.13 $\pm$ 1.13	11.35 $\pm$ 1.40
61 - 70	7	4.87 $\pm$ 3.31	90.61 $\pm$ 3.27	4.52 $\pm$ 1.68
Over 80	2	10.93 $\pm$ 10.93	89.07 $\pm$ 10.93	0.00 $\pm$ 0.00
<u>8 - 24h</u>				
11 - 20	4	0.00 $\pm$ 0.00	87.52 $\pm$ 3.16	10.17 $\pm$ 3.02
21 - 30	9	0.00 $\pm$ 0.00	86.07 $\pm$ 5.76	8.51 $\pm$ 5.38
31 - 40	7	1.11 $\pm$ 0.43	84.99 $\pm$ 3.99	7.16 $\pm$ 3.29
41 - 50	7	1.38 $\pm$ 1.22	93.07 $\pm$ 3.23	4.03 $\pm$ 1.64
51 - 60	5	6.13 $\pm$ 2.58	92.47 $\pm$ 2.27	1.40 $\pm$ 0.60
61 - 70	7	4.70 $\pm$ 2.40	90.34 $\pm$ 3.90	4.96 $\pm$ 2.11
Over 80	2	12.78 $\pm$ 12.78	87.21 $\pm$ 12.78	0.00 $\pm$ 0.00
<u>0 - 24h</u>				
11 - 20	4	4.56 $\pm$ 2.38	82.70 $\pm$ 3.72	11.24 $\pm$ 2.46
21 - 30	9	2.46 $\pm$ 1.32	85.00 $\pm$ 2.80	10.77 $\pm$ 2.55
31 - 40	7	6.69 $\pm$ 2.33	80.30 $\pm$ 3.77	10.22 $\pm$ 2.39
41 - 50	7	4.00 $\pm$ 1.12	86.59 $\pm$ 2.64	7.60 $\pm$ 1.64
51 - 60	5	4.34 $\pm$ 1.35	88.70 $\pm$ 1.13	6.97 $\pm$ 0.66
61 - 70	7	4.02 $\pm$ 2.14	89.80 $\pm$ 2.71	5.83 $\pm$ 0.96
Over 80	2	12.16 $\pm$ 12.16	87.84 $\pm$ 12.16	0.00 $\pm$ 0.00

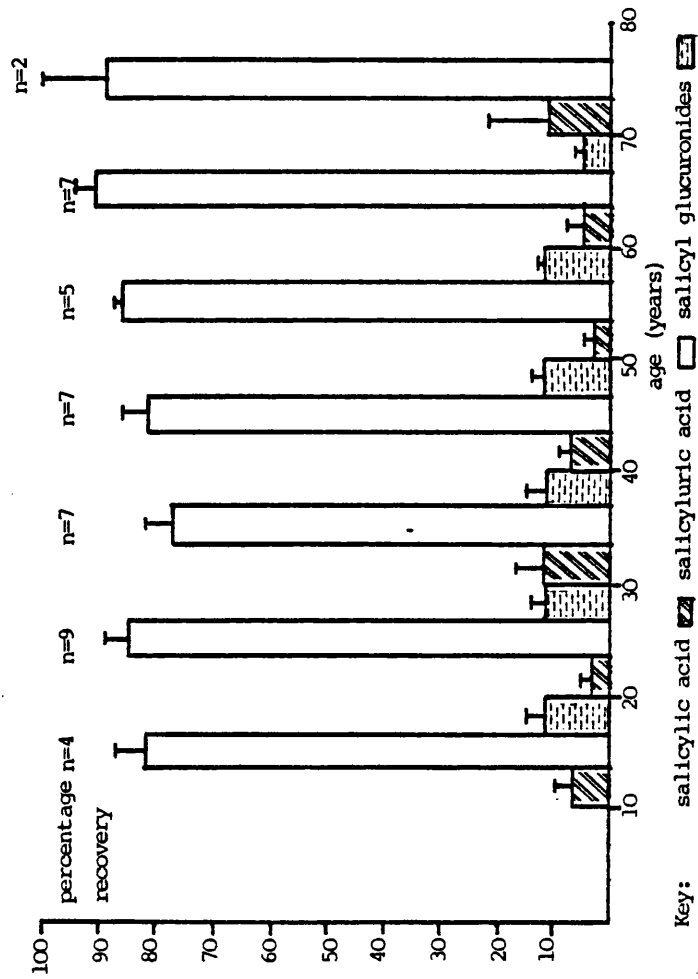


Fig. 7.1.3 Percentage recoveries of the metabolites of acetyl salicylic acid in 0 - 8 hour urine from normal volunteers who took aspirin 600mg by mouth (means  $\pm$  S.E.M.)

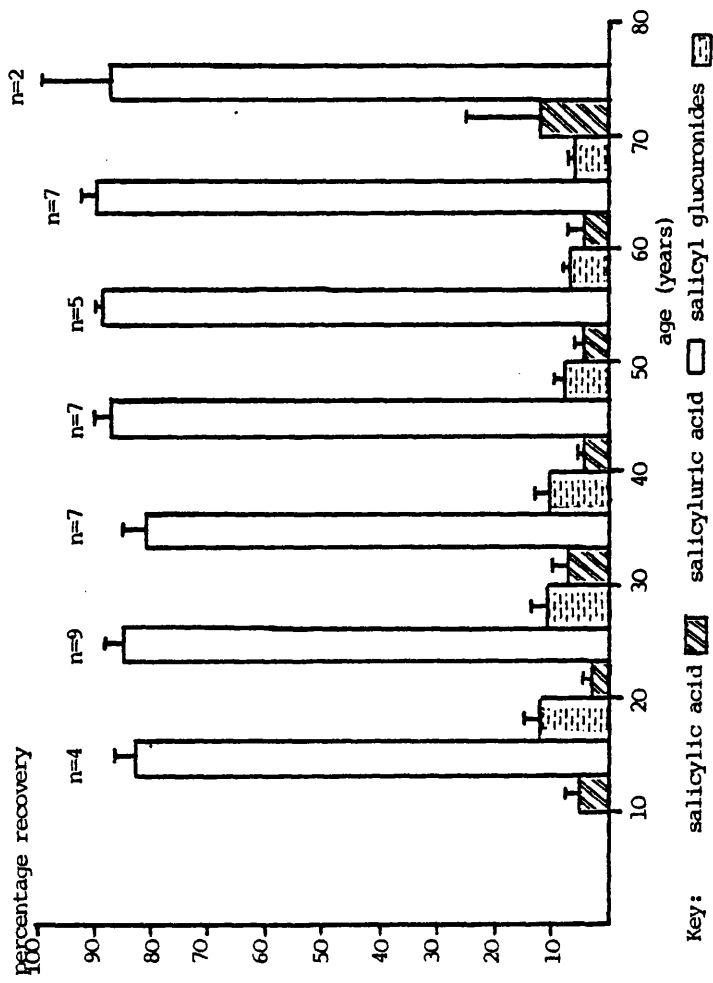


Fig. 7.1.4 Percentage recoveries of the metabolites of acetyl salicylic acid in 8 - 24 hour urine from normal volunteers who took aspirin 600mg by mouth (means  $\pm$  S.E.M.)

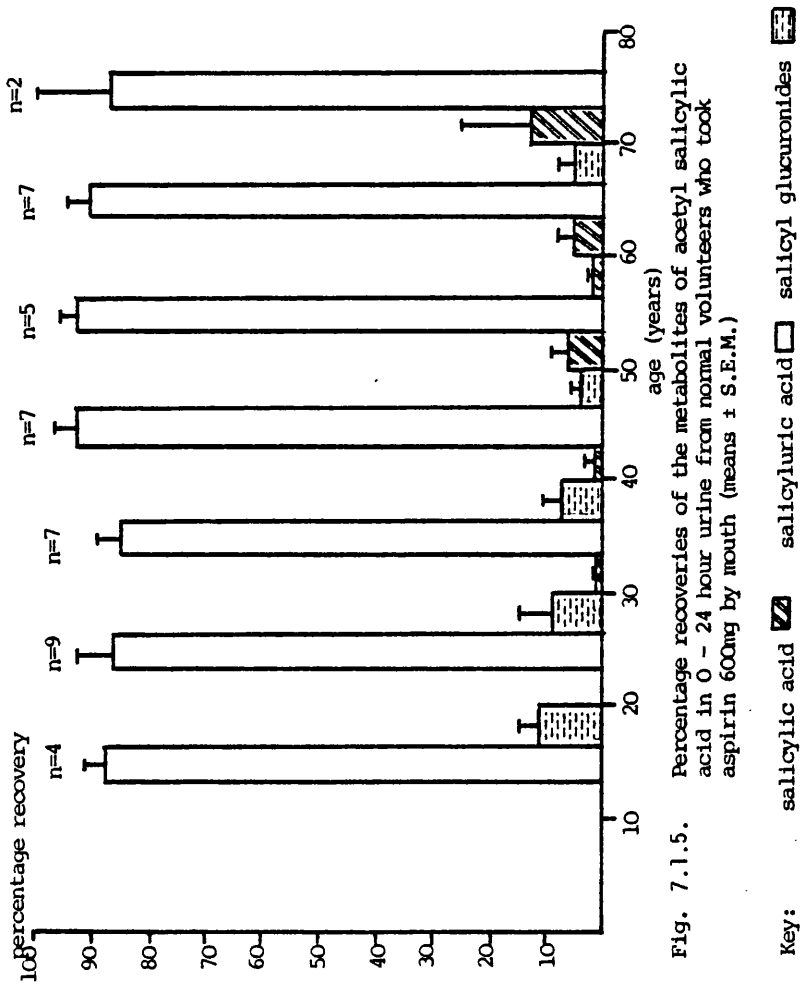


Fig. 7.1.1.5. Percentage recoveries of the metabolites of acetyl salicylic acid in 0 - 24 hour urine from normal volunteers who took aspirin 600mg by mouth (means  $\pm$  S.E.M.)

Key: salicylic acid salicylic acid glucuronides salicylic acid

Regression coefficients were calculated for the percentage recovery of each metabolite on age. The 8 - 24 hour proportional output of salicylic acid increased significantly with age ( $r = 0.524$ ,  $p < 0.001$ ) with a regression coefficient of  $0.135 \pm 0.035$  which is significantly different from zero ( $p < 0.001$ ). 0 - 8 hour and 0 - 24 hour percentage recoveries of salicylic acid were not related to age. Percentage recovery of salicyl glucuronides was negatively correlated with age in 0 - 8 hour urine ( $r = -0.334$ ,  $p < 0.05$ ) with a regression coefficient of  $0.117 \pm 0.053$  which is significantly different from zero ( $p < 0.05$ ). The same trend was observed in 8 - 24 hour urine but the standard error was large and statistical significance at the 5% level was not reached.

Overall in 0 - 24 hour urine there was a significant decrease in output of the salicyl glucuronides with age ( $r = -0.454$ ,  $p < 0.01$ ) with a regression coefficient of  $0.120 \pm 0.038$  which is significantly different from zero ( $p < 0.01$ ). Percentage recovery of salicyluric acid was not correlated with age. The regressions of each metabolite on age are plotted in Figs. 7.1.6, 7.1.7 and 7.1.8 for each urine collection period.

When the intercorrelations of each metabolite are examined it is found that salicylic acid recovery was negatively related to the percentage recovery of salicyluric acid ( $r = -0.679$ ,  $p < 0.001$  in the 0 - 8 hour urine,  $r = -0.316$ ,  $p < 0.001$  in the 8 - 24 hour and  $r = -0.649$ ,  $p < 0.001$  over the whole 24 hours). Percentage recoveries of salicyl glucuronides and salicyluric acid were also negatively related ( $r = -0.644$ ,  $p < 0.001$  for 0 - 8 hour urine,  $r = -0.656$ ,  $p < 0.001$  in 8 - 24 hour urine and  $r = 0.748$ ,  $p < 0.001$  in 0 - 24 hour urine). Percentage recovery of the salicyl glucuronides was unrelated to salicylic acid. Figs. 7.1.9 and 7.1.10 show the correlations between salicylic acid and salicyluric acid and between salicyluric acid and salicyl glucuronides respectively over each collection period.



Fig. 7.1.6.

Percentage recovery of salicylic acid from normal volunteers who took aspirin 600mg by mouth plotted against age.

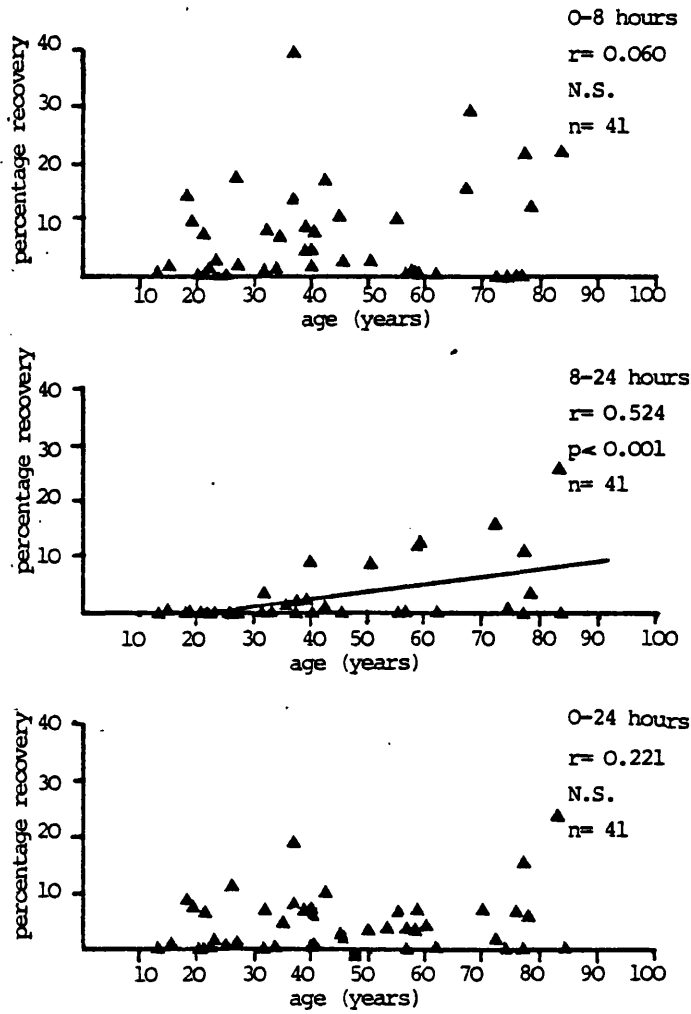


Fig. 7.1.7.

Percentage recovery of salicyl glucuronides from normal volunteers who took aspirin 600mg by mouth plotted against age

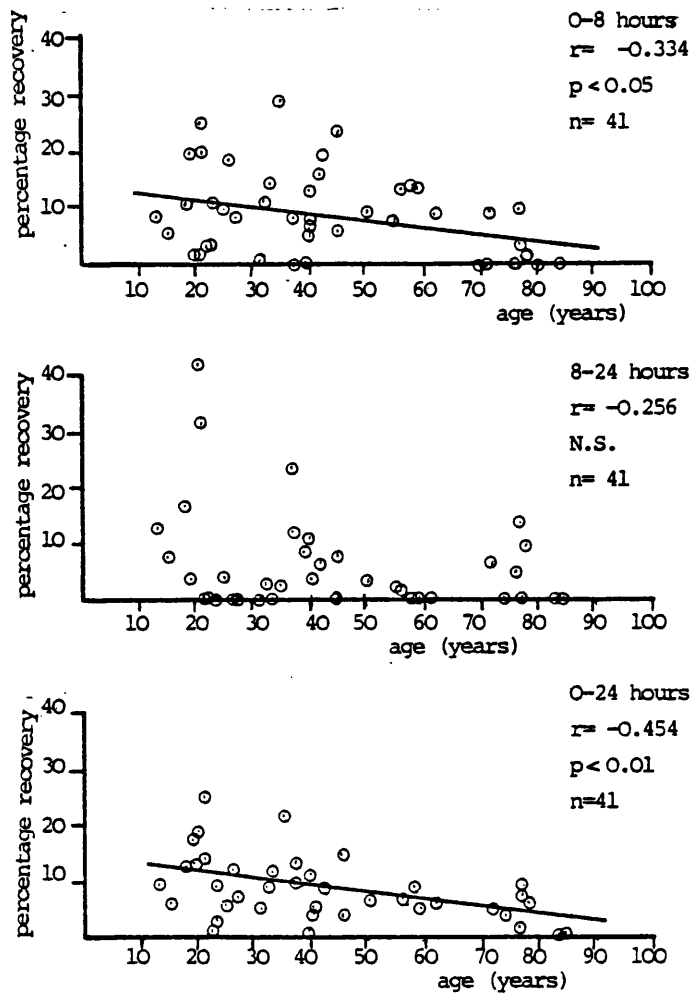


Fig. 7.1.8.

Percentage recovery of salicylic acid from normal volunteers who took aspirin 600mg by mouth plotted against age

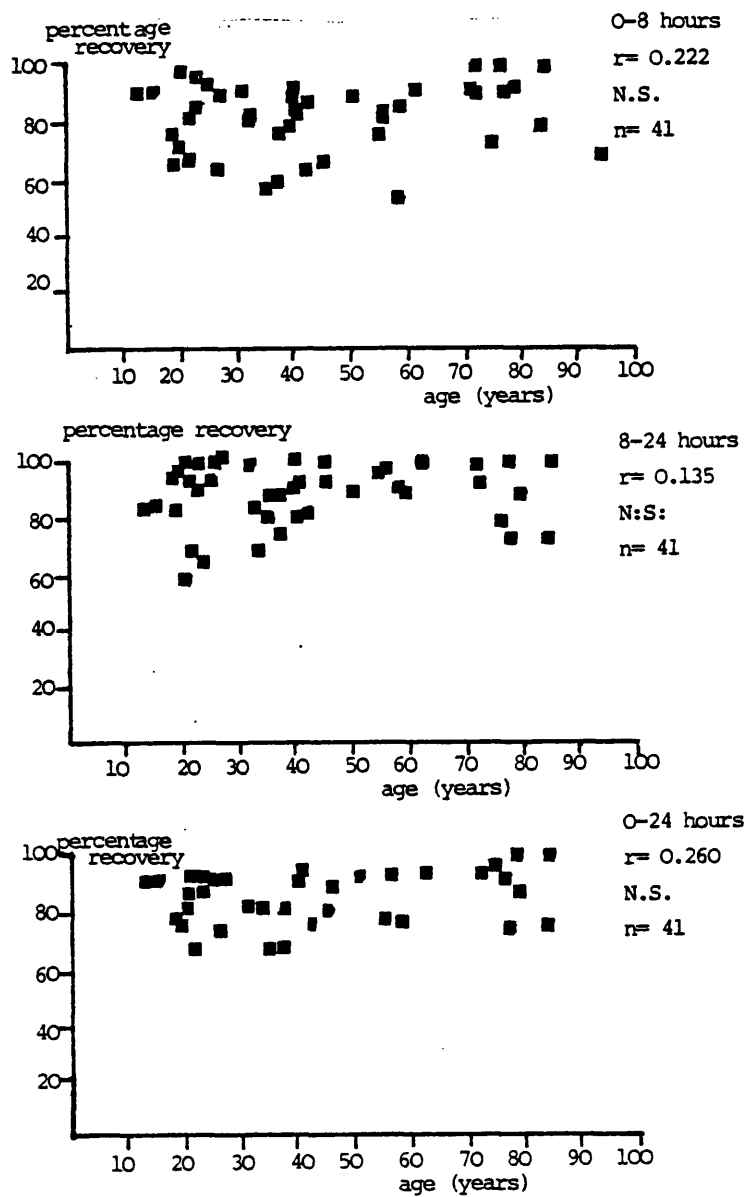


Fig. 7.1.9 Percentage recovery of salicylic acid against percentage recovery of salicylic acid from normal volunteers who took aspirin 600mg by mouth

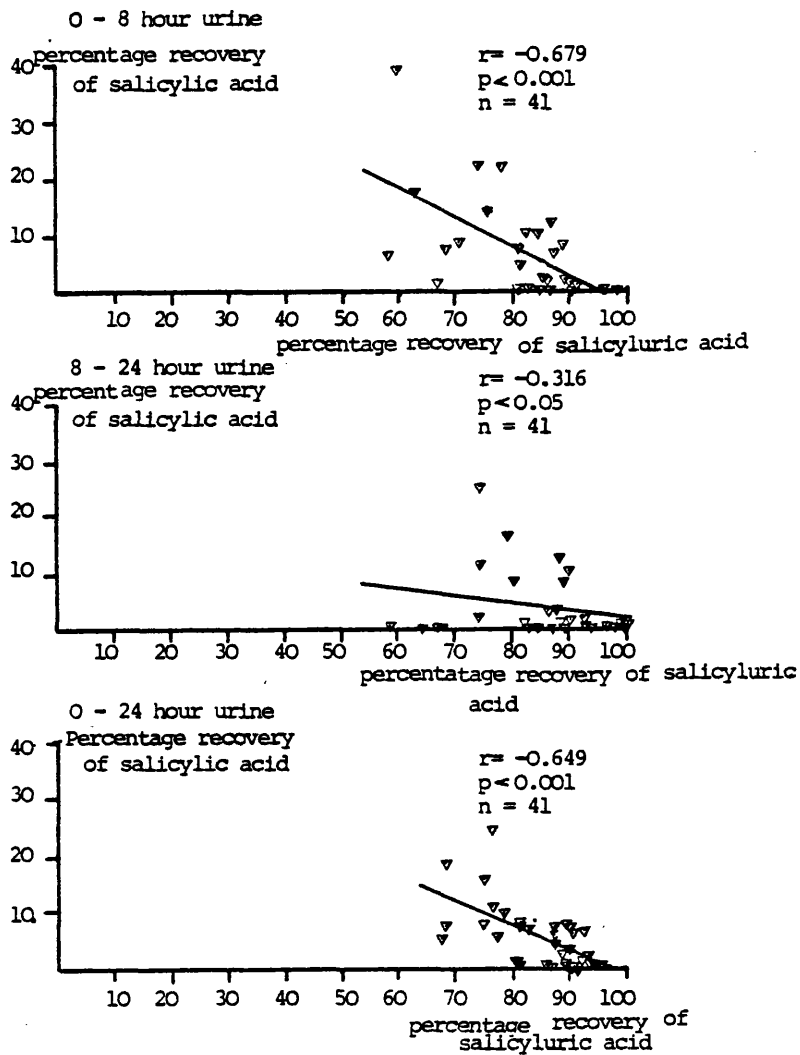
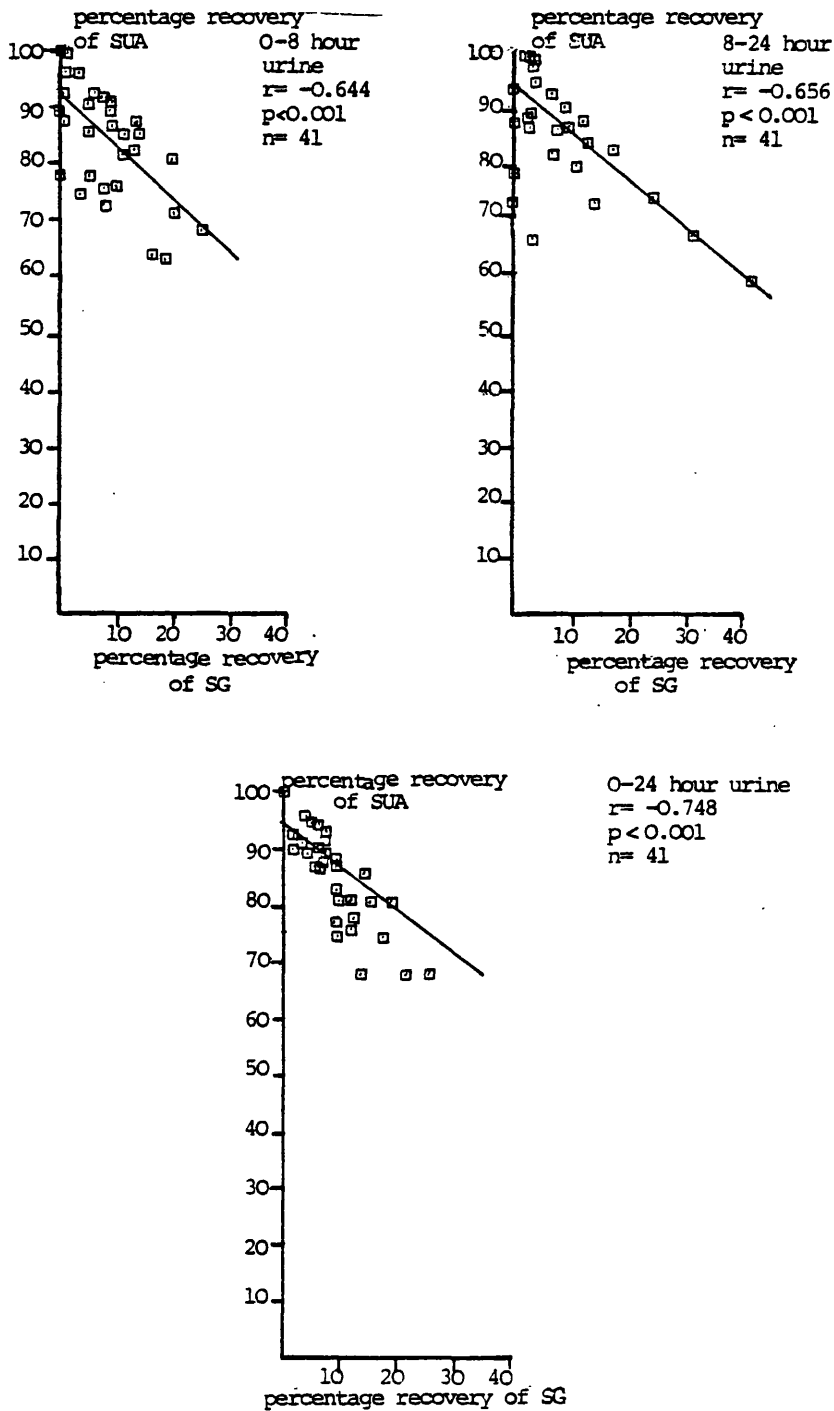


Fig. 7.1.10.

Percentage recovery of salicylic acid against percentage recovery of salicyl glucuronides from normal volunteers who took aspirin 600mg by mouth



## 7.2 Metabolism of aspirin in patients with rheumatoid arthritis

### Introduction

A study was undertaken to compare the metabolism of aspirin in patients with rheumatoid arthritis with the metabolism of aspirin in normal volunteers in the control study (Section 5.1). Rheumatoid patients constitute a group of likely chronic users of aspirin who are thus potentially at risk of aspirin related renal or hepatic disease. It was therefore of interest to record whether aspirin metabolism changes with chronic use. There were very few in-patients who regularly or occasionally took aspirin for analgesia at the time of the study in the Royal National Hospital for Rheumatic Diseases in Bath but 5 patients were investigated.

### Procedures

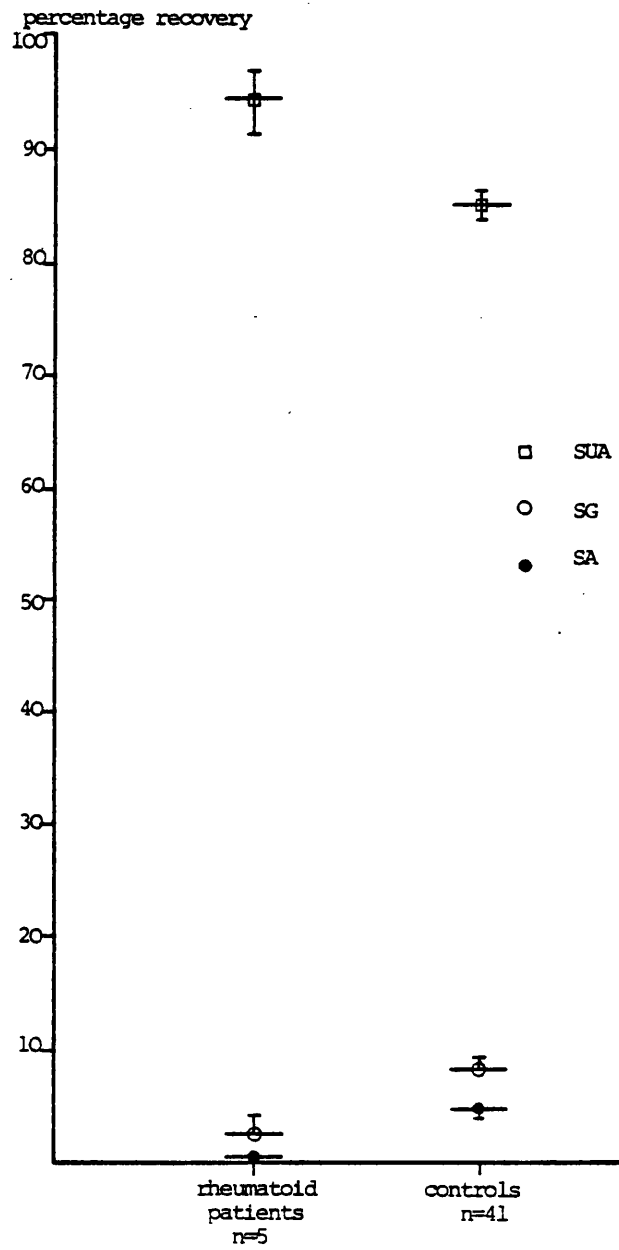
Five in-patients who were taking aspirin on a regular basis were studied. The patients took aspirin 600mg early in the morning between 6.30am and 7.30am and urine was collected for 8 hours (0 - 8h urine) and from 8 to 24 hours after dosing (8 - 24h urine) in acidified plastic bottles. During the 24 hour period of the study patients took additional aspirin and other drugs according to their normal dosing regimen. Details of other drugs being taken, age and sex for each patient are given in Appendix 26.

### Results

Percentage recoveries of salicylic acid, salicyluric acid and salicyl glucuronides were calculated for each urine collection period. Results are given in Table 7.2.1 and presented graphically in Fig. 7.2.1. Individual results of recovered metabolites expressed in mg corrected for molecular weight with respect to salicylic acid and as percentage recovery for each urine collection period are given in Appendix 27.

Fig. 7.2.1

Percentage recovery of the metabolites of acetyl salicylic acid in patients with rheumatoid arthritis and normal volunteers in urine collected over 24 hours (means  $\pm$  S.E.M.)



The urinary metabolic profile displayed by rheumatoid patients was similar to that of the controls. However, percentage recovery of salicylic acid in 0 - 8 hour urine ( $0.26 \pm 0.26$ ) and 0 - 24 hour urine ( $0.51 \pm 0.51$ ) but not 8 - 24 hour urine ( $0.64 \pm 0.64$ ) was significantly lower ( $p < 0.001$ ) than values for controls (0 - 8 hour:  $6.00 \pm 1.31$ , 8 - 24 hour:  $2.60 \pm 0.85$ ; 0 - 24 hour:  $4.68 \pm 0.85$ ). Percentage recovery of the salicyl glucuronides was also lower in rheumatoid patients in 0 - 8 hour urine ( $2.13 \pm 1.34$ ), ( $p < 0.001$ ) and in 0 - 24 hour urine ( $2.48 \pm 1.54$ ) ( $p < 0.002$ ) but not in 8 - 24 hour urine ( $3.24 \pm 2.31$ ) when compared with values in normal volunteers (0 - 8 hour  $9.16 \pm 1.14$ ; 8 - 24 hour  $5.79 \pm 1.42$ ; 0 - 24 hour  $8.35 \pm 0.87$ ). In the rheumatoid patients there was a greater percentage recovery of salicyluric acid in 0 - 8 hour ( $94.55 \pm 3.04$ ) ( $p < 0.01$ ) and 0 - 24 hour ( $94.24 \pm 8.77$ ) ( $p < 0.01$ ) but not 8 - 24 hour urine ( $3.24 \pm 2.31$ ) compared with control results (0 - 8 hour  $83.96 \pm 1.73$ ; 8 - 24 hour  $88.79 \pm 1.76$ ; 0 - 24 hour  $85.65 \pm 1.28$ ).

Table 7.2.1 Means  $\pm$  SEM of percentage recoveries of salicylic acid, salicyluric acid and salicyl glucuronides in 5 patients with rheumatoid arthritis after administration of aspirin 600mg.

Collection period	SA	SUA	SG
0 - 8h	$0.26 \pm 0.26$	$94.55 \pm 3.04$	$2.13 \pm 1.34$
8 - 24h	$0.64 \pm 0.64$	$93.04 \pm 3.52$	$3.24 \pm 2.31$
0 - 24h	$0.51 \pm 0.51$	$94.24 \pm 2.77$	$2.48 \pm 1.54$



### 7.3. Metabolism of aspirin in overdose

#### Introduction and Procedures

The metabolism of aspirin after overdose was examined in six patients. Five of the six were admitted within 7 hours of aspirin ingestion but one was not admitted until approximately 24 hours after overdose. Sequential urine samples were analysed by HPLC for unchanged acetyl salicylic acid, salicyluric acid, salicylic acid and the salicyl glucuronides as previously described (Section 2.7.b(iii) and 2.7c).

#### Results

Individual results and clinical details are given in Tables 7.3.1, 7.3.2, 7.3.3, 7.3.4, 7.3.5 and 7.3.6 below and are shown graphically in Figs. 7.3.1, 7.3.2, 7.3.3 and 7.3.4.

Table 7.3.1A1, female 20yr

Time	Event	mg expressed as equivalent of SA					
		SUA	ASA	SA	SG	$\frac{\text{SUA}}{\text{SA}}$	$\frac{\text{SG}}{\text{SA}}$
18.00	80 tablets of aspirin ingested						
19.55	Admitted to Casualty Dept.; gastric wash-out						
22.00		67.5	25.5	473.5	88.7	0.1	0.2
23.00		30.2	3.3	156.0	83.4	0.2	0.5
23.30	plasma salicylate 580mg/l						
24.00		20.1	2.8	178.4	65.3	0.1	0.4
01.00		25.2	3.4	303.4	9.2	0.1	0.0
02.00		39.4	5.1	618.6	19.6	0.1	0.1
04.00		25.7	0.0	155.6	38.4	0.2	0.2
04.30		34.3	0.0	498.8	0.0	0.1	0.0
05.15		20.2	0.0	204.4	0.0	0.1	0.0
08.10		120.6	0.0	158.6	0.0	0.8	0.0
10.00		70.0	0.0	201.0	53.5	0.4	0.3
11.15		29.7	0.0	24.2	9.2	1.2	0.4
14.00		108.2	0.0	60.8	21.1	1.8	0.3
20.15		70.4	0.0	2.9	24.5	24.3	8.5
22.15		83.5	0.0	28.8	N.M.	2.9	$\infty$
06.15		301.2	0.0	19.2	84.2	15.2	4.4
Total of individual metabolites		1046.2	40.1	3084.2	497.1		

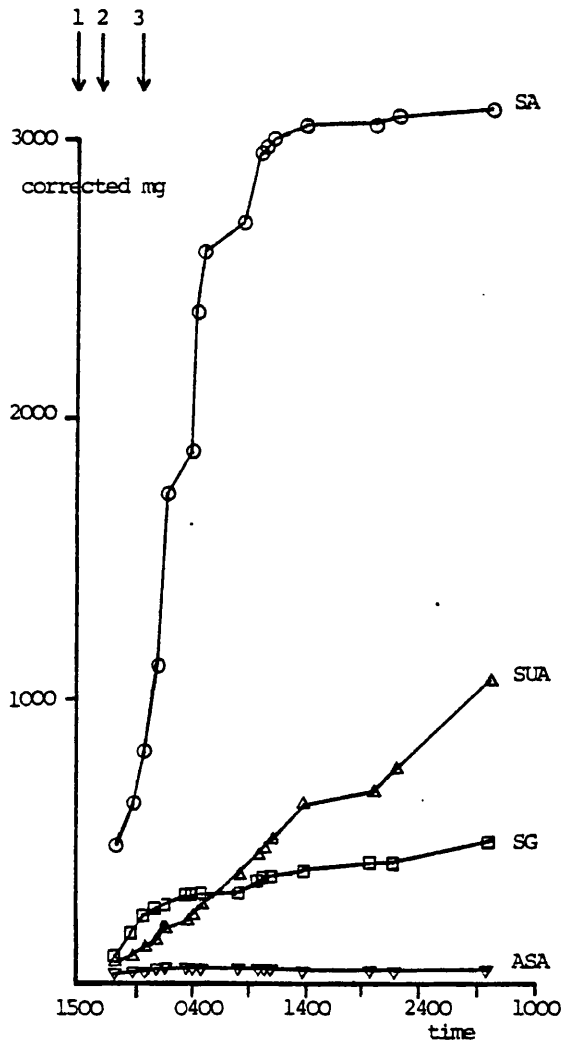
N.M. = not measured

Total recovery = 4667.6mg

The results are plotted in Fig. 7.3.1

Fig. 7.3.1.

Graph showing cumulative recovery of each metabolite corrected for molecular weight against time for patient shown in Table 7.3.1.



- 1 80 tablets of aspirin ingested
- 2 Admission
- 3 plasma salicylate level 580ug/ml

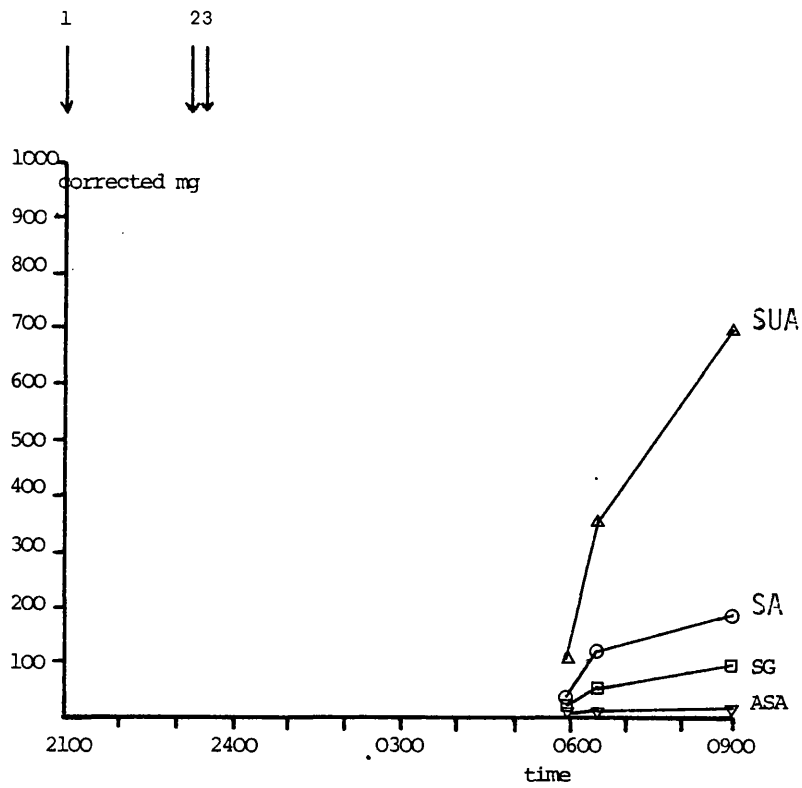
Table 7.3.2A2, Male 19yrs

Time	Event	mg expressed as equivalent of SA					
		SUA	ASA	SA	SG	$\frac{SUA}{SA}$	$\frac{SG}{SA}$
21.00	40 Anadin tablets plus 3 pints of lager ingested						
23.15	Admitted to Casualty Dept.						
23.30	Plasma salicylate 290mg/l						
06.00		105.2	4.3	36.4	22.4	2.9	0.6
06.30		249.3	5.6	79.7	31.1	3.1	0.4
09.00		341.6	6.1	70.0	39.5	4.9	0.6
Total of individual metabolites		696.1	16.0	186.1	92.9		

Total recovery = 991.1mg The results are plotted in Fig. 7.3.2.

Fig. 7.3.2.

Graph showing cumulative recovery of each metabolite corrected for molecular weight against time for patient shown in Table 7.3.2.



- 1 Ingested 40 Anadin
- 2 Admission
- 3 plasma salicylate 290 $\mu$ g/ml  
20ml ipecacuhana administered

Table 7.3.3

A3, male 82yr

Time	Event	mg expressed as equivalent of SA					
		SUA	ASA	SA	SG	$\frac{SUA}{SA}$	$\frac{SG}{SA}$
Overdose time not known but estimated to be about 24 hours before admission							
14.45	Plasma salicylate 790mg/l no gastric wash-out, urine alkalinisation						
18.15		116.2	0.0	55.1	111.4	2.1	2.0
20.15		82.2	0.0	34.2	47.2	2.4	1.4
22.15		66.3	0.0	164.4	46.4	0.4	0.3
00.15		53.9	0.0	66.0	27.5	0.8	0.4
02.15		52.8	0.0	13.7	32.1	3.8	2.3
04.15		64.0	0.0	99.4	46.4	0.6	0.5
06.15		67.7	0.0	172.4	13.5	1.0	0.2
08.30		74.0	0.0	77.5	17.3	1.0	0.5
10.15		56.3	0.0	57.6	27.8	1.0	0.5
12.30		113.5	0.0	51.3	44.3	2.2	0.9
14.30		84.4	0.0	2.1	21.4	40.2	10.2
16.30		86.8	0.0	0.6	7.1	144.7	11.8
18.30		85.6	0.0	0.7	27.5	122.3	39.3
20.30		55.1	0.0	0.5	13.0	110.2	26.0
23.30		94.7	0.0	1.4	25.5	67.6	18.2
01.15		68.1	0.0	0.0	22.8	$\infty$	$\infty$
03.15		76.9	0.0	0.0	18.1	$\infty$	$\infty$
05.30		60.6	0.0	0.0	18.3	$\infty$	$\infty$
Total of individual metabolites		1359.1	0.0	796.9	567.6		

Total recovery = 2723.6mg. The results are plotted in Fig. 7.3.3

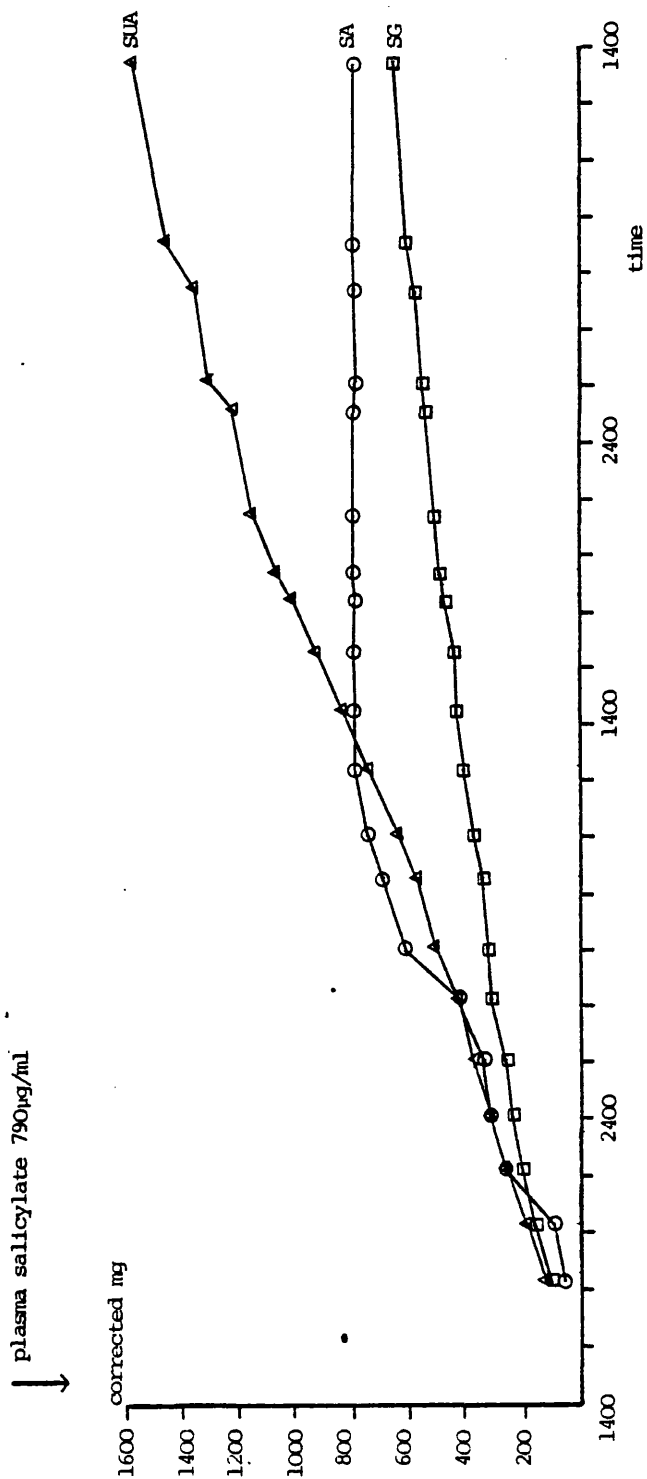


Fig. 7.3.3 Graph showing cumulative recovery of each metabolite corrected for molecular weight against time for patient shown in Table 7.3.3.

Table 7.3.4A4, female 28yr

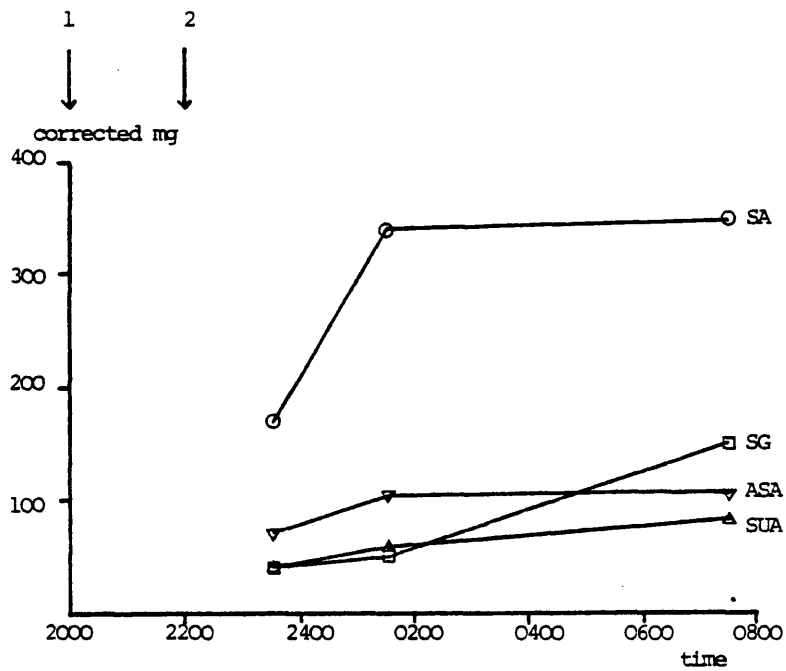
Time	Event	expressed as equivalent of SA					
		SUA	ASA	SA	SG	$\frac{SUA}{SA}$	$\frac{SG}{SA}$
20.00	36 Aspro tablets ingested						
21.50	Admitted to Casualty Dept.						
22.00	Plasma salicylate 340mg/l						
23.30		40.4	70.9	170.9	40.9	0.2	0.2
01.30		18.5	29.6	172.3	12.2	0.1	0.1
07.30		24.1	1.4	11.9	97.2	2.0	8.2
Total of individual metabolites		83.0	101.9	355.1	150.3		

Total recovery = 690.3mg. The results are plotted in Fig. 7.3.4



Fig. 7.3.4.

Graph showing cumulative recovery of each metabolite corrected for molecular weight against time for patient shown in Table 7.3.4.



1 36 Aspro ingested

2 plasma salicylate 340µg/ml

Table 7.3.5A5, Female, 15yr

Time	Event	mg expressed as equivalent of SA					
		SUA	ASA	SA	SG	$\frac{SUA}{SA}$	$\frac{SG}{SA}$
17.30	32 tablets of aspirin ingested						
00.23	Admitted to Casualty Dept. plasma salicylate 400ng/l						
06.20		200.4	0.0	266.7	138.0	0.8	0.5
10.00		156.0	0.0	320.0	217.1	0.5	0.7
11.00		61.9	0.0	353.5	253.7	0.2	0.7
13.30		81.2	0.0	343.3	309.0	0.2	0.9
19.45.		293.5	0.0	399.0	639.5	0.7	1.6
Total of individual metabolites		793.0	0.0	1682.5	1557.3		
Total recovery = 4032.8mg							

Table 7.3.6

A6, Female, 19yr

Time	Event	mg expressed as equivalent of SA					
		SUA	ASA	SA	SG	$\frac{SUA}{SA}$	$\frac{SG}{SA}$
17.00	Estimated time of overdose 32 tablets of aspirin ingested						
21.00	Admitted to Casualty Dept.; gastric wash-out						
07.15		394.1	0.0	207.5	188.9	1.9	0.9
09.55		59.7	0.0	3.4	62.4	17.6	18.4
10.00		508.1	0.0	36.9	194.4	13.8	5.3
16.00		296.2	0.0	3.4	132.8	87.1	39.0
22.00		370.5	0.0	138.3	18.7	2.7	0.1
Total of individual metabolites		1628.6	0.0	389.5	597.2		

Total recovery = 2615.3mg

It can be seen that in general the amount of salicylate recovered in urine was considerably less than the reported intake presumably as a result of the gastric wash-out for the patients described in Table 7.3.1 and 7.3.6 possibly also because of exaggeration by the patients of the number of tablets they had ingested. (The recovered dose is expressed in terms of salicylic acid - this should be multiplied by  $300/230 = 1.30$  to convert to the conventional 300mg aspirin tablets.

It can be seen from Tables 7.3.1, 7.3.4 and 7.3.5 that the major metabolite recovered was salicylic acid. The patients whose details are given in Table 7.3.2., 7.3.3. and 7.3.6 were admitted to Casualty only some 24 hours after overdose and the major metabolite present in urine was salicyluric acid presumably because salicylic acid had been metabolized and excreted by the time that urine collections were started. The percentages of the total recovery of salicylate recovered as salicyluric acid and the salicyl glucuronides in the six overdosed patients were compared with the percentage recoveries of these two metabolites in 24 hour urine from normal volunteers after aspirin 600mg. The recovery of salicyluric acid was found to be less ( $p < 0.001$ ) and the recovery of the salicyl glucuronides was greater ( $p < 0.01$ ) in the overdose patients. It can be seen from Tables 7.3.1 - 7.3.6 that initially the ratios SUA/SA and SG/SA were low but increased with time as salicylic acid was cleared and recoveries of salicyluric acid and the salicyl glucuronides rose.

## Chapter 8

### Discussion of Aspirin metabolism in man

#### 8.1. Control Study

In the present study the metabolism of aspirin was studied in normal volunteers. The percentage excretion of a single 600mg dose of aspirin was measured and the percentage recoveries of salicylic acid, salicyl glucuronides and salicylic acid in urine were quantified. Previous findings that these are the major metabolic products of acetyl salicylic acid excreted in urine were confirmed (Baldoni, 1915, Smith, Gleason, Stroll and Ogorzalik, 1946; Salassa, Bollman and Dry, 1948; Schachter and Manis, 1958; Bedford, Cummings and Martin, 1965; Levy, 1965).

It was found that the percentage of the total dose of aspirin excreted decreased significantly with age, the percentage recovery of the salicyl glucuronide also decreased significantly ( $p < 0.05$ ) with age in 0 - 8 hour urine. A decrease in kidney function with age may contribute to both effects since glomerular filtration rate and effective renal plasma flow diminish with increasing age (Davies and Shock, 1950). Davison (1971) suggested that active tubular secretion as well as glomerular filtration of the unbound salicylate occurs and reduction in effective renal plasma flow may thus limit loss by active tubular secretion.

The present results showed that there was substantial individual variation both in the total recovery of salicylate in 24 hours and in the percentage recoveries of each metabolite. Caldwell, O'Gorman and Smith (1979) reported similar variation in normal volunteers after acetyl salicylic acid 900mg by mouth. Levy (1965) calculated that above a threshold value of aspirin 360mg (equivalent to salicylic acid 276mg) saturation of the formation of salicylic acid occurred. Levy and Hollister (1964) found marked individual

differences in the apparent half life of salicylate elimination which was probably due to individual differences in the capacity to form salicyluric acid. Individual variation in the present study probably reflects the same phenomenon. Although it might have been tending towards zero order kinetics, the major metabolite recovered was salicyluric acid.

Results in the study also showed that the percentage recovery of salicylic acid was positively correlated with urinary pH but only in the first 8 hours probably because the percentage recovery of salicylic acid was so much lower in 8 - 24 hour urine as a result of further metabolism. Excretion of the acidic conjugates of salicylic acid would tend to lower the urinary pH. This in turn would tend to favour reabsorption of unionized free salicylate which would then be available to re-enter the circulation and reach the liver to be further metabolised.

The increase in percentage recovery of unchanged salicylic acid with age in 8 - 24 hour urine may have been a direct consequence of the negative correlation with age of the salicyl glucuronides in 0 - 8 hour and 0 - 24 hour urine if the formation of salicyluric acid was rate limited. Salicylurate recovery is unlikely to be correlated with age if saturation kinetics had indeed been approached.

Alternatively, ability to conjugate salicylate with glucuronic acid and glycine may diminish with increasing age or clearance by tubular transport of salicylate might not occur to such an extent in older people. Montgomery and Sitar (1981) found the serum concentrations of salicyluric acid (and gentisic acid) were increased in patients older than 60 years on chronic acetylsalicylic acid therapy.

However, these workers did not measure the urinary metabolites of acetylsalicylic acid so their study and the present work are not directly comparable.

The negative correlation of salicyl glucuronide recoveries in urine with age is in contrast to effects of age on paracetamol metabolism; i.e. that glucuronidation increased with age presumably as a consequence of the decreasing recoveries of paracetamol cysteine plus mercapturic acid with age. However, as conjugation with glycine is the major conjugation pathway for salicylate, the age effect seen in the recovery of salicyl glucuronides may well be secondary to other metabolic or renal effects, whereas for paracetamol, glucuronidation is a more important pathway.

## 8.2. Aspirin metabolism in patients with rheumatoid arthritis

In the present small study on the metabolism of aspirin in patients with inflammatory joint disease, it was not possible to measure the total percentage excretion of the administered dose of aspirin for reasons already described (Section 7.2). However the metabolic profile in these 5 patients does appear to differ from that observed in normal volunteers as the rheumatoid patients excreted much lower quantities of unchanged salicylic acid and salicyl glucuronides than did the normal volunteers. The findings of Burry and Dieppe (1976) and Kimberly and Klotz (1977) that serum creatinine concentrations were raised and creatinine clearance was diminished in volunteers who received aspirin for at least 7 days may be of relevance; that is, that the clearance of glucuronic acid and unchanged salicylate may also be decreased by the continuous salicylate therapy. Results in the present study raise the possibility that glycine availability may be increased in rheumatoid arthritis or that chronic therapy with aspirin may induce glycine N-acylase (the enzyme catalyzing the transfer of glycine to salicylic acid). Additional work needs to be carried out to measure plasma concentrations of salicylate and its metabolites and possibly to examine this system in vitro to understand these processes further.

8.3. Aspirin metabolism after overdose

Table 8.3. gives excretion rates of salicylic acid and the salicyl glucuronides in overdose patients compared with normal volunteers.

Table 8.3 mean rate of excretion of salicylic acid and the salicyl glucuronides in normal volunteers and overdose patients

1) Overdose patients

Overdose patient	Table	Period over which urine was collected	SUA mg*/h	SUA $\mu$ mol/h	SG mg*/h	SG $\mu$ mol/h	Total recovery mg*
A1	7.3.1	32h 0 min	32.4	235	17.03	123	4611.3
A2	7.3.2	3h 0 min	232.0	1681	30.98	224	991.0
A3	7.3.3	35h 15 min	38.6	280	16.10	117	3027.5
A4	7.3.4	8h 0 min	10.4	75	18.79	136	690.5
A5	7.3.5	13h 25 min	58.6	425	115.11	834	6226.1
A6	7.3.6	14h 45 min	110.4	800	40.49	293	2645.1

2) Normal volunteers (0 - 8 hour urine)

	SUA		Range	SG		Range
	Mean	SEM		Mean	SEM	
mg*/h	22.2 $\pm$ 1.7		5.3 - 60.7	2.7 $\pm$ 0.4		0.0 - 10.7
$\mu$ mol/h	161 $\pm$ 12		38 - 440	20 $\pm$ 3		0 - 78

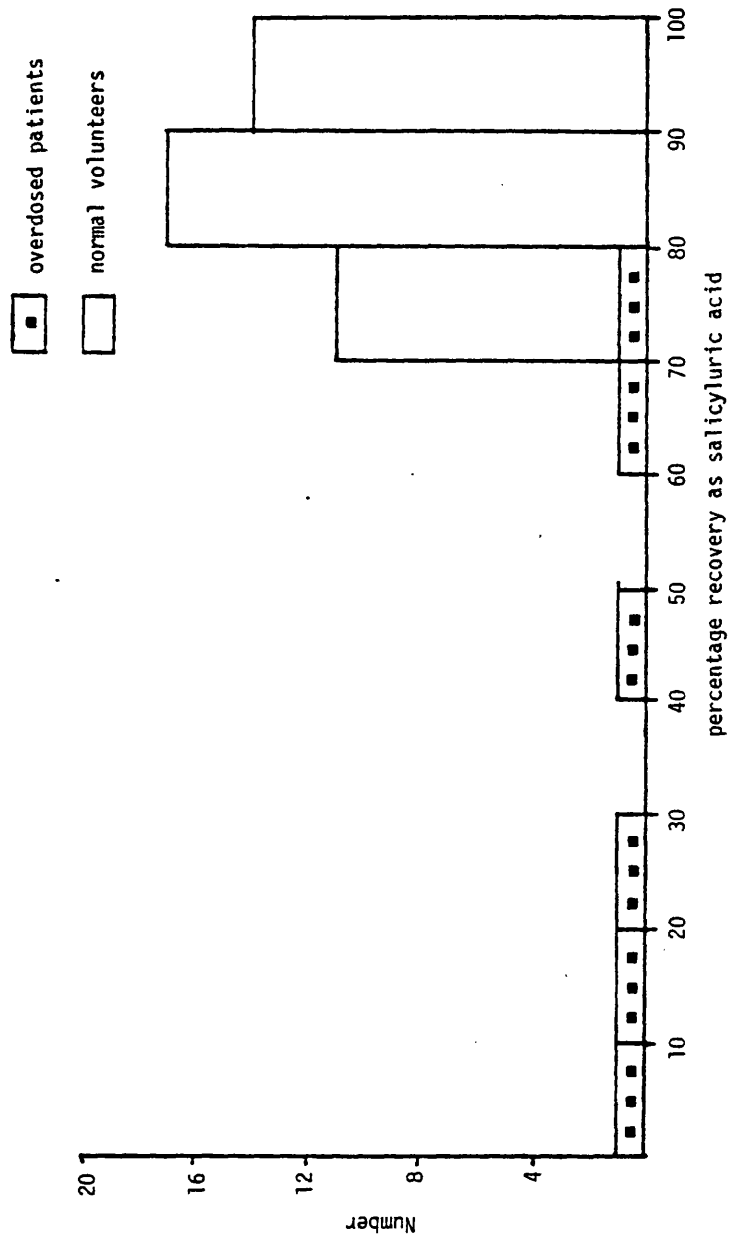
\* expressed as equivalent of SA



Values for excretion rates of salicylic acid in normal volunteers ranged from 38 - 440  $\mu\text{mol/h}$  (mean of  $161 \pm 12 \mu\text{mol/h}$ ) and are similar to values obtained by Levy (1965) which ranged from 250 - 400  $\mu\text{mol/h}$ . In Levy's study the maximum dose of salicylate ingested was 3.6g. In the overdose patients values ranged from 75 - 1681  $\mu\text{mol/h}$ . Thus both normal volunteers and overdose patients exhibit a great inter-individual difference in excretion rate of this metabolite of aspirin, a rate which does not appear to depend on the dose of aspirin recovered in urine. Results from the present study suggest that glycine depletion occurred after the high doses of aspirin ingested by these patients and that this may be compensated for by increased conjugation with glucuronic acid. Fig. 8.3 shows the frequency distribution of the percentage of salicylate recovered in urine as salicylic acid in normals and in overdose patients. It can be seen that salicylic acid recovery was lower in the overdose patients than after a therapeutic dose in normal volunteers. It has been known for many years that large doses of salicylate persist in the body for much longer periods than lower doses and half life may rise to as long as 30 hours because salicylic acid excretion is dose-dependent. This is apparently due to a limited ability to form the glycine conjugate because Levy, Amsel and Elliott (1969) showed that administration of salicylic acid to normal volunteers followed first-order excretion kinetics. Bedford, Cummings and Martin (1965) suggested that the limitation is not the supply of glycine because glycine 4g administered with 0.64g aspirin to volunteers did not affect the total salicylate excretion. However, at a dose of aspirin 0.64g it is unlikely that salicylate formation was capacity limited. At high doses however, glycine depletion may occur (as the body pool of glycine may be limited) or alternatively the enzyme catalyzing the transfer of glycine to salicylic acid (glycine N-acylase) may be operating maximally but the issue of which of these processes is occurring remains unresolved.

Fig. 8.3

Frequency distribution of the percentage dose of aspirin recovered as salicylic acid in normal volunteers after a therapeutic dose and in aspirin-poisoned patients



A further difference noted between the urinary metabolites in normal volunteers after a therapeutic dose and patients with aspirin overdose included the excretion of unchanged acetylsalicylic acid in three out of six of the overdose patients. The reason why no acetylsalicylic acid was detected in the urine of overdose patients A3, A5 and A6 (results presented in Table 7.3.3, 7.3.5 and 7.3.6 of Section 7.3) was probably because urines for analysis were collected from only 11 hours after reported overdosage time and possibly all acetylsalicylic acid has been excreted by then. It was not to be expected that significant quantities of unchanged acetylsalicylic acid would be excreted in the urine of normal volunteers because it is rapidly hydrolyzed in plasma but more may be seen in the overdose patients simply because the dose is greater and because urine collections were more frequent.

## Chapter 9

### Comparison of aspirin and paracetamol metabolism in man

The major routes of metabolism which are utilized by aspirin and paracetamol have been outlined in Chapter 1. After absorption, aspirin is deacetylated to salicylic acid which then conjugates with glycine to form salicyluric acid and with glucuronic acid to give the ester and ether glucuronides. Thus aspirin undergoes Phase I metabolism followed by Phase II metabolism and the major excretion products after therapeutic doses are salicyluric acid ( $85.65 \pm 1.28\%$ ) with the glucuronides accounting for  $8.35 \pm 0.87\%$ . In contrast, paracetamol forms mainly Phase II metabolites and the two major metabolites excreted are the glucuronide and sulphate conjugates which account for  $53.46 \pm 1.35\%$  and  $32.79 \pm 1.16\%$  respectively of the total recovery. However at least  $9.01 \pm 0.49\%$  of an administered dose of paracetamol is oxidised to an intermediate metabolite (a Phase I product) which then conjugates with glutathione to give paracetamol cysteine mercapturic acid or may react to give further oxidised metabolites (e.g. 2-hydroxy and 3-hydroxy paracetamol).

The present study permits the metabolism of aspirin and paracetamol to be compared in normal volunteers, in patients with rheumatoid arthritis and in overdosed patients. Although both drugs have been freely available without prescription for many years, a study on the metabolism of these analgesic anti-inflammatory drugs in relation to age in normal volunteers after a therapeutic dose has not previously been reported. The percentage dose of aspirin excreted in urine in 24 hours had a mean value of  $73.27 \pm 3.64\%$  and this is similar to the percentage of the dose of paracetamol that was found to be excreted in 24 hours ( $76.59 \pm 4.53\%$ ). It was found that the metabolic profiles of both drugs varied with age. The total dose of aspirin recovered in 0 - 8 hour urine expressed as a percentage decreased with increasing age. The percentage dose of paracetamol excreted did not vary with age. Although both drugs conjugate with

glucuronic acid, the percentage recovery of paracetamol glucuronide increased with increasing age in 24 hour urine while the percentage recovery of the salicyl glucuronides in 24 hour urine decreased with increasing age. This raises the possibility that the enzyme glucuronyl transferase which catalyses the conjugation of glucuronic acid with salicylic acid may be different from that which catalyses paracetamol glucuronidation. As the percentage recovery of paracetamol cysteine was found to decrease with increasing age, the increase in paracetamol glucuronide recovery with age might serve to compensate. It was also found that the percentage recovery of unchanged salicylic acid increased with increasing age in 8 - 24 hour urine and this may relate to a decrease in salicyl glucuronide recovery with age.

The metabolism of aspirin and paracetamol in patients with rheumatoid arthritis was also investigated. Rheumatoid patients might be considered to be at risk of toxicity from these drugs because of the large doses taken repeatedly over a period of time for analgesia (up to 5g of paracetamol and 1800mg of aspirin per day), and also because of concurrent therapy with other drugs possibly causing drug interactions. It is also possible that the disease state itself may alter the metabolism of these compounds. Although there was no indication of an increase in the recoveries of paracetamol cysteine plus mercapturic acid in these patients, there was a greater recovery of paracetamol glucuronides and lower recoveries of paracetamol sulphate and unchanged paracetamol than in the normal volunteers which could reflect conditions in which greater use may be made of the oxidation pathway. Similarly in RA patients there was a greater percentage recovery of salicyluric acid and lower percentage recoveries of the salicyl glucuronide and unchanged salicylate than in controls who took a therapeutic 600mg dose of aspirin. These results suggest that metabolism may be altered in rheumatoid patients. Endogenous sulphate available for conjugation with paracetamol may have been depleted as a result of the high doses being taken causing a compensatory increase in glucuronic acid conjugation. Alternatively induction of the enzymes

responsible for glycine and glucuronic acid conjugation might have occurred either as a result of the disease per se or by other drugs taken concurrently with aspirin and paracetamol.

The time courses of the urinary metabolic profile of aspirin and paracetamol after overdose were also followed. In aspirin overdose, the major metabolite recovered in urine initially was salicylic acid suggesting that the pathways of metabolism of salicylic acid were saturated or depleted. After paracetamol overdose, the recovery of unchanged paracetamol was only slightly higher (as a percentage) than the recovery in normal volunteers after a therapeutic 1g dose. By calculating the ratios paracetamol glucuronide/paracetamol sulphate (PG/PS) and paracetamol glucuronide plus paracetamol sulphate/paracetamol cysteine plus paracetamol mercapturic acid (PG + PS)/(PC + PM) it was possible to show that initial recoveries of paracetamol sulphate and paracetamol cysteine plus mercapturic acid were lower than after therapeutic doses. There was no suggestion of glucuronic acid depletion at the doses of paracetamol ingested by these patients. After overdose of aspirin there was evidence that glycine but not glucuronic acid depletion had occurred but recoveries of both these metabolites increased with time and it appears that conjugation with glucuronic acid might increase to compensate for the glycine depletion.

Mitchell, McMurtry, Statham and Nelson (1977) showed that aspirin is converted in the kidney to a highly reactive alkylating agent by cytochrome P-450 dependent microsomal mixed function oxidase. This intermediate is thought normally to be inactivated by conjugation with reduced glutathione and tubular necrosis only occurs when glutathione is depleted. Thus paracetamol and aspirin may cause toxicity in a similar fashion and protection against their toxicity may be effected by the body in similar ways. A further theory is that acidic anti-inflammatory drugs such as aspirin decrease renal blood flow, glomerular filtration rate and sodium and water excretion. This may be a result of inhibition of prostaglandin

synthesis and in particular prostaglandin E which is a renal medullary vasodilator. Prescott (1965) showed that ten individuals receiving 3.6g aspirin per day all showed a significant increase in the output of renal tubular cells and red blood cells after a latent period of about 24 hours. Paracetamol also has anti-prostaglandin activity and can cause renal damage in overdose.

It is surprising that although aspirin was first used clinically in 1899 and its effects on renal function first noted in 1917 (Prescott, 1979), it has only recently been reported that aspirin may be associated with hepatotoxicity (Russell, Sturge and Smith, 1971). It is therefore possible that salicylate is converted to a reactive metabolite in the liver as it is in the kidney (Mitchell et al, 1977). However, compared with paracetamol, hepatic injury due to aspirin is usually relatively mild, it is cumulative and appears many days after intake of large therapeutic doses (Konada, Kolling and Hindin, 1978).

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Appendix I

## Molecular weights and correction factors of paracetamol and its metabolites

	Molecular weight	Correction factor
paracetamol	151	1.000
paracetamol glucuronide	327	0.462
paracetamol sulphate	230	0.656
paracetamol cysteine	270	0.559
paracetamol mercapturic acid	312	0.484
2-hydroxy paracetamol	168	0.899
3-hydroxy paracetamol	168	0.899

## Molecular weights and correction factors of aspirin and its metabolites

	Molecular Weight	Correction factor
salicylic acid	138.18	1.000
acetyl salicylic acid	180.20	0.767
salicyluric acid	195.20	0.708
Gentisic acid	176.10	0.785

Thus a 600mg dose of aspirin is equivalent to 460.2mg salicylic acid. As the salicyl glucuronides were measured as salicylic acid after treatment with  $\beta$ -glucuronidase no correction factor was necessary.



## Appendix 2

Recovery of paracetamol and its metabolites in mg in each urine collection period corrected for molecular weight in the control study

Volunteer No.	0-8h				8-24h				0-24h						
	P	PG	PS	PC	PM	P	PG	PS	PC	PM	P	PG	PS	PC	PM
1	15.30	76.83	86.31	17.81	10.19	0.86	21.99	7.24	8.29	6.22	16.16	98.55	92.55	26.10	16.41
2	18.52	157.19	83.64	20.36	12.55	6.28	71.15	20.68	8.52	3.92	24.80	228.34	104.32	28.88	16.48
3	16.12	420.88	270.31	45.38	46.86	5.17	186.64	132.15	16.04	0.44	21.29	601.52	402.45	61.42	47.30
4	5.71	131.23	72.17	13.27	1.18	0.27	195.20	166.22	29.01	30.97	5.98	326.43	236.39	42.28	32.08
5	19.64	207.23	127.39	11.64	2.76	5.93	458.04	230.51	27.08	34.95	25.57	665.27	357.90	38.72	37.71
6	8.53	393.56	244.78	42.42	2.45	3.31	131.40	96.19	12.65	6.08	11.84	524.96	340.97	55.07	8.53
7	38.94	147.38	146.73	26.15	16.90	1.69	17.87	47.11	4.83	4.40	40.63	165.25	193.84	30.98	21.30
8	20.91	295.98	390.92	37.60	78.36	12.52	86.80	84.97	20.77	35.98	33.43	382.78	475.89	58.37	114.34
9	11.88	37.25	88.76	13.19	1.44	6.72	24.16	22.73	14.85	5.90	18.60	61.41	111.49	28.04	7.34
10	7.20	243.71	54.02	10.36	13.22	1.22	68.01	49.29	10.26	5.66	8.42	311.72	103.31	20.62	18.88
11	135.70	211.41	126.08	13.02	29.62	24.45	71.15	16.48	0.00	11.51	160.15	282.56	142.56	13.02	41.13
12	8.39	312.12	242.60	10.06	37.46	1.10	154.34	19.22	2.37	2.31	9.49	466.46	261.82	12.79	39.59
13	11.61	278.98	261.80	21.52	33.88	8.60	73.62	49.61	5.37	10.54	20.21	352.60	311.41	26.89	44.43
14	16.00	182.31	264.45	20.89	52.59	17.98	79.80	94.79	10.81	25.18	33.98	262.11	359.24	31.70	77.77
15	21.24	358.70	331.14	23.52	43.75	17.97	110.00	55.18	8.36	22.06	39.21	468.70	386.37	31.88	65.81
16	32.79	433.11	238.38	11.07	19.38	15.02	181.87	80.78	3.08	10.47	47.81	614.98	319.16	14.15	29.85

## Appendix 2

Recovery of paracetamol and its metabolites in mg in each urine collection period corrected for molecular weight in the control study

Volunteer No.	0-8h				8-24h				0-24h						
	P	PG	PS	PC	PM	P	PG	PS	PC	PM	P	PG	PS	PC	PM
17	67.04	387.01	234.61	29.78	18.19	20.44	230.56	112.05	50.29	14.86	87.48	617.57	346.66	80.07	33.05
18	39.48	365.03	124.24	26.31	31.53	8.42	282.84	28.88	13.61	26.25	47.81	647.87	153.12	39.92	57.78
19	22.04	89.62	10.78	1.75	2.46	5.99	180.36	197.00	47.62	18.53	28.03	298.01	207.78	49.37	20.99
20	30.22	234.22	128.94	6.10	22.08	27.12	188.99	103.78	2.78	21.33	57.34	423.31	232.31	8.88	43.41
21	9.03	117.02	102.70	8.80	0.85	9.64	202.31	148.94	27.94	2.94	18.67	319.33	251.64	36.74	3.79
22	15.30	158.28	145.78	7.62	18.29	5.03	52.63	8.75	1.62	4.21	20.33	210.91	154.53	9.24	22.50
23	15.94	206.77	97.95	7.30	6.63	19.28	191.33	63.95	10.94	16.32	35.22	398.10	161.90	18.24	22.95
24	22.26	206.82	264.36	27.07	47.79	3.88	18.54	17.09	2.43	5.13	26.14	225.36	281.45	29.50	52.92
25	26.84	367.21	205.56	11.74	14.22	12.82	49.08	8.86	1.66	5.42	39.66	416.29	214.42	13.40	19.64
26	26.60	393.36	249.02	9.50	6.87	15.31	153.97	94.46	6.71	7.73	41.91	547.33	343.48	41.91	14.60
27	94.50	429.82	139.48	36.83	39.86	75.40	187.57	65.14	15.43	19.48	169.90	617.39	204.62	52.26	59.34
28	1.70	135.55	95.78	12.69	2.61	18.46	276.06	58.11	40.04	8.62	20.16	411.61	153.89	52.73	11.23
29	42.86	315.02	59.04	13.58	13.83	2.03	50.18	12.99	15.71	5.70	44.89	365.20	72.03	22.29	19.53
30	11.30	171.94	133.46	7.77	16.46	3.40	78.54	54.36	7.36	17.48	14.70	250.48	178.83	15.13	33.94
31	10.25	299.06	188.60	9.68	19.66	3.60	169.28	85.75	4.53	16.33	13.85	468.34	274.35	14.20	35.99
32	11.84	295.38	159.87	48.21	43.21	5.61	142.55	61.05	17.10	16.04	17.45	437.93	220.92	65.31	59.26

Continued .....

## Appendix 2

Recovery of paracetamol and its metabolites in mg in each urine collection period corrected for molecular weight in the control study

Volunteer No.	0-8h				8-24h				0-24h						
	P	PG	PS	PC	PM	P	PG	PS	PC	PM	P	PG	PS	PC	PM
33	12.12	194.59	121.44	7.42	13.90	16.00	186.18	142.67	8.96	20.17	28.12	380.77	264.11	16.38	34.07
34	57.37	433.93	276.34	12.16	9.42	14.62	388.10	137.33	17.53	1.35	71.99	822.03	413.67	29.69	10.77
35	62.95	485.90	360.64	29.53	25.52	18.37	300.76	183.80	20.07	15.09	81.32	786.66	544.44	49.60	40.61
36	66.27	477.49	281.19	33.95	27.74	9.65	279.16	158.88	76.59	18.85	75.92	756.59	440.07	110.54	46.59
37	18.98	59.96	18.94	2.39	1.61	9.56	102.58	19.36	1.71	4.38	28.54	162.54	38.30	4.10	5.99
38	9.02	265.20	168.02	7.55	32.53	12.78	156.81	31.47	3.76	18.34	21.78	422.01	199.49	11.31	50.87
39	7.28	481.94	286.01	16.49	38.68	0.04	189.82	56.53	7.26	8.84	7.32	671.76	342.54	27.75	47.52
40	7.29	294.57	175.64	5.49	19.23	8.71	76.58	9.06	1.02	7.85	16.00	371.15	184.70	7.01	27.08
41	36.25	230.01	138.83	13.83	6.84	2.88	14.07	36.95	17.14	2.28	39.13	244.08	175.78	30.39	9.12
42	3.94	78.77	95.97	6.71	9.79	4.72	106.52	22.87	15.20	11.12	8.66	185.29	118.84	21.91	20.91
43	9.63	348.58	213.46	11.98	20.38	4.01	61.08	23.81	12.67	7.61	13.64	409.66	237.27	24.65	27.99
44	4.86	62.06	67.12	4.04	8.01	2.61	106.17	82.38	10.02	18.69	7.47	168.23	149.50	14.06	26.70
45	13.60	105.02	35.63	2.31	1.32	12.42	191.87	85.19	6.09	14.50	26.02	296.89	120.82	8.40	15.82
46	1.91	239.08	144.97	7.91	15.61	2.40	149.36	45.00	3.07	6.27	4.31	388.44	189.97	10.98	21.88
47	5.04	52.61	68.22	0.53	4.21	23.37	217.06	173.30	5.87	18.76	28.41	279.67	241.52	6.40	22.97
48	1.63	358.93	373.35	8.27	64.13	39.56	374.34	298.45	26.09	57.66	41.19	733.27	671.90	34.36	121.79
49	7.82	109.95	118.87	10.31	14.87	5.61	236.31	19.00	23.47	14.20	13.43	346.26	137.87	33.78	29.07

Appendix 3 Percentage recovery of paracetamol and its metabolites in each urine collection period

Volunteer No.	P	PG	0-8h PS	PC	PM	P	PG	8-24h PS	PC	PM
1	3.72	37.40	41.53	8.66	4.96	1.93	49.30	16.23	12.59	13.95
2	6.34	53.78	28.62	6.97	4.29	5.68	64.35	18.70	7.71	3.55
3	2.02	52.64	33.81	5.68	5.86	1.50	54.01	39.51	4.80	0.13
4	2.55	58.70	32.28	5.94	0.53	0.06	46.51	39.13	6.91	7.38
5	5.33	56.21	34.55	3.16	0.75	0.78	60.55	30.47	3.58	4.62
6	1.23	56.89	35.39	6.13	0.35	1.33	52.64	38.53	5.07	2.43
7	10.35	39.19	39.01	6.95	4.49	2.23	23.84	62.07	6.36	5.80
8	2.54	35.93	47.45	4.56	9.51	5.19	36.01	35.25	8.26	14.93
9	7.79	24.42	58.19	8.65	0.94	9.04	32.49	30.57	19.97	7.93
10	2.19	74.19	16.44	3.15	4.02	0.91	50.58	36.66	7.63	4.21
11	26.31	40.98	24.44	2.52	5.74	19.78	57.57	13.33	0.00	9.31
12	1.37	51.11	39.73	1.65	6.13	0.61	85.98	10.71	1.52	1.19
13	1.91	45.90	43.07	3.54	5.57	5.82	49.83	33.58	3.63	7.13
14	2.98	34.00	49.31	3.89	9.81	7.87	34.91	41.47	4.73	11.02
15	2.73	46.08	42.55	3.02	5.62	8.41	51.51	25.84	3.91	10.33
16	4.46	58.95	32.44	1.51	2.64	5.16	62.45	29.80	1.89	3.59
17	9.10	52.54	31.85	4.04	2.47	4.77	53.84	26.17	11.74	3.47
18	6.73	62.23	21.18	4.48	5.38	2.34	78.57	8.02	3.78	7.29
19	17.40	70.76	8.51	1.38	1.94	1.33	40.12	43.83	10.59	4.12
20	7.17	55.57	30.58	1.45	5.24	7.88	54.94	30.17	0.81	6.20
21	3.79	49.09	43.08	3.69	0.36	2.46	51.64	38.02	7.13	0.75
22	4.43	45.84	42.22	2.21	5.30	6.96	72.85	12.11	2.24	5.83
23	4.76	61.80	29.27	2.18	1.98	6.39	63.39	21.19	3.62	5.41
24	3.88	57.39	36.33	1.39	1.00	5.50	55.35	33.96	2.41	2.78

Appendix 3 continued:

Volunteer No.	P	PG	0-24h PS	PC	PM
1	6.46	39.52	37.01	10.44	6.56
2	6.16	56.69	25.90	7.17	4.09
3	1.88	53.04	35.49	5.42	4.17
4	0.93	50.75	36.75	6.57	5.00
5	2.27	59.13	31.81	3.44	3.35
6	1.26	55.77	36.22	5.85	0.91
7	8.99	36.56	42.88	6.85	4.71
8	3.14	35.95	44.69	5.48	10.74
9	8.20	27.07	49.14	12.36	3.24
10	1.82	67.33	22.32	4.45	4.08
11	25.05	44.19	22.29	2.04	6.43
12	1.20	59.03	33.13	1.62	5.01
13	2.67	46.67	41.22	3.56	5.88
14	4.44	34.27	46.97	4.14	10.17
15	3.95	47.25	38.95	3.21	6.63
16	4.66	59.94	31.69	1.38	2.91
17	7.51	53.02	29.76	6.87	2.84
18	5.06	68.44	16.18	4.22	6.10
19	4.86	46.86	36.06	8.57	3.64
20	7.49	55.28	30.39	1.16	5.67
21	2.96	50.67	39.93	5.83	6.01
22	4.87	50.52	37.01	2.21	5.39
23	5.53	62.55	25.44	2.87	3.61
24	4.25	36.62	45.74	4.79	8.60
25	3.81	59.18	30.48	1.90	2.78

Appendix 3 continued:

Volunteer No.	P	PG	0-8h PS	PC	PM	P	PG	8-24h PS	PC	PM
25	4.29	58.70	32.86	1.88	2.27	16.47	63.05	11.38	2.13	6.96
26	3.92	36.39	46.52	4.76	8.41	8.24	39.39	36.31	5.16	10.90
27	12.76	58.05	18.84	4.97	5.38	20.77	51.67	17.94	4.25	5.37
28	0.68	54.58	38.57	5.11	1.05	4.60	68.79	14.48	9.98	2.15
29	4.65	70.90	13.29	3.06	3.11	2.34	57.94	15.00	18.14	6.58
30	3.31	50.43	39.14	2.27	4.82	2.11	48.74	33.73	4.82	10.85
31	1.94	56.72	35.66	1.84	3.73	1.29	60.57	30.68	1.62	5.84
32	2.12	52.89	28.62	8.63	7.74	2.51	58.82	25.19	7.06	6.62
33	3.47	55.68	34.75	2.12	3.98	4.28	49.78	38.15	2.39	3.89
34	7.27	54.98	35.01	1.54	1.19	2.62	69.44	24.57	3.14	0.24
35	6.53	50.38	37.59	3.06	2.64	3.41	55.89	34.16	3.73	2.80
36	7.47	53.85	31.71	3.83	3.15	1.78	51.39	29.25	14.10	3.47
37	18.63	58.85	18.59	2.34	1.58	6.95	74.56	14.07	1.24	3.18
38	1.87	54.98	34.84	1.57	6.74	8.72	70.27	14.10	1.68	8.22
39	0.90	58.04	34.44	1.99	4.66	0.01	72.32	21.54	2.76	3.37
40	1.40	58.59	34.94	1.19	3.83	8.78	77.18	9.13	1.03	3.88
41	8.52	54.10	32.65	3.12	1.61	3.93	19.19	50.40	23.38	3.11
42	2.02	40.36	49.17	3.44	5.01	2.94	66.40	14.25	9.47	6.93
43	1.59	57.71	33.34	1.98	3.37	3.67	55.94	21.81	11.60	6.97
44	3.33	42.48	45.94	2.76	5.48	1.19	48.29	37.47	4.56	8.50
45	8.61	66.52	22.57	1.46	0.84	4.00	61.88	27.47	1.96	4.68
46	0.47	58.39	35.40	1.93	3.81	1.16	72.47	21.83	1.49	3.04
47	3.85	40.28	52.23	0.41	3.22	5.30	49.52	39.53	1.34	4.28
48	0.20	44.52	46.30	1.02	7.95	4.97	47.02	37.49	3.28	7.24
49	2.99	41.99	45.40	3.94	5.68	1.88	79.14	6.36	7.86	4.76

Appendix 3 continued:

Volunteer No.	P	PG	0-24h PS	PC	PM
26	4.35	56.80	35.65	1.68	1.51
27	15.40	55.95	18.54	4.74	5.38
28	3.10	63.36	23.69	8.12	1.73
29	8.45	68.78	13.57	5.52	3.68
30	2.93	49.89	37.41	3.01	6.76
31	1.72	58.05	34.01	1.77	4.46
32	2.18	54.68	27.58	8.15	7.40
33	3.89	52.63	36.51	2.26	4.71
34	5.34	60.97	30.68	2.20	0.80
35	5.41	52.35	36.23	3.20	2.70
36	5.31	52.92	30.78	7.73	3.25
37	11.92	67.87	15.99	1.71	2.50
38	3.09	59.82	28.28	1.60	7.21
39	0.67	61.47	31.34	2.17	4.35
40	2.65	61.66	30.68	1.16	3.83
41	7.85	48.96	35.26	6.10	1.83
42	2.43	52.10	33.42	6.16	5.88
43	1.91	57.44	33.27	3.46	3.92
44	2.04	45.97	40.85	3.84	7.29
45	5.60	63.44	25.82	1.79	3.38
46	0.70	63.10	30.86	1.78	3.55
47	4.99	47.40	42.45	1.12	4.04
48	2.57	45.76	41.92	2.14	7.60
49	2.40	61.79	24.60	6.03	5.19

Appendix 4

4.1 Dose of paracetamol given, smoking habits, other drugs being taken concurrently with paracetamol during the control study, and date of birth of each volunteer.

Volunteer No.	Tobacco, and other drugs	Dose of paracetamol	Sex	Date of birth
1	None	500mg	F	25.8.69
2	None	500mg	M	16.9.67
3	None	1g	M	2.5.57
4	None	1g	M	14.9.56
5	Antihistamines occasionally	1g	F	6.7.56
6	Not known	1g	M	1955
7	20 cigarettes/day	1g	F	18.10.58
8	None	1g	M	19.4.53
9	None	1g	F	13.4.56
10	Oral contraceptive	1g	F	19.9.52
11	None	1g	F	2.1.43
12	None	1g	M	22.11.42
13	None	1g	F	12.4.44
14	None	1g	M	14.2.49
15	None	1g	F	18.1.41
16	None	1g	M	3.1.45
17	None	1g	F	24.9.48
18	30 cigarettes/day	1g	F	11.9.46
19	None	1g	F	1.10.42



## Appendix 4 continued:

Volunteer No.	Tobacco, and other drugs	Dose of paracetamol	Sex	Date of birth
20	None	1g	F	28.5.39
21	None	1g	F	17.4.39
22	None	1g	F	21.1.31
23	phenytoin	1g	F	14.3.35
24	10 cigarettes/day	1g	M	9.9.39
25	None	1g	F	1.7.37
26	5-10 cigarettes/day	1g	F	24.11.32
27	None	1g	M	9.7.31
28	None	1g	F	15.8.31
29	None	1g	M	23.6.39
30	None	1g	F	13.1.24
31	None	1g	M	4.4.26
32	None	1g	F	11.8.25
33	Distalgesics	1g	F	20.12.26
34	30 cigarettes/day	1g	M	25.1.21
35	None	1g	M	6.5.21
36	½oz pipe tobacco/day	1g	M	16.11.23
37	None	1g	F	5.2.22
38	None	1g	M	16.5.17
39	None	1g	M	17.9.17
40	None	1g	F	23.7.10

## Appendix 4 continued:

Volunteer No.	Tobacco, and other drugs	Dose of paracetamol	Sex	Date of birth
41	diazepam 5mg nocte	1g	F	3.11.15
42	propranolol 10mg x 3, chlordiazepoxide 10mg nocte	1g	F	24.11.16
43	7-8 cigarettes/day	1g	M	21.12.11
44	2oz pipe tobacco/week	1g	F	4.6.05
45	7oz pipe tobacco/week	1g	M	3.2.06
46	None	1g	M	13.3.06
47	None	1g	F	22.12.08
48	None	1g	F	29.12.05
49	Lasikal 20mg OO, methyldopa 250mg BD	1g	F	11.7.02

## 4.2 Individual details of four volunteers who took paracetamol 2.5g by mouth

Initials	Sex	Age	H.G.O.			L.J.N.			P.N.B.			C.C.B.S.										
			P	PG	PS	P	PM	PC	P	PG	PS	P	PM	PC	P	PG	PS	PC	PM			
H.G.O.	F	24	21.29	140.75	81.43	7.99	2.46	24.02	106.43	57.95	13.00	2.30	37.45	414.74	190.93	32.46	8.37	37.83	505.88	102.91	19.61	10.35
L.J.N.	F	28	12.46	257.10	67.05	8.21	10.97	50.26	742.12	196.82	20.67	22.10	44.00	1687.37	267.61	32.63	28.70	28.70	269.71	72.85	23.10	9.78
P.N.B.	M	42	18.36	513.31	142.02	10.34	28.29	12.54	413.33	104.89	16.20	15.32	11.33	342.72	193.61	19.04	24.96	18.60	321.17	93.01	21.09	9.28
C.C.B.S.	M	28	7.48	426.50	91.24	16.95	14.10	8.66	353.15	84.32	21.71	18.05	7.41	285.32	127.70	17.34	20.63	-	-	-	-	-
			3.42	261.70	67.08	13.53	11.22	2.84	344.13	62.24	12.76	18.93	3.00	195.60	83.12	13.06	12.97	6.06	264.53	79.65	14.90	14.13

Recovery of paracetamol and its metabolites in mg in each 24h urine sample corrected for molecular weight.

## Percentage recovery of paracetamol and its metabolites in each 2h urine sample

	H.G.O.			L.J.N.			P.N.B.			C.C.B.S.										
	P	PG	PS	P	PM	PC	P	PM	PC	P	PM	PC	P	PM	PC					
0-2h	8.38	55.43	32.07	3.15	0.97	11.79	52.25	28.45	6.38	1.13	54.8	50.64	27.92	4.47	1.22	5.59	74.77	18.02	5.72	2.42
2-4h	3.33	73.60	17.94	2.20	2.93	4.07	71.91	19.07	2.00	2.14	2.14	31.90	12.99	1.58	1.39	7.10	66.74	18.02	5.72	2.42
4-6h	2.54	71.16	19.69	2.63	3.92	2.23	73.51	18.65	2.88	2.72	1.91	57.92	32.72	3.22	4.22	4.02	69.35	20.98	2.00	
6-8h	1.34	76.67	16.40	3.05	2.53	1.78	72.69	17.35	4.47	3.71	1.62	62.24	27.86	3.78	4.50					
8-10h	0.96	73.32	18.79	3.79	3.14	0.64	78.04	14.11	2.99	4.30	0.97	63.66	27.01	4.24	4.21	1.60	69.75	21.00	3.93	3.72

Appendix 55.1 Amount of paracetamol and its metabolites ( $\mu\text{g}$ ) in neonatal urine

Initials	P	PG	PS	PC	PM
L.D.	8.12	53.94	5.69	5.87	11.57
F.C.	26.04	30.79	2.98	0.00	0.00
R.M. (Twin)	2.91	17.71	3.58	3.36	0.00
R.M. (Twin)	85.05	309.25	56.48	35.80	0.00
D.C.	165.62	348.52	150.06	66.38	91.66
M.C.	432.40	302.57	74.68	75.53	36.74

5.2 Percentage recovery of paracetamol and its metabolites in neonatal urine

Initials	P	PG	PS	PC	PM
L.D.	9.53	63.32	6.68	6.89	13.58
F.C.	43.53	51.48	4.98	0.00	0.00
R.M. (Twin)	11.71	71.27	9.46	7.56	0.00
R.M. (Twin)	17.48	63.56	11.61	7.36	0.00
D.C.	20.14	42.39	18.25	8.07	11.15
M.C.	46.90	32.82	8.10	8.19	3.98

Appendix 6 American Rheumatism Association criteria (revised 1963)  
for diagnosis of rheumatoid arthritis

1. Morning stiffness
2. Pain on motion or tenderness in at least one joint (observed by a physician).
3. Swelling (soft tissue thickening or fluid - not bony overgrowth alone) in at least one joint (observed by a physician).
4. Swelling (observed by a physician) of at least one other joint (any interval free of joint symptoms between the two joint involvements may not be more than three months).
5. Symmetrical joint swelling (observed by a physician) with simultaneous involvement of the same joint on both sides of the body (bilateral involvement of mid-phalangeal joints is acceptable without absolute symmetry). Terminal interphalangeal joint involvement will not satisfy this criterion.
6. Subcutaneous nodules (observed by a physician) over bony prominences on extensor surfaces, or in juxta articular regions.
7. X-ray changes typical of RA (which must include at least bony decalcification localised to or greatest around the involved joints and not just degenerative changes) - degenerative changes do not exclude patients from any group classified as rheumatoid arthritis.
8. Positive agglutination test-demonstration of the 'rheumatoid factor' by any method that, in two laboratories has been positive in not more than 5 per cent of normal controls, or positive streptococcal agglutination test.

Appendix 7 Details of rheumatoid patients

Initials	Sex	Age (years)	Amount of paracetamol taken in the 24 hours of the study	Times when additional paracetamol was taken	Other drugs
A.S.	F	55	4	12.00, 22.00 06.30	Distalgesic, nitrazepam, naproxen
B.L.	F	53	2	12.30	nitrazepam, indomethacin, sodium aurothiomalate
R.G.	M	40	4	13.00, 17.00 22.00	prednisolone, naproxen
J.H.	F	60			indomethacin, penicillamine
M.P.	F	41	>1g	-	record not obtainable
K.P.	F	65	3.5	12.30, 22.00	indomethacin, sodium aurothiomalate
M.T.	F	78	4	12.00, 18.00	theophylline, naproxen, prednisolone
K.P.	F	65	5	13.30, 18.30 22.30, 06.45	sodium aurothiomalate, indomethacin, indomethacin
J.G.	F	59	>1g	-	record not obtainable
B.G.	F	65	4	12.30, 18.30 22.30	indomethacin, connalazine
P.C.	F	43	1	-	penicillamine, ibuprofen
S.T.	F	36	4	12.15, 18.20 22.30	diclofenac
T.S.	F	65	2	12.05	piroxicam, neomycin, aminopyphylline
J.H.	F	59	>1g	-	indomethacin, penicillamine
R.H.	F	71	>1g	-	prednisolone, azathioprine, indomethacin, distalgesic
E.P.	F	72	4	18.00, 22.00 06.00	ibuprofen, penicillamine, nitrazepam

Appendix 7 ContinuedDetails of rheumatoid patients

Initials	Sex	Age (years)	Amount of paracetamol taken in the 24 hours of the study	Times when additional paracetamol was taken	Other drugs
A.W.	F	49	4	12.30, 18.15 22.00	flurbiprofen
D.D.	F	42	3	12.10, 18.35	naproxen, chlormelanone
J.P.	F	73	4.5	12.30, 18.00 22.00, 06.00	ibuprofen, nitrazepam
D.F.	F	50	4	14.00, 18.00 22.00	clozic, flurbiprofen
V.H.	F	46	>1g	-	penicillamine
H.N.	F	69	2	12.10	nitrazepam, benoxoprofen, ferrous sulphate

Appendix 8 Individual values of mg recoveries of paracetamol and its metabolites in 22 rheumatoid patients

Initials	0-8h					9-24h					0-24h				
	P	PG	PS	PC	PM	P	PG	PS	PC	PM	P	PG	PS	PC	PM
V.H.	4.43	62.02	64.04	20.56	7.84	6.00	285.98	119.95	27.65	39.24	10.43	348.00	183.99	48.22	47.13
M.P.	53.46	1821.11	792.36	152.36	187.12	2.60	214.09	92.80	5.72	22.53	56.06	2035.20	885.17	158.08	209.65
J.G.	10.59	70.39	53.08	4.27	13.00	10.32	66.53	7.89	2.91	10.69	20.91	136.92	60.97	7.18	23.69
J.H.	2.22	135.06	82.75	5.52	8.63	9.70	281.83	114.14	17.50	34.69	11.92	416.89	196.89	23.02	43.32
R.H.	7.69	76.68	84.05	1.17	7.67	4.98	323.52	166.42	11.29	28.66	12.67	400.20	250.47	12.46	36.33
A.S.	60.45	901.59	304.29	33.64	305.10	58.51	1472.87	885.54	42.44	56.83	118.96	2374.46	689.83	76.08	87.34
B.L.	8.52	183.45	103.04	33.38	14.30	9.24	319.36	194.29	37.74	15.50	17.76	503.21	297.33	71.12	29.80
R.G.	9.62	431.12	104.34	15.94	14.67	21.42	1024.38	132.14	37.03	43.50	31.04	1455.50	236.48	52.97	58.17
J.H.	13.11	263.10	98.69	12.97	7.33	20.12	710.61	229.60	37.29	25.94	33.23	973.71	328.29	50.26	33.27
K.P.	5.75	230.29	85.66	19.45	13.78	45.95	972.46	232.51	89.09	68.77	51.70	1202.75	409.17	108.54	82.55
M.T.	43.87	371.90	139.63	21.97	12.92	142.45	1943.15	364.60	131.48	94.86	186.32	2315.05	504.23	153.45	107.78
K.P.	26.40	326.49	2.89	54.22	40.69	187.60	2587.60	995.77	494.99	417.91	214.00	2913.69	998.66	549.21	458.60
C.G.	26.31	1868.70	705.84	69.66	113.96	24.91	1551.83	554.62	55.66	86.60	51.22	3420.53	1260.46	125.32	200.56
P.C.	3.64	242.65	45.95	14.3	26.53	10.23	177.06	70.09	10.03	19.40	13.87	419.71	116.04	24.26	45.93
S.T.	19.53	624.54	109.72	34.78	24.75	29.18	1127.15	194.87	77.54	58.88	48.71	1751.69	304.59	161.85	83.63
I.S.	12.62	646.72	286.11	46.82	39.41	13.34	697.19	302.95	58.40	50.77	25.96	1343.91	589.06	105.22	90.18
E.P.	11.72	550.70	205.64	18.31	13.06	22.33	1153.22	368.64	46.82	41.35	34.05	1703.92	574.28	65.13	54.41
J.P.	5.84	89.58	66.94	4.01	7.01	29.38	1027.29	542.12	47.83	85.10	35.22	1116.87	609.06	51.84	92.11
D.F.	33.88	1163.43	1333.87	118.58	126.96	61.44	1407.45	723.89	142.56	204.49	95.32	2370.70	2057.70	677.13	331.45
H.M.	6.11	342.31	111.70	14.16	21.01	15.94	1139.10	365.92	47.41	62.56	22.05	1481.41	477.62	61.57	83.57
A.W.	17.97	1284.98	392.51	49.08	59.30	49.16	2643.23	871.90	101.21	133.32	67.13	3928.23	1264.44	595.53	192.62
D.N.	24.13	382.41	346.27	37.42	81.32	29.17	454.73	337.03	45.40	91.08	53.30	837.14	678.30	82.82	172.40



## Appendix 9

Individual values of percentage recoveries of paracetamol and its metabolites in 22 rheumatoid patients

Initials	0-8h				9-24h				0-24h					
	PG	PS	PC	PM	P	PG	PS	PC	PM	P	PG	PS	PC	PM
V.H.	2.79	39.03	12.94	4.93	1.25	59.73	25.05	5.77	8.20	1.64	54.57	28.85	7.56	7.38
M.P.	1.78	60.57	5.07	6.22	0.77	63.39	27.48	1.69	6.67	1.08	60.86	26.47	4.73	6.27
J.G.	7.00	46.51	2.82	8.59	10.49	67.65	8.02	2.96	10.87	8.37	54.84	24.42	2.88	9.49
J.H.	0.95	57.67	2.36	3.69	2.12	61.55	24.93	3.82	7.58	.17	60.24	28.45	3.33	6.26
R.H.	4.34	43.26	0.66	4.33	0.93	60.49	31.11	2.11	5.36	1.78	56.20	35.17	1.75	5.10
A.S.	4.54	67.76	2.53	2.29	2.90	73.05	19.12	2.10	2.82	3.55	70.95	20.61	2.27	2.61
B.L.	2.48	53.59	9.73	4.17	1.60	55.43	33.72	6.55	2.69	1.93	54.69	32.35	7.74	3.24
R.G.	1.67	74.88	2.77	2.55	1.70	81.40	10.50	2.94	3.46	1.69	79.36	12.89	2.89	3.17
J.H.	3.32	66.57	3.28	1.85	1.96	69.43	22.43	3.64	2.53	2.34	68.63	23.14	3.54	2.34
K.P.	1.62	64.97	5.49	3.89	3.06	64.84	21.57	5.94	4.59	2.79	64.86	22.07	5.85	4.45
M.T.	7.43	63.00	3.72	2.19	5.32	72.60	13.62	4.91	3.54	5.70	70.87	15.43	4.70	3.30
K.P.	5.86	72.44	12.03	9.03	4.01	55.24	21.26	10.57	8.92	4.17	56.75	19.45	10.70	8.93
C.G.	0.94	67.11	2.50	0.94	1.10	68.25	24.39	2.45	3.81	1.01	67.62	24.92	2.48	3.96
P.C.	1.09	72.87	4.27	7.97	3.57	61.73	24.44	32.50	6.76	2.24	67.72	18.72	3.91	7.41
S.T.	2.40	76.79	4.28	3.04	1.96	75.77	13.10	5.21	3.96	2.12	76.13	13.24	4.88	3.63
I.S.	1.22	62.69	4.54	3.82	1.19	62.10	26.98	5.20	4.52	1.20	62.38	27.34	4.88	4.18
G.P.	1.47	68.89	2.29	1.63	1.37	70.65	22.58	2.87	2.53	1.40	70.07	23.62	2.68	2.24
J.P.	3.37	51.67	2.31	4.04	1.70	59.32	31.30	2.76	4.91	1.85	58.02	31.97	2.72	4.83
D.F.	1.22	41.90	4.27	4.57	2.21	55.42	28.50	5.61	8.05	1.79	48.36	38.70	4.91	6.23
H.M.	1.23	69.11	2.86	4.24	0.98	69.84	22.44	2.91	3.84	1.04	69.67	22.46	2.90	3.93
A.W.	1.00	71.24	2.72	3.29	1.29	69.58	22.95	2.66	3.51	1.20	70.11	22.57	2.68	3.44
D.D.	2.77	43.88	4.29	9.33	3.06	47.74	34.86	4.77	9.56	2.92	45.90	37.19	4.54	9.45

Appendix 10      Total amounts of unchanged paracetamol and its metabolites (mg) in 7 hyperthyroid patients

Initials	0-8h						8-24h						0-24h									
	P	PG	PS	PC	PM	P	PG	PS	PC	PM	P	PG	PS	PC	PM	P	PG	PS	PC	PM		
A.M.	47.35	859.86	588.18	57.19	38.13	120.73	724.47	308.84	55.68	14.00	168.08	1584.33	897.02	112.87	52.13							
I.H.	31.43	272.44	81.37	11.24	5.23	13.71	187.57	39.84	10.36	1.74	45.14	460.01	121.22	21.60	6.97							
A.A.	13.37	88.52	46.51	3.04	8.71	6.93	25.71	8.51	0.63	1.74	20.30	114.23	55.02	3.67	10.45							
H.R.	19.37	71.90	66.83	6.81	5.23	19.74	136.23	120.97	11.62	15.08	39.1	208.13	187.80	18.43	20.3							
E.C.	14.18	239.64	184.94	18.56	32.95	5.20	28.86	18.32	4.93	9.35	19.38	268.50	203.26	23.49	42.3							
D.S.	17.41	448.80	106.25	15.27	21.79	4.25	157.03	29.14	7.51	14.39	21.66	605.83	135.39	22.78	36.1							
G.G.	3.09	111.61	95.00	4.44	13.17	7.14	108.79	59.37	5.83	10.57	10.23	220.40	154.37	10.27	23.7							

Appendix 11      Percentage recovery of paracetamol and its metabolites in 7 hyperthyroid patients

Initials	0-8h				8-24h				0-24h					
	PG	PS	PC	PM	P	PG	PS	PC	PM	P	PG	PS	PC	PM
A.M.	2.98	36.98	3.59	2.40	9.86	59.20	25.24	4.55	1.14	5.97	56.29	31.87	4.01	1.85
I.H.	7.82	20.26	2.86	1.30	5.41	74.07	15.74	4.09	0.69	6.89	70.24	18.51	3.30	1.06
A.A.	8.35	29.04	1.90	5.44	15.92	59.08	19.55	1.45	4.00	9.97	56.08	27.01	1.80	5.13
H.R.	11.35	39.30	4.00	3.07	6.50	44.865	39.84	3.83	4.97	8.25	43.93	39.64	3.88	4.29
E.C.	2.89	48.88	3.78	6.72	7.80	43.29	27.48	7.40	14.03	3.48	48.21	36.50	4.22	7.60
D.S.	2.86	73.63	2.50	3.57	2.00	73.96	13.72	3.54	6.78	2.64	73.72	16.47	2.77	4.40
G.G.	1.36	49.10	1.95	5.79	3.72	56.75	30.97	3.04	5.51	2.44	52.60	36.84	2.45	5.66

Appendix 12 Individual values of paracetamol and its metabolites excreted in urine (mg corrected for molecular weight) in Experiment 1 and Experiment 2

Experiment 1

Volunteer	P	0-8h			8-24h			0-24h							
		PG	PS	PC	PM	P	PG	PS	PC	PM	P	PG	PS	PC	PM
He	70.96	284.90	159.14	14.58	12.14	20.66	279.50	132.58	14.11	15.82	91.62	564.40	291.72	28.69	27.96
Ha	2.17	166.74	46.00	4.05	6.89	16.55	159.09	29.09	5.77	6.30	18.72	325.83	75.09	9.82	13.19
B	21.74	332.16	475.29	19.42	42.81	18.11	177.07	170.10	17.57	18.30	39.85	509.23	645.39	36.99	61.11
Pa	13.44	419.49	257.14	18.04	31.65	32.05	308.94	143.87	13.35	17.22	45.49	728.43	401.01	31.39	48.87
Pe	21.34	428.03	484.53	19.93	33.73	-	-	-	-	-	-	-	-	-	-
S	15.77	422.05	252.51	13.49	33.32	27.88	567.88	228.48	24.40	37.40	43.65	989.93	480.99	37.89	70.72

Experiment 2

Volunteer	P	0-8h			8-24h			0-24h							
		PG	PS	PC	PM	P	PG	PS	PC	PM	P	PG	PS	PC	PM
He	27.93	332.91	23.52	10.33	12.49	16.64	377.12	73.41	16.86	17.66	44.57	710.03	96.93	27.19	30.15
Ha	18.92	550.31	78.92	11.49	13.78	9.63	306.71	47.93	9.45	10.07	28.55	857.02	126.85	20.94	23.85
B	20.91	296.51	259.57	20.17	38.26	24.68	334.48	423.41	33.69	57.89	45.59	630.99	682.98	53.86	96.15
Pa	23.31	363.31	93.91	10.68	11.89	11.31	262.76	61.91	14.97	18.18	34.62	626.07	155.82	25.65	30.07
Pe	24.14	376.28	235.25	18.06	17.15	13.48	186.60	64.42	13.52	6.75	37.62	562.88	299.67	31.58	23.90
S	21.83	352.37	93.21	12.15	22.51	36.33	387.68	84.03	20.04	21.98	58.16	740.05	177.24	32.19	44.49

Appendix 13 Individual values of percentage recovery of paracetamol and its metabolites in urine in Experiment 1 and Experiment 2 (Section 3.5)

Experiment 1

Volunteer	P	0-8h				9-24h				0-24h					
		PG	PS	PC	PM	P	PG	PS	PC	PM	P	PG	PS	PC	PM
He	13.10	52.59	29.38	2.69	2.24	4.46	60.41	28.66	3.05	3.42	9.12	56.19	29.04	2.86	2.78
Ha	0.96	73.83	20.37	1.79	3.05	7.63	73.38	13.42	2.66	2.90	4.23	73.61	16.96	2.22	2.98
B	2.44	37.26	53.32	1.28	4.80	4.51	44.14	42.40	4.38	4.56	3.08	39.40	49.93	2.86	4.73
Pa	1.82	56.71	34.76	2.44	4.28	6.22	59.94	29.91	2.59	3.34	3.62	58.03	31.95	2.50	3.89
Pe	2.16	43.34	49.06	2.02	3.42	-	-	-	-	-	-	-	-	-	-
S	2.14	57.26	34.36	1.83	4.52	3.15	64.09	25.79	2.75	4.22	2.69	60.99	29.63	2.33	4.36

Experiment 2

Volunteer	P	0-8h				8-24h				0-24h					
		PG	PS	PC	PM	P	PG	PS	PC	PM	P	PG	PS	PC	PM
He	6.86	81.76	5.78	2.54	3.07	3.32	75.17	14.36	3.36	3.52	4.90	78.12	10.66	2.99	3.32
Ha	2.81	81.72	11.72	1.71	2.05	2.51	79.92	12.49	2.46	2.62	2.70	81.06	12.00	1.98	2.26
B	3.29	46.66	40.85	3.17	6.02	2.82	38.26	48.44	3.85	6.62	3.02	41.80	45.24	3.57	6.37
Pa	4.63	72.21	18.67	2.12	2.36	3.06	71.18	16.77	4.06	4.92	3.97	71.78	17.86	2.94	3.45
Pe	3.60	56.09	35.06	2.69	2.56	4.73	65.53	22.62	4.75	2.37	3.94	58.90	31.36	3.30	2.50
S	4.35	70.18	18.56	2.42	4.48	6.60	70.49	15.28	3.64	4.00	5.53	70.34	16.85	3.06	4.23

Appendix 14 Individual serum concentrations of paracetamol, paracetamol glucuronide and paracetamol sulphate (corrected for molecular weight) in Experiment 1 and Experiment 2 as described in Section 3.5

Experiment 1

Time (min)	Concentration $\mu\text{g/ml}$			Time (min)	Concentration $\mu\text{g/ml}$			Time (min)	Concentration $\mu\text{g/ml}$		
<u>He</u>	P	G	S	<u>Ha</u>	P	G	S	<u>B</u>	P	G	S
0	0.00	0.18	0.00	0	0.00	0.00	0.00	0	0.73	0.47	2.81
45	2.95	0.79	0.94	44	3.32	2.09	0.94	45	4.06	0.65	1.88
72	4.46	2.53	0.94	94	4.06	4.55	1.88	90	3.07	1.23	1.88
34	4.03	3.10	0.94	135	3.65	6.28	0.94	135	2.50	1.23	1.88
79	3.27	3.10	0.94	180	2.33	5.41	0.00	180	1.18	1.37	1.88
25	2.62	2.53	0.94	225	1.59	4.84	0.00	225	-	-	-
268	2.08	2.38	0.00	270	1.18	3.39	0.00	270	0.76	1.23	1.88
315	1.86	1.95	0.00	318	0.76	2.81	0.00	320	0.52	0.65	0.94

Time (min)	Concentration $\mu\text{g/ml}$			Time (min)	Concentration $\mu\text{g/ml}$			Time (min)	Concentration $\mu\text{g/ml}$		
<u>Pa</u>	P	PG	PS	<u>Pe</u>	P	PG	PS	<u>S</u>	P	PG	PS
0	0.24	0.33	0.00	0	0.00	0.05	0.00	0	0.00	0.00	0.00
45	3.81	1.66	0.94	47	3.15	0.50	0.94	46	3.32	1.37	0.94
91	2.50	2.87	0.94	94	2.58	1.37	1.88	91	2.74	2.53	0.94
135	2.16	3.10	0.94	135	2.74	2.24	1.88	136	2.16	3.10	0.94
178	1.01	2.53	0.00	180	2.41	2.09	1.88	180	1.34	2.67	0.94
224	0.76	2.09	0.00	228	1.51	1.81	0.94	226	1.09	1.95	0.94
268	0.60	1.95	0.00	268	1.18	1.66	0.94	271	1.01	1.81	0.00
313	0.52	1.66	0.00	316	0.93	1.23	0.94	317	0.60	1.37	0.00

Appendix 14 Cont'd Serum concentrations of paracetamol, paracetamol glucuronide and paracetamol sulphate (corrected for molecular weight) in Experiment 1 and Experiment 2 as described in Section 3.5

Experiment 2

Time (min)	Concentration $\mu\text{g/ml}$			Time (min)	Concentration $\mu\text{g/ml}$			Time (min)	Concentration $\mu\text{g/ml}$		
<u>He</u>	P	PG	PS	<u>Ha</u>	P	PG	PS	<u>B</u>	P	PG	PS
0	0.00	0.00	0.00	0	0.16	0.00	0.00	0	0.00	0.00	0.00
48	5.87	1.81	0.00	48	2.25	0.50	0.00	45	4.97	0.94	0.94
94	5.06	4.69	0.00	90	3.24	2.09	0.00	105	3.65	1.66	0.94
139	4.30	4.26	0.00	136	4.39	4.84	0.00	135	3.57	1.23	0.00
184	3.65	3.82	0.00	188	4.14	5.12	0.00	182	3.15	1.66	0.94
232	2.68	3.82	0.00	228	2.82	5.70	0.00	225	2.50	1.23	0.00
281	2.35	2.96	0.00	277	2.00	5.27	0.00	277	1.92	1.23	0.00
324	1.86	2.81	0.00	323	1.42	4.26	0.00	325	1.18	0.65	0.00

Time (min)	Concentration $\mu\text{g/ml}$			Time (min)	Concentration $\mu\text{g/ml}$			Time (min)	Concentration $\mu\text{g/ml}$		
<u>Pa</u>	P	PG	PS	<u>Pe</u>	P	PG	PS	<u>S</u>	P	PG	PS
0	0.00	0.00	0.00	0	0.41	0.00	0.00	0	0.00	0.00	0.00
46	4.22	1.23	0.00	49	3.07	0.94	0.00	44	3.73	1.08	0.00
93	3.65	2.81	0.00	90	2.91	1.87	0.00	90	2.58	2.38	0.00
139	2.91	1.67	0.00	141	4.06	2.87	0.94	137	2.41	2.38	0.00
183	2.66	2.67	0.00	188	3.24	2.67	0.94	187	2.00	2.24	0.00
230	2.00	2.38	0.00	228	2.16	2.38	0.00	227	1.51	2.24	0.00
280	-	-	-	275	2.00	2.38	0.00	271	1.09	1.81	0.00
323	1.09	2.09	0.00	320	2.00	2.09	0.00	319	0.85	1.23	0.00

Appendix 1515.1 Individual quantities (mg corrected for molecular weight) of paracetamol and its metabolites recovered in 24 hour urine after administration of 3 inducing agents in saline or saline alone to guinea pigs.

		P	PG	PS	PC	PM
paracetamol 150mg/kg	G1	4.26	31.70	0.85	0.00	0.00
	G2	4.38	32.20	1.73	0.00	0.00
	G3	6.32	0.00	0.83	0.00	0.00
	G4	4.62	57.14	1.42	0.00	0.00
paracetamol 150mg/kg after 7 day pretreatment with phenobarbitone 100mg/kg	G1	0.66	29.08	0.56	0.44	0.23
	G2	0.29	19.90	0.39	0.00	0.11
	G3	0.32	22.78	1.10	0.15	0.13
paracetamol 150mg/kg after 4 day pretreatment with phenylbutazone 100mg/kg	G1	0.41	22.95	0.43	0.05	0.24
	G2	0.40	31.47	0.84	0.10	0.00
	G3	0.84	51.07	1.22	0.06	0.34
	G4	0.56	13.97	0.40	0.09	0.00
paracetamol 150mg/kg after 5 day pretreatment with rifampicin 80mg/kg	G1	0.30	2.47	0.19	0.02	0.03
	G2	0.36	44.19	4.28	0.03	0.30
	G3	0.18	7.65	0.28	0.03	0.07
	G4	0.30	2.47	0.19	0.02	0.03
	G5	2.36	44.19	4.28	0.03	0.30
	G6	0.18	7.65	0.28	0.03	0.07



**15.2 Individual percentage recoveries of paracetamol and its metabolites in 24 hour urine after administration of 3 inducing agents in saline or saline alone to guinea pigs**

		P	PG	PS	PC	PM
paracetamol 150mg/kg	G1	11.57	86.12	2.31	0.00	0.00
	G2	11.43	84.05	4.52	0.00	0.00
	G3	88.39	0.00	11.61	0.00	0.00
	G4	7.311	90.44	2.25	0.00	0.00
paracetamol 150mg/kg after 7 day pretreatment with phenobarbitone 100mg/kg	G1	2.13	93.90	1.87	1.42	0.74
	G2	1.40	96.18	1.88	0.00	0.53
	G3	1.3	92.98	4.49	0.61	0.61
paracetamol 150mg/kg after 4 day pretreatment with phenylbutazone 100mg/kg	G1	1.70	95.31	1.78	0.21	1.00
	G2	1.22	95.92	2.56	0.30	0.00
	G3	1.57	95.40	2.28	0.11	0.64
	G4	3.73	93.01	2.66	0.60	0.00
paracetamol 150mg/kg after 5 day pretreatment with rifampicin 80mg/kg	G1	9.97	82.06	6.31	0.66	1.00
	G2	0.73	89.89	8.71	0.06	0.61
	G3	2.19	93.18	3.41	0.36	0.85
	G4	9.97	82.06	6.31	0.66	1.00
	G5	0.73	89.89	8.71	0.06	0.61
	G6	2.19	93.18	3.41	0.36	0.85

Appendix 16      16.1 Individual quantities (mg corrected for molecular weight) of paracetamol and its metabolites recovered in 24 hour urine after administration of 3 inducing agents in saline or saline alone to mice

		P	PG	PS	PC	PM
paracetamol 150mg/kg	M1	0.01	0.36	0.04	0.05	0.00
	M2	0.04	0.08	0.01	0.02	0.01
	M3	0.08	1.36	0.35	0.29	0.00
	M4	0.12	0.57	0.12	0.12	0.07
	M5		0.11	0.07	0.03	0.00
	M6	0.01	0.03	0.00	0.01	0.00
paracetamol 150mg/kg after 4 day pretreatment with phenobarbitone 100mg/kg	M1	0.07	0.60	0.01	0.24	0.08
	M2	0.07	0.43	0.00	0.16	0.06
	M3	0.08	0.88	0.20	0.33	0.21
	M4	0.05	0.42	0.01	0.26	0.14
	M5	0.05	1.85	0.15	1.21	0.49
	M6	0.07	0.81	0.03	0.32	0.15
paracetamol 150mg/kg after 7 day pretreatment with phenobarbitone 100mg/kg	M1	0.19	3.70	0.26	2.21	0.60
	M2	0.24	3.16	0.38	2.00	0.56
	M3	0.35	4.22	0.31	2.84	0.66
	M4	0.23	2.95	0.24	2.72	0.68
	M5	0.24	2.42	0.08	1.70	0.50
	M6	0.11	2.28	0.16	1.51	0.33
paracetamol 150mg/kg after 4 day pretreatment with phenylbutazone 100mg/kg	M1	0.84	6.19	0.62	1.62	0.42
	M2	1.49	13.33	1.05	2.92	1.33
	M3	1.24	8.50	0.67	2.04	0.47
	M4	2.84	21.73	2.48	4.77	0.70
	M5	1.15	9.86	1.11	2.38	0.70
	M6	1.54	12.46	1.35	3.63	0.62
paracetamol 150mg/kg after 5 day pretreatment with rifampicin 80mg/kg	M1	0.11	3.87	0.45	2.11	0.17
	M2	0.00	0.16	0.01	0.08	0.01
	M3	0.06	2.24	0.33	1.11	0.06
	M4	0.02	0.18	0.00	0.12	0.04
	M5	0.06	1.68	0.18	0.72	0.05

16.2 Individual percentage recoveries of paracetamol and its metabolites in 24 hour urine after administration of 3 inducing agents in saline or saline alone to mice

		P	PG	PS	PC	PM
paracetamol 150mg/kg	M1	1.19	78.98	7.98	11.85	0.00
	M2	26.97	50.94	6.57	11.06	4.47
	M3	4.00	64.99	16.95	14.06	0.00
	M4	12.58	59.32	12.58	12.19	3.32
	M5	10.56	64.83	6.43	18.18	0.00
	M6	15.15	64.34	0.00	20.52	0.00
paracetamol 150mg/kg after 4 day pretreatment with phenobarbitone 100mg/kg	M1	6.63	60.23	0.94	24.00	8.20
	M2	10.20	59.68	0.00	22.47	7.65
	M3	4.53	51.61	11.97	19.35	12.53
	M4	5.92	47.14	1.62	29.94	15.39
	M5	11.29	44.42	3.58	29.05	11.67
	M6	5.16	58.19	2.48	23.38	10.80
paracetamol 150mg/kg after 7 day pretreatment with phenobarbitone 100mg/kg	M1	2.73	53.16	3.74	31.75	8.62
	M2	3.78	49.84	5.99	31.54	8.83
	M3	4.18	50.36	3.70	33.89	7.88
	M4	3.37	43.26	3.52	39.88	9.97
	M5	4.86	48.99	1.62	34.41	10.12
	M6	2.50	51.94	3.64	34.40	7.52
paracetamol 150mg/kg after 4 day pretreatment with phenylbutazone 100mg/kg	M1	8.67	63.88	6.40	16.72	4.33
	M2	7.40	66.25	5.22	14.51	6.61
	M3	9.60	65.79	5.18	15.79	3.64
	M4	8.73	66.82	7.63	14.67	2.15
	M5	7.56	64.87	7.30	15.66	4.60
	M6	7.86	63.57	6.89	18.52	3.16
paracetamol 150mg/kg 5 day pretreatment with rifampicin 80mg/kg	M1	1.64	57.68	6.71	31.44	2.53
	M2	0.00	61.54	3.85	30.77	3.85
	M3	1.58	58.95	8.68	29.21	1.58
	M4	5.56	50.00	0.00	33.33	11.11
	M5	2.23	62.45	6.69	26.76	1.86

Appendix 17

Individual mg quantities corrected for molecular weight of paracetamol and its metabolites in 0-24 hour urine after paracetamol 100mg/kg in guinea pigs and mice

		P	PG	PS	PC	PM
guinea pig	1	2.56	6.95	0.00	0.07	0.19
guinea pig	2	2.78	7.28	0.00	0.06	0.39
guinea pig	3	7.26	0.55	0.09	0.02	0.05
guinea pig	4	0.50	0.29	0.00	0.00	0.00
mouse	1	2.69	3.61	0.355	2.07	0.50
mouse	2	1.66	7.31	0.34	2.62	0.46
mouse	3	1.35	11.20	1.01	3.22	0.31
mouse	4	6.61	1.74	0.34	2.77	0.86
mouse	5	0.59	0.94	0.00	0.61	0.05
mouse	6	7.48	3.44	0.39	2.92	0.50

Appendix 18

Individual percentage recoveries of paracetamol and its metabolites in 0-24 hour urine after paracetamol 100mg/kg in guinea pigs and mice

		P	PG	PS	PC	PM
guinea pig	1	26.20	71.41	0.00	0.72	1.94
guinea pig	2	26.45	69.27	0.00	0.57	3.71
guinea pig	3	91.09	6.90	1.13	0.25	0.63
guinea pig	4	63.29	36.71	0.00	0.00	0.00
mouse	1	29.18	39.15	3.80	22.45	5.42
mouse	2	13.40	59.00	2.74	21.15	3.71
mouse	3	7.90	65.54	5.91	18.84	1.87
mouse	4	53.65	14.12	2.76	22.48	6.98
mouse	5	26.94	42.92	0.00	27.85	2.28
mouse	6	50.78	23.35	2.65	19.82	3.39

Appendix 1919.1 Individual mg quantities correct for molecular weight of paracetamol and its metabolites after repeated dosing with paracetamol 100mg/kg twice daily in guinea pigs

		P	PG	PS	PC	PM
0-24h	G1	65.18	0.00	0.00	0.00	0.00
	G2	52.97	13.54	0.00	1.38	0.00
	G3	22.59	36.80	0.00	1.91	0.00
	G4	30.12	59.42	0.00	0.00	0.00
24-72h	G1	82.56	0.00	0.00	2.93	0.00
	G2	68.25	0.00	0.00	6.71	0.00
	G3	116.43	0.00	0.00	5.05	0.00
	G4	20.43	4.76	0.00	0.00	0.00
72-120h	G1	38.13	0.00	0.00	38.13	0.00
	G2	41.04	0.00	0.00	41.04	0.00
	G3	43.90	0.00	0.00	43.90	0.00
	G4	15.33	0.00	0.00	15.33	0.47
120-168h	G1	45.88	13.80	0.00	0.06	0.00
	G2	135.20	0.00	0.00	8.65	0.00
	G3	112.20	0.00	0.00	4.99	0.00
	G4	19.48	0.23	0.00	2.30	0.00
168-216h	G1	77.18	7.98	0.00	3.18	0.00
	G2	253.47	0.00	0.00	16.20	0.00
	G3	214.20	0.00	0.00	14.97	0.00
	G4	45.06	0.00	0.00	1.50	0.00

Appendix 19 cont'd19.2 Individual percentage recoveries of paracetamol and its metabolites after repeated dosing with paracetamol 100mg/kg twice daily in guinea pigs

		P	PG	PS	PC	PM
0-24h	G1	100.00	0.00	0.00	0.00	0.00
	G2	78.02	19.94	0.00	2.03	0.00
	G3	36.85	60.03	0.00	3.12	0.00
	G4	33.64	66.36	0.00	0.00	0.00
24-72h	G1	96.57	0.00	0.00	3.43	0.00
	G2	91.05	0.00	0.00	8.95	0.00
	G3	95.84	0.00	0.00	4.16	0.00
	G4	81.07	18.93	0.00	0.00	0.00
72-120h	G1	100.00	0.00	0.00	0.00	0.00
	G2	100.00	0.00	0.00	0.00	0.00
	G3	100.00	0.00	0.00	0.00	0.00
	G4	97.02	0.00	0.00	0.00	2.97
120-168h	G1	76.80	23.10	0.00	0.10	0.00
	G2	93.99	0.00	0.00	6.01	0.00
	G3	95.94	0.00	0.00	4.26	0.00
	G4	88.50	1.04	0.00	10.45	0.00
168-216h	G1	87.37	9.03	0.00	3.60	0.00
	G2	93.99	0.00	0.00	6.01	0.00
	G3	93.47	0.00	0.00	6.53	0.00
	G4	96.78	0.00	0.00	3.22	0.00

Appendix 2020.1 Individual mg quantities corrected for molecular weight of paracetamol and its metabolites after repeated dosing with paracetamol 100mg/kg twice daily in mice

		P	PG	PS	PC	PM
0-24h	M1	0.03	6.77	0.00	3.59	0.71
	M2	0.47	3.88	0.00	1.70	0.14
	M3	0.00	4.43	0.00	2.17	0.18
	M4	0.03	7.16	0.00	2.82	0.21
	M5	0.00	8.83	0.00	3.53	0.56
	M6	0.28	10.93	0.0	3.83	0.21
24-72h	M1	20.65	0.00	0.00	5.12	1.47
	M2	2.62	10.59	0.00	5.05	0.66
	M3	22.72	0.23	0.00	8.80	0.99
	M4	14.17	2.10	0.00	4.34	0.56
	M5	3.96	12.19	0.00	5.96	0.82
	M6	6.68	0.00	0.00	1.87	0.38
72-120h	M1	9.41	0.00	0.00	2.18	1.18
	M2	1.10	0.00	0.00	0.32	0.03
	M3	13.50	0.00	0.00	3.66	0.65
	M4	7.77	0.00	0.00	2.25	0.15
	M5	0.60	3.10	0.00	1.44	0.28
	M6	8.51	0.00	0.00	2.68	0.28
120-168h	M1	6.82	0.00	0.00	1.21	0.80
	M2	2.18	0.00	0.00	0.80	0.07
	M3	10.64	0.00	0.00	1.82	0.32
	M4	15.56	0.00	0.00	4.12	0.59
	M5	0.55	0.58	0.00	0.56	0.06
	M6	0.85	0.00	0.00	0.24	0.04

Appendix 2020.1 Individual mg quantities corrected for molecular weight of paracetamol and its metabolites after repeated dosing with paracetamol 100mg/kg in mice Cont'd

		P	PG	PS	PC	PM
168-216h	M1	13.82	0.00	0.00	5.79	1.49
	M2	5.02	13.65	0.00	8.74	1.09
	M3	19.40	0.00	0.00	9.64	0.50
	M4	2.24	12.56	0.00	6.03	0.38
	M5	2.39	16.00	0.00	10.34	0.83
	M6	17.90	0.00	0.00	7.58	1.42
216-264h	M1	11.58	0.00	0.00	5.89	1.09
	M2	15.31	0.00	0.00	8.17	0.48
	M3	11.01	0.00	0.00	5.77	0.23
	M4	1.41	7.67	0.00	4.87	0.18
	M5	1.48	11.64	0.00	7.00	0.52
	M6	12.33	0.00	0.00	5.02	0.22



Appendix 20 Cont'd20.2 Individual percentage recoveries of paracetamol and its metabolites  
after repeated dosing with paracetamol 100mg/kg twice daily in mice

		P	PG	PS	PC	PM
0-24h	M1	0.27	60.99	0.00	32.34	6.40
	M2	7.59	62.68	0.00	27.46	2.26
	M3	0.00	65.34	0.00	32.00	2.65
	M4	0.29	70.06	0.00	27.59	2.05
	M5	0.00	68.34	0.00	27.32	4.33
	M6	1.84	71.67	0.00	25.11	1.38
24-72h	M1	75.81	0.00	0.00	18.80	5.40
	M2	13.85	55.97	0.00	26.69	3.49
	M3	69.40	0.70	0.00	26.88	3.02
	M4	66.93	9.92	0.00	20.50	2.64
	M5	17.27	53.16	0.00	25.99	3.58
	M6	74.80	0.00	0.00	20.94	4.26
72-120h	M1	73.69	0.00	0.00	17.07	9.24
	M2	75.86	0.00	0.00	220.7	2.07
	M3	75.80	0.00	0.00	20.55	3.65
	M4	76.40	0.00	0.00	22.12	1.47
	M5	11.07	57.20	0.00	26.57	5.17
	M6	74.19	0.00	0.00	23.36	2.44
120-168h	M1	77.24	0.00	0.00	13.70	9.06
	M2	71.48	0.00	0.00	26.23	2.30
	M3	83.26	0.00	0.00	14.24	2.50
	M4	76.76	0.00	0.00	20.32	2.91
	M5	31.43	33.14	0.00	32.00	3.43
	M6	75.22	0.00	0.00	21.24	3.54

Appendix 20 Cont'd20.2 Individual percentage recoveries of paracetamol and its metabolites after repeated dosing with paracetamol 100mg/kg twice daily in mice cont'd

		P	PG	PS	PC	PM
168-216h	M1	05.50	0.00	0.00	27.44	7.06
	M2	17.61	47.89	0.00	30.67	3.82
	M3	65.67	0.00	0.00	32.63	1.69
	M4	10.56	59.22	0.00	28.43	1.79
	M5	8.08	54.13	0.00	34.98	2.81
	M6	66.54	0.00	0.00	28.18	5.28
216-264h	M1	62.39	0.00	0.00	31.73	5.87
	M2	63.90	0.00	0.00	34.10	2.00
	M3	64.73	0.00	0.00	33.92	1.35
	M4	9.98	54.28	0.00	34.46	1.27
	M5	7.17	56.40	0.00	33.91	2.52
	M6	70.18	0.00	0.00	28.57	1.25

Appendix 2121.1 Individual mg quantities (corrected for molecular weight) of paracetamol and its metabolites after concurrent administration of metabolic competing agents to guinea pigs

		P	PG	PS	PC	PM
paracetamol 100mg/kg	G1	2.56	6.95	0.00	0.07	0.19
	G2	2.78	7.28	0.00	0.06	0.39
	G3	7.26	0.55	0.09	0.02	0.05
	G4	0.50	0.29	0.00	0.00	0.00
paracetamol 100mg/kg + salicylamide 100mg/kg	G1	0.85	13.62	0.00	0.00	0.00
	G2	0.75	9.73	0.00	0.00	0.00
	G3	0.76	14.36	0.00	0.00	0.31
	G4	0.47	6.66	0.00	0.00	0.27
paracetamol 100mg/kg + L-ascorbic acid 100mg/kg	G1	2.03	16.93	0.85	0.00	0.00
	G2	0.86	13.09	0.00	0.00	0.00
	G3	1.64	32.81	0.85	0.00	0.00
	G4	1.40	11.90	0.00	0.00	0.00
paracetamol 100mg/kg + $\alpha$ -tocopherol 100mg/kg	G1	0.14	5.94	0.00	0.00	0.00
	G2	0.19	44.61	3.62	0.00	1.32
	G3	0.47	35.42	0.53	0.00	0.00
	G4	0.49	16.16	0.00	0.00	0.00

21.2 Individual percentage recoveries of paracetamol and its metabolites after concurrent administration of metabolic competing agents to guinea pigs

		P	PG	PS	PC	PM
paracetamol 100mg/kg	G1	26.20	71.14	0.00	0.72	1.94
	G2	26.45	69.27	0.00	0.57	3.71
	G3	91.09	6.90	1.13	0.25	0.63
	G4	63.29	36.71	0.00	0.00	0.00
paracetamol 100mg/kg + salicylamide 100mg/kg	G1	5.87	94.12	0.00	0.00	0.00
	G2	7.16	92.84	0.00	0.00	0.00
	G3	4.92	93.06	0.00	0.00	2.01
	G4	6.35	90.00	0.00	0.00	3.65
paracetamol 100mg/kg + L-ascorbic acid 100mg/kg	G1	10.25	85.46	4.29	0.00	0.00
	G2	6.16	93.84	0.00	0.00	0.00
	G3	4.64	92.95	2.41	0.00	0.00
	G4	10.53	89.47	0.00	0.00	0.00
paracetamol 100mg/kg + $\alpha$ -tocopherol 100mg/kg	G1	2.30	97.70	0.00	0.00	0.00
	G2	0.38	89.69	7.28	0.00	2.65
	G3	1.29	97.25	1.46	0.00	0.00
	G4	2.94	97.06	0.00	0.00	0.00

Appendix 2222.1 Individual mg quantities (corrected for molecular weight) of paracetamol and its metabolites after concurrent administration of metabolic competing agents to mice

		P	PG	PS	PC	PM
paracetamol 100mg/kg	M1	2.69	3.61	0.35	2.07	0.50
	M2	1.66	7.31	0.34	2.62	0.46
	M3	1.35	11.20	1.01	3.22	0.31
	M4	6.61	1.74	0.34	2.77	0.86
	M5	0.59	0.94	0.00	0.61	0.005
	M6	7.48	3.44	0.39	2.92	0.50
paracetamol 100mg/kg + salicylamide 100mg/kg	M1	0.74	1.85	0.00	1.45	0.27
	M2	1.12	0.37	0.00	1.02	0.19
	M3	2.37	9.55	0.30	3.88	0.33
	M4	2.99	6.00	0.24	3.20	0.45
	M5	3.10	10.05	0.08	3.41	0.54
	M6	4.51	5.98	0.37	4.34	0.79
paracetamol 100mg/kg + L-ascorbic acid 100mg/kg	M1	0.56	5.97	1.20	1.66	0.46
	M2	0.93	3.01	0.48	1.05	0.17
	M3	1.75	3.83	0.94	1.98	0.35
	M4	0.77	3.96	0.90	2.18	0.18
	M5	1.16	4.06	0.94	1.70	0.18
	M6	0.61	1.30	0.18	0.64	0.08
paracetamol 100mg/kg + $\alpha$ -tocopherol 100mg/kg	M1	0.21	3.83	0.08	1.53	0.18
	M2	0.81	8.04	0.30	3.93	0.63
	M3	0.19	1.47	0.04	0.52	0.08
	M4	0.35	4.56	0.02	2.15	0.37
	M5	0.58	5.28	0.02	2.26	0.28
	M6	0.82	11.30	0.86	4.86	0.70

22.2 Individual percentage recoveries of paracetamol and its metabolites after concurrent administration of metabolic competing agents to mice

		P	PG	PS	PC	PM
paracetamol 100mg/kg	M1	29.18	39.15	3.80	22.45	5.42
	M2	13.40	59.0	2.74	21.15	3.71
	M3	7.90	65.54	5.91	18.84	1.81
	M4	53.65	14.12	2.76	22.48	6.98
	M5	26.94	42.92	0.00	17.85	2.28
	M6	50.78	23.35	2.65	19.82	3.39
paracetamol 100mg/kg + salicylamide 100mg/kg	M1	17.17	42.92	0.00	33.64	6.26
	M2	41.48	13.70	0.00	37.78	7.04
	M3	14.42	58.12	1.82	23.62	2.01
	M4	23.21	46.58	1.86	24.84	3.49
	M5	18.04	58.50	0.46	19.85	3.14
	M6	28.20	37.40	2.31	27.14	4.94
paracetamol 100mg/kg + L-ascorbic acid 100mg/kg	M1	5.68	60.61	12.18	16.85	4.67
	M2	16.49	53.37	8.51	18.62	3.01
	M3	19.77	43.28	10.62	22.37	3.95
	M4	9.64	49.56	11.26	27.28	2.25
	M5	14.43	50.50	11.69	21.14	2.24
	M6	21.71	46.26	6.40	22.78	2.85
paracetamol 100mg/kg + $\alpha$ -tocopherol 100mg/kg	M1	3.60	65.69	1.37	26.24	3.09
	M2	5.91	58.64	2.19	28.66	4.60
	M3	8.26	63.91	1.74	22.61	3.48
	M4	4.70	61.21	0.27	28.86	4.97
	M5	6.89	62.71	0.24	26.84	3.32
	M6	4.42	60.95	4.64	26.21	3.78

Appendix 23

Details of volunteers including smoking and drinking habits, other drugs being taken concurrently with aspirin during the control study, and date of birth of each volunteer.

Subject No. 1 took 900mg aspirin but all other volunteers took 600mg aspirin.

Volunteer No.	Drugs and cigarettes taken concurrently with aspirin	Sex	Date of birth
1	Cigarette smoker	M	23/4/55
2	Paracetamol 1g 24 - 36 prior to aspirin	M	12/2/48
3	None	M	29/10/53
4	None	F	18/1/41
5	Contraceptive pill	F	14/10/54
6	None	M	14/7/49
7	None	F	17/4/38
8	None	M	14/4/58
9	Alcohol	M	16/12/47
10	None	M	3/1/45
11	Multivitamins	F	18/11/35
12	None	M	17/7/57
13	None	M	6/10/40
14	None	F	9/9/59
15	None	F	28/7/61
16	None	M	26/3/62
17	Cigarette smoker	F	20/6/40
18	None	F	1/11/25
19	None	F	7/6/22
20	None	F	26/12/67
21	None	M	7/6/65
22	nor-estorone, cigarette smoker	F	4/12/57
23	None	F	12/6/55
24	salbutamol periodically	F	16/5/43
25	None	F	10/6/24
26	Cigarette smoker	F	6/9/60

Appendix 23 continued

Volunteer No.	Drugs and cigarettes taken concurrently with aspirin	Sex	Date of birth
27	None	F	13/2/9/30
28	Cigarette smoker	F	31/1/40
29	Cigarette smoker	F	25/6/43
30	None	F	3/12/21
31	antihistamine	M	10/9/40
32	None	F	11/7/02
33	None	M	4/5/08
34	None	M	13/3/06
35	None	M	13/12/35
36	None	M	6/1/18
37	None	F	10/1/04
38	None	M	31/3/03
39	danthron	F	16/1/97
40	danthron	F	1/5/03
41	None	F	16/7/96



Appendix 24 Individual quantities of aspirin and its metabolites in mg in normal volunteers corrected for molecular weight in each urine collection period

Volunteer	0 - 8h			8 - 24h			0 - 24h								
	ASA	GA	SUA	ASA	GA	SUA	ASA	GA	SUA	SG					
1	8.90	0.00	0.61	186.02	19.88	8.05	0.00	0.00	266.88	10.45	16.95	0.00	0.61	452.90	30.33
2	0.00	0.00	31.18	324.18	43.84	9.97	0.00	3.64	106.49	3.64	9.97	0.00	34.82	430.51	47.48
3	0.00	0.00	5.97	336.59	30.03	0.00	0.00	0.00	51.95	0.00	0.00	0.00	5.97	388.54	30.03
4	11.77	0.00	46.83	482.83	0.00	0.00	0.00	2.21	120.61	11.30	11.77	0.00	46.83	603.28	11.30
5	3.45	0.00	52.70	190.84	55.92	0.00	0.00	0.00	164.64	0.00	3.45	0.00	52.70	355.48	55.92
6	4.22	0.00	1.54	292.97	19.66	0.00	0.00	0.00	120.18	2.45	4.22	0.00	1.54	413.15	22.11
7	2.49	0.00	12.53	46.54	11.62	23.22	0.00	2.31	179.90	14.11	25.71	0.00	14.84	226.44	25.73
8	0.00	0.00	2.03	209.94	7.22	19.94	0.00	0.00	173.04	0.00	19.94	0.00	2.03	382.98	7.22
9	8.63	0.00	3.15	305.86	51.41	24.07	0.00	0.00	52.21	0.00	32.70	0.00	3.15	358.87	51.41
10	16.18	0.00	19.66	170.17	85.44	9.20	0.00	1.68	108.20	3.36	25.38	0.00	21.34	278.37	88.80
11	11.66	0.00	4.26	106.54	36.86	0.00	0.00	0.00	162.33	12.82	11.66	0.00	4.26	268.87	49.68
12	0.00	0.00	0.00	130.90	4.90	10.24	0.00	0.00	18.51	0.00	10.24	0.00	0.00	149.41	4.90
13	0.00	0.00	2.66	238.10	16.24	0.00	0.00	0.00	117.81	4.34	0.00	0.00	2.66	355.91	20.88
14	0.00	0.00	20.95	200.09	73.50	0.00	0.00	0.00	9.26	4.17	0.00	0.00	20.95	209.35	77.67
15	1.06	0.00	30.24	240.26	67.57	0.00	0.00	0.00	58.16	1.96	1.06	0.00	30.24	298.42	69.43



Appendix 24 Individual quantities of aspirin and its metabolites in mg in normal volunteers corrected for molecular weight in each urine collection period

Volunteer	0 - 8h			8 - 24h			0 - 24h							
	ASA	GA	SUA	ASA	GA	SUA	ASA	GA	SUA	SA	SG			
30	0.00	0.00	104.77	0.00	0.00	21.68	155.43	0.00	0.00	0.00	21.68	260.20	16.05	
31	0.00	1.50	119.47	0.00	0.00	17.00	158.56	21.57	0.00	0.00	23.73	278.03	40.34	
32	0.00	0.00	18.50	131.21	1.11	0.00	8.40	249.43	27.72	0.00	1.50	26.90	380.64	28.83
33	0.00	0.00	42.66	0.00	0.00	6.85	344.02	23.44	0.00	0.00	6.85	386.68	23.44	
34	0.00	0.00	181.52	17.98	0.00	2.56	259.86	0.00	0.00	0.00	2.64	441.38	17.98	
35	0.00	0.00	18.30	152.30	9.71	0.00	0.00	74.30	0.00	0.00	18.30	226.60	9.71	
36	0.00	0.00	166.32	15.62	0.00	0.00	85.72	0.00	0.00	0.00	0.00	252.04	15.62	
37	0.00	0.00	94.87	0.00	0.00	10.14	49.52	2.69	0.00	0.00	10.14	144.39	2.69	
38	0.00	0.00	89.22	9.48	0.00	0.00	26.98	0.00	0.00	0.00	0.00	116.20	9.48	
39	0.00	0.00	20.61	73.69	0.00	0.00	47.67	138.74	0.00	0.00	68.28	212.43	0.00	
40	0.00	0.00	27.48	93.94	4.68	0.00	16.76	114.91	22.17	0.00	44.24	208.85	26.85	
41	0.00	0.00	161.83	0.00	0.00	0.00	34.32	0.00	0.00	0.00	0.00	196.15	0.00	

Appendix 25 Individual percentage recoveries of aspirin and its metabolites in normal volunteers in each urine collection period

Volunteer	0 - 8h			8 - 24h			0 - 24h								
	ASA	GA	SA	ASA	GA	SA	ASA	GA	SA						
1	4.13	0.00	0.28	86.36	9.23	2.82	0.00	0.00	0.00	3.38	3.66	0.00	0.12	90.44	6.06
2	0.00	0.00	7.81	81.20	10.99	8.06	0.00	2.94	86.06	2.94	1.91	0.00	6.66	82.35	9.08
3	0.00	0.00	1.60	90.34	8.06	0.00	0.00	0.00	100.00	0.00	0.00	0.00	1.41	91.52	7.07
4	2.17	0.00	8.65	89.17	0.00	0.00	0.00	1.65	89.93	8.42	1.75	0.00	6.96	89.62	1.68
5	1.14	0.00	17.40	63.00	18.46	0.00	0.00	0.00	100.00	0.00	0.74	0.00	11.27	76.03	11.96
6	1.32	0.00	0.48	92.02	6.17	0.00	0.00	0.00	100.00	0.00	0.96	0.00	0.35	93.68	5.01
7	3.40	0.00	17.12	63.60	15.88	10.58	0.00	1.05	81.94	6.43	8.78	0.00	5.07	77.36	8.79
8	0.00	0.00	0.93	95.78	3.29	10.33	0.00	0.00	89.67	0.00	4.84	0.00	0.49	92.92	1.75
9	2.34	0.00	0.85	82.88	13.93	31.55	0.00	0.00	68.44	0.00	7.34	0.00	0.71	80.40	11.54
10	5.58	0.00	6.74	58.39	29.32	7.51	0.00	1.37	88.37	2.74	6.13	0.00	5.16	67.26	21.45
11	7.32	0.00	2.67	66.87	23.14	0.00	0.00	0.00	92.68	7.32	3.49	0.00	1.27	80.39	14.85
12	0.00	0.00	0.00	96.39	3.61	35.62	0.00	0.00	64.38	0.00	6.22	0.00	0.00	90.80	2.98
13	0.00	0.00	1.04	92.64	6.32	0.00	0.00	0.00	96.45	3.55	0.00	0.00	0.70	93.87	5.43
14	0.00	0.00	7.11	67.93	24.95	0.00	0.00	0.00	68.95	31.05	0.00	0.00	6.80	67.98	25.22
15	0.31	0.00	8.92	70.84	19.92	0.00	0.00	0.00	96.74	3.26	0.26	0.00	7.57	74.74	17.42

Appendix 25 Individual percentage recoveries of aspirin and its metabolites in normal volunteers in each urine collection period

Volunteer	0 - 8h			8 - 24h			0 - 24h			SUA	SG					
	ASA	GA	SA	ASA	GA	SA	ASA	GA	SA							
16	0.00	0.00	14.52	75.45	10.03	10.03	0.00	0.00	82.94	17.06	0.00	0.00	9.61	77.98	12.40	
17	0.00	0.00	7.37	87.62	5.01	5.01	0.00	0.00	100.00	0.00	0.00	0.00	6.07	89.80	4.13	
18	0.00	0.00	10.10	82.45	7.45	7.45	0.00	0.00	97.80	2.20	0.00	0.00	7.15	86.93	5.92	
19	0.00	0.00	0.40	85.55	14.04	14.04	0.00	0.00	10.08	89.80	0.00	0.00	3.76	87.02	9.21	
20	0.00	1.34	0.00	90.50	8.16	8.16	0.00	3.26	0.00	83.91	12.84	0.00	1.85	0.00	88.74	9.40
21	0.00	2.90	1.51	90.62	4.98	4.98	0.00	5.99	0.00	86.49	7.52	0.00	3.85	1.04	89.34	5.76
22	0.00	0.91	2.62	85.21	11.25	11.25	0.00	0.00	100.00	0.00	0.00	0.00	0.72	2.09	88.23	8.96
23	0.00	0.00	0.00	80.27	19.73	19.73	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	85.99	14.01
24	0.00	2.34	14.01	75.83	7.81	7.81	0.00	0.00	88.19	11.81	0.00	1.37	8.19	80.96	9.47	
25	0.00	0.00	0.00	86.49	13.51	13.51	0.00	0.00	98.16	1.84	0.00	0.00	0.00	92.88	7.12	
26	0.00	0.00	0.00	98.43	1.57	1.57	0.00	0.00	58.10	41.90	0.00	0.00	0.00	81.08	18.92	
27	0.00	0.00	2.15	89.45	8.45	8.45	0.00	0.00	8.25	58.81	2.94	0.00	3.50	89.31	7.19	
28	0.00	0.00	1.25	91.53	7.22	7.22	0.00	0.00	100.00	0.00	0.00	0.00	0.77	94.81	4.43	
29	0.00	0.00	39.67	60.33	0.00	0.00	0.00	0.00	1.79	73.97	24.23	0.00	18.81	67.84	13.35	
30	0.00	0.00	0.00	86.72	13.28	13.28	0.00	0.00	12.24	87.76	0.00	0.00	7.28	87.34	5.39	

Appendix 25 Individual percentage recoveries of aspirin and its metabolites in normal volunteers in each urine collection period

Volunteer	0 - 8h			8 - 24h			0 - 24h								
	ASA	GA	SUA	ASA	GA	SUA	ASA	GA	SA	SUA	SG				
31	0.00	1.02	4.59	81.57	12.81	0.00	0.00	8.62	80.43	10.94	0.00	0.44	6.91	80.92	11.74
32	0.00	0.00	12.27	87.00	0.74	0.00	0.00	2.94	87.35	9.71	0.00	0.00	6.16	87.23	6.61
33	0.00	0.00	0.00	100.00	0.00	0.00	0.00	1.83	91.91	6.26	0.00	0.00	1.64	92.74	5.62
34	0.00	0.00	0.04	90.95	9.01	0.00	0.00	0.98	99.02	0.00	0.00	0.00	0.57	95.54	3.89
35	0.00	0.00	10.15	84.46	5.38	0.00	0.00	0.00	100.00	0.00	0.00	0.00	7.19	89.00	3.81
36	0.00	0.00	0.00	91.41	8.58	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	94.16	5.84
37	0.00	0.00	0.00	100.00	0.00	0.00	0.00	16.26	79.42	4.31	0.00	0.00	6.45	91.84	1.71
38	0.00	0.00	0.00	90.40	9.60	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	92.46	7.54
39	0.00	0.00	21.86	78.14	0.00	0.00	0.00	25.57	74.43	0.00	0.00	0.00	24.32	75.68	0.00
40	0.00	0.00	21.79	74.50	3.71	0.00	0.00	10.89	74.69	14.41	0.00	0.00	15.80	74.60	9.59
41	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	100.00	0.00

Appendix 26

Patient No.	Sex	Age (years)	Amount of aspirin taken over 24h of the study (mg)	Times at which additional aspirin was taken	Other drugs
1	M	69	1200mg	18.40	indomethacin, diclofenac, clobetasone butyrate ointment
2	F	63	1200mg	21.30	diazepam, indomethacin, bendrofluazide
3	F	45	1800mg	12.00, 22.00	phenylbutazone, prednisolone, penicillamine
4	F	71	1200mg	22.00	diazepam
5	M	41	1800mg	12.30, 18.30	indomethacin

Appendix 27 Individual quantities of aspirin and its metabolites in rheumatoid arthritis patients in mg corrected for molecular weight in each urine collection period

27.1

Patient	0 - 8h			8 - 24h			0 - 24h			SG		
	ASA	GA	SUA	ASA	GA	SUA	ASA	GA	SA		SUA	SG
1	0.00	0.00	178.51	0.00	0.00	260.74	0.00	0.00	0.00	0.00	439.25	0.00
2	0.00	0.00	205.64	0.00	0.00	264.61	0.00	0.00	0.00	0.00	470.25	0.00
3	0.00	0.00	213.72	14.33	0.00	242.69	11.02	0.00	0.00	0.00	456.41	0.00
4	24.08	0.00	131.25	0.00	29.53	253.62	0.00	53.61	0.00	11.43	384.87	0.00
5	0.00	0.00	272.00	12.44	8.82	137.60	19.66	8.82	0.00	0.00	409.60	32.10



Appendix 27.2 Individual percentage recoveries of aspirin and its metabolites in rheumatoid arthritis patients in each urine collection period.

Patient	0 - 8h		8 - 24h		0 - 24h		SG	SUA	SG	SUA	SG	SUA	SG
	ASA	GA	ASA	GA	ASA	GA							
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00
3	0.00	0.00	93.72	6.28	0.00	0.00	95.66	4.34	0.00	0.00	0.00	94.74	5.26
4	15.30	0.00	1.29	83.41	0.00	10.09	86.69	0.00	11.92	0.00	2.54	85.54	0.00
5	0.00	0.00	95.63	4.37	5.31	0.00	82.85	11.84	1.96	0.00	0.00	90.92	7.12