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PHARMACOKINETIC STUDIES WITH PIROXICAM

by

Peter A. Milligan B.Sc.(Hons.) M.Sc. M.R.Pharm.S.

this being a thesis submitted for the degree of

Doctor of Philosophy

in the Faculty of Medicine, University of Glasgow

Department of Medicine and Therapeutics

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DECLARATION

I declare that this thesis has been written by myself and is a record of work performed by myself. It has not been submitted previously for a higher degree.

The research was carried out in the Department of Medicine and Therapeutics, University of Glasgow, under the supervision of Professor B. Whiting and Doctor A. Kelman.

April, 1991 P. A. Milligan.

STATEMENT OF ETHICS

All study protocols received ethical approval from the Research and Ethics Committee, Stobhill General Hospital

All subjects were asked for written informed consent prior to inclusion in studies. Information sheets were always supplied

All studies were performed under the supervision of appropriately trained medical and nursing personnel

Full rescusitation facilities were available at all times

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LIST OF ABBREVIATIONS

A.D.R.	Adverse Drug Reaction
ANOVA	Analysis of Variance
AR	Attributable Risk
ATP	Adenosine Triphosphate
AUC	Area Under the Curve
B.C.D.S.P.	Boston Collaborative Drug Surveillance Programme
Cl	Clearance
ΔCl	Value by which Clearance is altered
Cmax	Maximum Plasma Concentration
^{51}Cr EDTA	^{51} Chromium labelled Ethylenediamine-tetra-acetate
C.S.M.	Committee on the Safety of Medicines
Cyclic AMP	Cyclic Adenosine Monophosphate
D	Dose
ERCP	Endoscopic Retrograde Cholangeopancreatography
F	Bioavailability
F.D.A.	Food and Drug Administration
HETE	Hydroxyeicostetraenoic acid
HPETE	Hydroperoxyeicosatetraenoic acid
HPLC	High Performance Liquid Chromatography
H.R.G.	Health Research Group
k_a	Absorption Rate Constant
k_e	Elimination Rate Constant
NANSAID	Non-Aspirin Non Steroidal Anti-Inflammatory Drug
NADPH	Nicotinamide Adenine Dinucleotide Phosphate (Reduced)
NSAID	Non Steroidal Anti-Inflammatory Drug
PG	Prostaglandin

LIST OF ABBREVIATIONS (cont'd)

PMNL	Polymorphonuclear Leucocytes
RR	Relative Risk
Tmax	Time to Maximum Concentration
$t_{1/2}$	Elimination half-life
V.A.S.	Visual Analogue Scale
V	Volume of Distribution
ΔV	Value by which Volume of Distribution is altered

SUMMARY

When one examines the scientific literature pertaining to the NANSAID, piroxicam, it is clear that controversy of one type or another has not been far away. Perhaps the most serious threat to the drugs existence concerned its reputed potential to induce a range of gastrointestinal toxicity, including perforation and haemorrhage. After intense investigation by the regulatory authorities, however, it was concluded that the data cited were flawed due to reliance on spontaneous Adverse Drug Reaction reports.

This controversy, together with the withdrawals of Opren® and Osmosin® during the 1980's, focused attention on prophylactic remedies for the potentially serious gastrointestinal toxicity exhibited, not only with piroxicam, but with all the NANSAIDs. Initial therapy was confined to the coadministration of H_2 receptor antagonists but now other treatments are available. Little interest, however, has been placed on the theoretical interaction between NANSAIDs and cimetidine, an H_2 receptor antagonist known to reduce the clearance of drugs that are metabolised via cytochrome P450 hepatic enzymes. Of the two studies that have examined this phenomenon, both have been of such a poor design that definite conclusions have been impossible. Before addressing the question of an interaction, therefore, simulation work was performed to design a study which would ensure the right degree of statistical confidence. The technique known as the Jackknife was introduced, its theory explained and its application to study design optimisation described. A prospective power calculation was performed to determine the number of subjects required to detect a 20% difference in the steady state AUC of piroxicam. The 20% value was chosen because it was considered that any alteration in the AUC, as a result of H_2 receptor antagonist coadministration, greater than this value would be clinically significant.

The interaction study was a randomised crossover design comparing the

coadministration of cimetidine and nizatidine in patients receiving chronic piroxicam therapy. No clinically significant interaction was found. There was no statistically significant alteration in the plasma AUCs of piroxicam and its metabolite 5-hydroxypiroxicam. However, the ratio of the two (5-hydroxypiroxicam:piroxicam) showed a statistically significant decrease in combination with either cimetidine or nizatidine. This indicated that a mild inhibition of piroxicam metabolism occurred. It is known that cimetidine binds to all isoenzymes of cytochrome P450 with low affinity, but to only a few with high affinity, resulting in a significant clearance alteration. Nizatidine has been shown to cause mild *in vitro* inhibition of cytochrome P450. The conclusion must therefore be that the H_2 receptor antagonists bind to the isoenzyme responsible for piroxicam metabolism with low affinity, and as a consequence no clinically significant interaction will occur as a result of this combination.

Another controversy, but by no means as crucial to the drugs continued availability for prescribing, was the question of whether piroxicam entered the bile and underwent enterohepatic circulation. Only one previous study has attempted to examine this, but it was flawed in its design, which made investigation of this phenomenon compelling.

Piroxicam's biliary excretion was examined in one patient with a naso-biliary drain which allowed complete collection of the biliary output. The patient received 80mg of piroxicam over 3 days and samples of bile, urine and plasma were collected over a 24 hour period and quantitated for piroxicam, 5-hydroxypiroxicam, and 5-hydroxypiroxicam glucuronide (after development of a suitable assay technique). Neither piroxicam nor 5-hydroxypiroxicam entered the bile in sufficient quantities to undergo significant enterohepatic circulation. However, 5-hydroxypiroxicam glucuronide was present in the bile in large amounts, but this is likely to become important only if reverse metabolism to reform parent drug takes place.

It can be concluded that the "multiple peak" phenomenon in piroxicam plasma

concentration/time curves was not a result of enterohepatic circulation. To investigate other possible mechanisms a volunteer study was performed in which careful consideration was made of the content and timing of food administration. A protocol rich in sampling points was employed to characterise these "multiple peaks" in greater detail. It was shown that the term "multiple peaks" was inaccurate as the perturbations formed were consistently the result of a rapid fall, followed by a more sustained rise in the plasma concentrations. Their appearance was not consistent with enterohepatic circulation. Investigation was therefore made of the possible physiological events that may accompany the consumption of food as the perturbations were coincident with particular meals. The proposed mechanisms included alteration in protein binding, and food induced alterations in clearance and volume distribution. Although likely displacement from albumin was demonstrated, the consequences of such a displacement would not give the results obtained. Piroxicam, in man, has a low clearance and any transient alterations as a result of an increase in unbound fraction, even up to 400% of the baseline value, would have little overall effect. Piroxicam also has a small total volume of distribution and again any alteration in fraction unbound, even up to 400% of the normal value, would have little or no effect on the total volume of distribution. However, alteration in volume of distribution can occur by other mechanisms. For example, an increase in plasma compartment volume might occur, and this was investigated by means of simulation studies. It was shown that alteration in plasma volume could result in a "dilution" of the concentration of drug present in the plasma. This situation would eventually normalise with homeostatic control, explaining the apparent fall and rise in the plasma concentrations witnessed in each subject. Clearance alterations could not explain the subsequent rise, and also could not explain the complimentary fall in plasma metabolite concentration, leading to the eventual conclusion that the perturbations found in plasma piroxicam concentration/time curves were the result of food induced alterations in the plasma compartment volume.

CHAPTER 1

REVIEW: THE EFFICACY, TOXICITY AND CLINICAL USE OF PIROXICAM AND THE NANSAIDs

1.1 INTRODUCTION TO CHAPTER 1

This chapter will review the pharmacological and pharmacokinetic properties of the non-aspirin non steroidal anti-inflammatory drug (NANSAID) piroxicam in man. In the later sections of this introductory chapter, examination will be made of the manifestations, epidemiology and prevention of adverse toxic reactions associated with piroxicam, and the other NANSAIDs. Particular emphasis will be placed on the incidence of NANSAID induced gastropathy, a well recognised and important side effect when the number of subjects likely to be exposed to these drugs is considered.

Where appropriate, distinction will always be made between the studies that examined NANSAIDs in isolation and those that simply examined NSAIDs (non steroidal anti-inflammatory drugs) i.e. whether aspirin derived data was excluded from the eventual study conclusions.

1.2 THE PLACE OF PIROXICAM IN RHEUMATOLOGY

Piroxicam was the first of the oxicam group of NANSAIDs to be introduced into the U.K., in 1980. By 1988 it had achieved eleventh place in terms of world sales with a gross return of the order of $350 million. Since the drug's introduction, the choice of NANSAIDs available for prescribing has changed dramatically with the withdrawal of benoxaprofen, zomepirac, alclofenac, Osmosin®, fenclocfenac, methrazone, indoprofen, isoxicam, and suprofen, with phenylbutazone only being available for use by hospital specialists.

The oxicams (Figure 1.1) were discovered in the late 1960's, and they are structurally unique, unrelated to the other groups (Figure 1.2, adapted from Calin 1988). Piroxicam has a pK_a of 6.5, the acidic moiety being the enol group in the benzothiazine ring which can undergo ionisation (Whitehouse 1986). The SO_2 group is lipophilic and enhances absorption as does the heterocyclic side-chain. The SO_2 group also exerts a steric hindrance on hydroxylation, with the result that clearance values for this drug, in man, are very low (Blocka et al 1988).

The slow elimination of piroxicam has contributed to its undoubted commercial success. The convenience of a once a day dosage regimen (perhaps in patients who are receiving numerous other drugs) proved to be commercially attractive. In a recent study (Kay et al 1987) it was shown that piroxicam was much more commonly prescribed by General Practitioners compared to hospital based doctors (including Rheumatologists).

Fig 1.1 Oxicam Derivatives

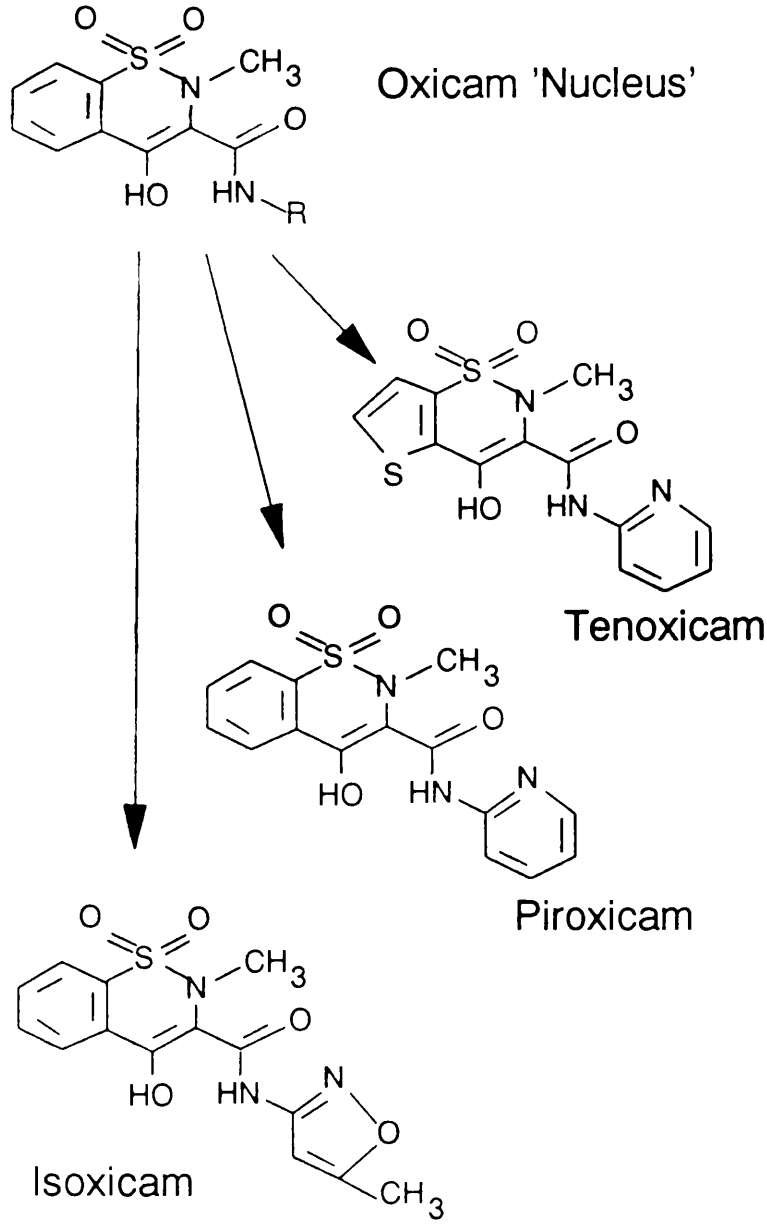
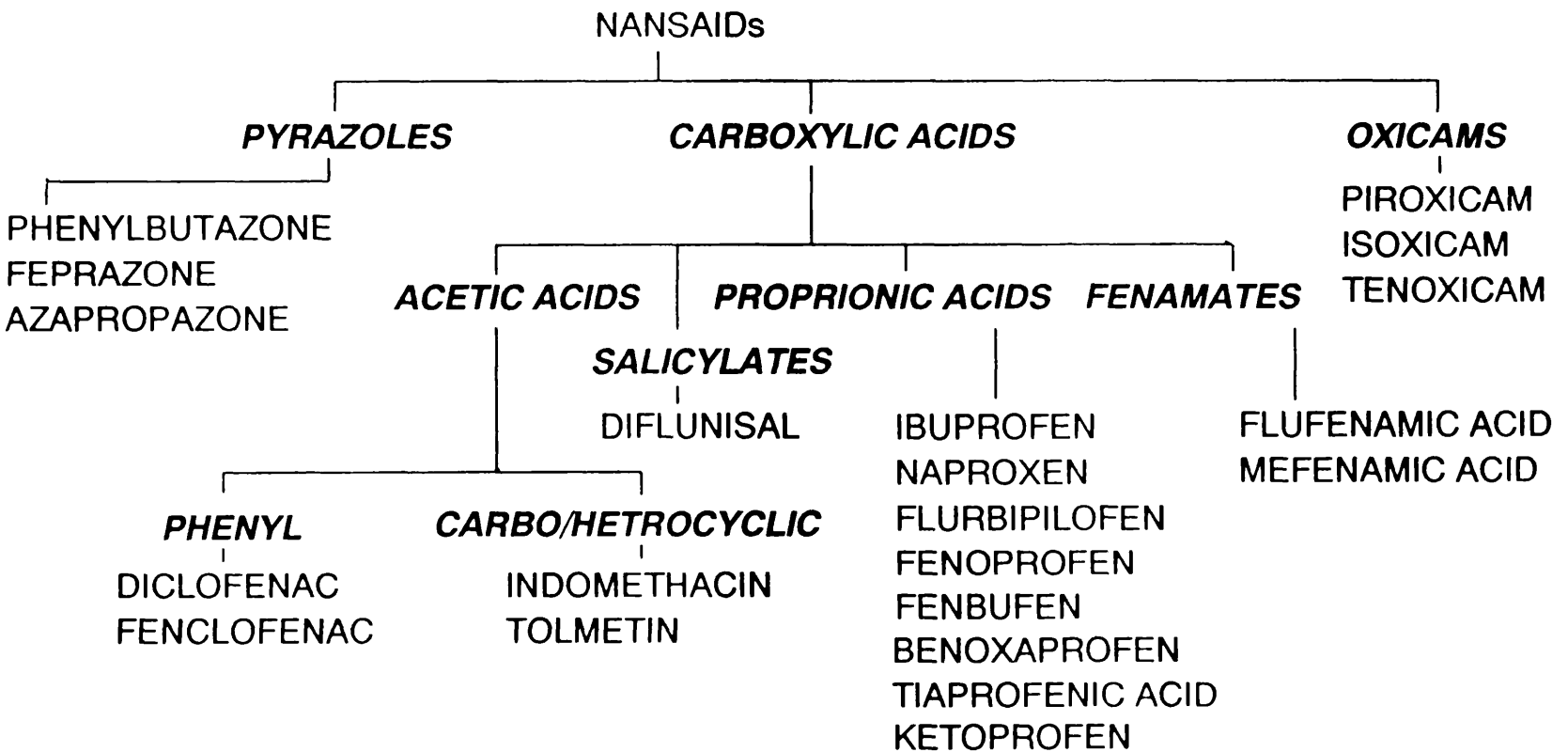


Fig 1.2 Chemical Classification of NANSAIDs



1.3 ANIMAL AND HUMAN PHARMACOLOGY

1.3.1 ANTI-INFLAMMATORY ACTIVITY

In a number of non specific animal models, piroxicam has been shown to possess anti-inflammatory activity. These include inhibition of carrageenan-induced paw oedema, adjuvant-induced arthritis and granuloma tissue formation in rats, urate crystal-induced synovitis in dogs, ultraviolet-induced erythema in guinea-pigs (Wiseman et al 1976) and leucocyte migration in inflammatory exudates (Schiantarelli et al 1981).

In the carrageenan-induced paw oedema test, piroxicam was found to be twice as potent as indomethacin (Schiantarelli et al 1981), 7 times more potent than naproxen, and 14 times more potent than phenylbutazone (Wiseman et al 1976). The anti-inflammatory activity of this drug is similar in adrenalectomised and intact animals, indicating that its mode of action is not due to adrenal stimulation.

1.3.2 ANALGESIC ACTIVITY

At a dose of 10-32mg/kg piroxicam, administered orally, inhibited phenylquinone induced writhing in mice to an extent similar to aspirin, 320mg/kg (Wiseman et al 1976). In this model, piroxicam 1.85mg/kg was less active than indomethacin (0.39mg/kg), but more active than fenoprofen (3.6mg/kg), ibuprofen (4.9mg/kg), naproxen (20.1mg/kg) and phenylbutazone (32mg/kg), in reducing writhing frequency by 50% (Milne & Twomey 1980).

Schiantarelli & Cadel (1981) chose as an index for investigation, a 70% reduction in writhing frequency. In this animal model piroxicam was 70 times more potent than phenylbutazone. Piroxicam was inactive in the "hot plate" and "tail flick" tests in mice and "flinch jump" and "tail pinch" procedures in rats, which indicated that the drug has no opiod-like analgesic effect (Milne & Twomey 1980).

1.3.3 EFFECT ON THE GASTROINTESTINAL MUCOSA AND PLATELET FUNCTION

The ulcerogenic effect of oral piroxicam in starved rats was 30-50% of that observed with indomethacin, and 4 times that of phenylbutazone when expressed in terms of the dose producing gastric ulcer in 50% of rats. Rectal administration halved the ulcerogenic effect of piroxicam administered orally, indicating that the drug may have a topical action on the gastrointestinal mucosa (Schiantarelli & Cadel 1981).

In man, the nature and time course of acute piroxicam-induced gastric mucosal injury was determined in 10 volunteers (Fellows et al 1989). Each received 10mg b.d. orally for 21 days and endoscopic assessments were made, together with luminal blood loss determinations via haemoglobin measurements in gastric washings. Acute mucosal injury consisted mainly of haemorrhagic lesions in the gastric body which developed to a maximum extent within 10 hours of the first dose of piroxicam, without any subsequent increase. On chronic administration intraluminal bleeding increased little for 10 days. However, by 21 days, there was a significant increase. These results suggest that the gastric mucosal injury was due to a topical action of the drug (maximum effect occurred at low plasma drug concentrations) and that the intraluminal bleeding may be a consequence of an inhibition of platelet aggregation (Weintraub et al 1978).

1.3.4 EFFECT ON PROSTAGLANDIN SYNTHESIS

In 1971 Vane showed that prostaglandins contributed to inflammation, fever and pain which could be inhibited by aspirin and NANSAIDs (Vane 1987). Apart from erythrocytes, all cells are capable of producing prostaglandins via the arachidonic acid cascade. Aspirin and the NANSAIDs were unable to inhibit the synthesis of the leukotrienes, and therefore, two pathways were proposed. One, the cyclooxygenase pathway, led to the formation of prostaglandins, thromboxanes etc., and could be inhibited by aspirin and the NANSAIDs. The other, the lipoxygenase pathway, led to

the formation of leukotrienes and could not be inhibited by these drugs (except by the dual pathway blockers e.g. benoxaprofen) (Figure 1.3). Unlike aspirin, NANSAIDs do not irreversibly acetylate the cyclooxygenase enzyme active site; they are selective, reversible inhibitors (Carty et al 1980). This action on prostaglandins provides an explanation for the analgesic effects of aspirin and the NANSAIDs, but it is likely that it only contributes partly to their anti-inflammatory effect *in vivo*.

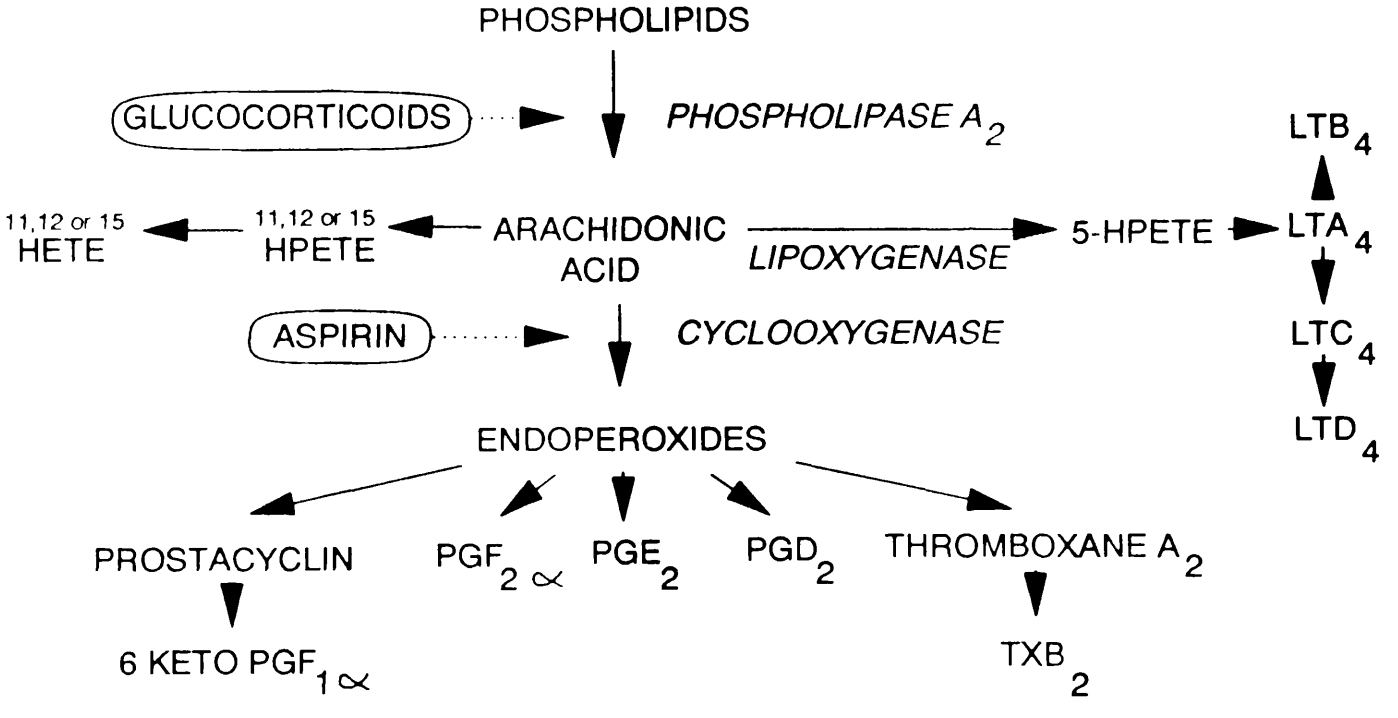
1.3.5 EFFECT ON CELLULAR FUNCTION AND IMMUNE RESPONSE

Polymorphonuclear leucocytes (PMNL) are believed to play an important role in both early and chronic stages of the inflammatory state. In rheumatoid arthritis and other inflammatory diseases, PMNL and macrophages are stimulated, resulting in the secretion of inflammatory mediators, including large amounts of superoxide ions (O_2^-) and hydrogen peroxide (H_2O_2). These cells produce superoxide via NADPH-oxidase (nicotinamide adenine dinucleotide phosphate, in its reduced form) an enzyme complex located in the plasma membrane and phagosome. In the non-phagocytosing cell the NADPH-oxidase is inactive, but when the cell is stimulated to phagocytose the enzyme is activated. Little is known about the molecular basis for this activation. Treatment with piroxicam *in vivo* has shown a 25% reduction in superoxide secretion by isolated granulocytes in both osteoarthritis and rheumatoid arthritis (Biemond et al 1986). *In vitro* experiments showed that piroxicam inhibited superoxide production of granulocytes by interference with the stimulation of NADPH-oxidase (Biemond et al 1986). It was noted, however, that piroxicam had to be present during stimulation of the enzyme in order to diminish superoxide production. Once the enzyme was stimulated piroxicam had no effect (Biemond et al 1986).

1.3.6 EFFECT ON ARTICULAR CARTILAGE

The rate of proteoglycan synthesis and turnover is increased in osteoarthritis. As the disease progresses, the chondrocytes are unable to replace the depleted proteoglycans, hastening cartilage degeneration. The treatment of osteoarthritis relies

Fig 1.3 Catabolic pathways of Arachidonic Acid



principally on the analgesic properties of NANSAIDs, although most have the capacity to modify the natural course of cartilage destruction in experimental animal models. However, some may adversely affect normal cartilage metabolism. In an *in vitro* study on weight-bearing porcine articular cartilage, the effects of NANSAIDs on normal cartilage glycosaminoglycan, collagen and non-collagen protein synthesis was examined. Indomethacin and sodium salicylate were shown to inhibit the synthesis of these substances whereas piroxicam had no effect (Hess & Herman 1986). Realistically, any NANSAID induced damage to chondrocytes must be considered preferable to the likely joint destruction obtained if the disease is allowed to progress untreated.

1.3.7 MECHANISMS OF ACTION

All the NANSAIDs inhibit prostaglandin synthesis, and it has up until recently been thought that their mechanism of action in rheumatoid disorders was a consequence only of this suppression. However, piroxicam and the other NANSAIDs also inhibit the activation of neutrophils, which provoke inflammation by releasing products other than prostaglandins (Weissmann et al 1985). Neutrophils are activated by a "twin signal" which is part of the general response to inflammatory stimuli. This "twin signal" consists of the mobilisation of intracellular calcium resulting in the activation of protein kinase C. Piroxicam has been shown to inhibit early steps in neutrophil activation by inhibiting calcium movements and increasing intracellular cyclic AMP levels (Weissmann et al 1985). Binding studies have shown that the mechanism of this effect appears to be an interference with the ligand receptor site modulated by the G protein (Weissmann 1987).

The inhibition of neutrophil activation, therefore, constitutes one of a number of possible modes of action of this and the other NANSAIDs. The other proposed mechanisms include inhibition of plasma prostaglandin synthesis (resulting in reduced synovial prostaglandin levels), reduced superoxide ion production via alteration of

PMNL function, and a decreased formation of IgM rheumatoid factor.

1.3.8 THERAPEUTIC TRIALS

In human efficacy studies, (examining both pain relief and anti-inflammatory properties in rheumatoid arthritis) piroxicam has been shown to be superior to placebo and comparable to aspirin (4.8g/day). Compared with other NANSAIDs piroxicam 20mg daily has been shown to be equivalent to indomethacin (75-200mg daily), ibuprofen (1.2-2.4g daily), naproxen (0.5-1g daily), diclofenac (75-150mg daily), ketoprofen (300mg daily), sulindac (400mg daily) and phenylbutazone (400mg daily).

When osteoarthritis is considered, favourable results were again obtained, with 20mg piroxicam shown to be as effective as diclofenac (150mg conventional formulation, 100mg sustained release, daily), fenbufen (600mg daily), indomethacin (75-125mg conventional formulation, 75mg sustained release, daily), and naproxen (0.5-0.75g daily).

Studies comparing piroxicam to indomethacin in the treatment of ankylosing spondylitis have shown piroxicam to be a suitable alternative. Results of open trials also suggest its usefulness in the treatment of gout, and placebo controlled trials have shown its efficacy in acute musculoskeletal injuries, dysmenorrhoea, post-surgical and traumatic pain.

For a more complete description of these studies the review paper by Brogden et al (1984) should be consulted.

1.3.9 GASTROINTESTINAL TOXICITY

Initially when piroxicam was introduced in the U.K. its potential to cause serious gastrointestinal side-effects was considered low. Indeed, early data sheets did not have pre-existing peptic ulcer disease as a contra-indication (Emery & Grahame 1982). Problems arose, however, as a result of the initial dosage form. The tablet preparation resulted in a number of gastrointestinal side effects being reported, and the drug was subsequently reformulated as a capsule. As pre-existing peptic ulcer disease was not a

contra-indication, a number of patients received piroxicam who were previously unable to tolerate an NANSAIDs. These patients were therefore most likely to be susceptible to the gastrointestinal side effects any NANSAID, with inevitable consequences.

Concern regarding the drug's safety emerged in 1985 (Fok et al 1985) when the suggestion was made that the methods used to assess gastrointestinal side effects were faulty, especially in the trials that reported a low incidence. The authors suggested that the incidence was underestimated because many patients remained asymptomatic. Gastroscopic studies have confirmed that up to 46% of patients with rheumatoid arthritis receiving a variety of NANSAIDs (and aspirin) have gastric lesions which are completely asymptomatic, possibly due to the analgesic effect of the drug (Bianchi Porro et al 1987). However, one problem in determining whether these lesions are drug-induced is that lesions similar in appearance are common in arthritic patients not receiving any drug treatment (Festen 1988). Interestingly, 19% of patients who complained of upper gastrointestinal symptoms were found to have no lesions.

The concern at the reported low incidence of piroxicam gastrointestinal side effects was supported by both the Swedish Committee on Adverse Drug Effects and the Belgian Centre for Drug Postmarketing Surveillance (Beerman et al 1985, Meyer & Thijs 1985).

O'Brien & Burnham (1985) also reported data from a study examining electrocoagulation treatment of bleeding peptic ulcers. Of the 204 bleeding ulcers, confirmed by gastroscopy, 53 (26%) had taken an NANSAID (or aspirin) prior to admission, although no data were given on the duration of therapy. Using a chi-square analysis they concluded that the observed frequency of bleeding episodes associated with piroxicam was statistically greater than would be expected, and of the 14 cases encountered with piroxicam, 12 were receiving no other medication. It must be stressed, however, that it is unknown whether the regional prescribing frequency was representative nationally.

Similar conclusions regarding the relationship between NANSAIDs and bleeding peptic ulcers were made by Somerville et al (1986). This study was important as it included both matched hospital and community-derived controls. Although the authors could not make firm statements about particular drugs, they contended that piroxicam, indomethacin, and naproxen were associated with bleeding complications particularly with patients over the age of 60. This view was generally supported. Furthermore, there were calls for drugs that exhibited a "long elimination half-life", such as piroxicam, to be contra-indicated in the elderly due to possible alterations in pharmacokinetics (Collier & Pain 1986, Christophidis & Louis 1983). Somerville's conclusion regarding the number of deaths associated with total NANSAID consumption was regarded by others to be an underestimate of the "true" incidence of approximately 3000 per annum (Armstrong & Blower 1986).

Pressure was placed on the regulatory bodies, both here and in the U.S.A., to limit, or ban the use of piroxicam in the over 60's. The Health Research Group in America petitioned the Food and Drug Administration (F.D.A.) on the grounds that gastrointestinal toxicity was more common with piroxicam than with other NANSAIDs. They contended that elderly patients exhibited reduced clearance. Furthermore, gastrointestinal toxicity was dose related and any reduction in clearance (as postulated in the elderly) would make this group particularly liable to suffer serious side-effects.

This petition, in common with the many other studies which reported that the gastrointestinal toxicity associated with piroxicam was greater than the other NANSAIDs, was flawed because it relied on spontaneous Adverse Drug Reaction (A.D.R.) reports. In rejecting the H.R.G.'s assertions, the F.D.A. stated that spontaneous reports could only provide "a signal of a potential problem .....they are not an appropriate basis for a final conclusion which must be based on epidemiological and other evidence" (F.D.A. Assessment of the Safety of Piroxicam 1986).

In response, to this result the H.R.G. cited a report by three F.D.A. epidemiologists which showed, after data adjustment to correct reporting rates over time, a "highly statistically significant increase in A.D.R. reports of bleeding, perforation, and ulcer for piroxicam" (Wolfe 1986). It is interesting to note, however, that when this study was eventually published, it concluded that there were no clinically significant differences in the frequency of cases of upper gastrointestinal bleeding, perforation, and ulcer between piroxicam and the other NANSAIDs (Rossi et al 1987).

Paulus, a rheumatologist who liaised with the F.D.A., highlighted some of the drawbacks of spontaneous A.D.R. reports (Paulus 1985). For example, the number of actual reports received represent only a fraction of the events actually occurring, but this fraction was unknown and can vary with time. Furthermore, difficulty lay in determining the number of patients actually exposed to the drug as data were only available for the total number of prescriptions dispensed. When the data for piroxicam and diclofenac were corrected for the length of time since its introduction (reporting rates during the second year of introduction can be up to five times greater than the level seen during the first) the results resembled the established agents far more closely. When the Committee on the Safety of Medicines (C.S.M.) examined "yellow card" reports, piroxicam was found to be no worse than any other NANSAID (apart from ibuprofen at low doses) available at that time (C.S.M. Update 1986). Confounding factors such as indications for use, marketing claims, media events, and reporting rates during the particular period of study were considered. It was concluded that the most toxic agents had already been withdrawn, and that the remainder were equally toxic.

Langman (1986) in the A.D.R. Bulletin stated that even although mucosal damage was evident from these agents, prolonged exposure did not mean that ulcers would develop as a result. This questioned the validity of endoscopic studies which had visualised the mucosa after short term exposure to an NANSAID. He emphasised that piroxicam's short marketing life-span may have influenced reporting rates. This

was supported by examining the Norwegian data gathered with sulindac (Giercksky 1986). It, like piroxicam when introduced, was claimed to be less gastrotoxic than the other NANSAIDs. Similar results, in terms of the number of A.D.R. reports sent to the regulatory bodies, were obtained with sulindac as were obtained after piroxicam's launch in the U.K..

The methods used to assess piroxicam toxicity have subsequently received much criticism, especially the papers that predicted likely gastrointestinal problems. Professor Inman at Southampton felt that the Swedish data relied too heavily on anecdotal reports (Inman & Rawson 1985). The post marketing surveillance scheme, which he set up with the co-operation of the C.S.M., indicated that the frequency of upper gastrointestinal bleeding or perforation in patients receiving benoxaprofen, fenbufen, zomepirac, indomethacin, or piroxicam ranged from 3-6 per 1000 patient years. Interestingly this range was the same whether the patient was receiving an NANSAID or not. He concluded that piroxicam could not be considered to be any more toxic than the other NANSAIDs examined in this study (benoxaprofen, fenbufen, zomepirac, and indomethacin).

Concerns, however, about the safety of this drug reappeared in 1987. Armstrong & Blower (1987a) gave details of a prospective study started in 1984. They concluded that there was a significantly higher frequency of ulcer complications observed in patients taking piroxicam compared to other NANSAIDs. Another paper indicated that A.D.R.'s were more commonly associated with NANSAIDs that exhibited a long elimination half-life in man (Adams 1987). Once again these studies were criticised due to the methodology employed, and their conclusions were questioned (Bortnichak et al 1987, Calin 1987, Stevens 1988).

The pharmaceutical company that manufactures piroxicam, Pfizer, obtained a great deal of information regarding the long term safety and tolerance of piroxicam from patient studies also performed in Norway (Husby 1986, Meisel 1986, Zizic et al

1986, Sachs & Bortnichak 1986, Bortnichak & Sachs 1986). However, assessment of side effects relied on Visual Analogue Scale (V.A.S.) data which may not be valid as many patients with mucosal damage may remain asymptomatic. Perhaps it was not surprising, therefore, that piroxicam was found to be no worse than any of the other NANSAIDs in terms of safety and efficacy.

In conclusion, it would appear that piroxicam has a side effect profile similar to the other NANSAIDs (Gerber 1987). Care must be taken to examine the source and methods employed in studies examining risks, with a particularly cautious emphasis placed on spontaneous A.D.R.'s as an index of toxicity (Langman 1989).

1.3.10 OTHER ADVERSE SIDE-EFFECTS

Side effects other than those affecting the gastrointestinal tract have been infrequently reported. As with other NANSAIDs, A.D.R. reports have implicated ankle oedema, dermatological side effects including rashes, pruritus, and photosensitivity, reversible elevations of blood urea, decrease in haemoglobin and haematocrit unassociated with gastrointestinal bleeding, increased serum transaminase, and bronchoconstriction in some aspirin hypersensitive asthmatic patients (Data Sheet Compendium 1990).

1.3.11 DOSAGE AND ADMINISTRATION

The initial adult dose in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis is 20mg once daily. In most subjects this is adequate for long term maintenance, but a few require 30mg daily. Administration of higher doses has been associated with increased side effects with no increased benefits. In acute gout an initial 40mg is given followed by 40mg daily for one week. In the treatment of acute musculoskeletal disorders, 40mg daily for two days is followed by 20mg daily.

1.4 PHARMACOKINETIC PROPERTIES

1.4.1 ABSORPTION

Although piroxicam is most often prescribed orally, it can also be administered intramuscularly, topically, and rectally (with efficacy equal to that of oral administration, Chantraine 1984). Two oral preparations are currently available, a capsule and a dispersible tablet. In the future, a pro-drug, piroxicam pivalic ester, may be available (Ligniere et al 1987).

Table 1.1 shows the absorption characteristics of piroxicam. Following oral administration of the capsule, peak plasma concentrations occur between 2 and 4 hours. Administration of piroxicam to the elderly yielded absorption characteristics similar to those obtained in younger subjects. One study (Ishizaki et al 1979) has published values for k_a, the absorption rate constant. The mean value obtained from 8 subjects was $7.60 \pm 5.32\ h^{-1}$.

The absolute bioavailability (F) has not been determined because no intravenous formulation exists. However, relative bioavailability, as determined by comparison of the area under the curve (AUC) following oral and rectal administration, is similar in extent, but different in the rate of absorption (Schiantarelli et al 1981). In the study by Kozjek et al (1987), however, 10 male volunteers received 20mg piroxicam orally and rectally in a crossover study and no significant differences were found in the AUC's obtained.

Concurrent administration of food appears to affect the rate of absorption of piroxicam but not its extent. Ishizaki et al (1979) studied 27 healthy subjects who were given either 30 or 60mg of piroxicam with or without food. Although food intake caused a significant decrease in maximum plasma concentration (Cmax) and increase in the time to maximum plasma concentration (Tmax), AUC was unaffected. In the study by Tilstone et al (1981) neither food nor a single ferrous sulphate tablet (iron is known to chelate certain drugs, therefore decreasing absorption) had any effect on AUC,

Table 1.1 Absorption Characteristics of Piroxicam

Reference	Study Design	Number	Age (Years)	Dose (mg)	C Max (mg/L)	T Max (h)	$AUC._{\infty}^{24}$ (mg/l/h)	$AUC._{\infty}^{\infty}$ (mg/l/h)
Ishizaki et al,1979	Dose Comparison	8 ♂	18-24	30 (n=4)	4.43 ± 0.42	2.5 ± 1.2		214.8 ± 24.9
(Mean ± S.E.M.)	(Pre-prandial)			60 (n=4)	7.23 ± 0.51	3 0 ± 1.7		388.4 ± 41.4
	Dose Comparison	19 ♂	18-24	30 (n=9)	2.98 ± 0.23[a]	4 3 ± 1 6 (n=19)[a]		189 ± 23.5
	(Post-prandial)			60 (n=10)	6.39 ± 0.49			312.8 ± 22.3
Schiantarelli et al, 1981	Single Dose Cross Over	6 ♂	Mean 35	40 p.o.	3.97 ± 0.43	2.7	72.4 ± 9.5	
	Oral vs Rectal			40 p.r.	3.00 ± 0.34	5 6	56.5 ± 8.1	
Darragh et al,1985	Single Dose vs	12 ♂ 32 ♀	30-59 (n=21)	20				196 ± 15
(Mean ± S.E.M.)	Steady State		30-69 (n=12)					179 ± 13
	After 36 days		70-80 (n=11)					153 ± 12
Edwards et al,1985	Single Dose Cross Over	6 ♂ 6 ♀	18-40	20	2.31 ± 0.38	2 01 ± 0.75		154 ± 52.2
	vs Isoxicam	7 ♂ 5 ♀	>65		2.29 ± 0.62	1 71 ± 0 68		163.6 ± 99.1

a = Sig.Diff. from Pre-prandial

clearance, volume of distribution, or elimination half-life.

Topical preparations of piroxicam show limited absorption into the systemic circulation (Norris et al 1985). After administering 3g of 0.5% gel for 8 hours in eight volunteers, the plasma levels obtained were less than 10% of those from either 20 mg orally or rectally.

An intramuscular preparation was studied by Fourtillan (1985) in 18 healthy males. Comparison was made between the 20mg orally and 20mg intramuscularly in a repeated dose crossover study. The two preparations had similar bioavailabilities, but the intramuscular administration resulted in higher plasma concentrations.

1.4.2 DISTRIBUTION: PROTEIN BINDING

Piroxicam is extensively bound to plasma albumin (>99%) (Richardson et al 1985: Blocka et al 1988). Consequently it is primarily confined to the extracellular fluid as reflected by a small apparent volume of distribution in both patients and volunteers (Table 1.2 and 1.3).

Piroxicam has been shown to have two binding sites on the albumin molecule. Matsuyama et al (1987) showed the association constants, after 6 hours equilibrium dialysis at 37°c against 0.1M phosphate buffer, to be $1.6 \times 10^5 \pm 0.5 \times 10^5$ l/mol for the high affinity site and $3.04 \times 10^3 \pm 0.5 \times 10^3$ l/mol for the low affinity site. Piroxicam binding was shown to be altered by both warfarin and fatty acids, but not by diazepam.

1.4.3 DISTRIBUTION: SYNOVIAL FLUID

In the synovial fluid, which is the proposed primary site of action for NANSAIDs in rheumatoid arthritis, piroxicam has been shown to be present in approximately half its plasma concentration (Trnavska et al 1984). Again there are two binding sites which have similar association constants to those in plasma after 20 hours equilibrium dialysis at 20°c against 0.1M phosphate buffer. For synovial fluid $K_1 = 2.38 \times 10^5 \pm 0.86 \times 10^5$ l/mol and $K_2 = 2.29 \times 10^3 \pm 0.75 \times 10^3$ l/mol (for plasma $K_1 = 1.93 \times 10^5 \pm 0.40 \times 10^5$ l/mol and $K_2 = 2.08 \times 10^3 \pm 0.70 \times 10^3$ l/mol). Bontoux et al (1984) examined the extent

Table 1.2 Piroxicam Pharmacokinetic Parameters in Volunteers

Reference	Number	Age (years)	Dose (mg)	t½ (h)	V/F (L)	CL/f (L/h/kg)
Ishizaki et al,1979 (Mean ± S.E.M.)	8 ♂ (Pre-prandial)	18-24	30 (n=4) 60 (n=4)	37.5 ± 2.4	8.48 ± 0.56	$2.68 \times 10^{-3} \pm 0.23 \times 10^{-3}$
	19 ♂ (Post-prandial)	18-24	30 (n=9) 60 (n=10)	32.9 ± 4.9	8.12 ± 0.55	$3.12 \times 10^{-3} \pm 0.16 \times 10^{-3}$
Hobbs & Twomey,1979	14 ♂		10,15,25,40, 70 (n=2) 100 (n=4)	45.6 ± 15.67 (n=12)	0.12 L/kg	
	20 ♂		40	56.8 ± 32.7		
Rogers et al,1981	4 ♂ 4 ♀	26-38	20 for 7 days	52.9 ± 20.1	10.4 ± 4.5	$136.7 \times 10^{-3} \pm 30.2 \times 10^{-3}$ L/h
Tilstone et al,1981	8 ♂	24-36	20 for 5 weeks	46.2	8.53	1.8×10^{-3}
Schiantarelli et al,1981	6 ♂	mean 35	40 p.o. 40 p.r.	31.2 ± 4.9 39.9 ± 4.4		

Table 1.2 Cont. Piroxicam Pharmacokinetic Parameters in Volunteers

Reference	Number	Age (years)	Dose (mg)	t½ (h)		V/F (L)	CL/f (L/h/kg)
Richardson et al, 1985 (Mean ± S.E.M.)	6 ♂ 6 ♀	20-31	20	51.9 ± 3 ♂		11.3 ± 0.3[b]	2.1 x10^{-3} ± 0.18 x 10^{-3}
				44.9 ± 3.1 ♀		9.2 ± 0.9	2.34 x 10^{-3} ± 0.18 x 10^{-3}
	6 ♂ 7 ♀	62-75	20	54.2 ± 4.8 ♂		10.8 ± 0.8[b]	1.92 x 10^{-3} ± 0.24 x 10^{-3}
				61.7 ± 3.8[a] ♀		7.8 ± 0.4	1.56 x 10^{-3} ± 0.12 x 10^{-3} [a]
				Stat.	**S.State**		
Darragh et al,1985 (Mean ± S.E.M.)	12 ♂ 32 ♀	30-59 (n=21)	20 Stat.	48.6 ± 4.2	55.2 ± 4.0	0.150 ± 0.012	
		60-69 (n=12)	20 for 36 days	40.0 ± 4.1	51.6 ± 3.9	0.142 ± 0.008	L/kg
		70-80 (n=11)		40.3 ± 3.2	45.4 ± 4.0	0.171 ± 0.018	
Edwards et al,1985	6 ♂ 6 ♀	18-40	20	57.1 ±16.4			143.4x10^{-3} ± 48x10^{-3} L/h
	7 ♂ 5 ♀	>65		57.8 ± 22.1			150.6x10^{-3} ± 54x10^{-3}
Richardson et al,1987	3 ♂ 3 ♀	23-33	20 for 15 days	54.9 ± 10.8		9.6 ± 2.4	121.2x10^{-3} ± 19.2x10^{-3} L/h
			[5-Hydroxypiroxicam	70.5 ± 11.3]			

a Sig diff. from young ♀
b Sig diff. from old ♀

Table 1.3 Piroxicam Pharmacokinetic Parameters in Patients

Reference	Disease	Number	Age (Year)	Dose (mg)	t½ (h)	V/F (L)	CL/F (L/h/kg)
Hobbs & Twomey,1979	R.A.	15		10,20,30 (n = 5) for 14 days	34.7 ± 20.43 38.3 ± 9.11 38.6 ± 8.33		
Woolf et al, 1983	R.A./O.A.	19	27-94	20 for 14 days	73.4 ± 24.6	0.31 ± 0.16 L/kg	$3.2 \times 10^{-3} \pm 1.8 \times 10^{-3}$
Blocka et al,1988	R.A.	10 ♂ 14 ♀	27-79	20 (n=23) 30 (n=1) for 6 weeks	53.0 ± 24.2	7.3 ± 2.0	$1.68 \times 10^{-3} \pm 0.78 \times 10^{-3}$
					[5-Hydroxypiroxicam 84.9 ± 41.0]		

to which synovial fluid concentration depended on the plasma concentration. Ten patients, mean age 58, received a single 20mg dose of piroxicam and in another study, 11 patients, mean age 53, received repeated doses of 20mg. In the single dose study the ratio of synovial fluid to plasma concentration was 0.48 $\pm$0.27 and in the multiple dose study this ratio was 0.41 $\pm$0.17 (the number of repeated doses ranged from 3-8 with one patient taking the drug for 25 days). Positive correlations were obtained between plasma and synovial fluid concentrations, independent of the duration of administration (R^2= 0.64, p<0.0001), and between synovial fluid protein concentration and the ratio of synovial fluid to plasma piroxicam concentrations (R^2= 0.53, p<0.03).

1.4.4 DISTRIBUTION: OTHER TISSUES

Piroxicam was detected in breast milk at about 1-3% of the maternal plasma concentrations (Ostensen et al 1988). In this study four women received 20mg of piroxicam daily. Steady state levels were achieved with a milk:plasma ratio of between 0.01-0.03. The relative dose ingested daily by a breast fed baby would therefore be 3.5-6.3 % of the maternal dose, which would approximate to 0.7-1.1mg for a 20mg daily dose.

1.4.5 ELIMINATION

Piroxicam is virtually completely metabolised. Only 2-5% is excreted in the urine as parent drug (Wisemen & Hobbs 1982). Clearance (Cl) and the elimination half-life ($t_{1/2}$) of piroxicam has been estimated in healthy volunteers (Darragh et al 1985), in patients (Hobbs & Twomey 1979), in young and old (Richardson et al 1985), and in single and multiple dose studies (Tables 1.2 and 1.3). Reported clearance values have ranged from 1.56 x10^{-3} $\pm$0.12 x10^{-3} to 3.12 x10^{-3} $\pm$0.16 x10^{-3} in these groups. The long elimination half-life (range 30-90 hours) has permitted a once daily dosage regimen, although it may take up to 18 days to achieve steady state concentrations (Fourtillan & Doubourg 1984). A loading dose of 40mg for two days will result in steady state levels being obtained much more quickly.

The early pharmacokinetic studies relied on a non specific degradative fluorometric assay which has subsequently been superseded by more sensitive chromatographic techniques, and this may have contributed to some of the differences between the pharmacokinetic parameters obtained in the early studies compared to recent studies (Verbeeck et al 1986).

1.4.6 METABOLISM

Piroxicam is extensively metabolised by hepatic cytochrome P450 enzymes, principally to the hydroxyl metabolite. Hydroxylation occurs at the five position in the pyridine ring and the hydroxylated metabolite undergoes subsequent glucuronidation. Figure 1.4 shows the major routes of metabolism in man (Hobbs 1986). Less than 5% of the parent drug is excreted in the urine, and, under steady state conditions, 75% of a dose is excreted as either 5-hydroxypiroxicam or 5-hydroxypiroxicam glucuronide in the urine and faeces (Verbeeck et al 1986). Richardson examined the proportions of these metabolites (Richardson et al 1987). On average 25% of a dose was recovered in the urine as 5-hydroxypiroxicam with two-thirds (17%) of this total obtained from hydrolysing the glucuronide metabolite. If 60-75% of a total dose is excreted as 5-hydroxypiroxicam or 5-hydroxypiroxicam glucuronide in the urine and faeces, the faecal content must therefore amount to 35-50% of this total.

1.4.7 BILIARY EXCRETION

Bile as a medium for the elimination of piroxicam has received much speculation but little experimental attention. Early studies witnessed a "multiple peak" phenomenon in the plasma concentration time curve and it was thought that drug entering the bile underwent enterohepatic circulation (Rogers et al 1981, Hobbs et al 1983, Richardson et al 1985, Edwards et al 1985, Fenner 1985) which contributed to the drug's long elimination half-life in man (Brune et al 1985). Figure 1.5 illustrates some typical plasma concentration/time curves which were considered to indicate the existence of piroxicam enterohepatic circulation. Only one published study has

Figure 1.4 Metabolic Pathways of Piroxicam in Humans

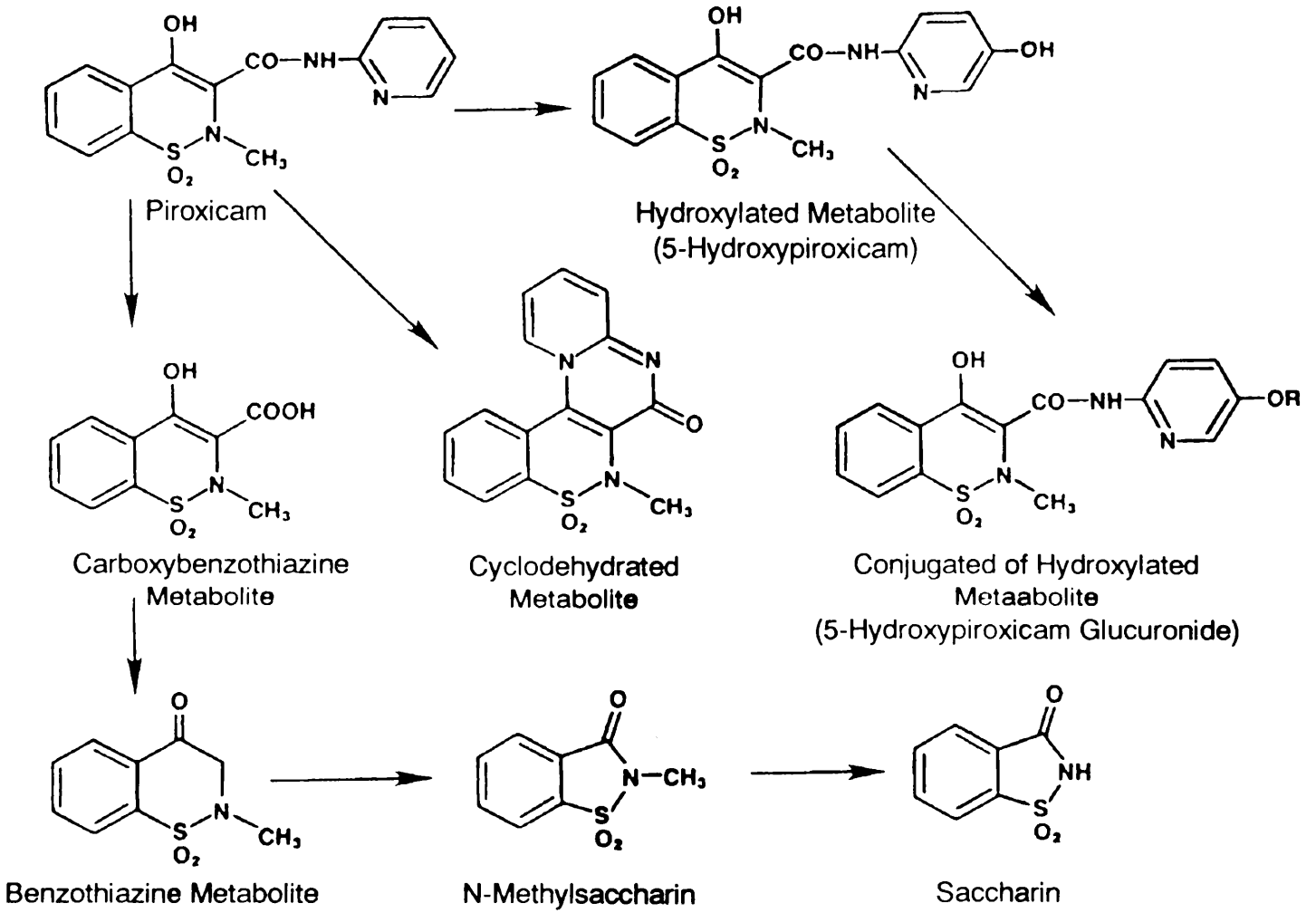
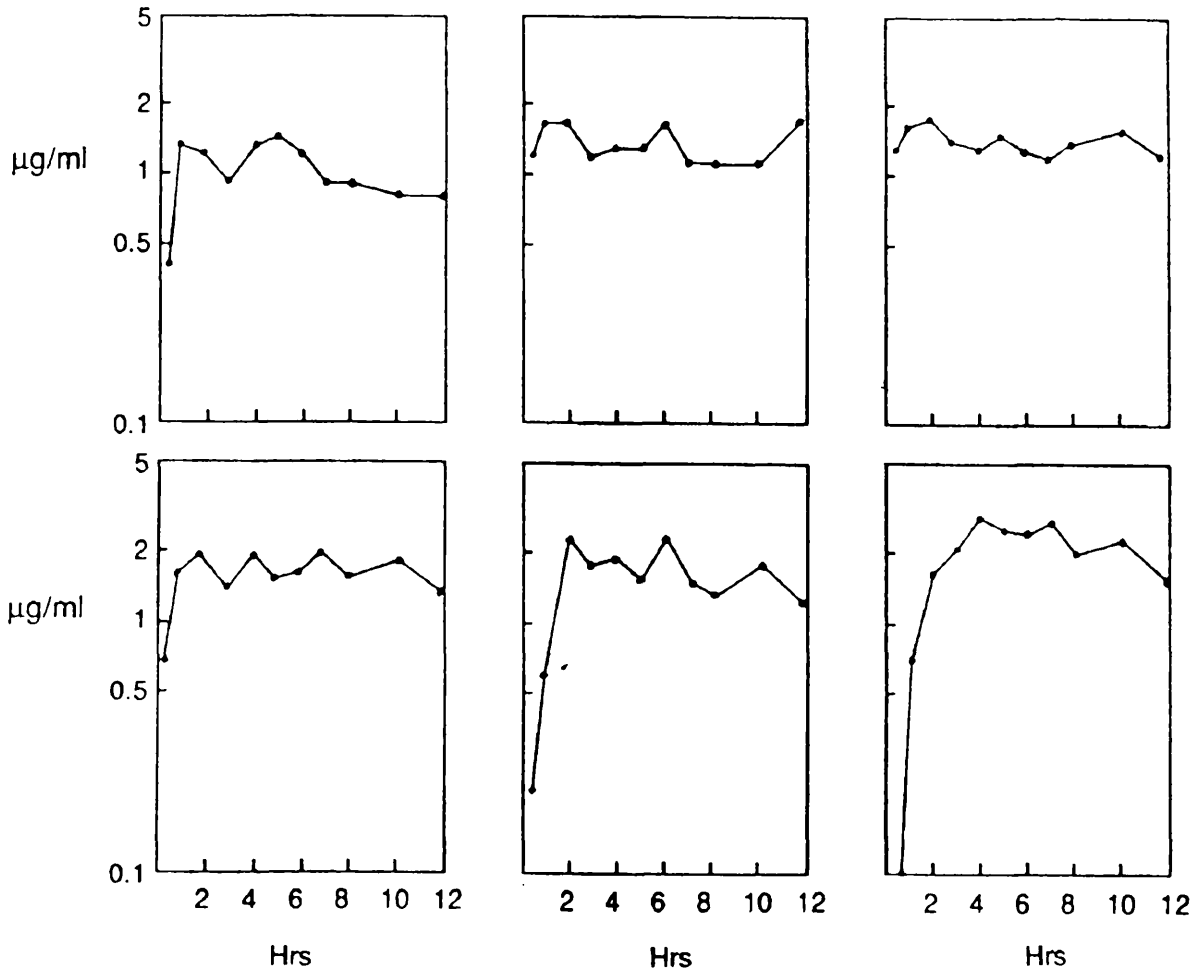


Figure 1.5 Literature Examples of Presumed Enterohepatic Circulation



attempted to quantify the biliary secretion of this drug (Verbeeck et al 1986). Fifteen hours after administration of a single 20mg dose of piroxicam to a healthy volunteer, bile was aspirated into a collecting tube following cholecystokinin infusion. No detectable levels of piroxicam or 5-hydroxypiroxicam were obtained, but no attempt was made to quantify possible glucuronides.

Indirect evidence of biliary secretion was provided by two studies which examined the effect of coadministration of cholestyramine (an anionic exchange resin) which binds bile salts (in addition to some drugs) to form an insoluble complex in the gastrointestinal lumen. This is likely to interrupt the enterohepatic circulation of bile and thus the disposition of any drug secreted in the bile. In these studies a single 20mg piroxicam capsule was administered with either cholestyramine or placebo in a crossover manner to healthy volunteers. Guentert examined the effect of 4g cholestyramine t.i.d. and Benveniste examined the effect of 8g t.i.d. (Guentert et al 1988: Benveniste et al 1990). The administration of cholestyramine was 3.5-4 hours after the piroxicam dose to avoid interference with the absorption of piroxicam. In both cases the clearance of piroxicam was increased with a reduction in the elimination half-life (46.8 to 28.1 hours, Guentert et al; 50.3 to 28.1 hours, Benveniste et al). Interestingly, in the Guentert study, the "multiple peaks" were not removed by the coadministration of cholestyramine. This casts doubt on the idea that the enterohepatic circulation was involved in the generation of these "perturbations". These results should, therefore, be examined closely as they cannot be used as direct evidence for enterohepatic circulation, as contended by Benveniste. The fact that the clearance is increased due to cholestyramine coadministration may indicate that piroxicam enters the bile and is cleared by that route in the faeces. It does not allow unequivocal statements to be made as to whether the drug (or drug metabolite) subsequently leaves the bile (undergoes reverse metabolism to form parent compound) and then re-enters the systemic circulation.

Boylan et al (1987) have shown, with cholestyramine, that drugs can be removed from the systemic circulation and excreted in the faeces via mechanisms other than those involving biliary excretion. Cholestyramine (and activated charcoal, Berlinger et al 1983) creates a "sink condition" whereby there is an artificially increased flux of drug from the blood into the intestinal lumen with a net increase in excretion via the faeces. This would result in increased drug clearance, irrespective of whether it entered the bile or not. This may be the case with piroxicam, because as stated before there little evidence that it is present in the bile. Speculation about "multiple peaks" in the plasma concentration time profiles resulting from enterohepatic circulation therefore may be erroneous.

1.5 IMPLICATIONS OF PHARMACOKINETIC PROPERTIES FOR THERAPEUTIC USE

1.5.1 DOSE AND THERAPEUTIC RANGE/SIDE EFFECTS

The usual dose for piroxicam in inflammatory joint conditions is 20mg once a day, but evidence is scarce for a "therapeutic range" for plasma piroxicam concentrations with respect to anti-inflammatory effects in humans. Wiseman noted that in patients with chronic polyarthritis, correlations were evident between plasma concentrations and duration of morning stiffness and pain (Wiseman & Hobbs 1982). In both indices, improvement was noted as steady state levels were attained, and as concentrations declined exacerbation of the clinical symptoms were seen. Pavelka in a study of 24 rheumatoid arthritic patients noted "therapeutic results" were significantly better in the patients who attained plasma levels greater than 5.4mg/l in comparison to those who were below this level (Pavelka et al 1988). In the large multi-centre crossover study by Rugstad et al (1986), 640 patients with osteoarthritis (192 males, 448 females), mean age 67, had a single blood sample taken within 3-12 hours of their 20mg dose at monthly intervals. Adverse events were assessed by direct questioning and visual analogue scales during the first 4 and 12 weeks of therapy. No correlations were apparent between drug concentrations and adverse events in this study, and no differences were seen between the 4 week and 12 week data.

Using 8 different measures of disease activity, Blocka et al (1983) examined 23 patients with rheumatoid arthritis (9 males, 14 females) aged between 27-79 in a six week study using the standard dose of 20mg piroxicam daily. The degree to which the patients responded did not correlate with either piroxicam plasma concentrations, bound or unbound, or with synovial fluid levels. However, synovial fluid concentrations were only determined in 6 patients. Of the patients who experienced mild side effects (9 out of 23) the average steady state plasma levels were in fact slightly lower than the group as a whole (7.04 ±2.78mg/L versus 9.20 ±4.43mg/L).

1.5.2 DISEASE AND PIROXICAM PHARMACOKINETICS

Tables 1.2 and 1.3 show the pharmacokinetic parameters obtained in patient and volunteer studies. These parameters are similar irrespective of which group is studied. However, Woolf et al (1983) found in 19 patients with rheumatoid or osteoarthritis that the volume of distribution values were twice those previously reported. This was considered to be the result of low plasma albumin concentrations, but it is not clear whether the low albumin was due to the disease itself (causing hypoalbuminaemia), or due to the study being performed during an acute "flare-up" resulting in a disruption of piroxicam protein binding.

The influence of renal impairment on piroxicam pharmacokinetics was studied by Darragh et al (1985). In 18 patients with mild to moderate impairment no differences in pharmacokinetic parameters were found between patients with and without renal impairment. Woolf et al (1983) examined the relationships between half-life, steady state plasma concentrations, plasma albumin levels, and creatinine clearance, in 19 patients. No significant correlations were obtained. This is not surprising because of the drug's extensive hepatic metabolism.

Limited data are available on the effects of liver disease on piroxicam pharmacokinetics. Preliminary reports indicate that "plasma concentrations are increased" in severe liver insufficiency (cirrhosis) but not in mild liver disease (fatty infiltration) (De Schepper & Heynen 1984).

1.5.3 INFLUENCE OF AGE AND SEX

There is no conclusive evidence that the pharmacokinetics of piroxicam are either sex or age related, although studies are contradictory (Tables 1.2 and 1.3). Edwards et al (1985) examined single dose pharmacokinetics in 24 volunteers, 12 young (<40) and 12 elderly (>65). There were no differences in the pharmacokinetic parameters between the groups. Richardson et al (1985) also studied single dose pharmacokinetics. Significant differences in volume of distribution disappeared once volumes were

corrected for body weight. However, significant differences existed for both clearance and elimination half-life between young and elderly women. These results may, however, be spurious: multiple comparisons were performed in 4 small groups.

Woolf et al (1983) also examined multiple dose pharmacokinetics (14 days) in 19 patients between 27 and 94 years of age. In this study, there were no correlations between clearance or steady state plasma concentration and age. The study by Darragh et al (1985) in which 21 healthy volunteers and 23 patients with osteoarthritis received 20mg daily for 36 days had similar conclusions. Plasma concentrations, elimination half-life, AUC, and volume of distribution were unaffected by age. The large retrospective study by Hobbs & Gordon (1984) also did not show any age related effects on the steady state plasma concentrations in 279 patients ranging from 21 to 83 years.

However, two studies disagreed with these findings. Rugstad et al (1986) showed an increase in plasma piroxicam concentrations with increasing age, with females showing higher piroxicam concentrations than males. After regression analysis, however, only 15% of the total intersubject variability was accounted for by age or sex. In the study by Blocka et al (1988) 23 rheumatoid arthritis patients (9 male, 14 female) aged between 27 and 79 received 20mg piroxicam for 6 weeks. Marginally significant correlations were obtained between steady state concentrations and age ($r= 0.43$, $p= 0.043$); half-life and age ($r= 0.41$, $p= 0.050$) and plasma clearance and age ($r= 0.52$, $p= 0.011$).

The difficulty lies in interpreting the importance of the latter results as methodological differences exist between the studies. For this reason, conclusive statements cannot be made. It would appear, however, that if age or sex exerts any effect on the clearance of piroxicam, the well documented intersubject variability would overwhelm any marginal age or sex relationships.

1.6 PHARMACOKINETIC DRUG INTERACTIONS

1.6.1 ASPIRIN

Hobbs & Twomey (1979) examined the influence of aspirin coadministration on the plasma piroxicam AUC in healthy volunteers. Two groups of eight healthy male volunteers received an initial 40mg dose of piroxicam followed by 20mg daily for 2-5 days. One group also received aspirin 972mg q.i.d. on day 4 or 5. No significant effects were obtained as a result of coadministration.

1.6.2 ANTACIDS

In another study in 20 males, Hobbs & Twomey (1979) examined, in a cross over manner, the effect of two proprietary antacid preparations, Amphogel® (aluminium hydroxide and magnesium hydroxide) and Mylanta II® (aluminium hydroxide, magnesium hydroxide and activated dimethicone). Again no significant effects on piroxicam pharmacokinetics were obtained. However, as in the previous study, the authors point out that, due to the large intersubject variability, and the small numbers involved, these studies could only detect large effects (>45%) with any statistical certainty.

1.6.3 RIFAMPICIN

Rifampicin is a well documented inducer of liver enzymes, and 6 healthy volunteers entered a trial in order to determine if single dose piroxicam pharmacokinetics were altered as a result of coadministration (Patel et al 1988). Rifampicin 600mg was given for 7 days prior to administration of a single 40mg dose of piroxicam, and the results were compared to another single piroxicam dose given after a washout period. No significant differences were noted in the pharmacokinetic parameters elimination half-life, Cmax, Tmax, and AUC.

1.6.4 RANITIDINE

In a double-blind two way crossover study, piroxicam 20mg plus ranitidine 150mg b.d. for 10 days was compared to piroxicam plus placebo given in random order

28 days apart to 18 healthy male subjects (Dixon et al 1989). Mean results showed no significant differences between treatments.

1.6.5 CIMETIDINE

Two studies have been performed examining the coadministration of cimetidine in man. Cimetidine has been shown to inhibit the clearance of drugs that undergo cytochrome P450 metabolism. In the study by Mailhot et al (1986), 10 male volunteers (23-35 years) received 20mg piroxicam after one week's treatment with cimetidine 300mg q.i.d. and the results were compared to those obtained from piroxicam 20mg without any pretreatment with cimetidine. Statistically significant differences were obtained for AUC_0^{∞}, clearance, volume of distribution and Cmax. However, Tmax, $t_{1/2}$ and k_e were not statistically different. The authors contended that the differences in AUC could be due to an increase in bioavailability, a decrease in the volume of distribution, or a decrease in k_e. However, no change in k_e was noted in this study, and the increase in AUC is more likely to be due to a decreased clearance or increased bioavailability. As k_e is unaltered, both the clearance and volume of distribution must have altered to the same degree since $k_e = Cl/V$.

In the study by Said and Foda (1989) 12 males (21-36 years) were divided into two groups of six. One group received 20mg piroxicam while the other received 200mg cimetidine t.i.d., with this group receiving their piroxicam dose two hours after the cimetidine dose. Six blood samples were taken within the 24 hour period and analysed by the degradative fluorometric assay technique. AUC_0^{24}, $t_{1/2}$ and k_e were calculated from semilogarithmic plots. A statistical difference in AUC was detected between the two groups, with the cimetidine group having an increased AUC. No statistically significant differences existed between k_e and $t_{1/2}$. The paucity of data points, however, is likely to have contributed to this result. Extrapolations from 6-24 hours is likely to have defined the slope, and hence k_e, and as this entailed only 3 points the margin for error was great if one (or more) of these points was inaccurate.

Furthermore, given the well known pharmacokinetic variability of piroxicam and the study design employed (two parallel groups with no crossover) retrospective power analysis indicates that 33 subjects per group would be required to detect the differences observed with an 80% power. The results can therefore at best be regarded as inconclusive.

1.7 NANSAID INDUCED GASTROPATHY: MANIFESTATIONS

Endoscopic reports suggest that lesions and ulcers associated with NANSAIDs show consistent differences from the peptic ulcers in the general population (Roth 1986). For this reason, the term "NANSAID gastropathy" has been introduced to differentiate between the NANSAID induced lesion and classical peptic ulcer disease. In many instances, significant damage only occurs when other agents such as bile or ethanol are present.

In the absence of such agents, NANSAIDs cause mucosal reddening, vasoconstriction and shedding of surface epithelial cells, but few breaks in the mucosa. This implies that NANSAIDs may act by impairing the prostaglandin-mediated defence mechanisms (Somerville & Hawkey 1986).

Indomethacin and piroxicam appear to have a topical irritancy (like aspirin), which can be reduced by formulation alterations. However, some novel formulations have been disastrous, e.g. Osmosin®, which merely moved the problem further down the gastrointestinal tract.

As these drugs inhibit the cyclooxygenase pathway, there may be diversion of arachidonic acid production down the lipoxygenase pathway (Figure 1.3). This would result in an increased production of hydroperoxyeicosatetraenoic acid (HPETE), hydroxyeicosatetraenoic acid (HETE), and leukotrienes, which could lead to cellular damage. Rainsford (1984) proposed that since most of these drugs are weak acids, intracellular accumulation would occur, resulting in the release of oxygen free radicals and lysosomal enzymes, which, in combination with the drug's prostaglandin synthesis inhibition, would cause local ischaemia, microhaemorrhage with further production of oxygen free-radicals potentiating their effects. Another mechanism includes an impairment of the mucosal metabolism of ATP (adenosine triphosphate) which disrupts cell function, regeneration and synthesis of mucus.

Lange et al (1986) concluded that drug induced gastric injury was directly related

to inhibition of local prostaglandin synthesis. Their study in volunteers showed that the endoscopically derived gastric injury scores were higher for aspirin and indomethacin compared to placebo, but that no significant difference was found between placebo and piroxicam. Inhibition of gastric mucosal prostaglandin synthesis exhibited a similar pattern of results. A significant correlation was shown between the gastric mucosal injury and decreased mucosal prostaglandin synthesis. However, no such relationships were seen in the duodenum indicating that the duodenal mucosa was not affected by the NANSAIDs to the same extent as the gastric mucosa. Goldin et al (1988), in a patient study, refuted these findings. Endoscopic assessment of gastroduodenal mucosal damage in addition to gastric PGE_2 and 6-ketoPGF$_{1\alpha}$ (stable metabolite of PGI_2, prostacyclin) levels were obtained before and after treatment with indomethacin at a daily dose of 150mg. After one week, the concentrations of both prostaglandins were significantly lower, irrespective of the presence or absence of mucosal damage. Cimetidine 400mg twice daily or ranitidine 150mg twice daily reduced the mucosal damage but had no effect on the prostaglandin concentration.

Indomethacin enterohepatic circulation has been studied as a possible factor in both gastric and duodenal toxicity (Brune et al 1987). A correlation was obtained between biliary excretion of several NANSAIDs, including indomethacin, ibuprofen, diflunisal, diclofenac, and naproxen with ileal perforation in rats. Reducing the extent of enterohepatic circulation caused a reduction in the degree of small intestinal erosions. However, studies need to be carried out in humans, as data on the degree of biliary excretion of these drugs and the incidence of intestinal side-effects are unavailable.

Intestinal permeability tests have been carried out by Bjarnason et al (1986) in patients receiving aspirin or NANSAIDs. There are at least three permeability pathways across the small intestine with the most important one, with regard to macromolecular transport, being defective intercellular tight junctions between adjacent

enterocytes. ^{51}Chromium labelled ethylenediamine-tetra-acetate (^{51} Cr EDTA) and lactulose, with molecular weights of 340 and 342 respectively, test specifically for the integrity of these junctions. Intestinal permeability may be important in rheumatoid arthritis because antigens may gain access to the body if intestinal permeability is increased. This may initiate disease either directly or indirectly with the production of circulating immune complexes.

Twelve patients with rheumatoid arthritis untreated by aspirin or an NANSAID had normal test results, whereas 12 aspirin or NANSAID treated patients had increased intestinal permeability to ^{51}Cr EDTA. An increase in permeability was also found in volunteers taking these drugs. Furthermore, indomethacin suppositories also increased intestinal permeability, indicating that it was a systemically mediated effect. As no change was detected in untreated rheumatoid arthritis patients, the suggestion was made that aspirin and the NANSAIDs cause increased permeability and enhanced macromolecular absorption (assuming the same pathway is used by these macromolecules as ^{51} Cr EDTA).

The use of ^{111}Indium labelled leucocyte scintiscans and faecal collections showed that aspirin and NANSAIDs produced small intestinal inflammation on long term treatment (Bjarnason et al 1987). The study in 90 rheumatoid arthritis and 7 osteoarthritic patients showed that inflammation of the small intestine occurred in two thirds of the patients examined and could persist for up to sixteen months after discontinuation of therapy.

The data reviewed here refer to the upper gastrointestinal tract. Evidence is also available which implicates NANSAIDs in the development of lower gastrointestinal disorders (Rampton 1987, Langman et al 1985). This implies that these drugs may have toxic effects throughout the whole of the alimentary tract, based on a toxicity mechanism similar to that observed in the stomach. The small intestine is likely to bear the main brunt of NANSAID induced side-effects, probably due to high local

concentrations of NANSAID at the site of absorption, and continuous re-exposure by enterohepatic circulation (if it exists), but many colonic side-effects may go unreported or unrecognised.

In conclusion, it can be seen that the surface mucosal-bicarbonate barrier (which maintains a pH gradient in order to neutralise any hydrogen ions back-diffusing from the lumen) has a limited capacity to resist injury by luminal contents once a primary insult has "breached" the barrier. Disruption of this barrier appears to be equivalent to destroying the superficial epithelium, and if the noxious stimulus continues, the defence and repair mechanisms are overwhelmed, and damage occurs to the proliferative zone (the area where epithelial cell migration commences and which normally replaces desquamed superficial epithelial cells within three days). Factors that maintain a high intracellular pH play a role in preventing deeper damage. It appears that aspirin and NANSAIDs have the ability to disrupt this barrier by a variety of mechanisms including both topical and systemic actions.

1.8 NANSAID INDUCED GASTROPATHY: EPIDEMIOLOGY

NANSAID induced gastropathy has been described for many years, and more recent endoscopically derived evidence has tended to implicate these drugs further in the generation of gastrointestinal toxicity. Reports of gastrointestinal complications have increased to both the C.S.M. and the F.D.A. (Roth 1986) with bleeding reported twice as often as ulcers. This is a source of concern as it is known that approximately 22 million prescriptions were written for these drugs in the U.K. in 1983 (Walt et al 1986).

In this section the attributable risk (AR) and relative risk (RR) are reported. The AR provides an estimate of the number of patients with the endpoint of interest e.g. haematemesis and/or melaena directly caused by NANSAID consumption. This differs from relative risk which would be the risk of patients developing, for example, haematemesis and/or melaena whilst receiving NANSAIDs compared to patients not receiving NANSAIDs.

Caruso and Bianchi-Porro (1980) examined endoscopically patients on a variety of NANSAIDs. When the group was considered as a whole, lesions occurred in 23% of patients taking a single drug but in 51% when two or more were taken. A further endoscopic study by Lanza et al (1983a) showed that the combination of NANSAIDs and aspirin or alcohol resulted in an additive toxic effect on the gastric mucosa.

Acute superficial erosions caused by a drug do not necessarily mean that chronic administration with the same drug will cause frank ulceration. Large studies would be required to prove causal links, such as that mounted for aspirin by the Boston Collaborative Drug Surveillance Programme (B.C.D.S.P.) which examined data from over 16,000 admissions. In order to get round the expensive and time consuming nature of such trials, Clinch et al (1983) limited themselves to the question of whether anti-inflammatory drugs, as a whole, were associated with adverse gastrointestinal effects. They used a pre-existing "active" endoscopy service, i.e. endoscopies at

regular intervals in elderly patients, regardless of the presence or absence of dyspepsia, and carried out a retrospective study in over 200 patients with a mean age of 75 years. When aspirin was excluded there was a highly significant association between "lesions" and NANSAID use, with a significantly higher proportion of females in the "lesion group". The "lesion group" in this study included patients with endoscopic evidence of oesophagitis, benign gastric ulceration, gastritis, duodenal ulceration, or duodenitis. There were no differences in terms of age, smoking or drinking habits between the control and "lesion groups". However, in the "lesion group" there was an increased incidence of patients with either rheumatoid arthritis or osteoarthritis, all of whom received NANSAIDs.

As an alternative to examining patients receiving NANSAIDs and looking for associations between drug use and gastric lesions, Langman's group in Nottingham examined the consumption of anti-inflammatory drugs in patients who had been admitted with small or large bowel perforation or haemorrhage (Langman et al 1985). These patients were matched for age and sex with a group of patients who had uncomplicated lower bowel disease. It was shown that patients with perforation or haemorrhage were more than twice as likely to have been taking anti-inflammatory drugs .

Bartle et al (1986) found 42.1% of patients with upper gastrointestinal bleeding admitted to hospital were taking NANSAIDs compared to 18.3% of the controls. Furthermore the patients whose bleeding was associated with NANSAID use were significantly older. The relative risk of developing an acute nonvariceal upper gastrointestinal tract bleeding episode was estimated in this study as 3.5 for any NANSAID.

O'Brien & Burnham (1985) looked at their gastroscopic data for patients admitted with a presumptive diagnosis of upper alimentary bleeding. Of the 204 patients examined, 24% had taken NANSAIDs before admission.

Duggan et al (1986) showed that associations previously described for aspirin also existed for NANSAIDs i.e. an association between chronic use and gastric ulceration but not duodenal ulceration. This was a case control study involving 180 matched pairs (85 duodenal,95 gastric ulcers) with the cases obtained from surgical wards and the controls from dermatological wards. There was a RR of 5.0 between the regular use of anti-inflammatory drugs and gastric ulcer shown in this study. No relationships were obtained between NANSAID intake and duodenal ulcer.

Another important study from Langman's group (Somerville et al 1986) matched patients admitted with bleeding peptic ulcer not only to hospital in-patient controls but also community-derived controls. This was the first study to do this and it also specifically examined patients who were over the age of 60. It was discovered that 38% of the patients admitted with bleeding peptic ulcers used NANSAIDs. Indeed, NANSAIDs were taken over twice as often by the patients as by the community derived controls (RR 2.7) and nearly four times as often as the hospital controls (RR 3.8). An interesting finding was that these relative risks not only applied to gastric ulcer, as seen in the earlier studies, but also to duodenal ulcer.

The AR was also derived. When comparison was made to community controls the AR was 0.22, and compared to hospital controls, it was 0.24. Using the number of patients studied (290 over 2 years) and an AR 0.22, about 60 patients would have been admitted with bleeding due to their medication, a serious and worrying number if the population as a whole is considered. The view that NANSAIDs predispose patients to perforation was supported by Collier & Pain (1985). Although the incidence was not as common as gastrointestinal haemorrhage derived from other studies, their case control study found that 47% of a consecutive series of patients over 65 years admitted to hospital with peptic ulcer perforation took NANSAIDs, compared to 7% in the surgical control group. The methodology has been criticised in the respect that there might have been greater identification of NANSAID intake in the perforation group,

but this is unlikely to account completely for the differences observed.

Carson et al (1987) carried out a retrospective study using a computerised data base to evaluate the risk of developing upper gastrointestinal bleeding 30 days after "exposure" to an NANSAID. Over 47,000 "exposed patients" were compared to over 44,000 "unexposed patients". The relative risk, once adjusted for confounding factors, was only 1.5, which is small compared to the previous studies. Patients were followed-up for six months after a prescription for a single NANSAID had been dispensed. However, they did not take into account the length of exposure to the drug prior to the study period, and they only excluded those patients with a previous diagnosis of upper gastrointestinal bleeding.

A further note of caution was made by Montgomery (1987). He accepted Somerville's and Collier & Pain's data showing an association between NANSAIDs and haemorrhage and perforation in elderly patients with gastric ulcer, but he considered that the case that these drugs caused ulcers was not proven. Although an agent could cause ulcer bleeding or perforation, Montgomery contended that it could not be assumed that it would also cause ulcers.

The data for Adverse Drug Reactions associated with NANSAIDs reveal that gastrointestinal diseases constitute a large percentage of all "yellow cards" submitted to the C.S.M. (C.S.M. update 1986). It is only when national figures like these are considered that the scale of the problem can be appreciated, as it is likely that only a faint impression of the problem would be apparent to an individual General Practitioner (Cockel 1987). However, more recent reports from the B.C.D.S.P. suggest that the risks associated with NANSAID consumption may be overestimated. Beard et al (1987) examined hospitalisation due to bleeding from the stomach or the oesophagus in patients over 64 who had received a prescription for an NANSAID within the previous 90 days, and this included long term as well as short term users. The observed difference in rates (NANSAID users= 1.3 x non users) appeared to indicate that

NANSAID use in this group would be unlikely to lead to any major increase in hospitalisation. However, it should be noted that these data were generated from only 29 patients taking NANSAIDs and 28 matched controls. In another study from the same group, Jick et al (1987) found that the frequency of hospital admission for perforated ulcer was not affected by concomitant NANSAID use. They examined patients over 10 years of age whose diagnosis on discharge was perforated ulcer and who had presented a prescription for any NANSAID within 90 days prior to admission.

Later, Jick's standpoint was supported by Henry et al (1987). This was a case control study which found that NANSAIDs were taken by 39% of patients who had died of ulcer complications. Similar rates were also found for aspirin use. The authors were keen to note that relative risks could not have been determined in this study as they did not include a control group without ulcers. However, they concluded that the data indicated that neither aspirin nor NANSAIDs could be associated with an increase in mortality due to ulcer complications.

This study, together with that of Jick et al, appears to be at variance with the large body of evidence which concludes that NANSAIDs are causal for not only ulcer complications but also for the development of peptic ulcer in susceptible patients. Henry et al contradicted the evidence that aspirin intake was associated with frank ulceration and perforation. This view is hard to justify as no data were presented on the amount and duration of aspirin intake in either cases or controls. This omission of data is important as a body of evidence had been built up which states that aspirin is a problem in chronic heavy use, and only under these conditions are serious adverse effects likely to be seen. Not only does the paper contradict other workers' studies, it also contradicts earlier work by their own group. In 1986 Duggan et al showed that an association existed (RR 5.0) between the regular use of NANSAIDs and the development and complications of gastric ulcer. Associations between regular aspirin consumption, either used singly or in combination with NANSAIDs, and gastric

ulceration were also described, in keeping with previous studies. Obviously, this more recent paper helped to confuse rather than clarify the issue.

A prospective longitudinal study - American Rheumatism Association Medical Information System (Fries et al 1989) was performed in 22,000 patients in 17 centres throughout the USA and Canada. It is the most recently published epidemiological study. They noted that patients receiving any NANSAIDs (or aspirin) had a hazard ratio for any gastrointestinal symptoms severe enough to require hospitalisation that was 6.45 times that of patients not taking these drugs. Characteristically, older patients, or those who had previous symptoms requiring either discontinuation of therapy or coadministration of H_2 receptor antagonists or antacids, were at greatest risk. The authors estimated that anti-inflammatory drug induced gastropathy accounted for 2,600 deaths and 20,000 hospitalisations each year in patients with rheumatoid arthritis alone.

Some interesting work has been published by surgeons dealing with the consequences of haemorrhagic and perforated peptic ulcers (Armstrong & Blower 1987b). They strongly disagreed with the studies that considered aspirin and NANSAID gastrointestinal toxicity as rare, and more controversially, benign. They showed that 60% of patients with life threatening complications of peptic ulcers took these drugs. Furthermore, 78.2% of the patients who died were shown to have received anti-inflammatory drugs. The mortality rate associated with complications of peptic ulcer disease in patients taking NSAIDs was more than twice that expected in patients not receiving NSAIDs. If this mortality rate was extrapolated to the national level it would indicate that 4,000 people died each year due to NSAID induced upper gastrointestinal complications. It must be noted, however, that the cases and controls in this study were not matched, casting some doubt on the value of the results. Furthermore, questioning of the two groups took place at different times and by different people.

Dewer (1985), another surgeon, showed similar data. In this study 50% of the

over 60s (who have the worst prognosis for emergency surgery) took aspirin or an NANSAIDs compared to 20% of the under 60s. The incidence in females was twice that in males, although fewer females were studied. This was also in agreement with Armstrong & Blower's results. The association was greater with bleeding than with perforation, except in the over 60s where perforation rates were higher than in the under 60s.

Table 1.4 summarises all the above studies. Studies have been excluded if their methodology did not allow relative risks to be calculated. If relative risks were not stated, but could be calculated, the following formula was employed:-

$$RR = \frac{a}{a+b} + \frac{c}{c+d}$$

where:

a = number of patients receiving drug with lesions

b = number of patients receiving drug without lesions

c = number of patients not receiving drug with lesions

d = number of patients not receiving drug without lesions

Differences therefore exist as to the perceived risk of gastrointestinal complications associated with NANSAID use. This may be accounted for largely by the differences in size of studies, patient populations, statistical methods, study design and study outcomes. This makes any attempt to formalise a meta-analysis impossible. If individual risk is low, it is necessary to consider whether risk is concentrated in any specific group e.g. the elderly. Langman noted that, by case control studies, it was possible to show an increased risk of ulcer complications in individuals aged over 60 (Langman 1989). Collier & Pain (1985) demonstrated a highly statistically significant correlation between annual number of elderly patients with perforated peptic ulcer who were taking an NSAID and the annual number of prescriptions issued for these drugs.

Table 1.4 Summary of Epidemiology Studies

Author	Year	Patient Number	Control Number	Gastropathy Measure	Design	Drugs including Aspirin	Subjects	Summary statistics	Conclusions
Clinch et al	1983	100	50	Mucosal Lesions	Patient drug History ,Case Notes,Physician Interview (Retrospective)	No	Ulcer NSAID users vs Nonusers: also control group of nonulcer hospitalised patients	% and No Deaths, Operations per group. % NANSAID users for Control Group	Apparent Relationship between NSAID use and life threatening Ulcer complications (RR = 6.1)
Collier & Pain	1985	269	269	Perforated Peptic Ulcer	Case Notes (Retrospective)	Yes	Ulcer vs Age/Sex matched nonulcer hospitalization	No NSAID users per Group	Significant Difference between NSAID use in cases and control for elderly (RR : 2.3 [<65], 11.5 [>65])
Langman et al	1985	268	268	Small or Large Bowel Perforation or Haemorrhage	Case Notes (Rectrospecive)	Yes	Perforation/ Haemorrhage vs Age/Sex matched uncomplicated lower bowel disease	No NSAID users per Group	Relationship between NSAID use & complications Perforation RR = 2.6 Haemorrhage RR= 2.6
Duggan et al	1986	180	180	Gastric/Duodenal Ulcer	Questionnaire (Prospective)	No	Endoscopy determined G.U./D.U. vs Age/sex/ Social class/matched hospitalized controls	NANSAID Consumption and G.U./D.U.	Association exists for Gastric Ulcer (RR =5) but not for Duodenal Ulcer (RR = 1.1)
Somerville et al	1986	230	230(Comm) 230(Hosp)	Bleeding Peptic Ulcer	Questionnaire (Prospective)	No	Elderly,ulcer bleeding hospitalized vs Age/sex matched hospital and community controls	No.NANSAID users per group	Bleeding strongly associated with NANSAID use Community RR =2.7 Hospital RR =3.8

Table 1.4 Cont. Summary of Epidemiology Studies

Author	Year	Patient Number	Control Number	Gastropathy Measure	Design	Drugs including Aspirin	Subjects	Summary statistics	Conclusions
Bartle et al	1986	57	123	Acute Non-Variceal Upper Gastrointestinal Bleeding	Physician Interview (Prospective)	No	Upper G.I. bleeding vs Age/sex matched non bleeding	No.NANSAID Users per group	Patients whose bleeding was associated to NANSAID (RR =3.5) Were Sig. older than patients who bled without NASAID
Beard et al	1987	29	28	Bleeding from stomach or oesphagus	Computerised = outpatient, in-patient,discharge prescriptions (Retrospective)	No	Elderly NANSAID exposed and Nonexposed cohorts	Hospitalized rates for bleeding per group (Person days at risk) incidence ratio estimate	No increase in bleeding (RR =1.3)
Jick et al	1987	54	324	Perforated Peptic Ulcer	As above	No	NANSAID exposed and nonexposed cohorts; case control study	Hospitalised rates for perforation per group (Person days at risk)	Little association between NASAID and perforated Peptic ulcer (RR = 1.2)
Carson et al	1987	47136	44634	Upper Gastro-Intestinal bleeding	As above	No	NANSAID exposed and nonexposed cohorts	Risk of developing gastrointestinal bleeding within 6 months of Initial (30 day) exposure	Small increase risk of bleeding Due to NASAID Consumption (RR=1.5)
Armstrong & Blower	1987	235	1246	Life threatening Peptic Ulcer	Case notes (6 months retrospective) 30 month follow up (Prospective)	Yes	Ulcer NSAID users vs Nonusers: also control group of Nonulcer hospitalization	% and No. Deaths/ operations per group: % NSAID users for contol group	Apparent relationship between NSAID use and life threatening ulcer complications (RR =6.1)

Jick et al (1987) also showed that perforation rates increased sharply with age.

Another problem with the data is the significant occurrence of "silent ulceration". Many ulcers in patients receiving NSAIDs are asymptomatic (Roth 1986). In the absence of routine endoscopy, clinical estimates of ulcer frequency are probably too low. Many ulcers first present with a catastrophic bleed or perforation. Almost 60% of the cases of complicated ulceration reported by Armstrong & Blower had no gastrointestinal symptoms. It may be argued that silent ulcers are more dangerous than symptomatic ulcers because of the absence of warning symptoms. Even when symptoms are present, they are not necessarily indicative of ulceration, confounding further the difficulty in recognising the presence of potentially life threatening ulceration.

Maintenance treatment with H_2 receptor antagonists appears to some extent to decrease the frequency of asymptomatic ulcers (Pounder 1989). Curiously, it also appears to prevent asymptomatic ulcers becoming symptomatic. Another confounding factor is that ulcers can heal despite continued drug intake (Dewer 1985). Dewer noticed that patients who had symptoms of peptic ulcer for less than three months were more likely to be taking NSAIDs than not. However, if they had experienced the symptoms for more than 1 year they were less likely to be taking NSAIDs (Dewer 1985). This could indicate refractoriness, or adaptation to the drugs' gastrointestinal toxicity.

In conclusion, the development of NANSAID induced gastropathy is a well recognised phenomenon. There is, however, disagreement as to the extent of the problem. Chronic use appears to be associated with the development of duodenal, and especially, gastric lesions which can progress into more serious conditions. The heterogeneous nature of the individual studies described in this section has made formal meta-analysis almost impossible.

Examination of Table 1.4 shows that the prospective studies consistently found

higher relative risks compared to the retrospective studies which may be due to the removal of some confounding factors and bias exhibited in retrospective studies. The attributable risk may not be as high as previously thought. In general, there would be a reduction of mortality and morbidity by the decreased prescribing of these agents, especially in conditions where their clinical benefit is doubtful such as low back pain. Their use, however, remains disturbingly great.

1.9 NANSAID INDUCED GASTROPATHY: TREATMENT AND PREVENTION

Whether or not peptic ulcer is drug related, its therapy tends to be similar. However, if ulcers in patients receiving NANSAIDs have a different aetiology, does it follow that they will have a similar response rate if traditional anti-ulcer treatment is employed?. In this section examination will be made of the treatments available, the studies carried out in both normal subjects and patients, and the effects of continuing or discontinuing the NANSAID during therapy. When consideration of the results of studies are made, it should be borne in mind that the body's own intrinsic healing capabilities also play a role. The extent of this has been shown in "classical" peptic ulcer disease where placebo groups in some studies have been shown to have healing rates between 30 and 60% (Clearfield & Borowsky 1986). Furthermore, healing rates can differ between populations, with Europeans showing faster healing rates than North Americans.

1.9.1 H_2 RECEPTOR ANTAGONISTS

Since the back diffusion of acid into the gastric mucosa has been shown to cause acute injury, initial therapy was aimed at decreasing acid secretion or neutralising intra-luminal acid. Of the H_2 receptor antagonists available, both cimetidine and ranitidine have been marketed for a number of years with an enviable safety record. Recently two newer agents, nizatidine and famotidine, have been introduced, and although they differ structurally, they do not appear to offer any specific advantages over existing agents.

Lanza et al (1983b) carried out a double blind endoscopic study in two groups of 15 volunteers. One group received aspirin 650mg q.i.d., the other, flurbiprofen, 50mg q.i.d.. After a one week endoscopy, four subjects from each group exhibited significant gastric and/or duodenal damage. Those eight continued their particular drug for another month but with the addition of cimetidine 300mg q.i.d.. On re-endoscopy significant improvement was seen only in the subjects with duodenal damage.

When consideration is made of the coadministration of H_2 receptor antagonists in

order to prevent or heal NANSAID induced gastropathy, studies involving healthy volunteers may not be able to predict this situation in patients. For example, patients with rheumatoid arthritis have a higher incidence of peptic ulceration even when they are not receiving any medication (O'Brien 1983). Therefore, the disease can influence the response and direct comparisons may not be valid.

Golan & Keren (1982) showed a reduction in upper gastrointestinal symptoms with cimetidine as a single night time dose in arthritic patients receiving indomethacin or diclofenac. Loiudice et al (1981) showed cimetidine coadministration healed NANSAID induced gastric ulcers. Croker et al (1980) also looked at arthritic patients with anti-inflammatory drug induced ulcers and found similar healing and recurrence rates to non arthritic ulcer patients. In duodenal ulcer, expected healing rates with H_2 receptor antagonists are 75% within 4 weeks and up to 90% at 8 weeks; gastric ulcer healing rates tend to be slower (Howden & Hunt 1990). However, most ulcers recur when treatment stops indicating that these drugs do not alter the natural history of the condition.

These studies all showed a reduction in severity of gastrointestinal damage once the anti-inflammatory drug had been discontinued. It would be more appropriate to examine the situation when NANSAIDs are continued. Rachmilewitz et al (1986a) showed cimetidine decreased the mucosal damage caused by indomethacin despite its continued use. Another study (Rachmilewitz 1988) showed that cimetidine could prevent damage due to short term courses of aspirin 650mg q.i.d., naproxen 500mg b.d., or indomethacin 50mg t.i.d.. Roth (1982) showed cimetidine to be significantly better than placebo in decreasing the frequency of endoscopically determined peptic ulceration in arthritic patients who had been receiving a number of different NANSAIDs (and aspirin) on a long term basis. An improvement was also seen in rheumatoid arthritis patients on long term salicylate therapy with cimetidine despite continued aspirin therapy.

In the large double blind multicentre study from Norway comparing piroxicam and naproxen, 63 out of 2000 osteoarthritis patients experienced serious gastrointestinal side effects (16 diffuse gastritis, 17 gastric ulcers, and 30 duodenal ulcers). Cimetidine 400mg every eight hours was given in order that they could continue their medication. Of the 47 who had either gastric and duodenal ulcers, 43 healed within eight weeks. None of these patients, or the remaining sixteen with diffuse bleeding gastritis experienced further symptoms (Giercksky et al 1988).

Cimetidine 800mg given as a single night time dose to treat anti-inflammatory drug induced erosions was examined by Bijlsma (1988a) in 187 patients who continued their medication. Patients with ulcers had a healing rate of 49% at four weeks which increased to 81% by eight weeks. Erosions had a four week and eight week healing rate of 82% and 97% respectively. Maintenance treatment with 400mg daily was continued in 113 patients with endoscopically-healed lesions. During six months of observation with endoscopy at one monthly intervals, the cumulative probability of recurrence of lesions was 12%, with a mean time to recurrence of 116 days. This figure is similar to the relapse rates obtained in non drug induced peptic ulcer disease.

Similar results were found by the author in 127 patients receiving NANSAIDs, and aspirin, who experienced gastrointestinal symptoms which were significant enough to warrant endoscopy but which subsequently proved negative (Bijlsma 1988b). In this double blind placebo controlled study, the four week healing rates were 72% on cimetidine and 49% on placebo. Relief from heartburn was 87% in the cimetidine group and 60% on placebo. In the maintenance phase, 11% in the cimetidine group relapsed (mean time 120 days) and 10% in the placebo group (mean time 35 days). Cimetidine was therefore considered useful in the treatment of both endoscopically positive, and negative, drug induced gastrointestinal symptoms despite continued use.

Few of these studies address the differing effects of cimetidine on gastric ulcers and duodenal ulcers associated with NANSAIDs. It cannot be assumed that their

healing rates are the same. For example, Stalnikowicz et al (1988) showed that although cimetidine reduced the amounts of endoscopically scored mucosal lesions in patients receiving indomethacin (50mg three times a day), it only achieved statistical significance in the duodenum. This difference between healing rates for gastric and duodenal lesions has been an area of exploitation for the manufacturers of synthetic prostaglandin analogues (see section 1.9.2).

Overall, although cimetidine has been demonstrated to be beneficial in the situations outlined above, care must be taken when examining individual studies. Reliance on endoscopic scoring systems may be problematical (Graham 1989). Many authors provide separate scores for each type of lesion e.g. erythema, petechiae, erosions and acute ulcers. Lesion scores may be given for different areas e.g. oesophagus, gastric body/antrum, and duodenum and the totals for each lesion type in each area may be added to give a total that is statistically compared. There are therefore so many comparisons made that finding a statistical significance by chance is highly likely.

When ranitidine was considered, similar results to cimetidine were obtained. Bianchi Porro et al (1987) showed impressive trends in favour of ranitidine healing gastric mucosal lesions in arthritic patients, either continuing or discontinuing their NANSAID treatment. However, the power of this study may have been so low that conclusive statements would be difficult to make as only 48 rheumatoid patients were investigated and they, in turn, were subdivided into four equal groups. Furthermore, ranitidine did not prevent gastric damage. Musi et al (1984) performed endoscopy on patients with obvious gastrointestinal symptoms from a rheumatology clinic. NANSAIDs and aspirin (if appropriate) were continued and patients were given either ranitidine or aluminium hydroxide. Patients treated with ranitidine achieved improved endoscopic lesion scores, but, this was only significant when severe lesions were present.

These findings have been confirmed by studies from Scandinavia (Manniche et al 1987, Malchow-Moller et al 1985, Malchow-Moller 1987). These authors examined the effects of different ulcer treatments in rheumatoid patients with peptic ulcers who had either continued or discontinued their medication. There was no statistical difference in the healing times for ranitidine or sucralfate (section 1.9.3) either in the presence or absence of anti-inflammatory drug therapy. This indicated that ulcer healing could be brought about despite continued intake, and furthermore, the healing rates found corresponded to those obtained in non-rheumatoid patients.

Rachmilewitz et al (1986b) showed that ranitidine increased the synthesis of PGE_2 and 6-keto$PGF_{1\alpha}$ in both antral and fundal gastric mucosa. This effect occurred at non-antisecretory doses indicating that ranitidine may also have an effect on prostaglandin synthesis.

As far as the newer agents are concerned there are insufficient data on their use in NANSAID induced gastropathy at present.

In conclusion, only full doses of H_2 receptor antagonists have been shown to heal ulcers and prevent recurrences. They do reduce severe acute injury and also reduce non-ulcer gastrointestinal disturbances. However, there appears to be differences between their effectiveness in the treatment of NANSAID induced gastric ulcer when compared to duodenal ulcer, (McCarthy 1989) and as their efficacy in prophylaxis has not been established, their routine use in all cases would appear to be unjustified.

1.9.2 PROSTAGLANDINS

The cyclooxygenase enzyme may exist as different isoforms in various body tissues (Whittle et al 1980). The possibility therefore existed for the development of prostaglandin analogues to reverse the NANSAID induced cyclooxygenase inhibition in selective tissues e.g. the gastric mucosa. In this section, therefore, discussion will be made of the role of exogenous prostaglandins in the treatment of NANSAID gastropathy. These agents have been called "cytoprotective", a term which implies that

they will protect against mucosal injury by mechanisms other than reduction of acid secretion. This has been criticised as a misleading term because the individual cells are not "strengthened" or "cytoprotected" (cell protected) (Guth 1987). Nevertheless, the term has come into widespread use. The problem with it is that, by definition, cytoprotection is a preventative effect of an agent given prior to the administration of a necrotising substance, resulting in a reduction in damage caused by that substance. This action cannot be extrapolated to a completely separate action that heals an already established lesion. It may be logical to assume that if an agent has one of the above actions it will have the other. What cannot be assumed is that the same mechanisms are involved in each case.

It has been show that prostaglandins have two main actions. At lower doses they are considered to be cytoprotective whereas at higher doses they suppress acid secretion. Prostaglandins are thought to protect against all ulcerogenic agents as they prevent the appearance of lesions after exposure to necrotising agents in animal studies (Holt & Hollander 1986). Restitution of the mucosa appears to be more rapid after prostaglandin pretreatment than when there is no pretreatment (Hawkey & Walt 1986). This may be a consequence of preserving the cells that initiate mucosal repair, rather by than direct stimulation of the repair processes. These findings have, by and large, not been reproduced in human studies (Charlet et al 1985). The ability to state unequivocally that certain actions occur at non-antisecretory doses has also proved difficult. This is due to a lack of standardised methods and the use of techniques which were not sensitive enough to differentiate between the two proposed activities with regard to the dose. Animal studies have indicated that prostaglandins may be involved in the "adaptation" seen after chronic therapy with aspirin and the NANSAIDs; nevertheless this remains controversial (Hawkey & Rampton 1985).

If prostaglandins are important in the integrity of the mucosal barrier, does a deficiency predispose to peptic ulceration?. Wright et al (1982) noted that patients

with chronic gastric ulcers had reduced mucosal PGE levels compared to controls. Other studies indicate that there may also be abnormalities in duodenal ulceration (Hawkey & Rampton 1985, Hawkey & Walt 1986).

The synthetic prostaglandins are based on either PGE_1 (e.g. misoprostol) or PGE_2 (e.g. enprostil). Diarrhoea is a common side effect although it varies between drugs. Abdominal pain and possible abortifacient effects further mitigate against their use. The accumulating evidence suggests that misoprostol and enprostil are of similar potency to H_2 receptor antagonists in healing both "classical" gastric and duodenal ulcers. Furthermore, there is little evidence to suggest that their healing rates are any higher than those expected from their antisecretory activity. For this reason it appears that "cytoprotection" may not be a major factor in ulcer healing by prostaglandins (Hawkey & Walt 1986).

Hawkey et al (1986) examined some strategies to reduce anti-inflammatory drug induced gastropathy. This study involved looking at healthy volunteers who had been taking either aspirin or an NANSAID for over two days. Mucosal damage was quantified by determining blood content in the gastric washings. Experimental agents used in this study included enprostil, ranitidine, milk, and a linoleic acid and gamma linolenic acid preparation (Efamol®). These were given for 20 - 60 minutes before the dose or alternatively, Laboprin®, a lysine containing aspirin preparation, was given instead of regular aspirin. Only enprostil and ranitidine reduced mucosal bleeding and increased the pH of the washings. This suggested that acid inhibition was the mechanism by which mucosal damage was reduced.

Rachmilewitz et al (1986b) examined PGE_2 and 6-keto$PGF_{1\alpha}$ levels in fundal and antral mucosa obtained from patients with active duodenal ulcer, healthy controls, and patients on long-term anti-inflammatory therapy. Levels were significantly reduced in both the patient groups. There was a greater reduction in the group receiving medication in comparison to the duodenal ulcer group. The decreased prostaglandin

content in the duodenal ulcer group was not affected by one month's treatment with misoprostol, arbaraprostil, sucralfate, pirenzepine or placebo. However, ranitidine significantly increased the levels, especially of PGE_2. Unfortunately, these drugs were not given to the anti-inflammatory drug treated group.

The ability of prostaglandins to protect against NANSAID induced damage was examined in two studies. In the first Lanza et al (1988) compared misoprostol (200μg q.i.d.) and cimetidine (300mg q.i.d.) to placebo in protecting tolmentin induced (400mg q.i.d.) gastric and duodenal injury in normal volunteers. After six days treatment endoscopic assessment showed a success rate for preventing overall damage to be 26.7% in the placebo group, 63.3% in the cimetidine group and 93.1% in the misoprostol group ($p < 0.001$). When the different sites were compared independently, the success rates for cimetidine and misoprostol did not differ in the duodenum, but there was a statistically higher success rate in the gastric mucosa with misoprostol.

This finding was confirmed in a long term study using piroxicam, ibuprofen, or naproxen in 420 patients with osteoarthritis (Graham et al 1988). Endoscopy was performed at entry and after one, two, and three months of continuous treatment with either 100 or 200μg misoprostol q.i.d. or placebo. Treatment failure was defined as the development of gastric ulcer. 5.6% of the 100μg group and 1.4% of the 200μg group developed gastric ulcers compared to 21.7% in the placebo group ($p < 0.001$). In these two studies diarrhoea was very common (two to three times more common in the treatment group compared to placebo).

Another feature of the Graham study was the low occurrence of duodenal ulcer. In patients taking misoprostol, this only occurred in 2.5% compared to 3.6% in the placebo group. This placebo rate is lower than most other studies and may reflect exclusion of patients with prior ulceration. Even if it is true, at a rate of 3.6% it will be very hard to show that any drug could better it.

In a study by Dammamm et al (1989), the high prevalence of diarrhoea in the

misoprostol group was associated with a 20-60% reduction in the steady state indomethacin concentration when compared to volunteers receiving ranitidine or placebo. It was the authors contention that this reduced absorption could have played a role in the lower gastroduodenal lesion scores found in their study in the misoprostol group. This area, therefore, requires further investigation.

In conclusion, the role of prostaglandins in the treatment of NANSAID induced gastropathy is still controversial. They do not heal ulcers with any greater efficiency than would be predicted from their inhibition of acid secretion. The ability to prevent damage by necrotising agents seen in animal studies, the so-called cytoprotective effect, has not been well demonstrated in humans. Their side effects and healing rates indicate that they possess no therapeutic advantage over the H_2 receptor antagonists in ulcer healing, although they have demonstrated efficacy in preventing gastric ulcers in patients receiving anti-inflammatory drugs, but this needs further investigation. At present, positive indications for these drugs are few.

1.9.3 SUCRALFATE

This is a molecule of sucrose with multiple substitutions for aluminium salts. It has been shown to be effective in the treatment of both gastric and duodenal ulcers (Holt & Hollander 1986). At low pH values some aluminium hydroxide ions dissociate and the residual compound becomes negatively charged. This then polymerises to form a viscous paste-like substance which is the "active" form of sucralfate. This substance adheres to the mucosa. However, when the mucosa is damaged, sucralfate binds with six times its normal affinity as the negatively charged sucralfate binds to the positively charged denatured proteins for at least 6 hours. Sucralfate therefore protects the mucosa by insulating it from the luminal acid, by adsorbing bile salts, and by reducing peptic activity. It is not an antacid; however the aluminium hydroxide may provide local buffering against gastric acid. Sucralfate also appears to stimulate local prostaglandin production (Tarnawski et al 1987).

Caldwell (1985) examined the use of sucralfate in reducing gastrointestinal symptoms associated with NSAID intake. Sucralfate was given for two weeks, while NSAIDs were continued. Sucralfate administration resulted in a disappearance of heartburn, epigastric pain and distress, despite continued anti-inflammatory drug intake. This result was in agreement with the Scandinavian studies described earlier (Manniche et al 1987, Malchow-Moller et al 1985, Malchow-Moller 1987) which showed that sucralfate healed ulcers at the same rate irrespective of whether an NANSAID was continued or not.

One factor that mitigates against the drug's success is that on long term treatment aluminium absorption may be a problem (Allain et al 1990) and for this reason it is only licensed in the U.K. for up to 12 weeks continuous treatment.

1.9.4 BISMUTH CHELATE

The proprietary preparation of De Nol® (Tripotassium dicitrato bismuthate = colloidal bismuth subcitrate = bismuth chelate) has recently come back into favour for the treatment of peptic ulceration due to its actions on Helicobacter pylori, and its ability to reduce ulcer relapse rates. It selectively chelates the proteinaceous material of an ulcer base forming a protective coat which adheres for about 6 hours. In addition, it directly inactivates pepsin, via chelation, and may also increase luminal PGE_2 levels (Konturek et al 1987). It was also shown that this increased luminal release of PGE_2 was completely abolished by aspirin in man, and by indomethacin in dogs (Konturek et al 1987). The role therefore for this agent in NANSAID gastropathy will only become clear once the appropriate studies are performed.

Bismuth cannot be used for maintenance therapy as absorption may lead to encephalopathy (Gavey et al 1989). It darkens the teeth, tongue and stools, the liquid formulation smells of ammonia, and needs to be taken q.i.d.. For these reasons a more palatable tablet formulation with a convenient b.d. dosage regimen has been introduced. The maximum length of the course is 56 days with at least 1 month between courses.

1.10 CONCLUSIONS

All NANSAIDs decrease prostaglandin synthesis, with the result that mucosal prostaglandin levels fall. NANSAID induced gastropathy is a well recognised phenomenon, although the heterogeneous nature of the studies thus far performed make it is impossible to say whether or not a causal relationship exists.

Prophylactic treatments e.g. H_2 receptor antagonists, prostaglandins can heal and delay the recurrence of gastropathy, however, it is unclear whether they can prevent the condition. The "cytoprotective" effect of prostaglandins has not been well recognised in humans, and although the current prostaglandins appear to heal NANSAID induced gastric ulcers more effectively than H_2 receptor antagonists their side effect profile will restrict their use.

Piroxicam is no better or no worse, in terms of toxicity, than the other NANSAIDs currently available despite assertions to the contrary. Its long elimination half-life has ensured its commercial success, as General Practitioners (and patients) prefer the simplicity of a once a day dosage regimen, and this popularity has been reflected in terms of sales, making it one of the most profitable branded NANSAIDs ever developed.

There are, however, a number of unresolved questions regarding the pharmacokinetics of piroxicam e.g. the outcome when it is combined with known hepatic enzyme inhibitors (e.g. H_2 receptor antagonists), the implications of any clearance alterations, the extent of it's biliary excretion, and the nature of the "multiple peaks" present in plasma concentration time curves. These questions, warrant further investigation and consideration.

CHAPTER 2

MATERIALS AND METHODS

2.1 INTRODUCTION TO CHAPTER 2

In this chapter, examination is made of the existing methods available to determine piroxicam and 5-hydroxypiroxicam in plasma and urine. Their drawbacks are outlined, and the need for a specific assay to quantify trace amounts of drug and metabolite not only in plasma and urine, but also in bile, is described. The methods developed, with the materials used, are listed, in addition to a description of the assay's reliability in terms of extraction recovery, accuracy and precision (both inter- and intra-assay). Finally, discussion is made of the advantages of these methods and some of the problems that had to be overcome in the assay development.

2.2 RATIONALE FOR ASSAY DEVELOPMENT

Early studies on the pharmacokinetics of piroxicam employed a degradative fluorometric "wet" chemical analytical technique. This involved strong acid hydrolysis of piroxicam to generate 2-aminopyridine followed by fluorometric assessment of this product after purification. External standards were used to determine the concentration of drug present in the sample. This technique was not ideal as it lacked sensitivity and selectivity. By using chromatographic techniques improved assay systems were developed. Chromatography could be performed on biological samples containing piroxicam without chemical modification of the drug. Furthermore, they were simpler analytically, more rapid, and as they incorporated internal standards, they were also more accurate. Table 2.1 lists the published chromatographic methods for the analysis of this drug (and in some instances 5-hydroxypiroxicam). It was considered essential that the assay system employed in this work would be flexible enough to cope with a variety of biological samples (plasma, urine and bile) with sufficient accuracy and precision to be able to detect quantities of both piroxicam and 5-hydroxypiroxicam at concentrations as low as 50ng/ml in each of these body fluids. Table 2.1 shows that no published assay system has yet fulfilled these criteria.

An HPLC (High Performance Liquid Chromatography) system was envisaged for the reasons outlined above. From the point of view of simplicity, use of a single column to enable analysis of piroxicam and 5-hydroxypiroxicam in all biological samples, without sacrificing accuracy, was desired. None of the published assays met these conditions for both plasma and urine and no published reports were available for the analysis of this drug and/or metabolite in bile. Method development therefore proceeded with the objective of establishing an assay technique which would fulfil all the above mentioned criteria.

TABLE 2.1

PUBLISHED ASSAY METHODS FOR PIROXICAM QUANTITATION IN HUMAN BIOLOGICAL SAMPLES

Reference	Year	Column Type	Plasma /Urine	Sample Volume (ml)	Internal Standard (µg)	Extraction Agent	Injection Volume (µl)	Detector (nm)	Recovery (%)	Linear Range (mg/L)
Twomey et al	1980	H.P.L.C. (µBondapack CN)	P	0.1	Isoxicam (10)	Diethylether	100	UV (365)	IS(74) Piroxicam (77)	0.5-20
Riedel & Laufen	1983	H.P.T.L.C.	P/U (and tissues)	0.5	Piretanide (0.5)	Dichloromethane	50	Fluorescence (excitation 366)	IS(85) Piroxicam (95)	0.1-15
Fraser & Woodbury	1983	L.C. (Lichrosorb RP-8)	P	1.0	Naproxen (100)	Chloroform	10	UV (330)	IS(85) Piroxicam (88)	0.5-30
Dixon et al	1984	H.P.L.C. (Lichrosorb RP-18)	P	1.0	Tenoxicam (100)	Dichloromethane	20	UV (361)	IS(81) Piroxicam (81)	0.2-20

Tsai et al	1985	H.P.L.C. (Lichrosorb RP-18)	P/U	0.5 (Plasma) 2.0(Urine)	Indomethacin (10mg)	Diethylether	50	UV(365)	5-Hydroxy Piroxicam (65) Piroxicam (70)	0.5-25
Richardson et al	1986	H.P.L.C. (μBondapack CN-Plasma) (μBondapack C18-Urine)	P/U	0.5 (Plasma) 1.0(Urine)	Isoxicam (10)	Diethylether	50	UV(365)	IS(90)	
								Plasma	5-Hydroxy Piroxicam(90) Piroxicam(85)	0.05-5
								Urine	5-Hydroxy Piroxicam(84) Piroxicam(86)	0.1-5
Macek & Vacha	1987	H.P.L.C. (Separon Six CN)	P	0.2	X	X (Precipitation)	50	UV(360)	X	0.27-10.8
Boudinot & Ibrahim	1988	H.P.L.C. (Econosphere)	P	1	6 Methyl piroxicam(500)	Methylene Chloride	5-200	UV(360)	Piroxicam(91)	0.002-50
Gillian et al	1989	H.P.L.C. (Spherisorb C18)	P	0.5	Naproxen(10)	Diethylether	100	UV(360)	Piroxicam(100)	0.25-5

2.3 MATERIALS

2.3.1 CHEMICALS

The following chemicals were used in all analyses: acetic acid (Pronalys AR, May and Baker); acetonitrile (HPLC Far UV, Fisons); citric acid (AnalaR, FSA); diethlyether (Pronalys AR, May and Baker); disodium phosphate (AnalaR, BDH); β-glucuronidase enzyme (Bovine Type BI, Sigma); hydrochloric acid (Pronalys AR, May and Baker); orthophosphoric acid (Pronalys AR, May and Baker); sodium acetate (AnalaR FSA); sodium dihydrogen phosphate (Pronalys AR, May and Baker); sodium hydroxide (AnalaR BDH).

2.3.2 CONSUMABLES

The following items were uniformly used in all analyses: Waters 4ml sample vials (cat. no. 72710); Waters compression spring (cat. no. 72708); Waters H-style caps (cat. no. 72711); Waters PTFE septa (cat. no. 73005); Microsert limited volume inserts (Jones Chromatography, cat. no. 150710); Pyrex disposable culture tubes 13 x100mm (Corning); Centrifree® micropartition system (Amicon); Gilson Pipetteman with Gilson polypropylene tips; Techsil 10 CN column packing (HPLC Technology).

2.3.3 DRUGS

Piroxicam was a gift from Pfizer (Sandwich U.K.) and 5-hydroxypiroxicam was a gift from Pfizer (Groton, Conn. U.S.A.). Isoxicam was a gift from Warner-Lambert/Parke-Davis (Gwent, U.K.).

2.3.4 EQUIPMENT

The following apparatus were used: Waters autosampler WISP 710B; Waters pump M45; Shimadzu UV detector SPD6A; Shimadzu Chromatopack Integrator CR3A; Gallenkamp orbital shaker; MSE Mistral 2l centrifuge; Techne Dri-block DB3; SMI Multitube Vortexer 2601; Dawe Sonicleaner; Grant Waterbath.

2.4 SOLUTIONS

2.4.1 0.1M ACETIC ACID

0.58ml glacial acetic acid was made up to volume (100ml) in a volumetric flask with freshly distilled and filtered water.

2.4.2 0.1M SODIUM ACETATE

0.8203g sodium acetate was dissolved in a 100ml volumetric flask with freshly distilled and filtered water.

2.4.3 0.1M ACETATE BUFFER pH 5.0

To 14.8ml acetic acid 0.1M was added 35.2ml sodium acetate 0.1M and made up to 100ml in a volumetric flask with freshly distilled and filtered water.

2.4.4 0.1M CITRIC ACID

2.1g citric acid was dissolved in a 100ml volumetric flask with freshly distilled and filtered water and made up to volume.

2.4.5 0.2M DISODIUM PHOSPHATE

3.56g disodium phosphate was dissolved in a 100ml volumetric flask with freshly distilled and filtered water and made up to volume.

2.4.6 CITRIC ACID-PHOSPHATE BUFFER pH 4.9

To 50.5ml citric acid 0.1M was added 49.5ml disodium phosphate 0.2M in a 100ml volumetric flask.

2.4.7 HYDROCHLORIC ACID 1.0M

8.6ml glacial hydrochloric acid was added to 70ml freshly distilled and filtered water in a 100ml volumetric flask before making up to final volume.

2.4.8 SODIUM HYDROXIDE 0.01M

0.4g sodium hydroxide was made up to volume (1000ml) in a volumetric flask with freshly distilled and filtered water.

2.4.9 MOBILE PHASES

In order to analyse piroxicam and 5-hydroxypiroxicam in plasma, urine and bile,

on the same chromatography column, specific mobile phases were employed to optimise the separation of the various chromatographic peaks. For the analysis of plasma and urine samples, a mobile phase containing 22% acetonitrile gave optimal results. For the preparation of this solution, 440ml acetonitrile was made up to 2 Litres with freshly distilled and filtered water. 3.423g sodium dihydrogen phosphate was added, and after mixing for 5 minutes, the final pH was adjusted to 3.5 with orthophosphoric acid. Helium was then bubbled through for 20 minutes to degas the solution. If patients were also receiving sulphasalazine, the final pH was adjusted to 2.5 and not 3.5 (see section 2.5).

For the analysis of bile samples, a mobile phase consisting of 10% acetonitrile was employed. For its preparation, 200ml acetonitrile and 1.560g sodium dihydrogen phosphate was used. Otherwise the method was identical to that described above.

2.5 METHODS

2.5.1 HPLC ANALYSIS OF PIROXICAM AND 5-HYDROXYPIROXICAM IN PLASMA, URINE AND BILE

2.5.1.1 EXTRACTION PROCEDURE IN PLASMA

To 0.5ml of plasma, in a 12ml heavy duty glass centrifuge tube (manufactured by Glass Blowing Department, Glasgow University) was added: 1ml of 0.01M sodium hydroxide (containing piroxicam and 5-hydroxypiroxicam if spiking samples in order to obtain calibration line), 0.1ml internal standard solution (0.1mg/ml isoxicam in 0.01M sodium hydroxide), 0.25ml of 1M hydrochloric acid, and 5ml diethylether. The tubes were stoppered and shaken for 6 minutes at 240 revs/min using a Gallenkamp Orbital Shaker and centrifuged at 2000 revs/min for 10 minutes at 4°C (MSE Mistral 2L). The ether phase was transferred to a pyrex tube (Corning) and evaporated to dryness (37°C) under nitrogen (Techne Dri-block DB3). The residue was reconstituted in 250μl of acetonitrile/water (50:50) and vortexed for 60 seconds (SMI multitube vortexer 2601) immediately prior to injection (50μl) on to the column.

2.5.1.2 EXTRACTION PROCEDURE IN URINE AND BILE

The extraction procedure for unconjugated piroxicam and 5-hydroxypiroxicam in the urine and bile was identical to that used in plasma, with the addition of one extra stage. After collection of the ether phase, a back-extraction was performed to purify the sample. 2ml citric acid-phosphate buffer (pH 4.9) was added to the ether in a clean centrifuge tube. This was again shaken and centrifuged before collecting the ether phase. The ether was then evaporated and reconstituted in the above manner.

For the measurement of 5-hydroxypiroxicam glucuronide, 5ml urine or bile was adjusted to pH 5.0 with an equal volume of 0.1M acetate buffer. This was incubated for 24 hours in a water bath at 37°C, in the presence of 500 I.U. Bovine Liver β-glucuronidase. 1ml of this mixture was then acidified by 0.25ml 1.0M hydrochloric acid after addition of internal standard (0.1ml 0.1mg/ml isoxicam in 0.01M sodium

hydroxide). Extraction was performed as per the unconjugated 5-hydroxypiroxicam.

In all instances, residues were reconstituted in 250µl acetonitrile/water (50:50) and vortexed for 50 seconds (SMI multitube vortexer 2601) prior to injection (50µl) on to the column.

2.5.1.3 REVERSED-PHASE CHROMATOGRAPHY

Instrumentation included a Waters autosampler WISP 710B, a Waters M45 pump, a fixed wave length UV detector (Shimadzu SPD6A) and an integrator (Shimadzu CR3A chromatopack). For simultaneous quantitation of piroxicam and 5-hydroxypiroxicam in plasma, urine and bile a Techsil CN column (20cm x3.9mm, 10µm particle size, HPLC Technology) was used. For the analysis of plasma and urine the mobile phase consisted of 50 mM sodium dihydrogen phosphate in acetonitrile/water (22:78) with a final pH of 3.5. For the analysis of bile the mobile phase was similar except that the acetonitrile concentration was 10%.

The flow rate in all instances was 2.5ml/minute and the eluate was monitored at 365nm. A guard column using Techsil CN was employed in all instances and replaced after each run.

In each run, spiked samples were included over the concentration range 0.05 to 30mg/l. This was used to construct a calibration line for that particular run. In addition, 0.5mg/l standard samples were dispersed throughout the patient samples in order to evaluate assay performance throughout the analysis.

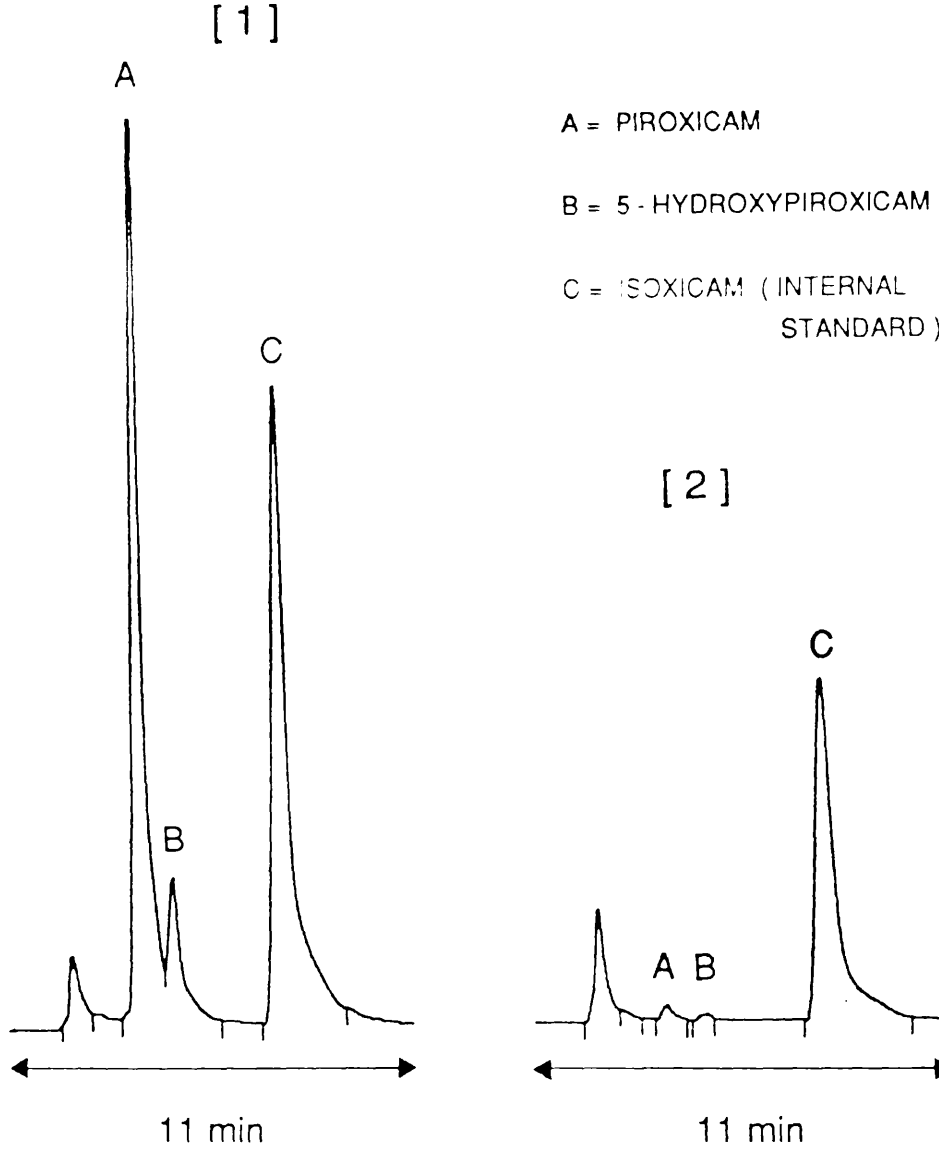
Figure 2.1 shows an example of a typical plasma (both bound and unbound) chromatogram. Figures 2.2 and 2.3 show an example of a chromatogram obtained both pre- and post-glucuronidase incubation in urine and bile respectively.

2.5.2 HPLC ANALYSIS OF UNBOUND PLASMA CONCENTRATION OF PIROXICAM AND 5-HYDROXYPIROXICAM.

2.5.2.1 INTRODUCTION

As was outlined in Chapter 1, piroxicam is greater than 99% bound to plasma

FIGURE 2.1 EXAMPLE OF PLASMA CHROMATOGRAPHS



1. TYPICAL CHROMAGRAPH OBTAINED IN PLASMA (BOUND AND UNBOUND)

2. TYPICAL CHROMAGRAPH OBTAINED IN PLASMA (UNBOUND)

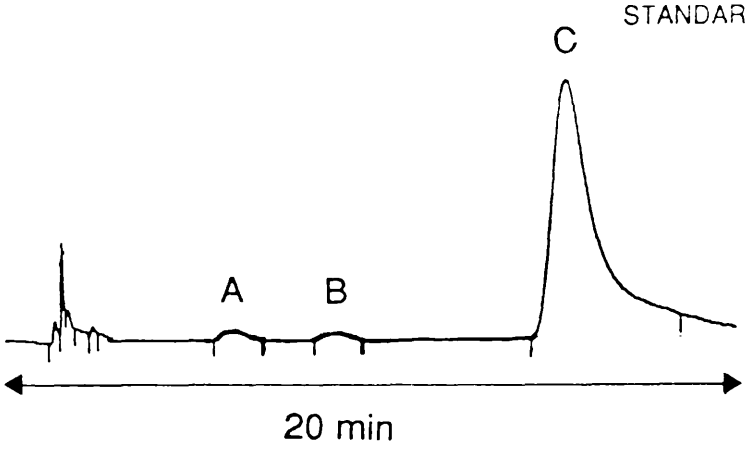
FIGURE 2.2 EXAMPLE OF BILE CHROMATOGRAPHS

A = PIROXICAM

B = 5 - HYDROXYPIROXICAM

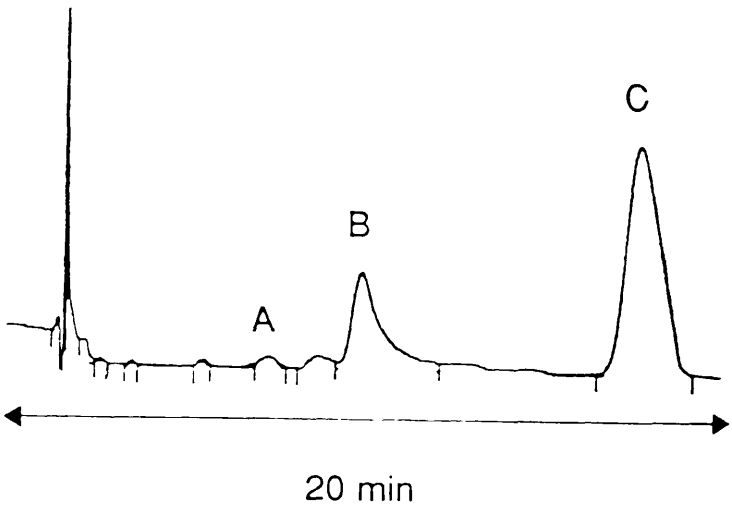
C = ISOXICAM (INTERNAL STANDARD)

[1]



TYPICAL CHROMAGRAPH OBTAINED IN BILE PRE-GLUCURONIDASE INCUBATION.

[2]



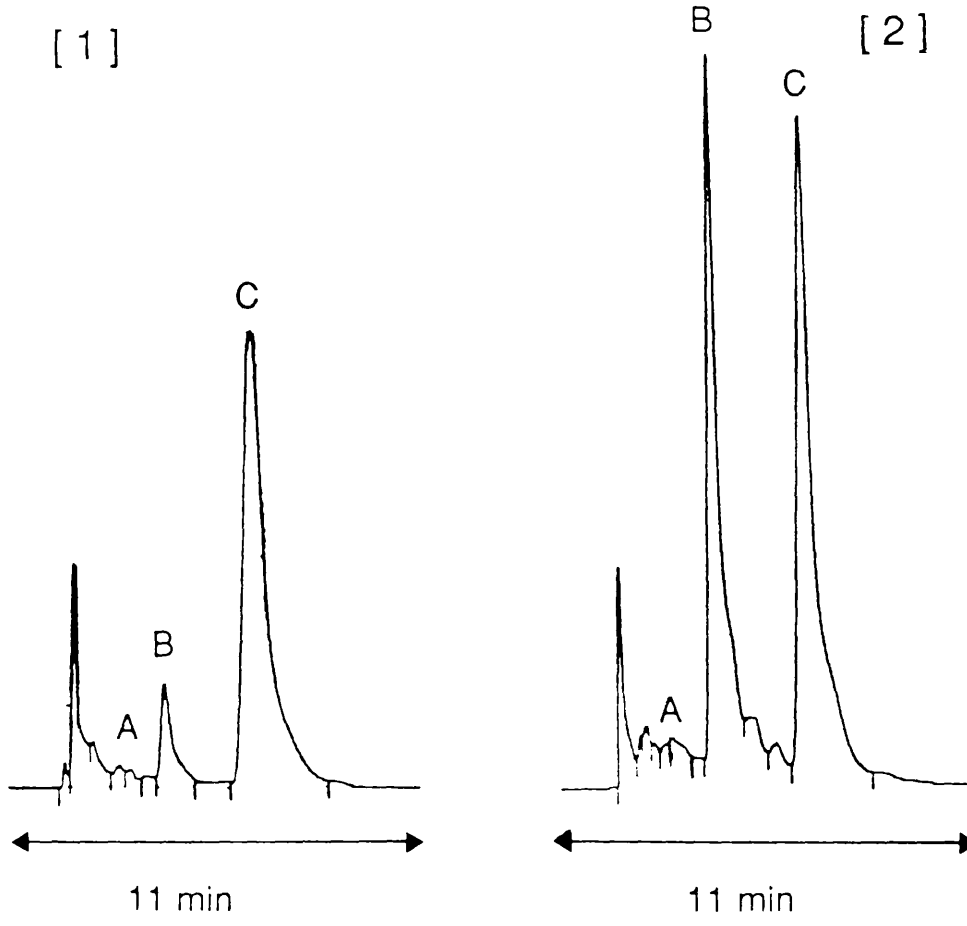
TYPICAL CHROMAGRAPH OBTAINED IN BILE POST-GLUCURONIDASE INCUBATION.

FIGURE 2.3 EXAMPLE OF URINE CHROMATOGRAPHS

A = PIROXICAM

B = 5 - HYDROXYPIROXICAM

C = ISOXICAM (INTERNAL STANDARD)



1. TYPICAL CHROMAGRAPH OBTAINED IN URINE PRE - GLUCURONIDASE INCUBATION

2. TYPICAL CHROMAGRAPH OBTAINED IN URINE POST- GLUCURONIDASE INCUBATION

albumin, and as a consequence the circulating unbound concentration of this drug is low. Determination of unbound drug by ultrafiltration methods confers advantages over other techniques such as equilibrium dialysis (Lin et al 1987). It is very rapid, therefore eliminating the disadvantages of long equilibrium times which may degrade unsuitable ligands or proteins. Furthermore, long dialysis times may result in adsorption to the membrane and dilution of plasma compartment by buffer which would result in a false equilibrium being set up. Ultrafiltration methods do not show these drawbacks. Unbound drug concentration determined in the ultrafiltrate is independent of the fractional volume of the sample filtered (Lin et al 1987). For these reasons ultrafiltration methods were employed in the analysis of unbound concentrations of piroxicam and 5-hydroxypiroxicam.

2.5.2.2 ULTRAFILTRATION METHODS

1.0ml of plasma (either patient unknowns or spiked volunteer samples) was pipetted in to a Centrifree® micropartition system (Amicon) at room temperature. This was centrifuged at 2000 revs/min for 2 hours at 25°C until 0.25ml of ultrafiltrate was obtained.

2.5.2.3 EXTRACTION PROCEDURE

To 0.25ml of ultrafiltrate, in a 12ml heavy glass centrifuge tube (manufactured by Glass Blowing Department, Glasgow University) was added: 0.5ml of 0.1M sodium hydroxide, 0.05ml of internal standard solution (0.1mg/ml isoxicam in 0.01M sodium hydroxide), 0.125ml 1.0M hydrochloric acid and 5ml diethylether. The extraction procedure was then identical to that listed for plasma in section 2.5.1.1 with the exception that 100µl of the reconstituted residue was injected on to the column. Figure 2.1 shows a typical chromatogram.

2.6 ASSAY VALIDATION

2.6.1 EXTRACTION RECOVERIES

Table 2.2 lists the extraction recoveries consistently obtained in each biological sample. The addition of a purification step to both urine and bile samples improved the recovery of low concentration samples as the influence of interfering peaks was lessened. The results obtained are in agreement with the previously published values shown in Table 2.1. The extraction recoveries obtained for isoxicam, the internal standard, were 88%, 94%, and 90% in plasma, urine and bile samples respectively.

2.6.2 LINEARITY

The assay linearity for drug and metabolite was determined by performing linear regression analysis on the plot of the peak-height ratios of either piroxicam or 5-hydroxypiroxicam over the internal standard, versus concentration in the range 0.05 to 30mg/l. No measured concentrations were ever greater than 19mg/l. Table 2.3 lists the correlation coefficients obtained in the assay of both piroxicam and 5-hydroxypiroxicam using known standards in the range 0.05 to 20mg/l. Correlation coefficients obtained were typically 0.9999.

2.6.3 ASSAY PRECISION

Bulk control standards were prepared by adding known amounts of piroxicam and 5-hydroxypiroxicam to blank plasma and urine. Pre-dose bile was collected from the individual and this was used as the control. Table 2.4 summarises both the intra- and the inter-assay coefficients of variation obtained over the range 0.05mg/l to 30mg/l. Only at the extreme end of the concentration range does the inter-assay variability approach 20%. For concentrations greater than 0.1mg/l inter-assay variability is for most instances less than 10%.

TABLE 2.2

EXTRACTION RECOVERIES (%) FOR PLASMA, URINE AND BILE SAMPLES

CONCENTRATION (mg/L)	PLASMA PIROXICAM	PLASMA 5-HYDROXY PIROXICAM	URINE PIROXICAM	URINE 5-HYDROXY PIROXICAM	BILE PIROXICAM	BILE 5-HYDROXY PIROXICAM
0.05	80	93	100	100	94	100
0.1	82	87	100	97	86	100
0.5	98	94	100	100	86	100
5	97	88	100	92	93	100
20	100	90	100	89	91	96
30	100	88	100	88	91	91

TABLE 2.3

TYPICAL CORRELATION COEFFICIENTS FOR PLASMA, URINE AND BILE SAMPLES

	PLASMA PIROXICAM	PLASMA 5-HYDROXY PIROXICAM	URINE PIROXICAM	URINE 5-HYDROXY PIROXICAM	BILE PIROXICAM	BILE 5-HYDROXY PIROXICAM
r	0.9999	0.9999	0.9999	0.9999	0.9997	0.9999
Slope	0.4633	0.2362	0.4175	0.2198	0.6583	0.2674
Intercept	-4.7×10^{-3}	-1.1×10^{-3}	0.0199	9.1×10^{-3}	-0.0795	8.1×10^{-3}

TABLE 2.4

INTRA - AND INTER - ASSAY COEFFICIENT OF VARIATION (%) FOR PLASMA, URINE AND BILE SAMPLES

CONC. (mg/L)	PLASMA				URINE				BILE			
	PIROXICAM		5-HYDROXY PIROXICAM		PIROXICAM		5-HYDROXY PIROXICAM		PIROXICAM		5-HYDROXY PIROXICAM	
	Intra (n=4)	Inter (n=6)	Intra (n=4)	Inter (n=6)	Intra (n=4)	Inter (n=6)	Intra (n=4)	Inter (n=6)	Intra (n=4)	Inter (n=6)	Intra (n=4)	Inter (n=6)
0.05	2.9	5.4	8.0	19.1	4.9	3.3	2.0	2.0	3.4	24.0	3.9	21.2
0.1	2.4	6.8	2.6	2.9	2.1	11.6	4.5	3.4	3.9	6.7	1.4	3.0
0.5	0.8	1.5	0.7	4.7	1.8	3.2	2.9	6.3	3.0	8.6	3.4	8.6
5	0.3	1.5	0.3	1.4	0.6	2.7	0.3	1.9	1.3	8.1	0.7	7.6
20	0.6	2.8	0.6	2.5	0.9	2.5	0.6	1.8	1.2	9.9	0.7	9.3
30	0.2	1.6	0.1	2.3	0.7	1.9	0.5	1.8	3.2	6.4	2.5	11.6

2.7 DISCUSSION OF ASSAY DEVELOPMENT

As has been previously outlined, the development of an HPLC assay system conferred advantages over other analytical techniques. However, problems were encountered in the development of this assay. Initially disposable plastic test tubes (not glass) were employed for economic reasons. It was discovered that adsorption of the drug and internal standard occurred into the plastic if left in contact with the plastic container walls. For this reason, disposable and heavy duty re-usable glassware was essential in this assay system.

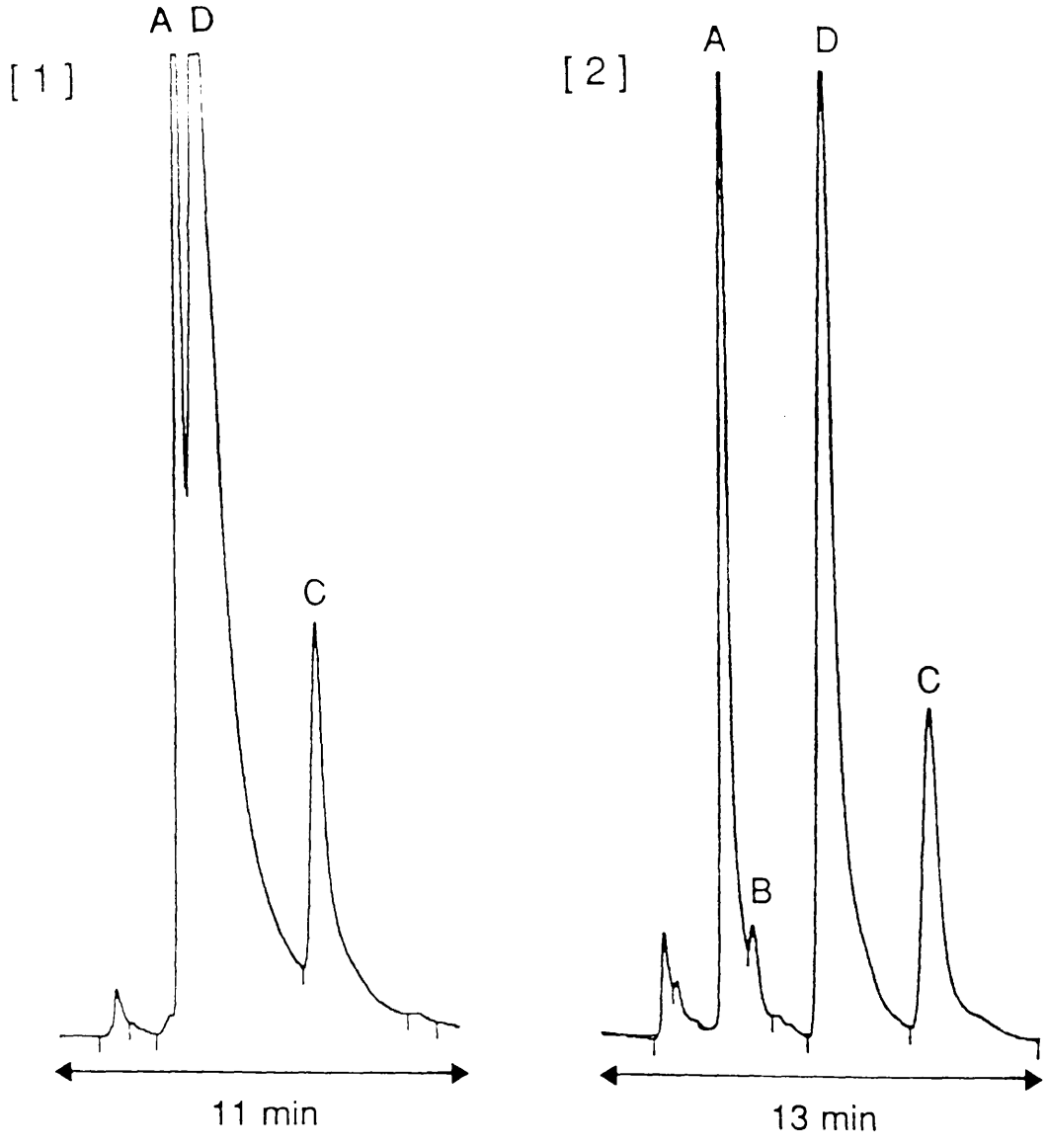
Another problem was encountered with patients receiving the second line antirheumatic agent, sulphasalazine. It is used in high doses (2 to 4g daily) and, in man, is excreted in the urine after azo-reduction to sulphapyridine and 5-aminosalicylic acid, as their hydroxylated metabolites. The UV max of sulphapyridine is 359nm and piroxicam is 360nm, as determined by scanning UV measurements. Therefore as sulphasalazine is taken in such large amounts, interference and partial obliteration of both the piroxicam and 5-hydroxypiroxicam chromatographic peaks was obtained in both plasma and urine samples. By adjusting the pH of the mobile phase to pH 2.5, the polarity of the interfering material was increased and as a consequence the peak was eluted after both the piroxicam and 5-hydroxypiroxicam peak but before the internal standard allowing detection and quantitation to occur as before. Figure 2.4 shows the effect of altering the mobile phase pH.

Ratios of drug to internal standard peaks were quantified in terms of heights and not areas as it was found that height was less variable. It also conferred the advantage that manual assessment of individual chromatograms could be performed at all stages which proved to be of particular benefit in the analysis of bile.

Initially bile analysis was performed in a 22% acetonitrile mobile phase as discussed earlier. However, after glucuronidase incubation, interfering peaks were obtained which made detection of small quantities of piroxicam and 5-

FIGURE 2.4 EXAMPLE OF PLASMA CHROMATOGRAPHS IN PATIENTS RECIEVING SULPHASALAZINE

A = PIROXICAM
B = 5 - HYDROXYPIROXICAM
C = ISOXICAM (INTERNAL STANDARD)
D = SULPHASALAZINE METABOLITE



1. TYPICAL CHROMAGRAPH OBTAINED IN MOBILE PHASE pH 3.5

2. TYPICAL CHROMAGRAPH OBTAINED IN MOBILE PHASE pH 2.5

hydroxypiroxicam difficult. By decreasing the acetonitrile concentration to 10%, the retention times of these drugs relative to each other was increased and good separation was obtained. However, instead of tall, narrow peaks, short, broad peaks were obtained which the integrator had difficulty in quantitating satisfactorily. For this reason, peak heights were always reported.

CHAPTER 3

AN INVESTIGATION OF THE PERTURBATIONS IN PLASMA PIROXICAM CONCENTRATIONS EXHIBITED IN MAN

3.1 INTRODUCTION TO CHAPTER 3

As discussed in Chapter 1, the extent of enterohepatic circulation of piroxicam has been the subject of much speculation, but it has not been investigated to any great extent. In the work described in this chapter, the nature and extent of the perturbations exhibited in plasma piroxicam concentration/time curves were examined by carefully designed experimental and simulation studies. The effect of food consumption on the disposition of piroxicam was also examined in healthy volunteers with particular emphasis on possible alterations of protein binding, and both clearance and volume of distribution. Furthermore, the appearance of piroxicam and 5-hydroxypiroxicam in a variety of biological fluids, including bile, was investigated in order that unequivocal statements could be made about the role of enterohepatic circulation in the disposition of this drug in man.

3.2 THE INFLUENCE OF FOOD CONSUMPTION ON PLASMA PIROXICAM CONCENTRATIONS

3.2.1. INTRODUCTION

Several studies in man have reported "multiple peaks" in plasma piroxicam concentration/time curves, as described in Chapter 1. It has been suggested that these "multiple peaks" indicate that the drug undergoes enterohepatic circulation, and that biliary recycling contributes to the drug's long elimination half life. In this study, we wished to examine this contention in greater detail. The nature and extent of these "multiple peaks" was unclear. If they were the result of enterohepatic circulation, food consumption might well result in stimulated release of bile, reflected by a post-prandial increase in the plasma concentration of the recycled material. This study, therefore, standardised the timing of food administration and employed a blood sampling schedule which attempted to characterise these "multiple peaks" in greater detail.

The content of food was standardised as various nutrients have been shown to have differential effects on physiological functions. In the study by Moneta et al (1989) superior mesenteric artery blood flow, as determined by Duplex ultrasound, was increased to varying degrees after a protein, fat, carbohydrate or mixed constituent meal. When Indocyanine Green (ICG) clearance was employed as an indicator of hepatic blood flow, a post-prandial increase was shown by Svensson et al (1983) after a high protein meal. The maximum increase (69%) was obtained 40 minutes after consumption and was still 36% above control values after 100 minutes. This had important implications for drugs whose hepatic elimination is flow dependent.

Alteration in haemodynamics due to food consumption is not exclusively confined to the gastrointestinal tract and surrounding tissues. In the studies by Fagan et al (1986a,b) both volunteers and patients exhibited post-prandial increases in supine/standing heart rate and cardiac output, together with a decrease in supine/standing diastolic blood pressure and total systemic resistance. Consumption of

a high carbohydrate meal also reduced supine systolic blood pressure.

It is was therefore important to know the constituents of the meals provided and to standardise the constituents on each study day.

3.2.2. SUBJECTS

Table 3.1 lists the demographic details of the six healthy volunteers who participated in this study. The six had a mean age of 26 years and were all within 20% ideal body weight. Renal and hepatic function tests and a full haematological screen including, serum ferritin were performed. Subjects were excluded if they had any current, or previous history of peptic ulceration, any allergy, or adverse reaction to aspirin or NANSAIDs.

3.2.3 METHODS

After an overnight fast, the volunteers received 1 piroxicam 20mg capsule (Feldene® Pfizer U.K.). Blood samples (5ml) were obtained for six days according to the sampling schedule shown in Figure 3.1. The content (Table 3.2) and timing of food administration was standardised with volunteers requested not to eat or drink outwith the allotted times and to fast after 10 p.m. each night.

Plasma piroxicam concentrations were measured by the HPLC method detailed in Chapter 2.

3.2.4. RESULTS

Figures 3.2 and 3.3 show the individual plasma piroxicam concentrations obtained during the interval 0-14 hours with Figure 3.4 showing the mean of all six. Figures 3.5 and 3.6 show the individual results obtained during the interval 14-120 hours with Figure 3.7 showing the mean results. The time of food administration has been annotated. It appears that perturbations do occur, and that they correspond to food consumption. In most instances a greater effect is observed following lunch rather than the evening meal which may be a consequence of the different meals have different nutritional constituents (Table 3.2).

TABLE 3.1

VOLUNTEER DEMOGRAPHIC DETAILS

VOLUNTEER	M/F	AGE (years)	WEIGHT (kg)	ALBUMIN (g/l)
1	M	29	72.1	45
2	M	23	76.2	46
3	M	20	73.9	49
4	M	35	77.1	43
5	M	25	72.6	52
6	M	26	79.8	51
MEAN		26	75.3	

FIGURE 3.1: VOLUNTEER STUDY BLOOD SAMPLING SCHEDULE

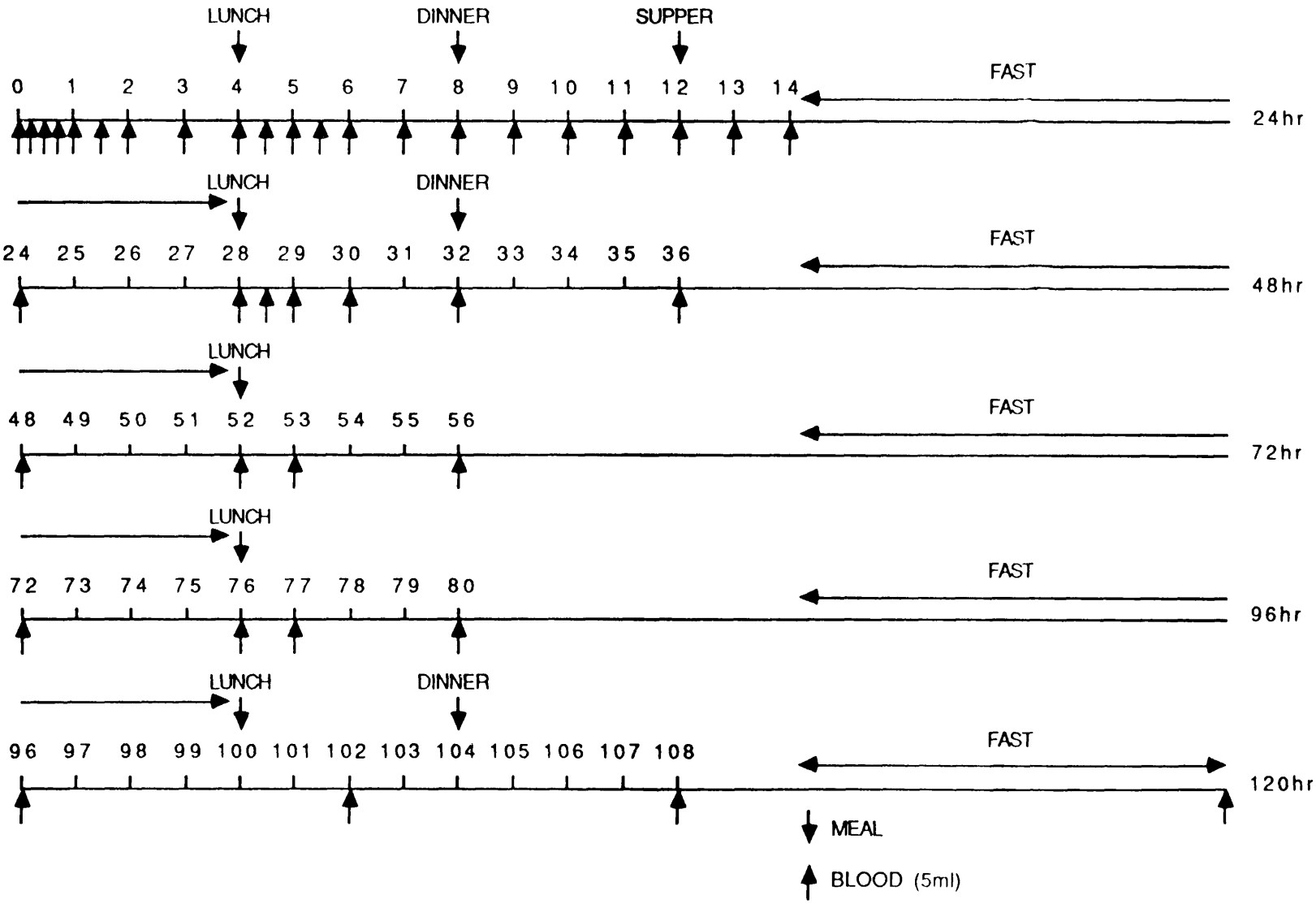


TABLE 3.2

FOOD CONSTITUENTS

	WATER (g)	PROTEIN (g)	FAT (g)	CARBOHYDRATE (g)	FIBRE (g)	CALORIES (Kcal)
Lunch	666	18	36	74	7	678
Dinner	467	6.5	4	20	1	145
Supper	115	3	1	12	1	85

FIGURE 3.2 : PLASMA PIROXICAM CONCENTRATIONS (SUBJECTS 1 - 3) 0 - 14 HOURS

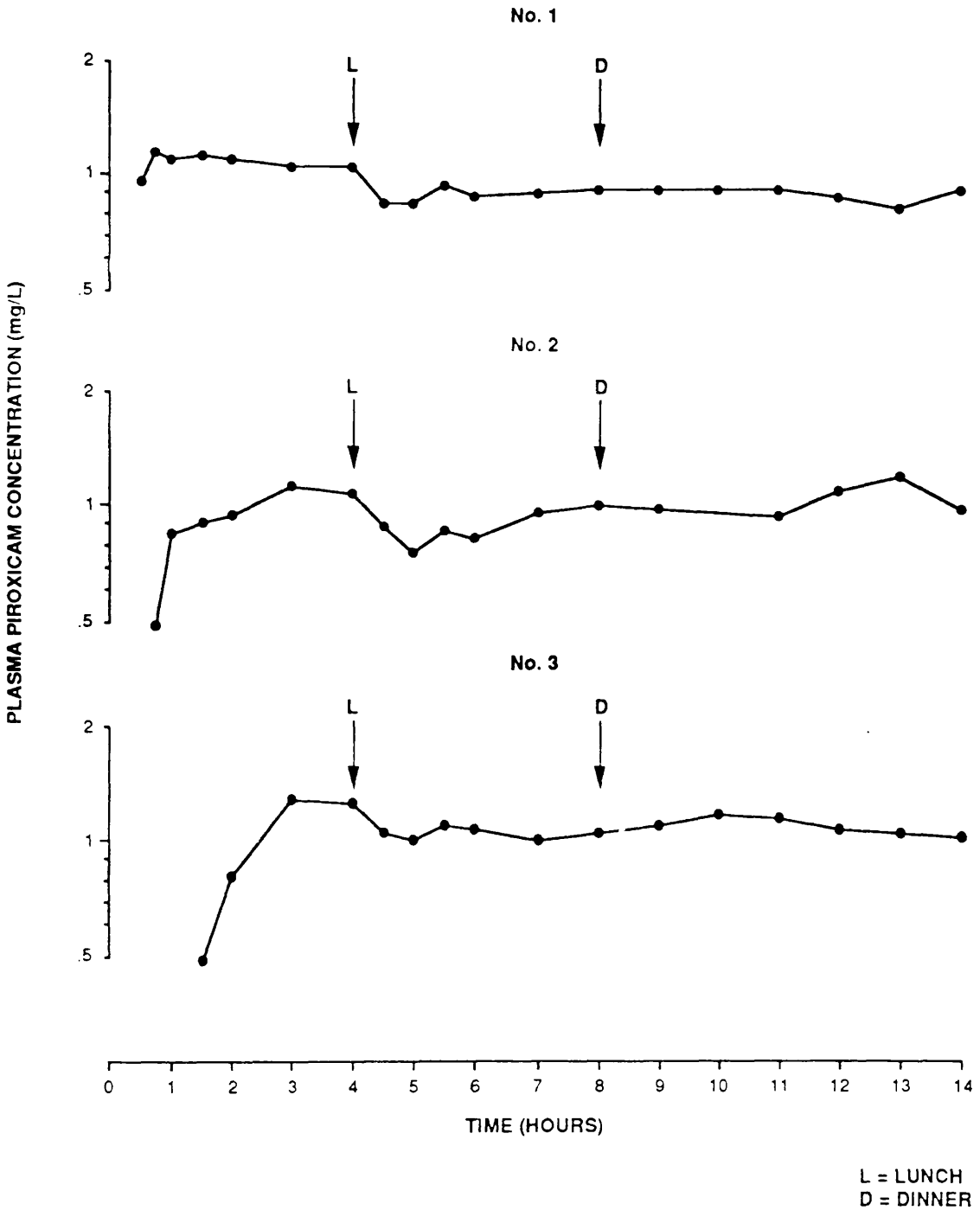


FIGURE 3.3 : PLASMA PIROXICAM CONCENTRATIONS (SUBJECTS 4 - 6) 0 - 14 HOURS

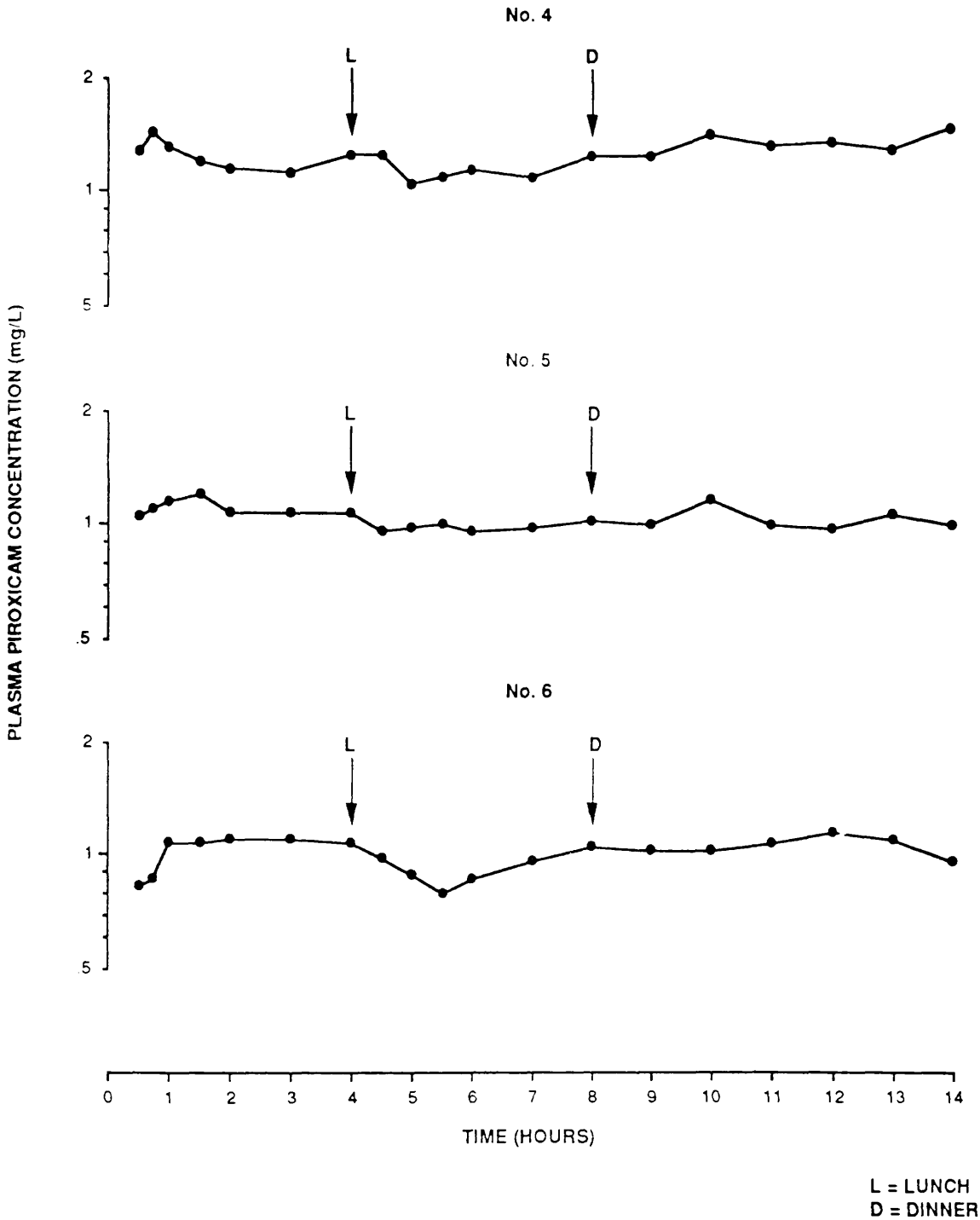


FIGURE 3.4: MEAN ± S.D. OF PLASMA PIROXICAM CONCENTRATIONS IN ALL SUBJECTS FROM 0 - 14 HOURS

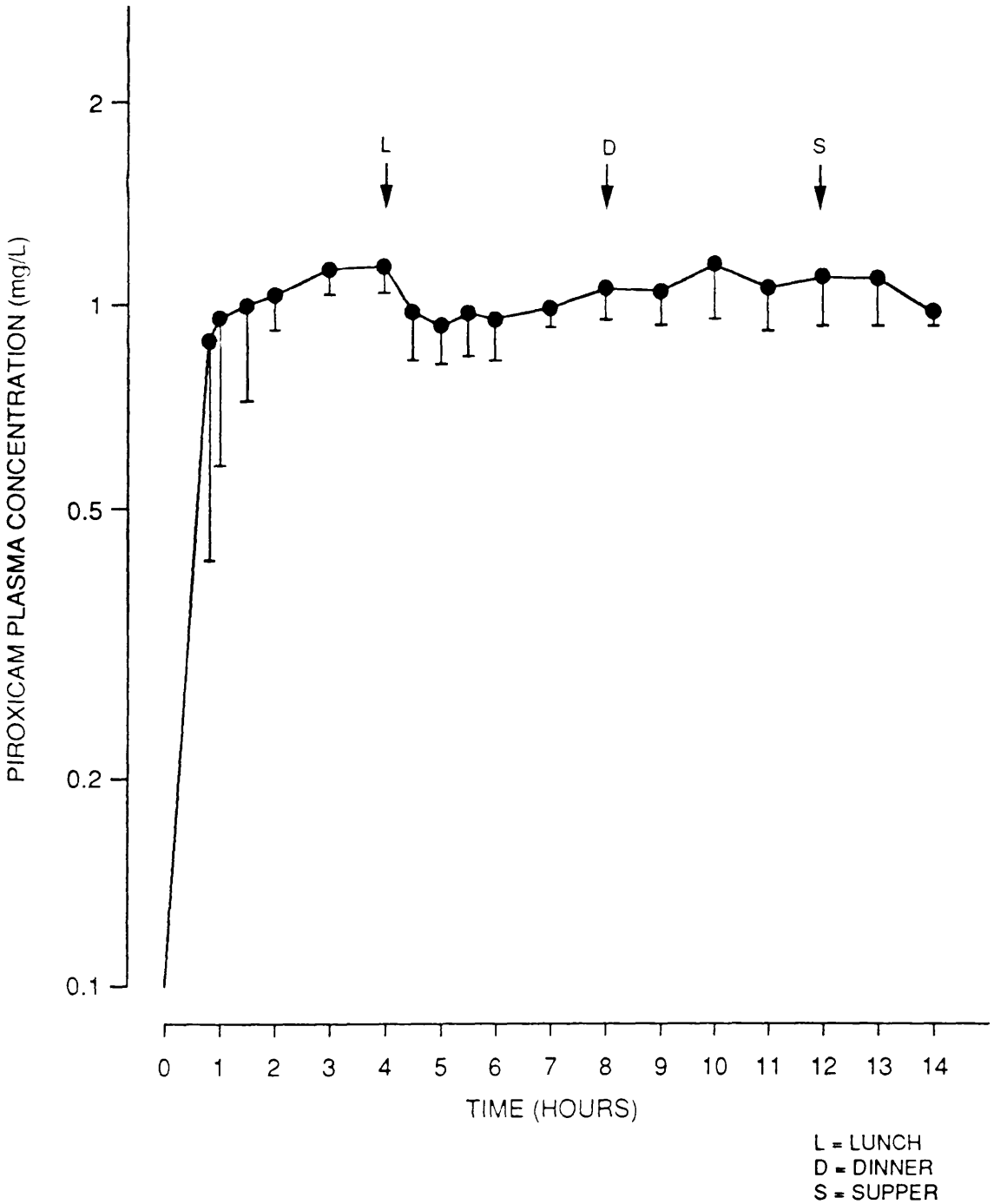


FIGURE 3.5 : PLASMA PIROXICAM CONCENTRATIONS (SUBJECTS 1 - 3) 14 - 120 HOURS

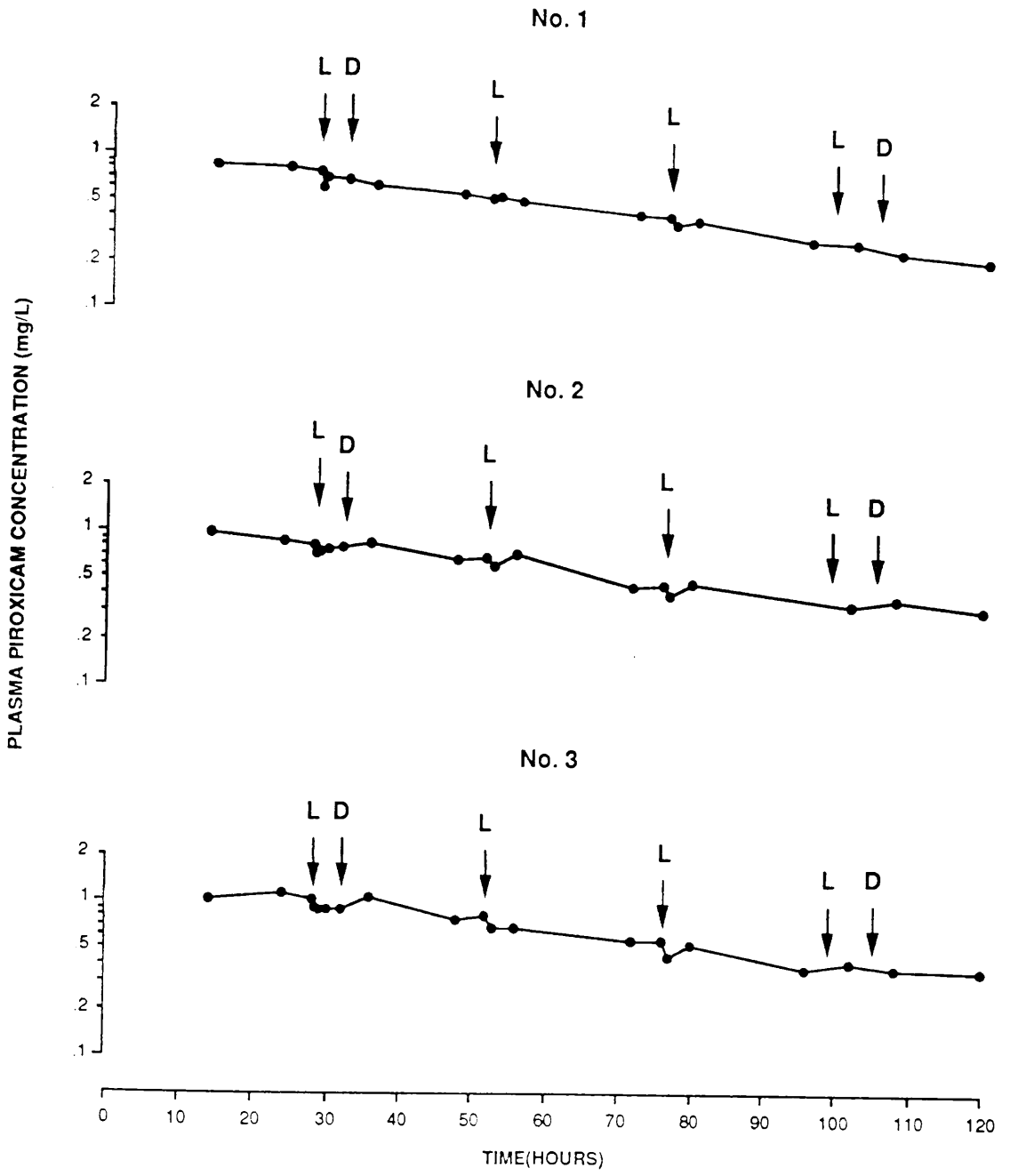


FIGURE 3.6 : PLASMA PIROXICAM CONCENTRATIONS (SUBJECTS 4 - 6) 14 - 120 HOURS

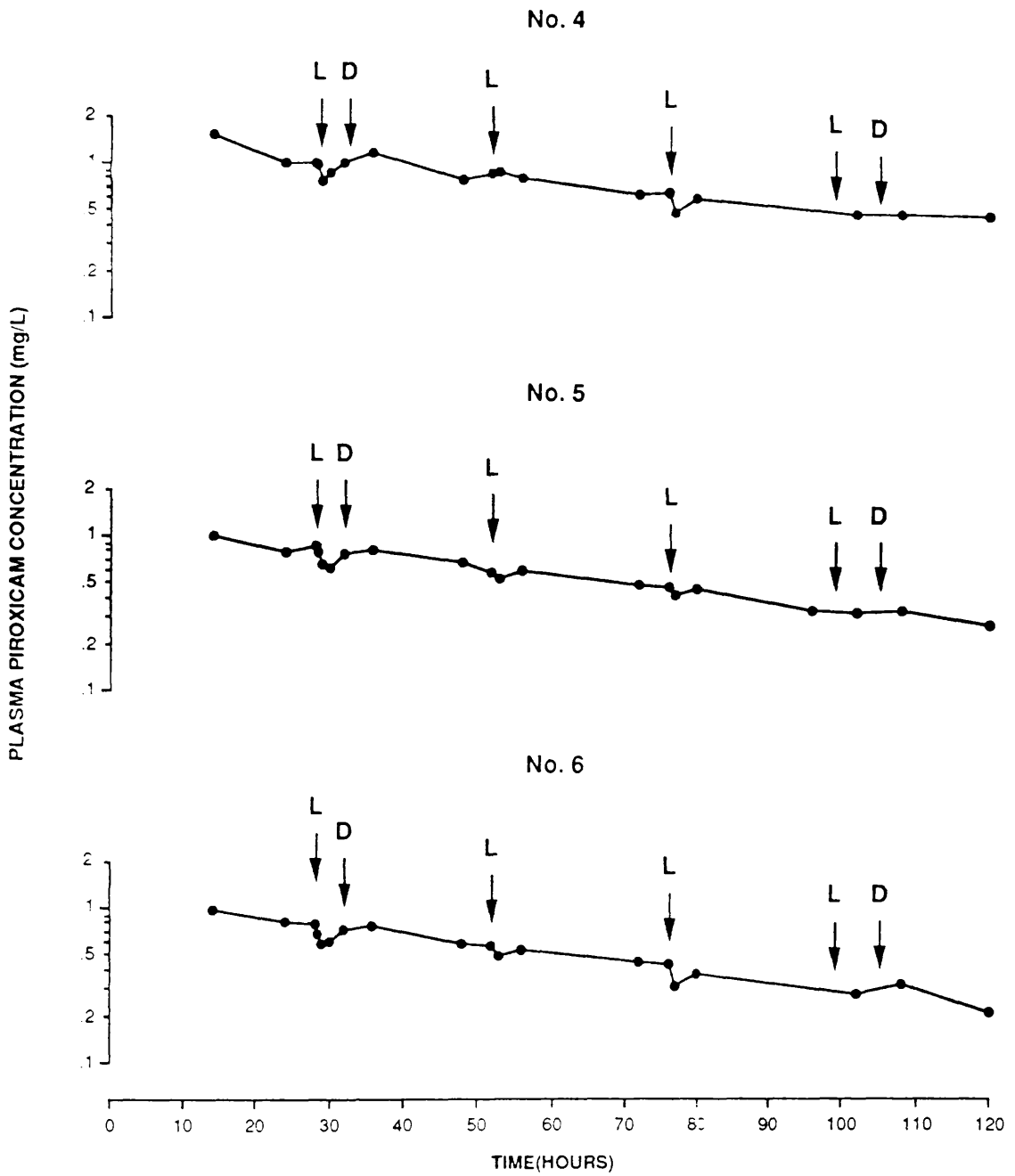
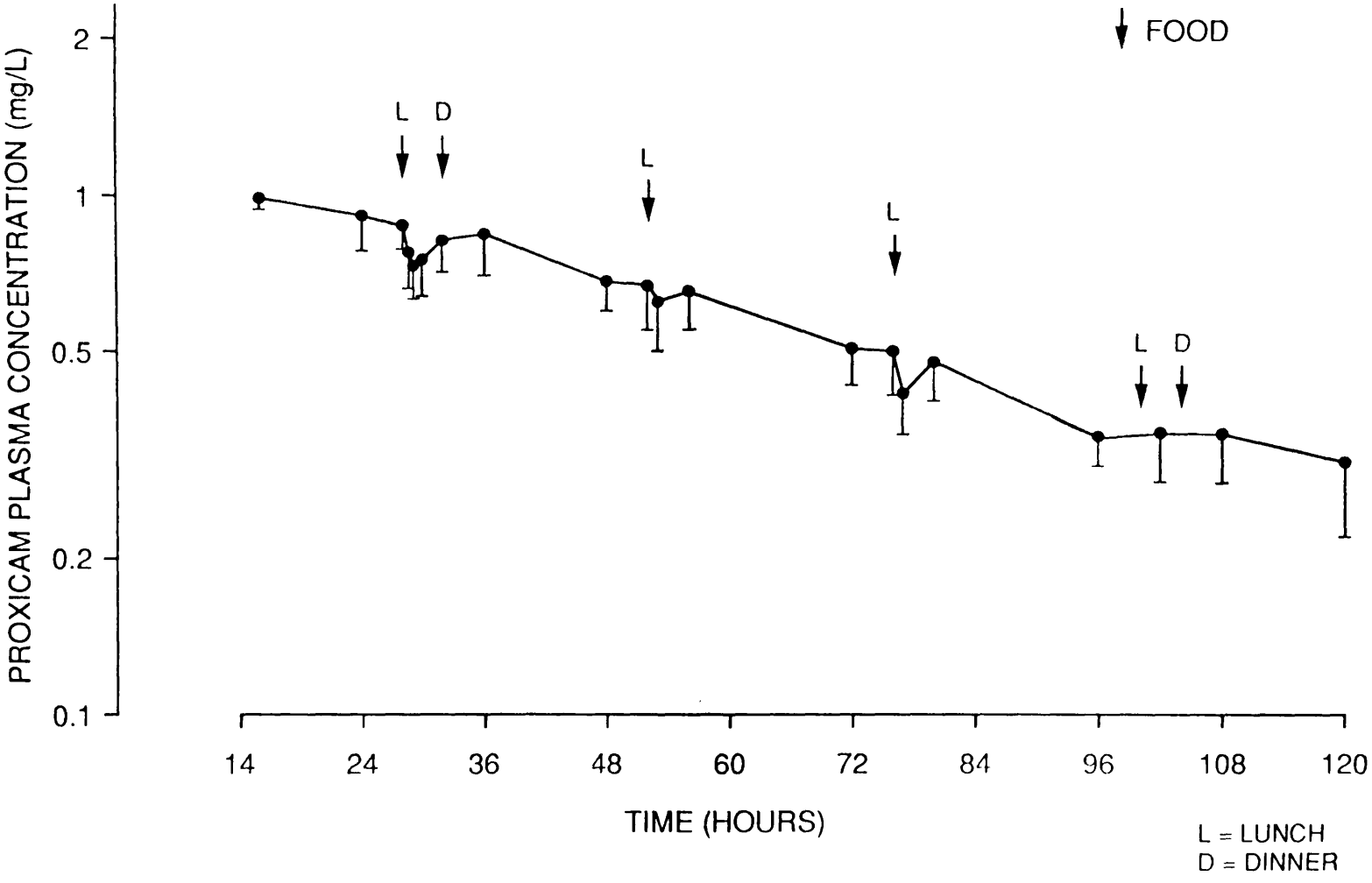


FIGURE 3.7: MEAN ± S.D. OF PLASMA PIROXICAM CONCENTRATIONS IN ALL SUBJECTS FROM 14 - 120 hours



3.2.5 DISCUSSION

The results of this study are consistent with previous observations which highlighted perturbations within the first 24 hours of piroxicam administration. Extensive sampling was continued beyond this interval and showed the presence of perturbations throughout the whole elimination phase of this drug. The perturbations, however, showed a sharp decrease followed by a slower return and the term "multiple peaks" is, therefore, inappropriate. The timing and content of food was controlled, and this, together with an extensive sampling schedule, made this study design unique in relation to all previous published work with this drug. The lack of secondary peaks after food consumption, therefore, adds little support to the view that this drug enters the bile and undergoes enterohepatic circulation to a significant degree, although unequivocal statements cannot be made until direct quantitation of biliary piroxicam concentrations are made. It is more likely that some physiological event brought about by the consumption of food (or some constituent of food) causes an alteration in the disposition of piroxicam.

Although this work has characterised the appearance of these perturbations, no information is available regarding the underlying mechanism of this phenomenon, except that the perturbations accompany certain meals. Without prior information as to the nature of the basic mechanism(s) it would be impossible to "model" these events. Consideration must therefore be made of likely events (which accompany food consumption) that could mimic the results obtained. These events may include biliary recycling, protein binding alterations, modified clearance, or volume of distribution alterations. These will be explored further in the following sections.

3.3 THE DISPOSITION OF PIROXICAM AND ITS METABOLITES IN THE PLASMA, URINE, AND BILE IN MAN

3.3.1 INTRODUCTION

As was shown in Chapter 1, there is little direct experimental evidence of the extent, if any, of piroxicam's (or its metabolite's) clearance via the biliary route in man. The only reported study (in a single healthy volunteer) failed to detect either piroxicam or 5-hydroxypiroxicam in aspirated bile 15 hours after ingestion of a single 20mg dose of piroxicam (Verbeeck et al 1986). This lack of information (which extends to the biliary excretion of all drugs in man) is partly due to the relative inaccessibility of the biliary tract and the limitations of the methods used to collect bile samples. Furthermore, the development of suitable assay techniques with which to quantitate drug concentrations is hampered by the relatively high concentration (up to 1000 times plasma concentration) achieved in the bile by compounds such as bile salts and bilirubin. This may have prevented Verbeeck from attempting to quantify piroxicam glucuronides as enzymic degradation of all glucuronides present in the bile will result in massive interfering peaks unless a suitable assay technique is employed (see Chapter 2).

The need, therefore, existed for a study to be performed, to determine the extent of the biliary excretion of piroxicam, 5-hydroxypiroxicam, and 5-hydroxypiroxicam glucuronide in man, after development of a suitable assay, with careful consideration given to the methods employed in collecting the samples.

Initially, T-tube sampling was not considered a suitable method for bile collection as the subject would probably have disturbed hepatic function, and altered piroxicam metabolism. Moreover, complete bile collection is impossible with a T-tube (Rollins & Klaassen 1979). For these reasons, it was intended to study subjects who had a naso-biliary drain inserted after endoscopic retrograde cholangeopancreotography (ERCP) for the relief of biliary obstruction. Such patients (medical, not surgical) would not

suffer post-operative trauma. Furthermore, liver function would quickly improve after insertion of the drain to relieve the obstruction. Also the technique would allow total collection of biliary output during the study period. Initially, recruitment of four subjects was considered feasible (during the time available) but logistical problems reduced this number to only one. We therefore reverted to subjects with T-tubes inserted after surgery, with the clear understanding that the quality of information obtained from such subjects would not be as conclusive. Unfortunately even these subjects proved to be extremely elusive. Insertion of T-tubes only occurred if investigation of the biliary tree was performed during the operation. Only two T-tubes were inserted during an 8 month period and both patients were unsuitable for inclusion in this study. It can therefore be concluded that studies investigating biliary excretion are not only hampered by the inherent inaccessibility and collection problems posed in man, but perhaps more significantly, the lack of suitable subjects in which these investigations can be readily performed. This is true of all drugs, not only piroxicam, and until such studies are performed much of the data regarding the biliary excretion of drugs in man will remain largely speculative (Rollins & Klaassen 1979).

3.3.2 SUBJECTS

As outlined above, the initial plan was to study four male or female patients with obstructive jaundice who required naso-biliary drains to be inserted at ERCP. Patients were excluded if:

a) they had a current or past medical history of peptic ulceration, confirmed by endoscopic examination during insertion of the drain

b) there was a history of allergy or adverse reaction to aspirin or NANSAIDs

c) blood coagulation was abnormal

d) they were of child-bearing age and pregnant

e) any other significant past medical history was present.

ENZYME	VALUE	NORMAL RANGE
Alk. Phos.	154	40-115 IU/I
Gamma-G.T.	54	0-40 IU/I
Bilirubin	20	5-20 µmol/L
AST	30	13-42 IU/I
ALT	59	11-55 IU/I
L.D.	219	1-300 IU/I

The patient eventually studied was a 72 year old female with common bile duct stones, a history of obstructive jaundice and pyrexia on admission. The study was performed 5 days after insertion of the drain at a time when her liver function tests were practically normal (see opposite).

3.3.3 METHODS

As a single dose of piroxicam, 20mg would produce levels of 5-hydroxypiroxicam in plasma approaching the assay detection limits, the patient received 30mg piroxicam daily for 2 days prior to the study so that detectable levels of metabolites might be obtained. After an overnight fast, 20mg piroxicam was given at time zero (Figure 3.8). Blood samples (5ml) were obtained at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 24 hours. Total urine output was collected for the 24 hour period in addition to total bile output which was collected in aliquots from -2 to 0, 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, and 12 to 24 hours.

Plasma samples were assayed for both piroxicam and 5-hydroxypiroxicam. Urine and bile were assayed for piroxicam, 5-hydroxypiroxicam, and 5-hydroxypiroxicam glucuronide.

3.3.4 DATA ANALYSIS

Linear and logarithmic trapezoidal AUCs were calculated for both piroxicam and 5-hydroxypiroxicam in the plasma, and for piroxicam and 5-hydroxypiroxicam (before and after glucuronidase incubation) in bile and urine.

3.3.5 RESULTS

Table 3.3 shows the concentrations achieved and the amount of piroxicam and 5-hydroxypiroxicam excreted in the bile for each time interval. Figure 3.9 illustrates this diagrammatically. It can be seen that very little piroxicam enters the bile (0.036mg) and biliary excretion therefore cannot be considered an important route of elimination for piroxicam in this patient. However, 5-hydroxypiroxicam is present in twice the piroxicam concentration (0.073mg), in marked contrast to the situation in plasma,

FIGURE 3.8 : BILE STUDY SAMPLING SCHEDULE

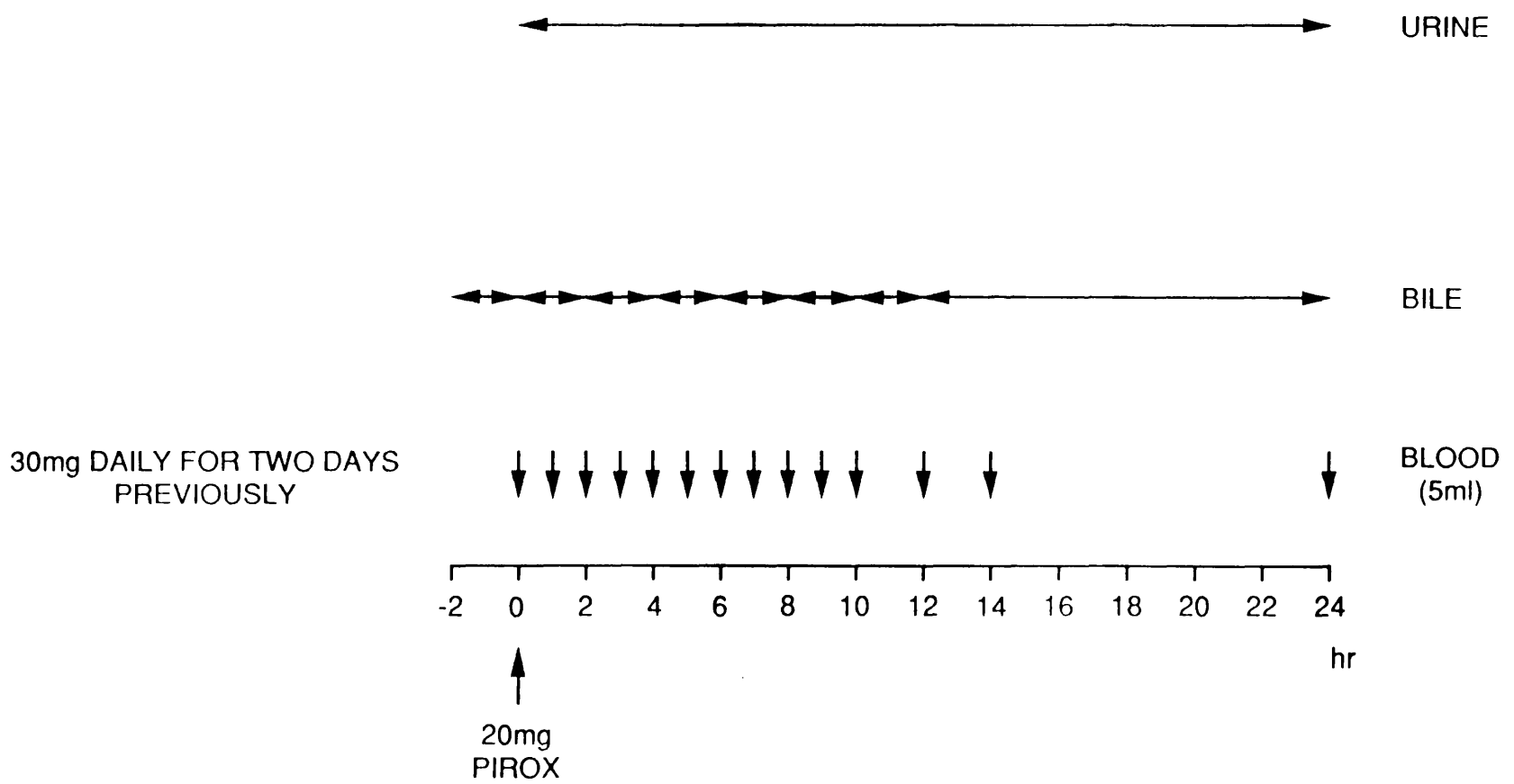
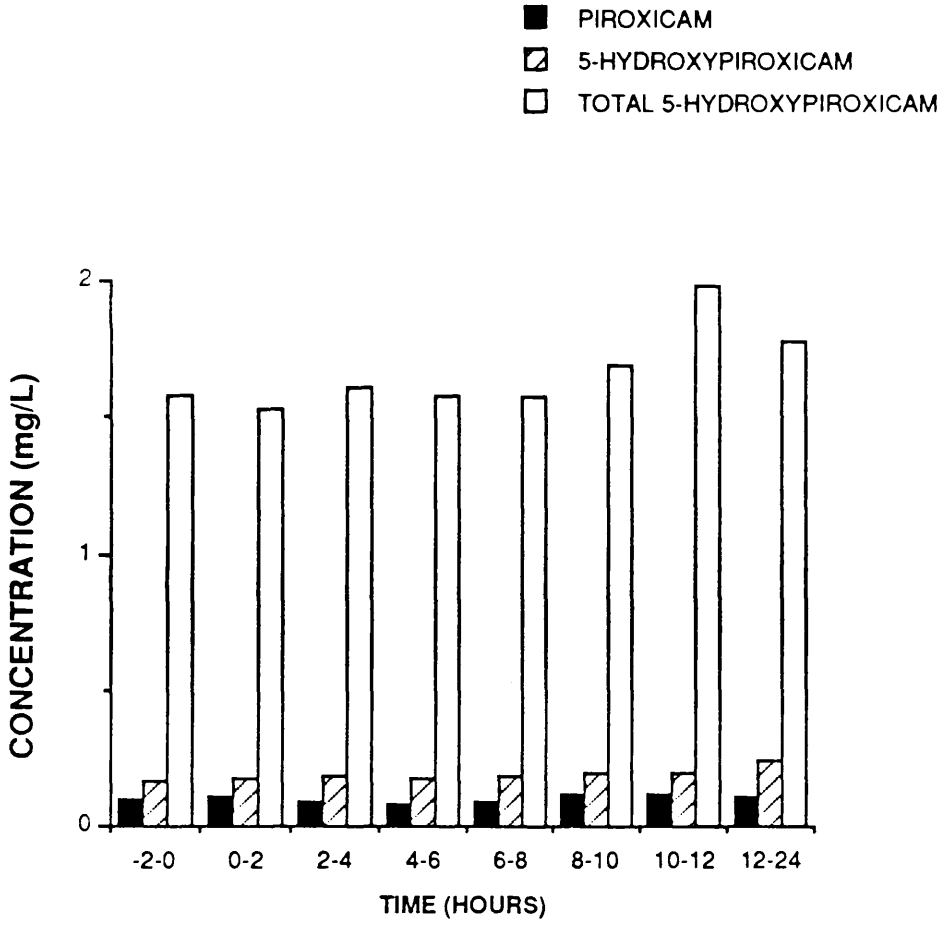


TABLE 3.3

PIROXICAM AND 5-HYDROXYPIROXICAM CONCENTRATIONS IN BILE

TIME INTERVAL (h)	VOLUME BILE (L)	CONCENTRATION PIROXICAM (mg/l)	AMOUNT PIROXICAM (mg)	CONCENTRATION 5-HYDROXY PIROXICAM (mg/l)	AMOUNT 5-HYDROXY PIROXICAM (mg)	CONCENTRATION (TOTAL) 5-HYDROXY PIROXICAM (mg/l)	AMOUNT (TOTAL) 5-HYDROXY PIROXICAM (mg)
Pre	0.025	0.097	0.0024	0.169	0.0042	1.577	0.0344
0-2	0.035	0.106	0.0037	0.178	0.0062	1.530	0.0536
2-4	0.040	0.086	0.0034	0.186	0.0074	1.609	0.0644
4-6	0.037	0.079	0.0029	0.175	0.0065	1.577	0.0584
6-8	0.034	0.086	0.0029	0.186	0.0063	1.577	0.0536
8-10	0.030	0.124	0.0037	0.199	0.0060	1.702	0.0511
10-12	0.029	0.119	0.0034	0.195	0.0057	1.997	0.0579
12-24	0.150	0.106	0.0159	0.235	0.0353	1.789	0.2683
TOTAL	0.380		0.0361		0.0734	0.6073	

FIGURE 3.9 : BILIARY CONCENTRATIONS OF PIROXICAM AND 5-HYDROXYPIROXICAM



where piroxicam concentrations are approximately 10 times the 5-hydroxypiroxicam concentrations. Furthermore, it would appear from this subject that 5-hydroxypiroxicam (as the glucuronide) was excreted into the bile in significant amounts (0.607mg). Piroxicam, in the form of glucuronides, was not detected in the bile.

Table 3.4 lists the AUCs obtained for piroxicam, 5-hydroxypiroxicam and the total 5-hydroxypiroxicam (after glucuronidase incubation) in the plasma, urine and bile in this subject. The extensive metabolism of piroxicam is shown by the very small levels of the parent drug in either the urine or the bile. When 5-hydroxypiroxicam is considered, it is present in both the urine and plasma to a similar extent, with bile only contributing 15% of the total AUC value. However, when glucuronidated metabolite is considered, it can be seen that, similar to the situation found in the urine, the majority of 5-hydroxypiroxicam is glucuronidated. In the urine, approximately 20% of the total metabolite is unconjugated. In the bile this only accounts for 11%, indicating that the bile must be considered as a significant route of elimination for the metabolite in its conjugated form.

3.3.6 DISCUSSION

The Verbeeck study discussed earlier (Verbeeck et al 1986) failed to detect either parent or metabolite in the bile, possibly because only 20mg of piroxicam were given to the subject. Glucuronides were not measured in the bile.

In the patient described above, piroxicam, as parent drug, did not enter the bile to any significant extent, confirming that this drug undergoes extensive metabolism (Chapter 1). However, the glucuronidated metabolite was present in large quantities, and it is known that glucuronidated drugs present in the bile are likely to be subjected to enterohepatic circulation (Rollins & Klaassen 1979). The potential, therefore, exists for the metabolite, and not the parent drug, to undergo enterohepatic circulation. This will, however, have little consequence because the metabolite has only a fraction of the parent drugs anti-inflammatory activity (Lombardino 1981). Only if reverse

TABLE 3.4

PIROXICAM AND 5-HYDROXYPIROXICAM AUC's IN PLASMA, URINE AND BILE

	AUC_0^t (mg/L/h)		
	PIROXICAM	5-HYDROXY PIROXICAM	5-HYDROXY PIROXICAM (TOTAL)
Plasma	113.76	12.97	-
Urine	1.39	16.56	81.36
Bile	2.52	4.77	42.28
TOTAL	117.67	34.30	123.64

metabolism of the metabolite occurs (to reform parent drug) could this become important.

It must be stressed that this study has shown the extent to which parent drug and metabolite enter the bile. However, as a result of collecting the total biliary output during the study period, any enterohepatic circulation was interrupted and no comment can be made about the eventual fate of the metabolite. It does appear, however, that the perturbations found in piroxicam plasma concentration/time curves are not a consequence of enterohepatic circulation. Other mechanisms, therefore must be sought.

3.4 FOOD CONSUMPTION AND PLASMA PROTEIN BINDING ALTERATION

3.4.1 INTRODUCTION

As discussed in Chapter 1, more that 99% of piroxicam in the plasma is bound to albumin. Any alteration in the unbound fraction, therefore, may have important implications for the disposition of this drug. It is the unbound fraction that is free to distribute into the extracellular space, which contains 60% of total body albumin (Rowland & Tozer 1989) and it is the unbound fraction of piroxicam that is available for hepatic metabolism. Any alteration in the plasma protein binding of this drug (as a result of food consumption) should therefore be investigated, bearing in mind that there may be a number of confounding factors. For example, the binding characteristics of albumin may change with age. Plasma albumin concentration decreases with increasing age (Veering et al 1990) and patients with rheumatoid arthritis tend to have decreased plasma albumin concentrations (Wanwimolruk et al 1983). Examination of food related events must therefore include patients, across a wide age range.

3.4.2 SUBJECTS

Two healthy volunteers who took part in the original single dose study were available to return for this investigation (no.'s 4 and 6). Also, two rheumatoid arthritis patients who took part in the steady state interaction study to be described in Chapter 5 (no.'s 13 and 14) agreed to participate in this study. Table 3.5 lists their demographic details. In the steady state study, the consumption of a standard lunch resulted in a similar perturbation of plasma piroxicam (and 5-hydroxypiroxicam) concentrations to that observed in the single dose volunteer study.

3.4.3. METHODS

The methods used differed between patients and volunteers. As the volunteers only received single doses of piroxicam, detection of unbound plasma concentrations was impossible as a result of the levels obtained being below the assay's limit of

TABLE 3.5

PROTEIN BINDING STUDY SUBJECT DEMOGRAPHIC DETAILS

SUBJECT		M/F	AGE (Years)	ALBUMIN (g/l)
Volunteer	NO.4	M	35	43
Volunteer	NO.6	M	26	51
Patient	NO.13	F	71	38
Patient	NO.14	F	57	39

detection. If the subject was at steady state, however, detection of unbound concentration is possible. In order to avoid the volunteers taking large amounts of piroxicam to achieve steady state concentrations each was given the standard lunch, as before, and the samples obtained were subsequently spiked prior to analysis. The volunteers therefore did not receive any piroxicam.

Venous blood samples (5ml) were obtained in each volunteer at the following times after consumption of the standard lunch (Table 3.2) -1/2, 0, 1/4, 1/2, 3/4, 1, 1 1/4, 1 1/2, 1 3/4, 2, 2 1/2, 3, 3 1/2, and 4 hours and frozen until analysis. On the day of analysis, 0.9ml plasma was spiked with 0.1ml piroxicam standard solution (0.1mg/ml) which was equivalent to a final plasma concentration in each aliquot of 11.11mg/l. The spiked samples were mixed thoroughly and centrifuged before determination of unbound concentration by ultrafiltration, as described in Chapter 2.

In the patients, who were already at steady state, spiking was unnecessary. Furthermore any alterations in the binding of the metabolite, 5-hydroxypiroxicam, could be investigated in these subjects. As before, patients were fasted before receiving the standard lunch. Venous blood samples (5ml) were obtained at -1, 0, 1/4, 1/2, 3/4, 1, 1 1/2, 2, 2 1/2, 3 and 4 hours with 1ml plasma samples being subjected to ultrafiltration as described earlier.

Plasma piroxicam and 5-hydroxypiroxicam concentrations were determined by HPLC as described in Chapter 2.

3.4.4 DATA ANALYSIS

In the patients, the unbound concentration was divided by the total concentration to obtain the percentage unbound concentration of piroxicam and 5-hydroxypiroxicam for the interval studied. In the volunteers, only the unbound piroxicam concentration was reported.

3.4.5 RESULTS

Figure 3.10 shows the post-prandial increase in unbound piroxicam concentration

obtained in the volunteers. Table 3.6 lists the results. There are some missing values as a result of inadequate collection of ultrafiltrate (less that 0.25ml). In both volunteers there was an increased unbound piroxicam concentration to approximately 260% within 1 to 2 hours which then declined to baseline value by about 4 hours. Figure 3.11 shows the results obtained in the patients and Tables 3.7 and 3.8 list the data. It can be seen that the changes in the unbound concentration of piroxicam were similar in extent to those obtained in the volunteers, however, they did not appear to return to baseline values within the timescale studied. It is also evident that there is an increased unbound concentration of 5-hydroxypiroxicam which also does not return to baseline values within the 4 hour interval studied. This may reflect an age difference in the binding characteristics of NANSAIDs to albumin (or a result of the different methodologies employed in the derivation of the unbound concentrations).

When the patients are considered, it can be seen that not only do the unbound concentrations of both piroxicam and 5-hydroxypiroxicam rise, but their total plasma concentration falls. Therefore, when the percentage unbound is calculated, there is a relatively greater increase (between 300 and 400%) obtained in comparison to the unbound concentration alone. This was not a unique result. Figure 3.12 shows the results obtained in the interaction study (Chapter 5) where subjects received the standard lunch on three separate occasions. Although the concentrations shown are total plasma values, it can be seen that they are in very close agreement with the results presented here.

3.4.6 DISCUSSION

The consumption of the standard lunch in all subjects caused an alteration of unbound piroxicam in this study, indicating that a displacement of drug from its binding sites occurs. This drug is bound only to albumin, and the differences in plasma albumin concentrations (patients 38, 39g/l; volunteers 43, 51g/l) may have a role in both the extent and the duration of the displacement shown. In both the single dose

FIGURE : 3.10 UNBOUND PIROXICAM CONCENTRATIONS IN VOLUNTEERS

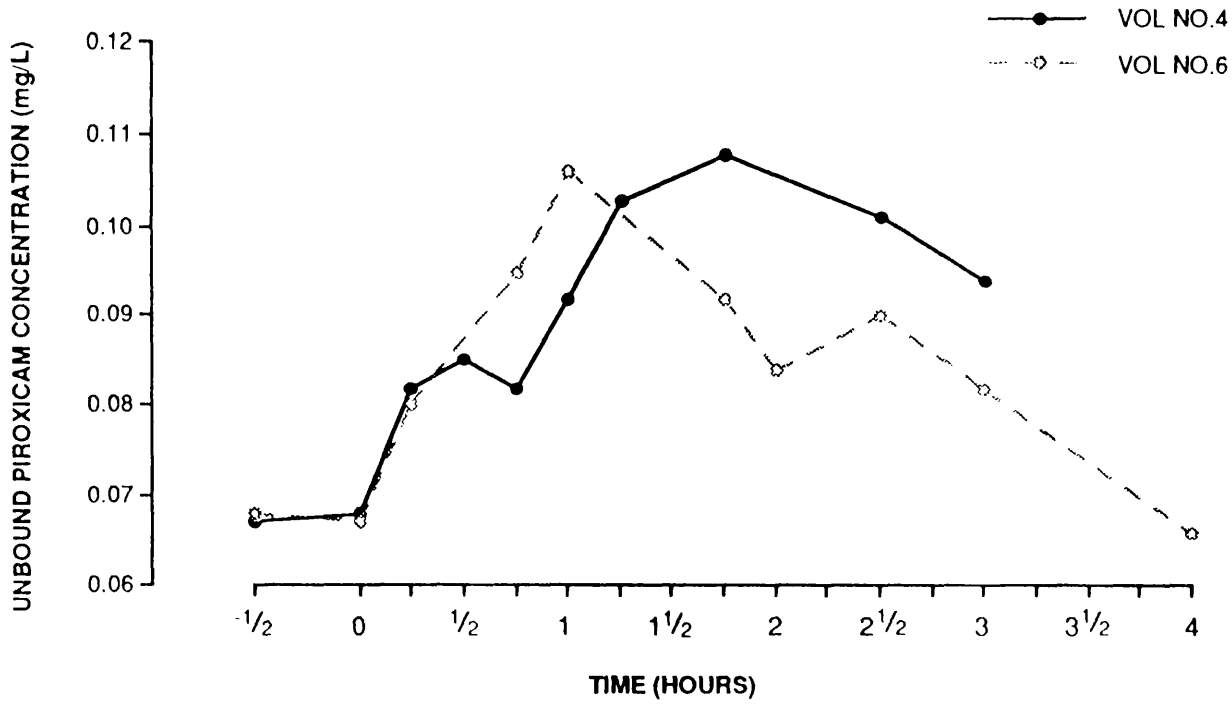


TABLE 3.6

VOLUNTEER PIROXICAM UNBOUND CONCENTRATIONS

TIME (h)	Unbound Piroxicam Concentrations (mg/L) NO. 4	NO. 6
-1/2	0.067	0.068
0	0.068	0.067
1/4	0.082	0.080
1/2	0.085	-
3/4	0.082	0.095
1	0.092	0.106
1 1/4	0.103	-
1 3/4	0.108	0.092
2	-	0.084
2 1/2	0.101	0.090
3	0.094	0.082
4	-	0.066

FIGURE: 3.11 % UNBOUND PIROXICAM AND 5-HYDROXYPIROXICAM IN PATIENTS

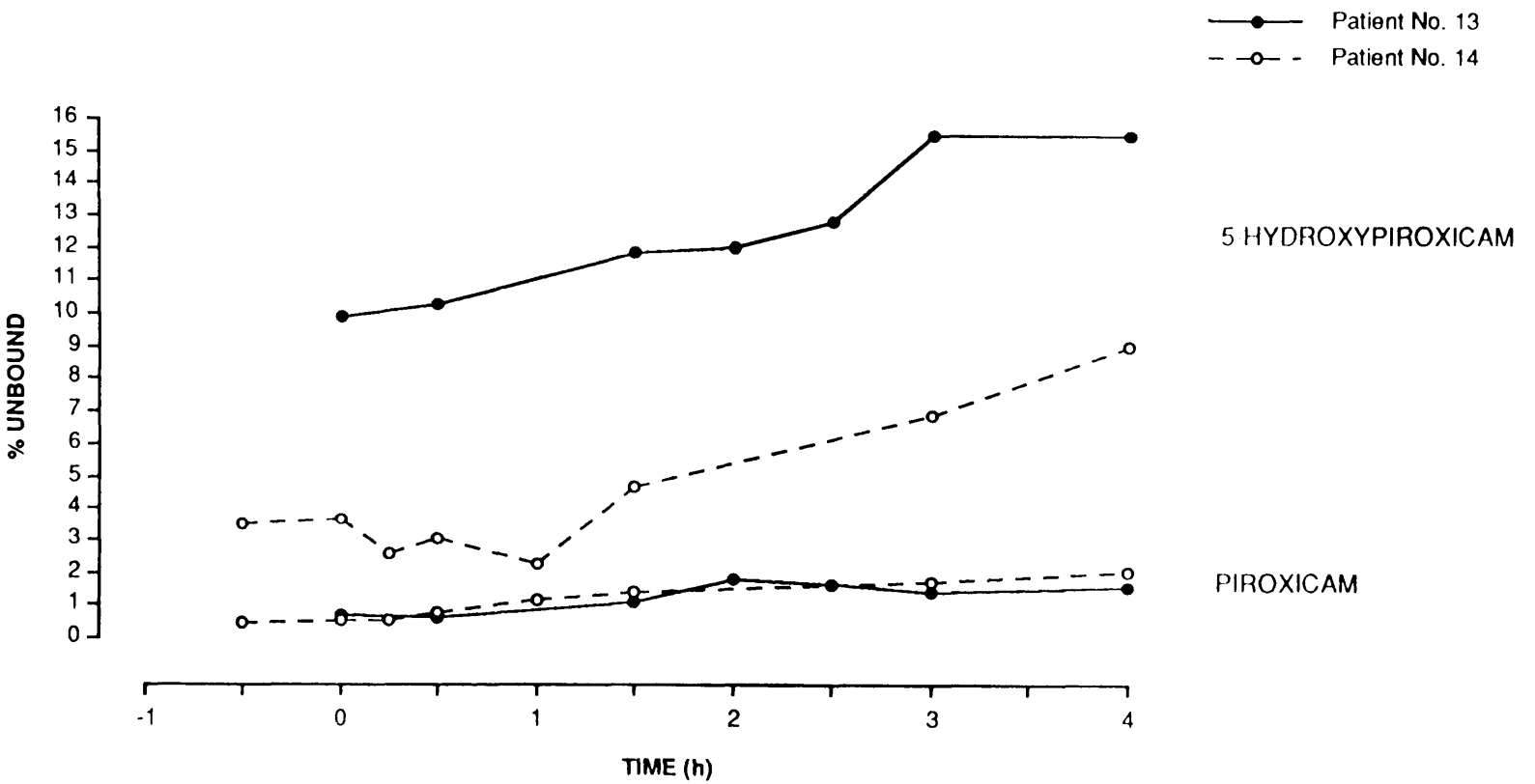


TABLE 3.7

PATIENT NO.13 BOUND AND UNBOUND PIROXICAM AND 5-HYDROXYPIROXICAM CONCENTRATIONS

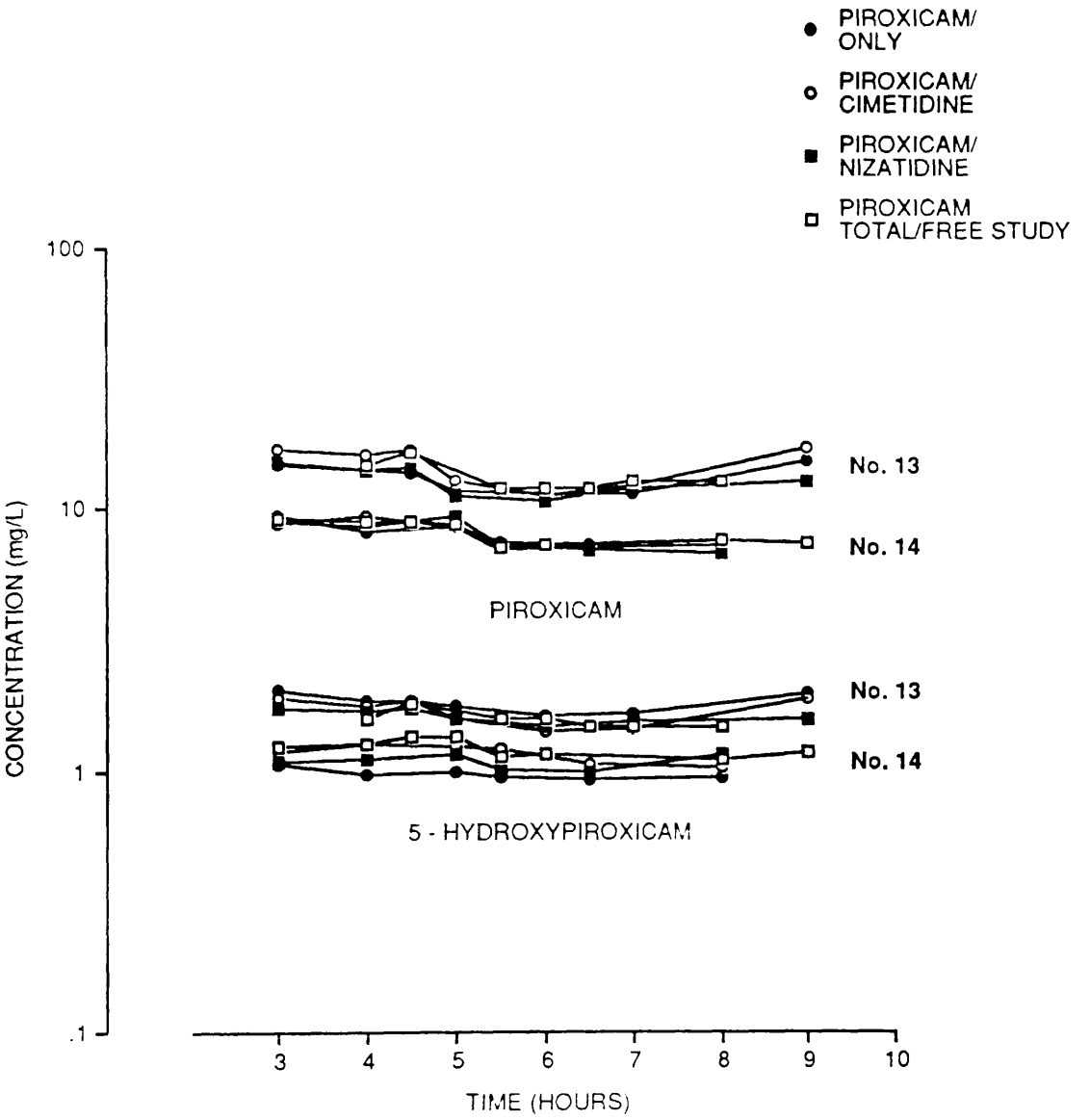
TIME	PIROXICAM (mg/L)			5-HYDROXYPIROXICAM (mg/L)		
(h)	(Total)	(Free)	(%)	(Total)	(Free)	(%)
0	14.85	0.11	0.74	1.61	0.16	9.93
1/2	16.54	0.11	0.66	1.84	0.19	10.33
1 1/2	12.14	0.13	1.07	1.60	0.19	11.88
2	12.08	0.22	1.82	1.58	0.19	12.02
2 1/2	12.16	0.20	1.64	1.48	0.19	12.84
3	12.93	0.18	1.39	1.48	0.23	15.54
4	12.83	0.20	1.56	1.48	0.23	15.54

TABLE 3.8

PATIENT NO. 14 BOUND AND UNBOUND PIROXICAM AND 5-HYDROXYPIROXICAM CONCENTRATIONS

TIME	PIROXICAM (mg/L)			5-HYDROXYPIROXICAM (mg/L)		
(h)	(Total)	(Free)	(%)	(Total)	(Free)	(%)
-1	9.18	0.043	0.47	1.23	0.044	3.58
0	8.95	0.049	0.55	1.26	0.047	3.73
1/4	8.99	0.052	0.58	1.35	0.035	2.59
1/2	8.85	0.066	0.75	1.35	0.041	3.04
1	7.19	0.086	1.19	1.13	0.026	2.30
1 1/2	7.31	0.105	1.44	1.15	0.054	4.69
3	7.66	0.134	1.75	1.12	0.078	6.96
4	7.46	0.153	2.05	1.19	0.108	9.08

FIGURE : 3.12 TOTAL PIROXICAM AND 5-HYDROXYPIROXICAM IN PATIENTS ON FOUR OCCASIONS



volunteer study and the steady state interaction study, the perturbations were associated with the consumption of the standard lunch, but not the standard evening meal. The phenomenon may therefore depend critically on the constituents of a meal. The fat content may be important. Fats are hydrolysed to fatty acids (2-monoglycerides and glycerol) which are absorbed only from the small intestine once micelles are formed with bile. These micelles are then carried across the epithelial cells by pinocytosis, the bile salts are reabsorbed via another active transport mechanism. The long chain fatty acids and the 2- monoglycerides are resynthesised to triglycerides and glycerol phosphatides in the epithelial cells of the intestine. Minute globules of fat incorporating cholesterol and phospholipids are coated with protein forming chylomicrons of 0.5μm diameter. These are then transferred into lymph from where they enter the systemic circulation via the thoracic duct. Their half-life is approximately 15 minutes, and they remain in the blood for a few hours after consumption of a fat containing meal. Once in the blood, they can either be stored by tissues or used immediately, after hydrolysis to triglycerides by the enzyme lipoprotein lipase which is produced in the endothelial cells of capillaries. This is a rapid process. If less than 30g total fat is ingested, no alteration in plasma lipids and related substances will be observed. In the above study, only the standard lunch had a fat content above 30g, and after consumption of such an amount, it would be expected that total serum fatty acid levels would reach a maximum 2-4 hours after the meal. Shorter chain fatty acids, however, are not absorbed in this manner. They are more hydrophilic than the longer chain molecules, and absorption is possible from the stomach. They would appear in the portal circulation much more quickly than the fat that is absorbed by the above mechanisms, and the possibility that these short chain fatty acids could result in displacement of drugs from plasma albumin has to be considered.

It has been shown that fatty acids bind to plasma albumin via 2 high affinity binding sites located in the 1st domain, one for carbon chains of less than 10 and

another for carbon chains greater than 10 (Kragh-Hansen 1981). The NANSAIDs binding sites however, are located in the 6^{th} domain, making direct competition for binding sites unlikely. However, increased fatty acid binding can result in conformational changes in the binding sites of other domains together with a physical masking of other binding sites due to the fatty acids' long carbon chain preventing receptor-ligand complexes forming (Kragh-Hansen 1981).

The possibility that fatty acids could disrupt piroxicam binding to albumin was examined by Matsuyuma et al (1987). In this *in vitro* study, human serum albumin binding to various ligands was investigated using equilibrium dialysis and significant alterations of piroxicam binding was shown as a result of increased fatty acid content of human serum albumin. It appears likely that the differential effects of the meals could be due to the different nutritional composition of the meals, with displacement brought about as a result of the higher fat content of the standard lunch.

3.5 FOOD CONSUMPTION AND ALTERATIONS IN PIROXICAM CLEARANCE AND VOLUME OF DISTRIBUTION

3.5.1 CLEARANCE ALTERATIONS : INTRODUCTION

Piroxicam is a low extraction drug, i.e., only unbound drug is available for metabolism. In the protein binding study, there was up to a 400% increase shown in the percentage of unbound drug in the plasma. This implies that more drug is available to be cleared and the consequences of this should be considered further. It is clear, however, that any alterations in clearance as a result of food induced displacement will be transient. The prediction of the overall effects on the clearance of piroxicam was estimated by means of a simulation study.

3.5.2 CLEARANCE ALTERATIONS: SIMULATION STUDY METHODS

Piroxicam exhibits first order absorption and elimination characteristics in man, and one compartment models have been adequately fitted to plasma concentration/time data (Chapter 1, Chapter 4). A simulation programme was written (A.W.Kelman, a copy of which appears in the Appendix) that allowed a step-up in clearance to be introduced at any time during the elimination of a drug with the above pharmacokinetic characteristics. The new clearance value then declined exponentially to its original value at a pre-selected rate. The programme required values for the following parameters: clearance, a value by which clearance is altered (ΔCl), a rate constant for the recovery of clearance values, a volume of distribution, an absorption rate constant, an initial plasma concentration, a dose, and the time after the dose when the clearance was altered. These parameters were calculated or estimated for the four subjects who took part in the protein binding study, and Table 3.9 lists the individual parameters for each subject. For the volunteers, parameter values were obtained after fitting a one compartment, first order absorption, first order elimination model to the individual subject's plasma concentration/time data (Chapter 4). Mean values of k_a and V (corrected for weight) were used to estimate the patient parameters. This was

TABLE 3.9

SUBJECT PHARMACOKINETIC PARAMETERS

SUBJECT	CL (L/h)	V (L)	K_a (h^{-1})	INITIAL CONC. (mg/L)	DOSE (mg)	PRE FOOD F_u	POST FOOD F_u
Volunteer No. 4	0.140	15.2	45	0	20	6.1×10^{-3}	9.7×10^{-3}
Volunteer No. 6	0.240	18.3	3	0	20	6.1×10^{-3}	9.5×10^{-3}
Patient No.13	0.063	14.7	15	13.2	20	7.4×10^{-3}	18.2×10^{-3}
Patient No.14	0.105	15.4	15	8.3	20	4.7×10^{-3}	20.5×10^{-3}

considered justified as little or no differences in piroxicam pharmacokinetic parameters have been shown between patients and volunteers in numerous pharmacokinetic studies (Chapter 1). Patient clearance values (Cl/F) were estimated from individual trapezoidal AUC assessments i.e. D/AUC_0^{24} (Chapter 5).

Clearance values in each subject were altered four hours after the dose had been introduced. In each subject, clearance was increased by 100, 200, 300, and 400% to try to match the results obtained in the protein binding study. Rates at which initial clearance values were resumed were set at 0.1, 0.5, 1.0, and 2.0 h^{-1} to cover the range of values obtained from each individual.

3.5.3 CLEARANCE ALTERATIONS: SIMULATION STUDY RESULTS

Figures 3.13 to 3.19 show the results obtained in this study. In order to allow a comparison between actual and simulated results, the actual decline in plasma piroxicam concentrations (solid line) is superimposed on the upper graph. The dotted line represents the simulated fall in plasma piroxicam concentration assuming there is no alteration in clearance. Also shown on this upper graph is the effect on plasma piroxicam concentrations of increasing the initial clearance value by 100-400%, and then allowing recovery at the various rates detailed in the Figure title. The lower graph illustrates the extent of the clearance alteration and its return to initial values.

When patient no.13 is considered (Figures 3.13 to 3.16) at high recovery rates (1, $2h^{-1}$) there is a negligible change in the simulated plasma concentrations as a result of small alterations in clearance. It is also evident that the profile is flat, a consequence of relatively low clearance values. From the actual profile, it can be seen that there is a rapid decline in plasma concentrations, followed by a slower increase. This rapid decline does not appear to be a result of a change in clearance as the simulated profiles show greatest effect at later time points. Even with the slow rates of recovery, the massive (400%) clearance changes can only account for a decrease in plasma concentration of about 1mg/l whereas the actual levels had decreased by more than

FIGURE 3.13 EFFECT OF CHANGING CLEARANCE BY 100 - 400 % AT A RATE OF RECOVERY OF 2.0h^{-1} IN PATIENT NO.13

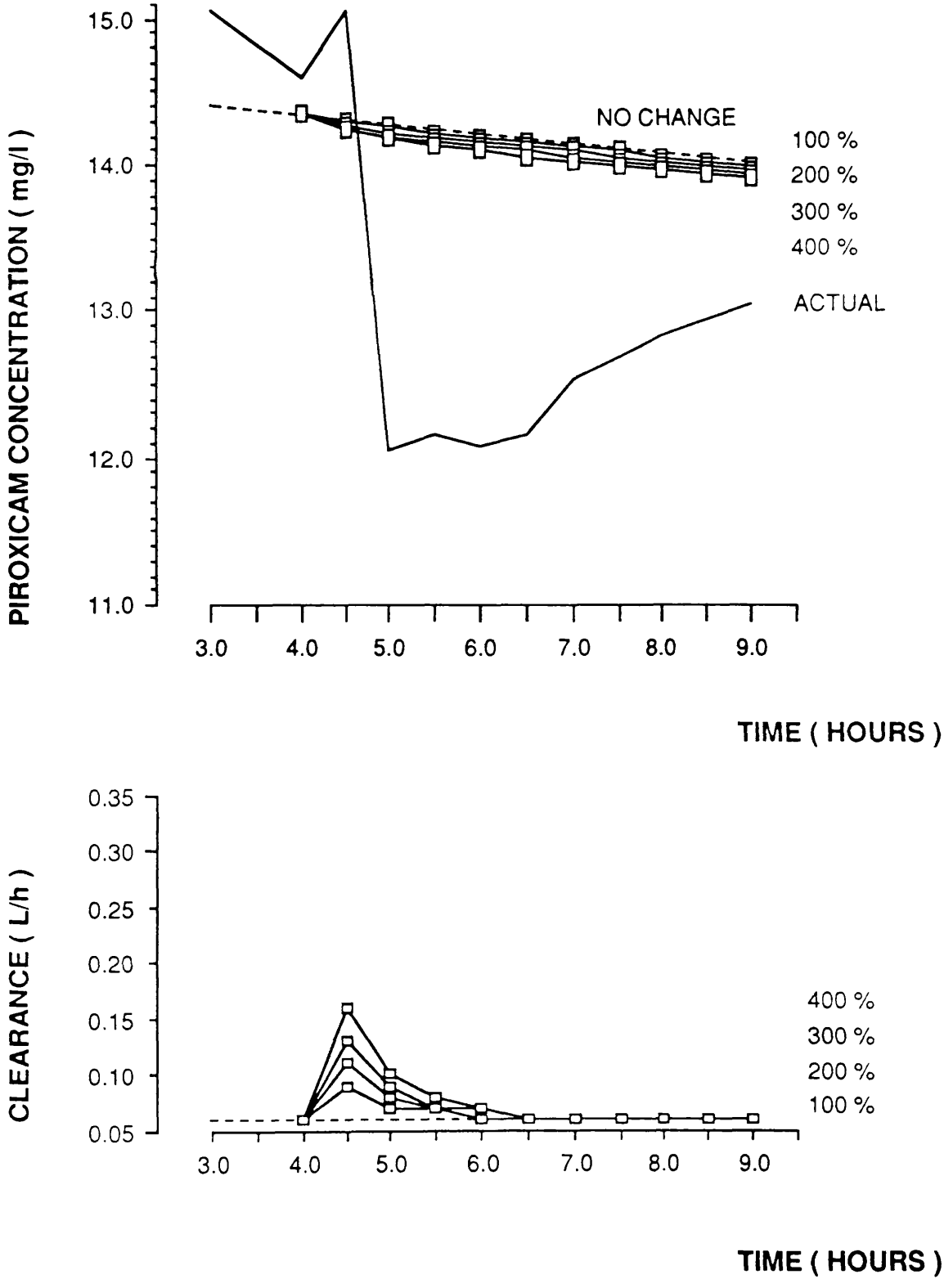


FIGURE 3.14 EFFECT OF CHANGING CLEARANCE BY 100 - 400 % AT A RATE OF RECOVERY OF 1.0h^{-1} IN PATIENT NO.13

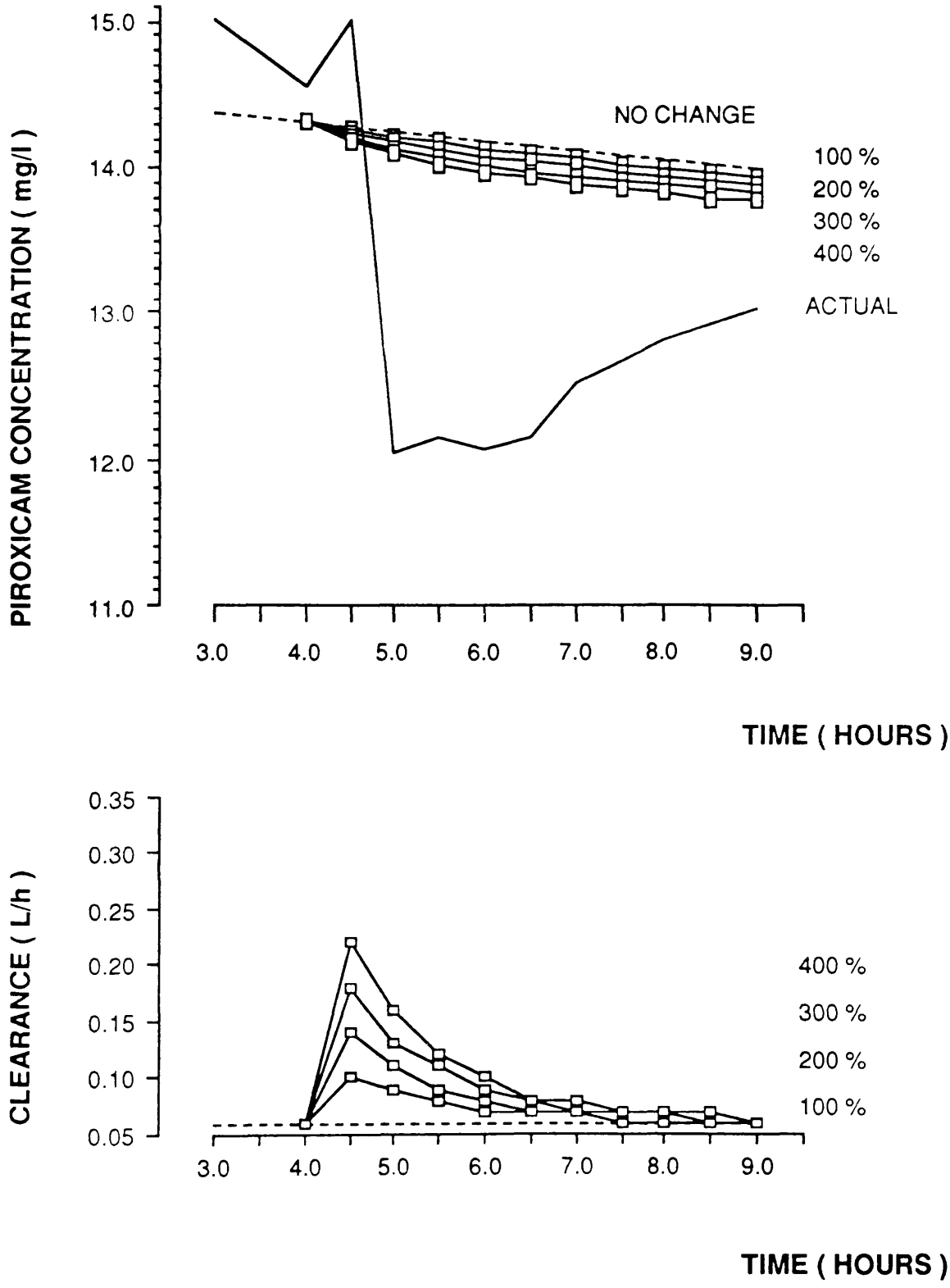


FIGURE 3.15 EFFECT OF CHANGING CLEARANCE BY 100 - 400 % AT A RATE OF RECOVERY OF 0.5h $^{-1}$ IN PATIENT NO.13

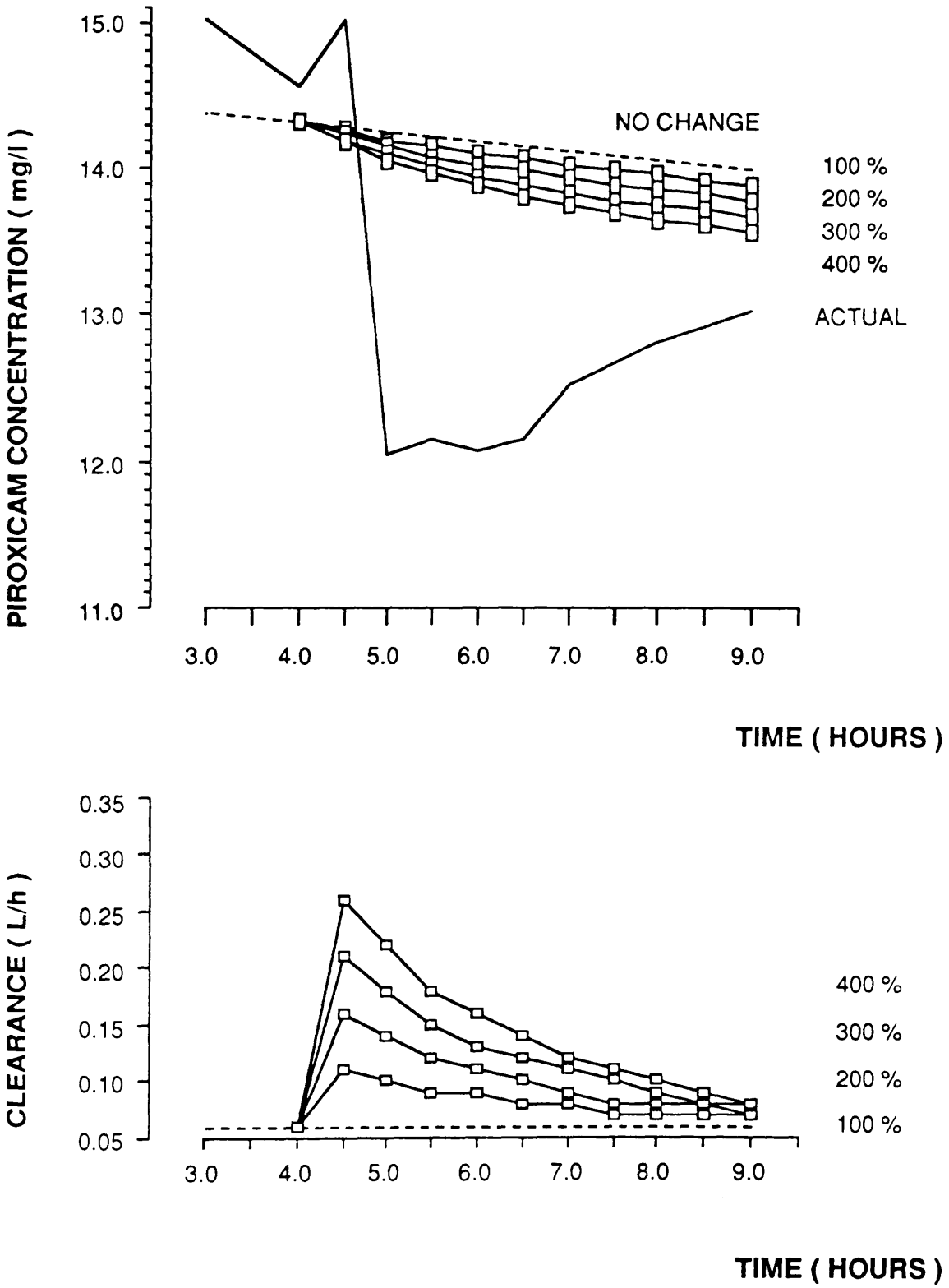


FIGURE 3.16 EFFECT OF CHANGING CLEARANCE BY 100 - 400 % AT A RATE OF RECOVERY OF $0.1h^{-1}$ IN PATIENT NO.13

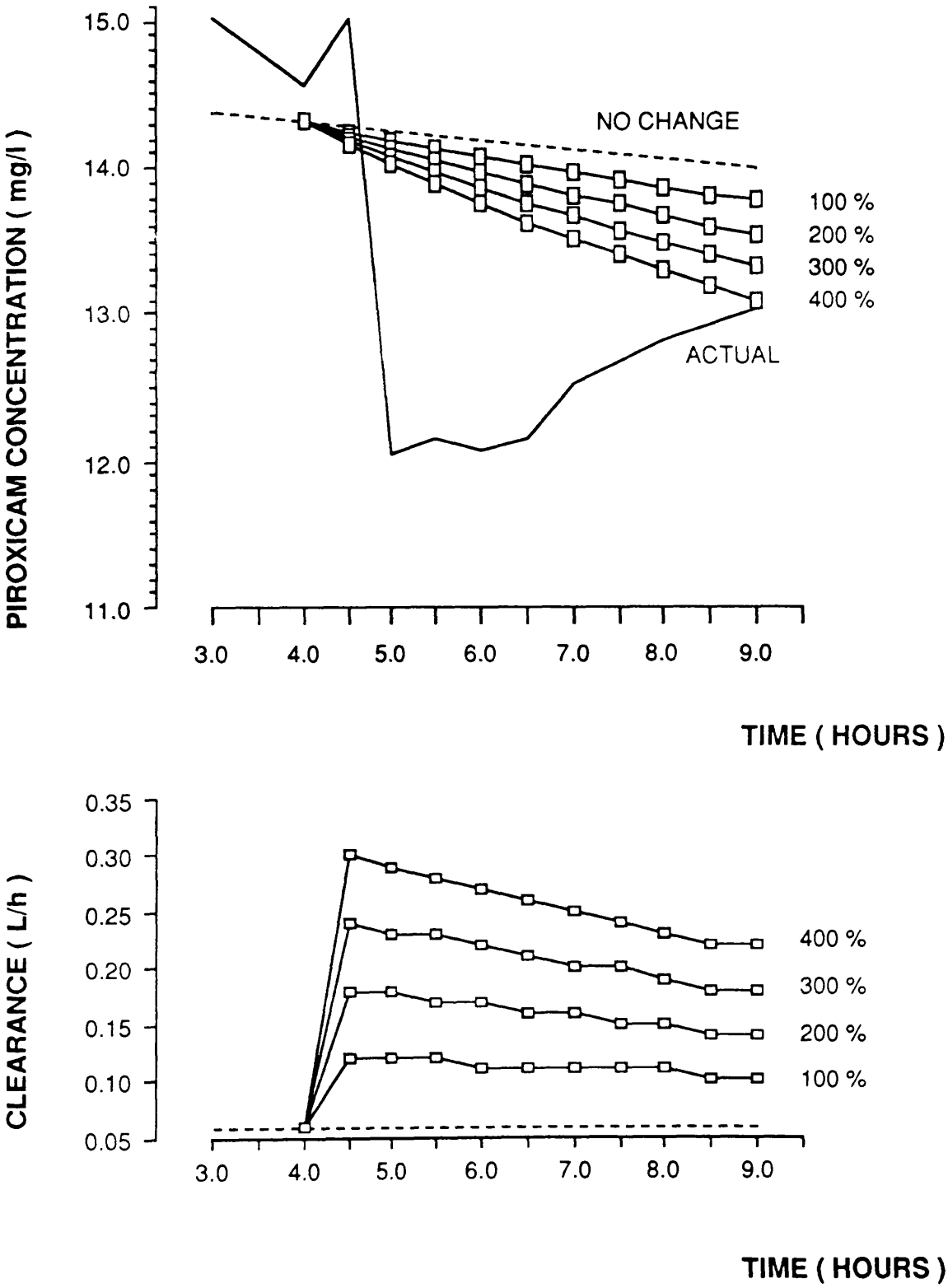


FIGURE 3.17 EFFECT OF CHANGING CLEARANCE BY 100 - 400 % AT A RATE OF RECOVERY OF 0.1h^{-1} IN PATIENT NO.14

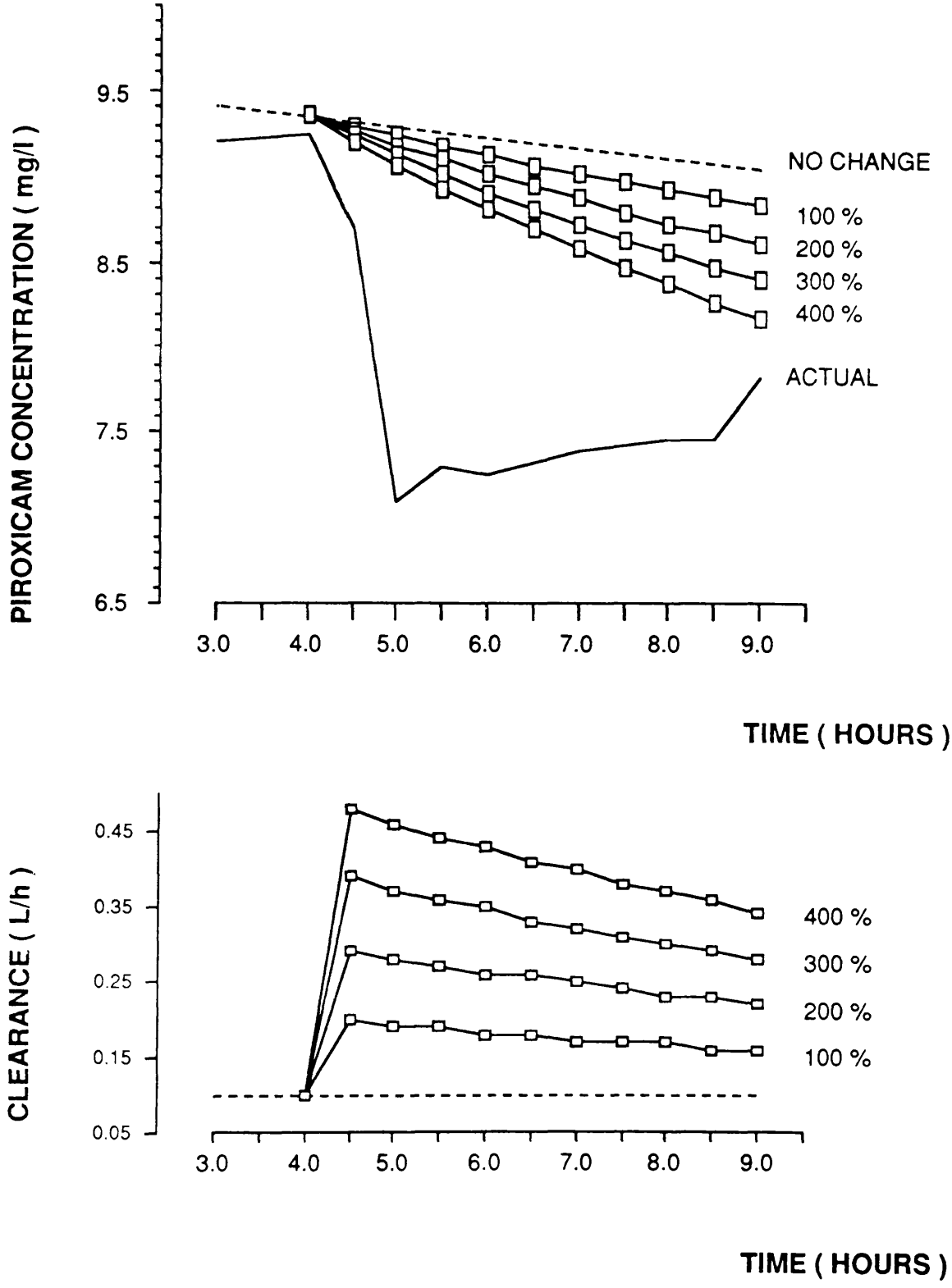


FIGURE 3.18 EFFECT OF CHANGING CLEARANCE BY 100 - 400 % AT A RATE OF RECOVERY OF $0.1h^{-1}$ IN VOLUNTEER NO.4

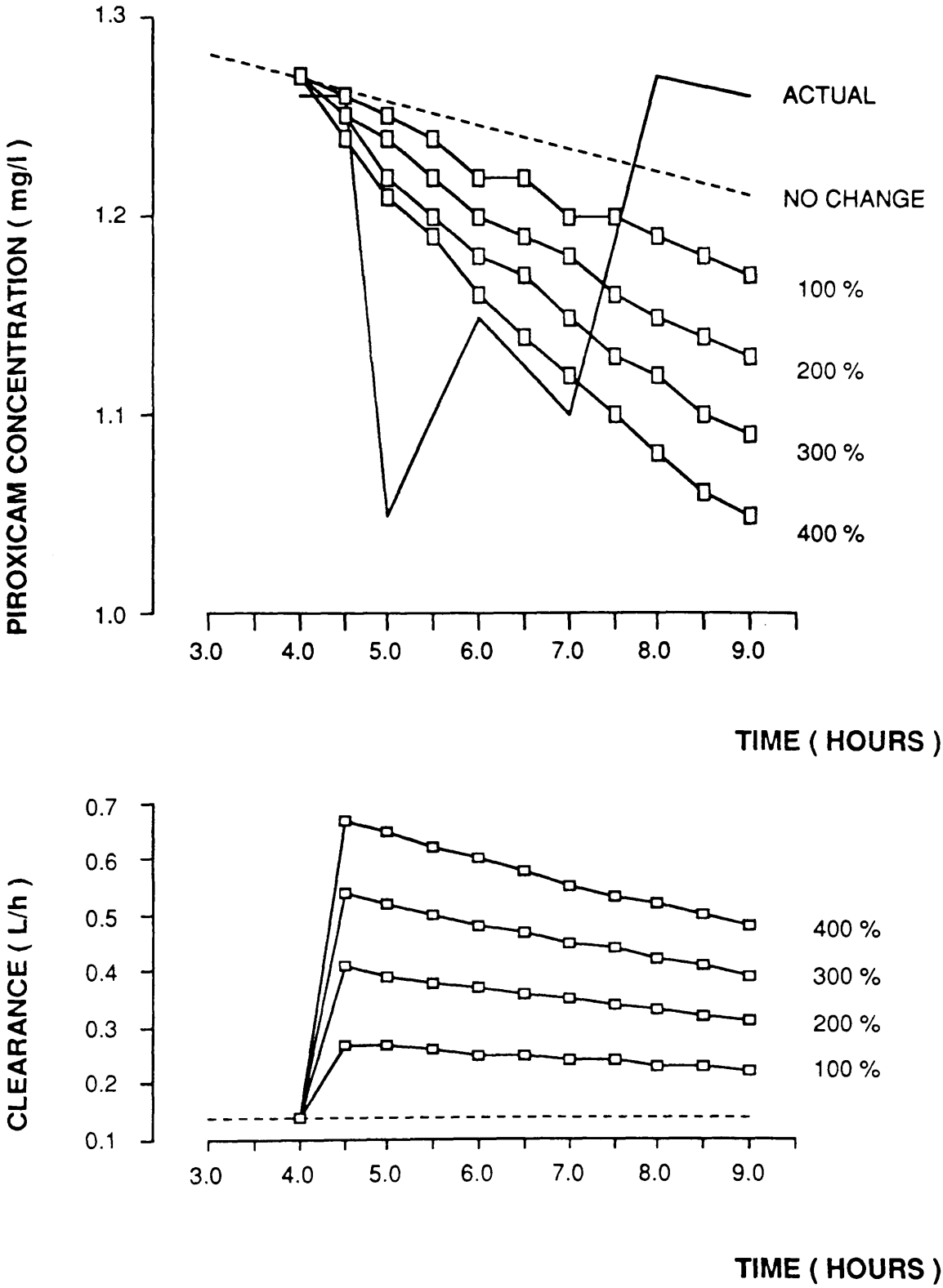
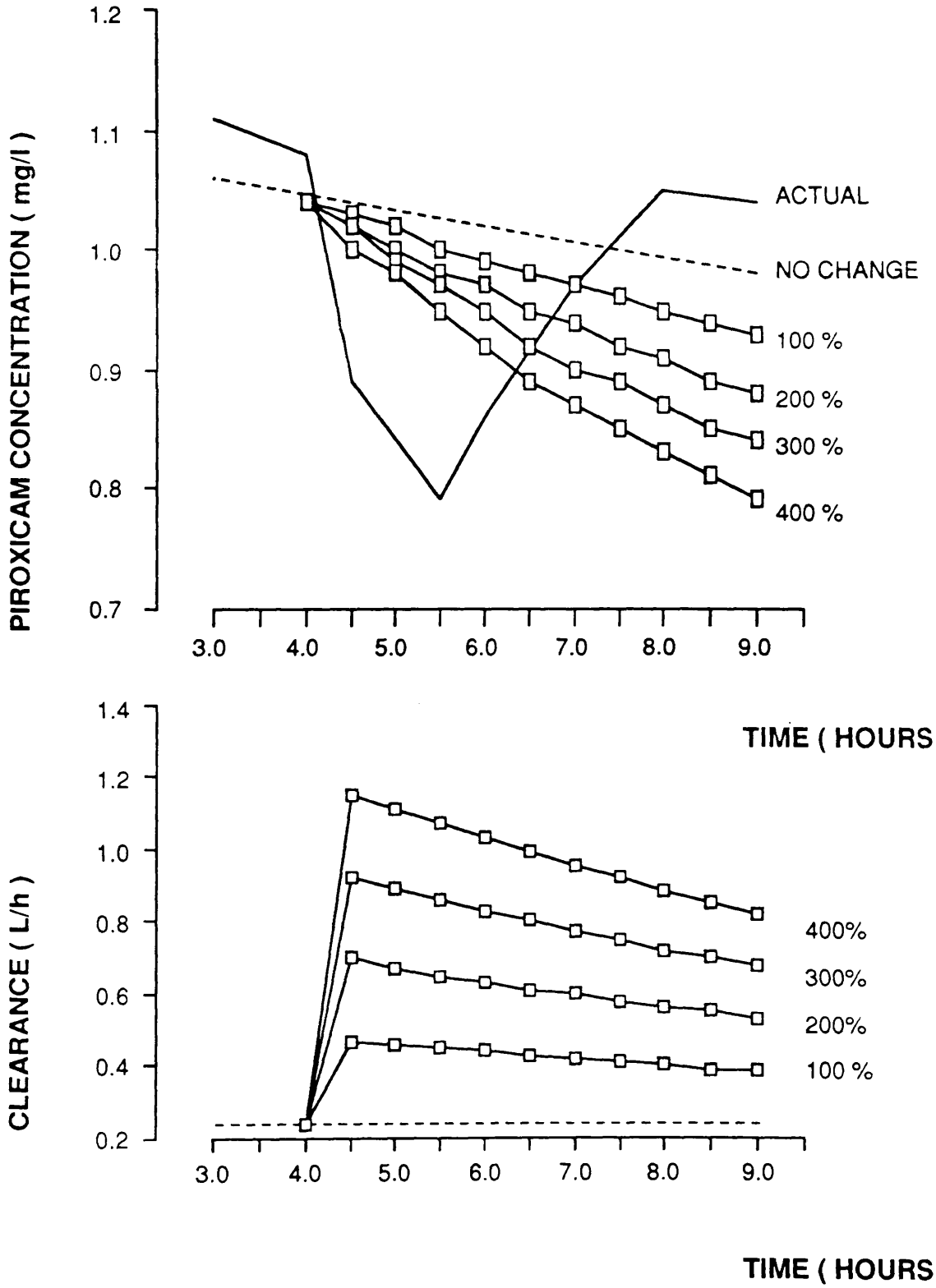


FIGURE 3.19 EFFECT OF CHANGING CLEARANCE BY 100 - 400 % AT A RATE OF RECOVERY OF $0.1h^{-1}$ IN VOLUNTEER NO.6



twice this amount by four hours. When patient no. 14 was considered, similar patterns were found (Figure 3.17) with the initial decline unlikely to be accounted for by an alteration in clearance, although it may contribute to the later changes.

Figures 3.18 and 3.19 show the results in each volunteer at the slowest recovery rate. It appears that increasing the clearance in these subjects has a greater effect on the plasma piroxicam profile than it did in patients. Once again, however, alterations in clearance appear to be poor predictors of the initial rapid decline in actual plasma concentrations. However, unlike the patients, the decline and rise in actual concentrations was virtually symmetrical implying that clearance alterations were likely to have even less of an impact than that seen in patients. The patients certainly had lower initial clearance values compared to the volunteers, and they also had lower plasma albumin concentrations, as already discussed. These factors may have contributed to the slower "rise" in actual plasma concentrations.

From the simulations performed, it would appear that in all instances, even a massive increase in clearance (400%) and a slow rate or recovery ($0.1h^{-1}$) would be inadequate to explain the actual results in both patients and volunteers. Consideration should therefore be made of alterations in volume of distribution as a result of food consumption.

3.5.4 VOLUME OF DISTRIBUTION ALTERATIONS:INTRODUCTION

Piroxicam has a small volume of distribution (approximately 0.2L/kg) and binding to albumin can occur both in the plasma (40% total body albumin) and in the extracellular space (60% total body albumin). As a consequence, any alteration in binding to plasma proteins, as shown earlier, will be reflected throughout the whole of the extracellular space and not only in the intravascular site.

A model is therefore required to predict the consequences of changes in plasma protein binding on both the apparent volume of distribution and the overall pharmacokinetics of piroxicam.

3.5.5 VOLUME OF DISTRIBUTION ALTERATIONS: MODEL CHARACTERISTICS

Figure 3.20 shows the model. A more complete description of its application can be found in Rowland & Tozer (1989), Chapter 25. The amount of drug in plasma is the product of the plasma volume and the plasma drug concentration. Using this model it is possible to predict the alteration in total volume of distribution as a result of increasing the fraction unbound (Figure 3.21). It can be seen that by increasing the fraction unbound even up to 400% of its original value, little or no changes occur in the total volume of distribution of a drug with a normal unbound fraction of 0.01. Patient no. 13 had a normal unbound fraction of 0.007, patient no. 14, 0.005, and volunteers 4 and 6 both had an unbound fraction of 0.006. It can therefore be anticipated from this model that despite an increase in unbound fraction of up to 400%, no change in total volume of distribution would occur. This is in agreement with Lin et al (1987).

With the model, the volumes of the intravascular, extravascular, and intracellular spaces were fixed at 3, 12, and 27 litres respectively, a reasonable approximation as all subjects were about 70kg in weight. However, the model did not account for alterations in these volumes as a result of food consumption.

The volumes of these compartments are brought about by an equilibrium between opposing forces. Pressure in the capillaries forces fluid and its dissolved substances through the capillary pores into the extravascular space. Osmotic pressure exerted by plasma proteins results in fluid movement from the extravascular space into the intravascular space which will prevent the continued loss of fluid volume from the blood into the extravascular space. This simplified situation is complicated by the influence of the lymphatic system which returns to the intravascular space the small amounts of fluid and protein that continually leak into the extravascular space.

The consumption of liquid causes an increase in the intravascular volume as it is rapidly absorbed from the stomach. This results in the intravascular space becoming

FIGURE 3.20 : DISTRIBUTION MODEL

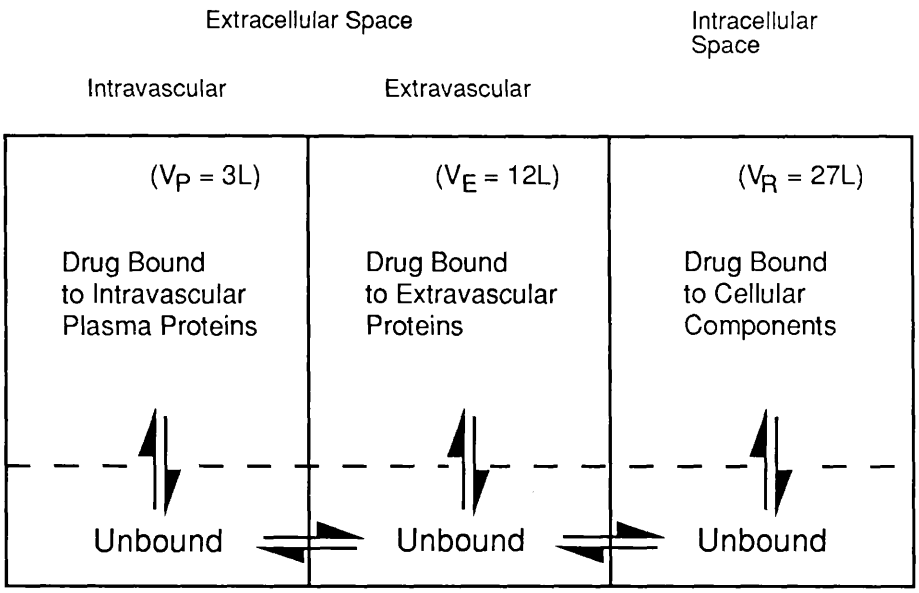
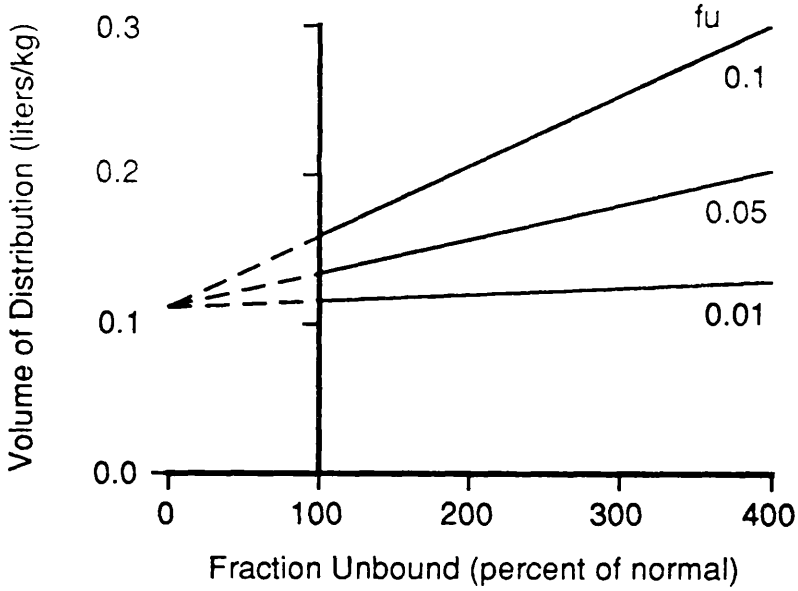


FIGURE 3.21 : EFFECT OF INCREASING UNBOUND FRACTION IN PLASMA ON (TOTAL) VOLUME OF DISTRIBUTION



hypotonic relative to the extravascular space. Osmosis begins immediately at all membranes with large amounts of water moving from one space to another with the result that, within minutes, the extra volume is distributed evenly between the intra- and extravascular spaces. It would therefore appear that the fluid content of the meals would only result in an transient alteration in plasma volume.

Utilisable carbohydrates in the diet are hydrolysed to mono- or disaccharides. The monosaccharides are absorbed by active transport mechanisms whereas the disaccharides are absorbed passively into the portal circulation. The rapid reformation of isotonic conditions described in the situation of fluid consumption would also quickly reverse the hypertonicity induced by the increase in carbohydrate concentration after absorption from the gastrointestinal tract. As a consequence, the influence of carbohydrate on the overall plasma volume would, like the other food constituents that disrupt isotonicity, only result in a transient alteration of plasma volume.

Proteins are enzymatically hydrolysed to amino acids and absorbed (98% total intake) by active transport if they are the L-enantiomer, and passive transport if they are the D-enantiomer. Proteins are the only dissolved substances of the plasma and the extravascular fluid which do not readily diffuse through the capillary membrane. Furthermore, when small quantities of protein do diffuse through the membrane into the extravascular fluid they are soon transferred to the lymph. The net effect is that the total protein concentration of plasma averages about three times that of the extravascular fluid (73g/l versus 20-30g/l). This inequality in concentration gives rise to the osmotic pressure described earlier. The magnitude of this effect is not linear with protein concentration, as an additional force exists, the so called Donnan effect. This is a result of proteins possessing a negative charge which requires small cations (Na^+) to flux from the extravascular space in order to surround the protein molecule. This increases the number of osmotically active substances with the result that the osmotic effect is increased by another 50%. As already stated, the consequences of this would

only be felt in the intravascular space as these charged molecules do not readily pass through the capillary membrane.

It was therefore considered appropriate to perform a simulation study where alterations were made to the total volume of distribution after food consumption. Theoretically it is possible that protein absorption from a meal could cause an increase in the total intra- and extravascular volume, with the increased osmotic effect of proteins (or amino acids) in the intravascular space causing a flux of fluid from the extravascular space which in turn would cause a flux from the lymphatic system.

3.5.6 VOLUME OF DISTRIBUTION ALTERATIONS: SIMULATION STUDY METHODS

As with the previous simulation study it was assumed that piroxicam exhibited first order absorption and elimination characteristics. The effect of altering the volume from its baseline value by pre-set amounts (5, 15, or 25%) was investigated. Unlike the situation with the simulated clearance alterations both the rate of change and the rate of recovery of the individual's initial volume of distribution were varied. The programme (a copy of which appears in the Appendix) therefore required values for the following parameters: clearance, initial volume of distribution, a value by which volume of distribution was altered (ΔV), a rate constant by which this alteration occurred, a rate constant for the recovery of the original volume of distribution, an absorption rate constant, initial plasma concentration, dose, and the time after the dose when the volume was altered. Table 3.9 lists the pharmacokinetic parameters used in this simulation study.

Volume of distribution values were altered in each subject by 5, 15, and 25% four hours after the dose had been introduced. Rates of alteration varied between 0.1 to $2h^{-1}$ and rates of recovery ranged from 0.05 to $1h^{-1}$ in order to include all the actual values obtained from each subject.

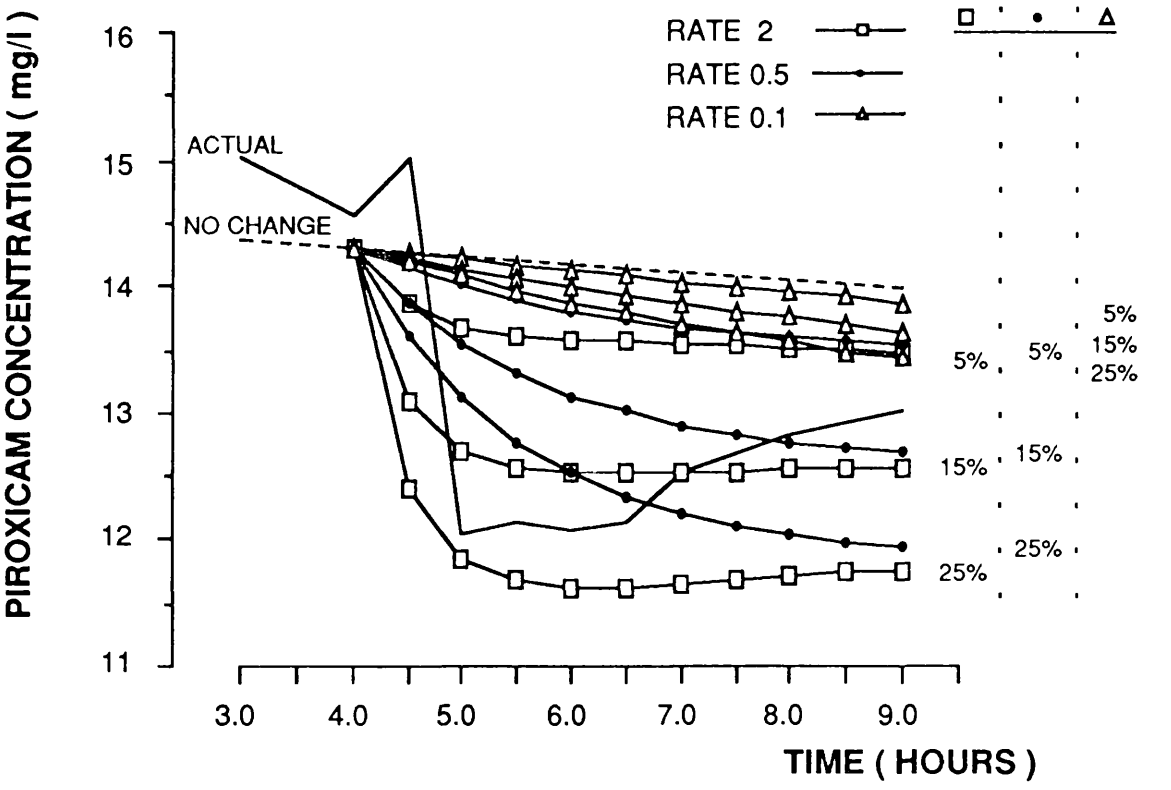
3.5.7 VOLUME OF DISTRIBUTION ALTERATIONS: SIMULATION STUDY RESULTS

Figures 3.22, 3.23, 3.24 show the results obtained in patient no.13 at various rates and extent of volume changes. As with the Figures illustrating clearance alterations, the actual plasma profiles (solid line) and the unchanged situation (dotted line) have been superimposed on all graphs. If one examines the upper graph of Figure 3.22 it can be seen that the rate of change of volume of distribution has been allowed to alter by $0.1h^{-1}$ (triangles), $0.5h^{-1}$ (dots), and $2.0h^{-1}$ (squares) but the rate of recovery has been fixed in all instances at $0.05h^{-1}$. The extent of the volume change varied from 5, 15, to 25%. The legend on the right hand side allows identification of the individual profile corresponding to a particular combination of rate of change and extent of change. The subsequent Figures, whilst using the same nomenclature and symbols, have different recovery rates. In the lower graphs the combination of recovery rate and extent of change on the initial volume of distribution value is illustrated. Once again the same nomenclature and symbols have been employed for consistency.

Unlike the situation found with the simulated clearance alterations, volume of distribution alterations, of the magnitude examined in this study, could explain the actual changes exhibited in the individual i.e. a rapid initial decline in plasma concentrations followed by a more sustained rise. However, large alterations in volume would be required i.e. 15, 25% and slow recovery rates (≤ 0.25). Similar results were found with patient no. 14 (Figure 3.25) and it would therefore appear that in these two subjects volume changes alone could describe the plasma concentration alterations exhibited, providing that the volume of the intra- and extravascular spaces increased by greater than 15%.

Figures 3.26 and 3.27 show the results in each volunteer at the slowest recovery rate. Once again the initial rapid decline found in each subject was adequately

FIGURE 3.22 EFFECT OF CHANGING TOTAL VOLUME OF DISTRIBUTION BY 5 - 25 % AT A RATE OF 0.1 - 2h $^{-1}$ WITH RECOVERY RATE OF 0.05h $^{-1}$ IN PATIENT NO. 13



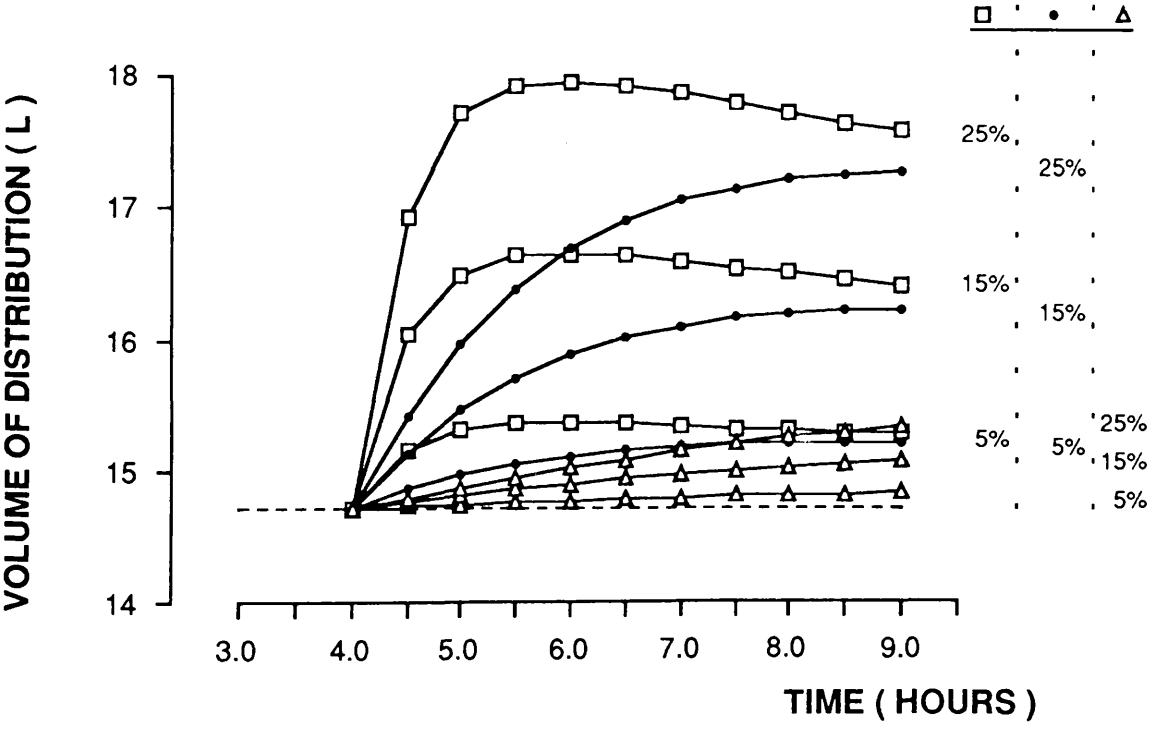
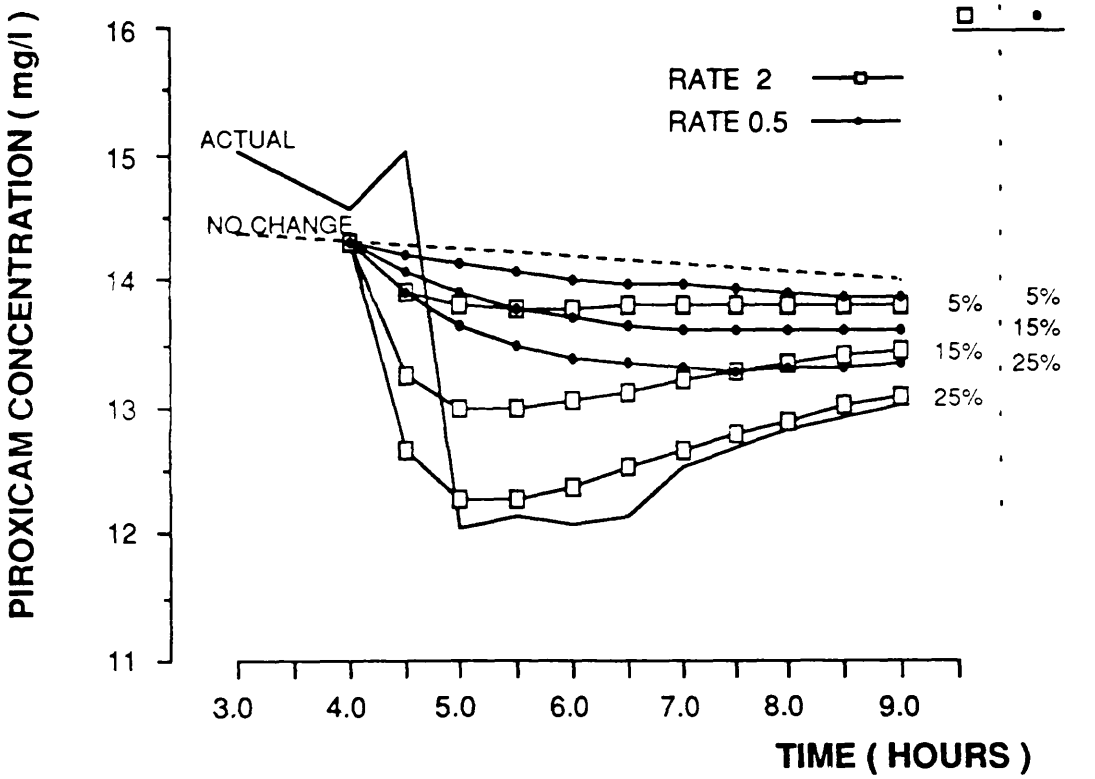


FIGURE 3.23 EFFECT OF CHANGING TOTAL VOLUME OF DISTRIBUTION BY 5 - 25 % AT A RATE OF 0.5 - 2h $^{-1}$ WITH RECOVERY RATE OF 0.25h $^{-1}$ IN PATIENT NO. 13



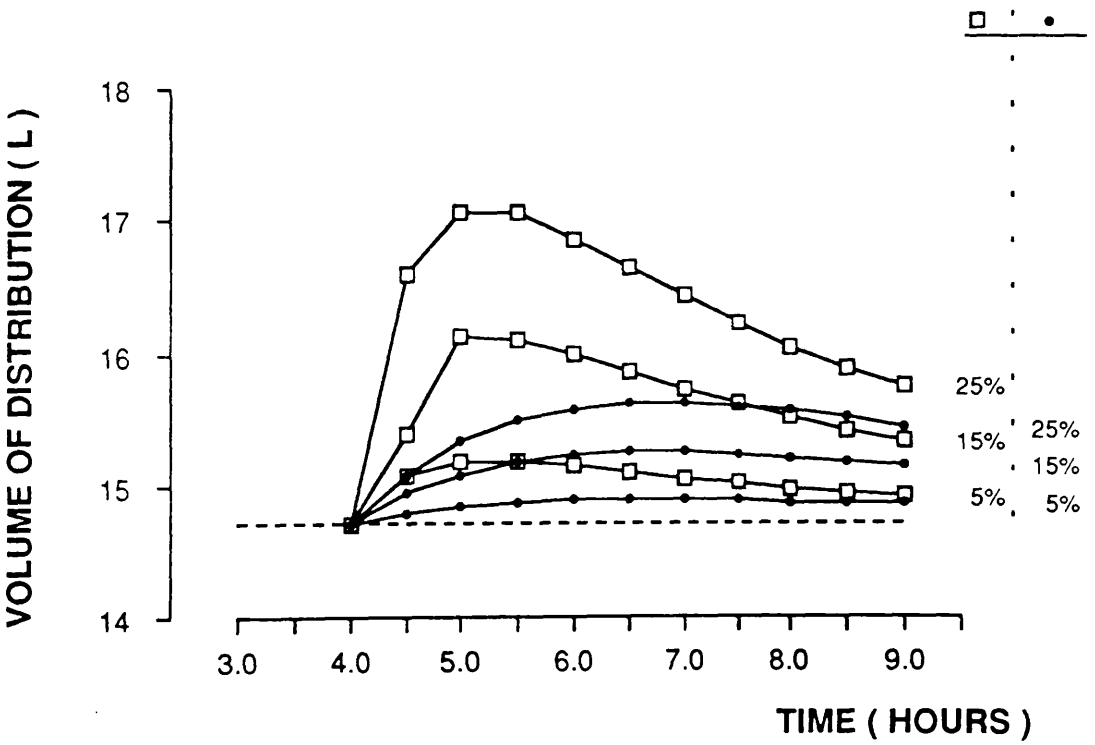
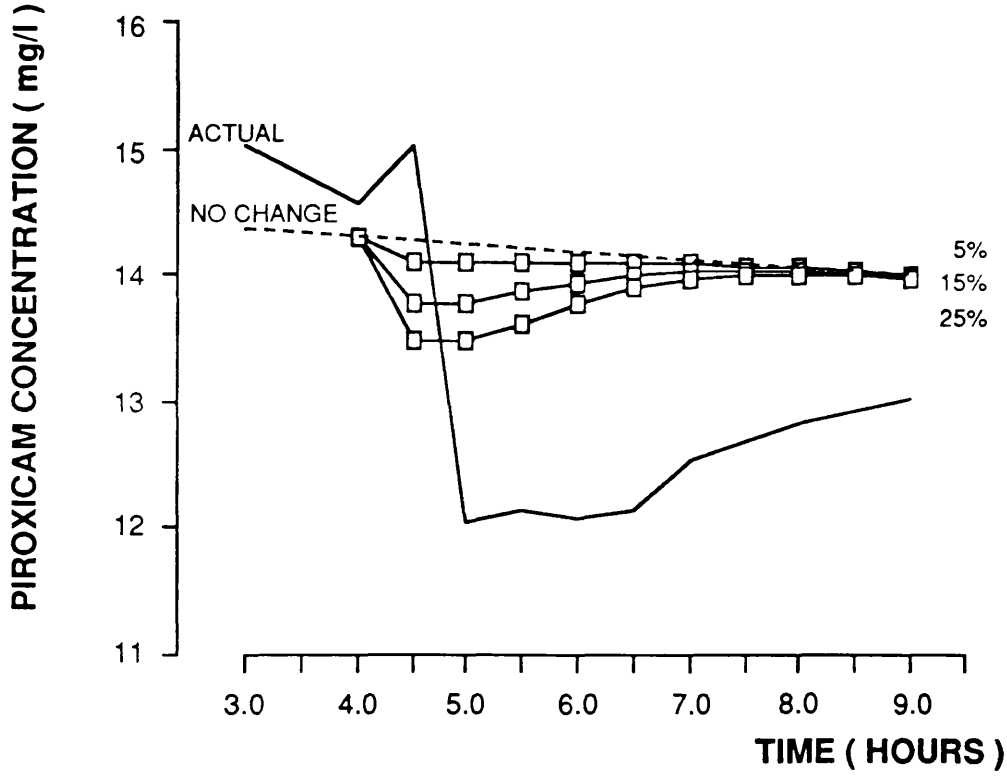


FIGURE 3.24 EFFECT OF CHANGING TOTAL VOLUME OF DISTRIBUTION BY 5 - 25 % AT A RATE OF $2h^{-1}$ WITH RECOVERY RATE OF $1.0h^{-1}$ IN PATIENT NO. 13



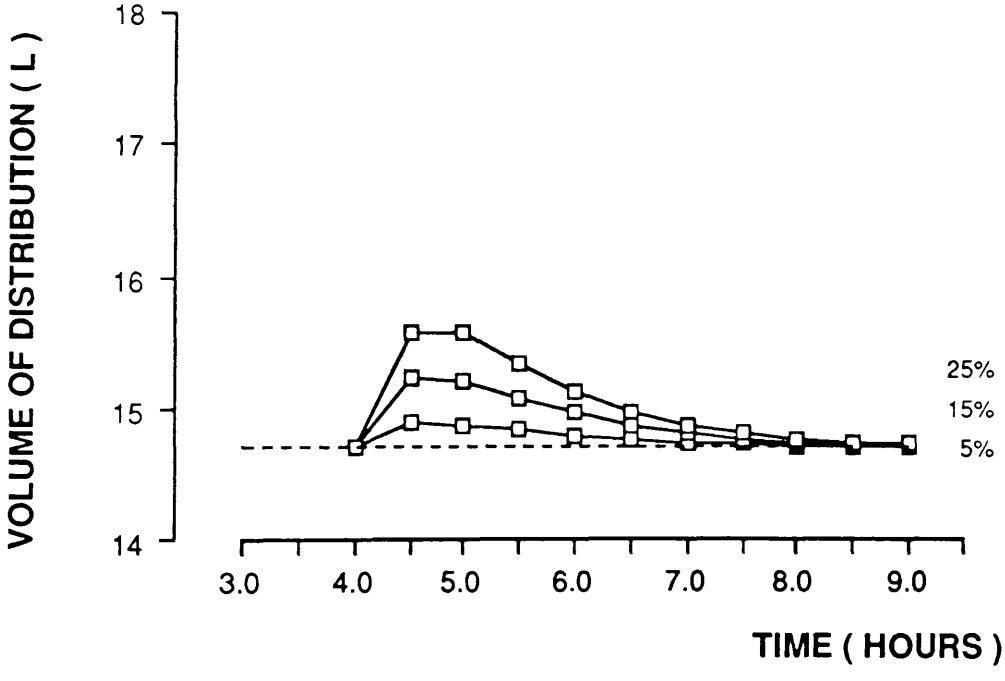
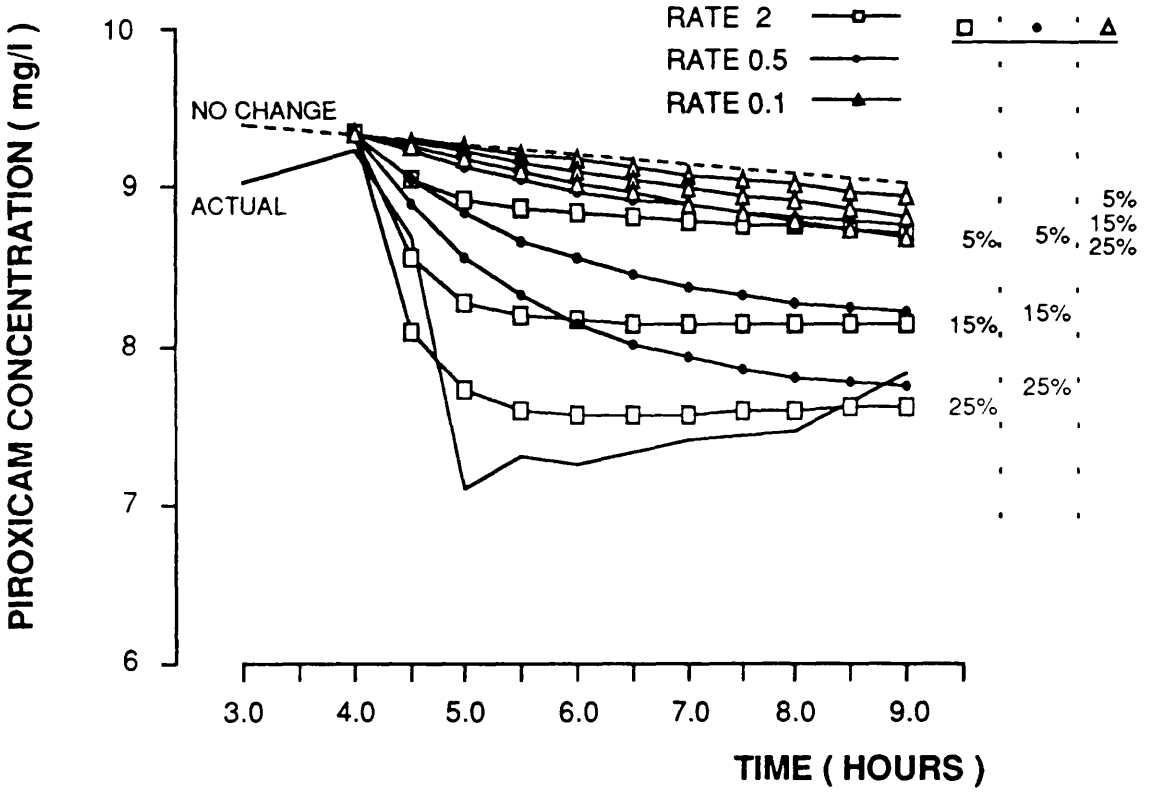


FIGURE 3.25 EFFECT OF CHANGING TOTAL VOLUME OF DISTRIBUTION BY 5 - 25 % AT A RATE OF 0.1 - 2h $^{-1}$ WITH RECOVERY RATE OF 0.05h $^{-1}$ IN PATIENT NO. 14



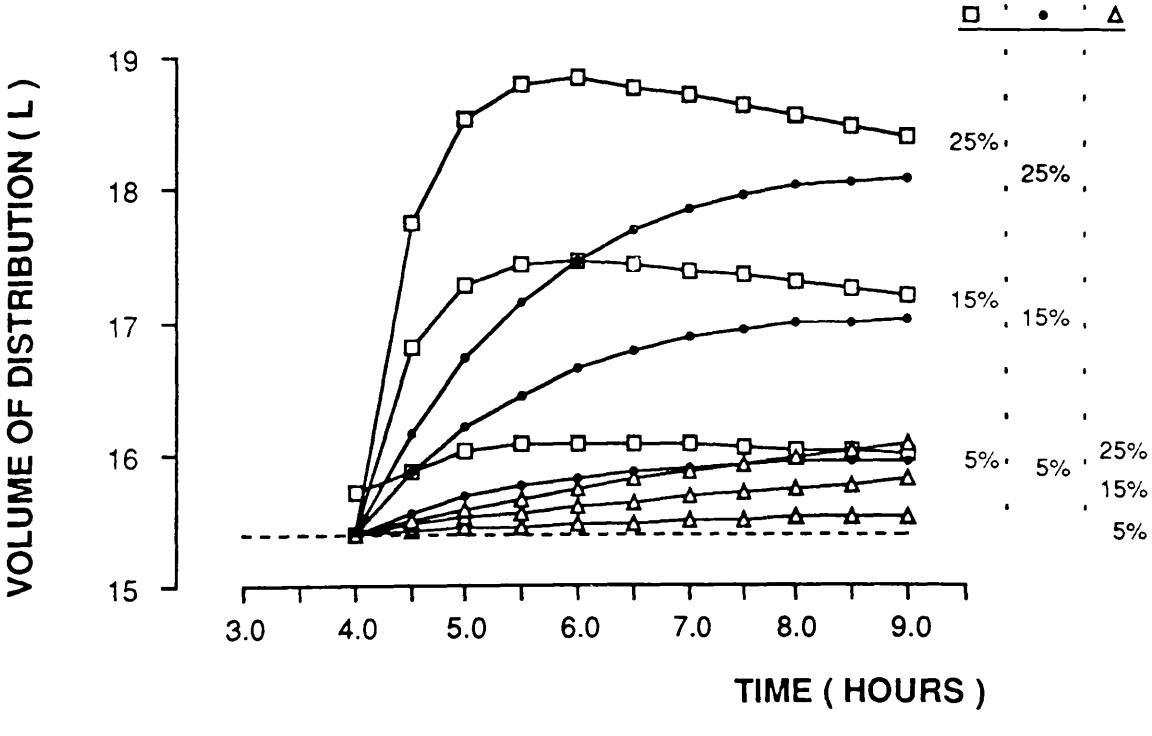


FIGURE 3.26 EFFECT OF CHANGING TOTAL VOLUME OF DISTRIBUTION BY 5 - 25 % AT A RATE OF 0.1 - 2h $^{-1}$ WITH RECOVERY RATE OF 0.05h $^{-1}$ IN VOLUNTEER NO. 4

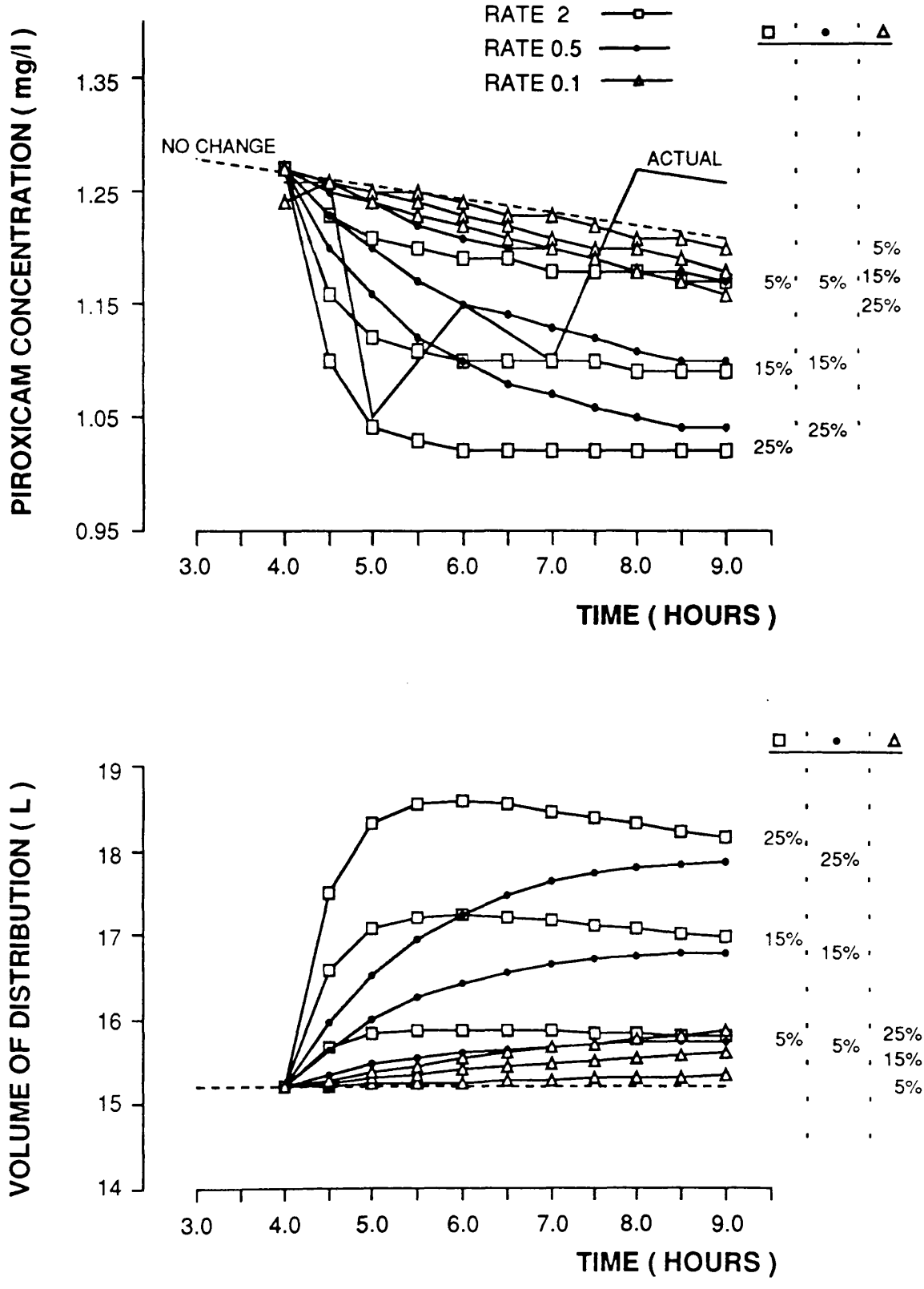
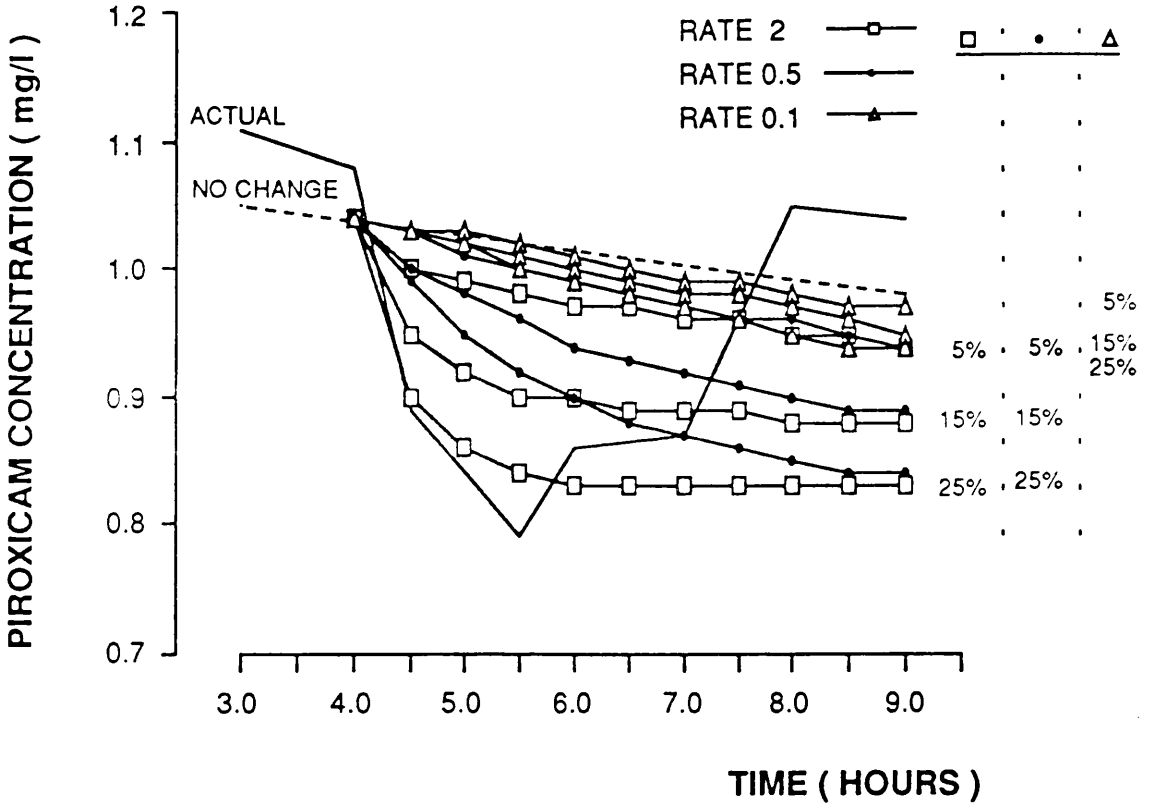
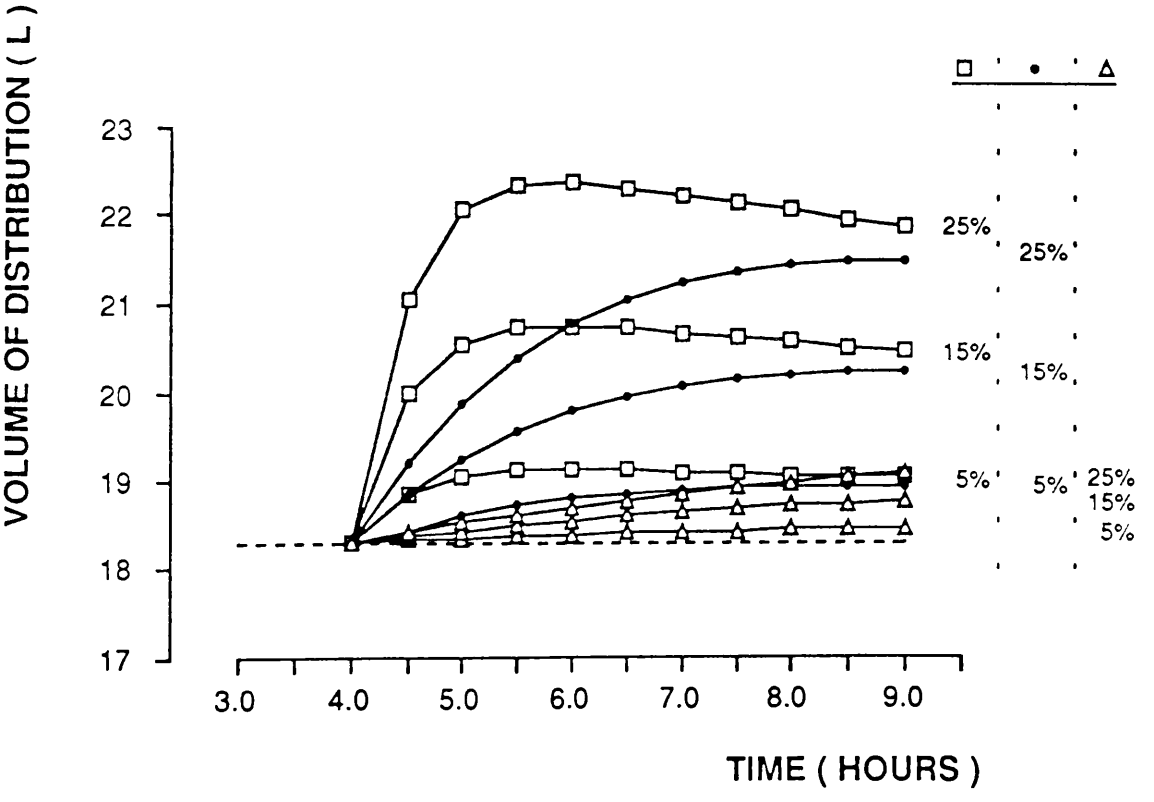


FIGURE 3.27 EFFECT OF CHANGING TOTAL VOLUME OF DISTRIBUTION BY 5 - 25 % AT A RATE OF 0.1 - 2h $^{-1}$ WITH RECOVERY RATE OF 0.05h $^{-1}$ IN VOLUNTEER NO. 6





described by the model, but the plasma concentrations remained lower for longer in the simulated profiles than were actually found. This, once again, may be a consequence of the volunteers being younger and their homeostatic mechanisms being quicker to reform pre-prandial conditions.

3.6 DISCUSSION

In this chapter it has been suggested that food consumption is implicated in the perturbations in plasma piroxicam concentration/time profiles found in both healthy volunteers and patients with rheumatoid arthritis. It has been suggested that a displacement from albumin binding sites occurs as a result of fatty acid binding to the albumin molecule. However, this may not be relevant due to the theoretical considerations developed above. Clearance alterations may contribute to these perturbations, but only if there is a massive increase in clearance, and this is an unlikely response to food. Furthermore, decreased piroxicam concentrations were mirrored by parallel decreases in 5-hydroxypiroxicam concentrations. Increased clearance of parent drug would lead to an increase in plasma metabolite levels, not the situation found in any of the patients studied.

Despite the increased unbound fraction, no alteration in apparent total volume of distribution would be anticipated with this drug. Therefore only an alteration in the plasma compartment volume could explain the results found. In the model, this volume was fixed at 3 litres but the consumption of food and drink would result in a transient expansion of plasma volume during absorption. This idea helps to explain the results obtained and a simple "dilution" of the plasma volume after food consumption may be all that is involved.

CHAPTER 4

STUDY DESIGN OPTIMISATION AND THE JACKKNIFE TECHNIQUE

4.1 INTRODUCTION TO CHAPTER 4

In this chapter the design characteristics of an interaction study (Chapter 5) examining the combination of H_2 receptor antagonists and piroxicam will be closely scrutinised. As this study will rely on AUC assessments to reveal any changes in clearance and/or bioavailibility, as a result of the combination, consideration has to be made of the limitations of the methods commonly used to calculate AUC's (i.e. the trapezoidal rule). Although errors in trapezoidal AUC determinations are not normally calculated it does not mean that they do not exist. This is important when considering a study's power to detect pre-set endpoints.

One method to calculate the error in a trapezoidal AUC estimation is to use the Jackknife technique. The theory of this statistical technique is illustrated. Also shown are some examples its use in a variety of situations involving AUC assessments.

4.2 JACKKNIFE THEORY

This technique has been extensively reviewed by Efron & Gong (1983). It has been used in the determination of the errors associated with the estimation of model parameters, using a two compartment pharmacokinetic model for illustration (Nimmo et al 1981).

The essential steps, in the Jackknife technique, are as follows and are illustrated in Figure 4.1.

1. Assume a data set, e.g. concentration/time points, consists of n observations, x_i, t_i where i= 1,...,n, and that a parameter, Θ, e.g. AUC, is to be estimated using these data. Using all data points the AUC is determined by the trapezoidal method to provide the best estimate for that area, $\bar{\Theta}$.

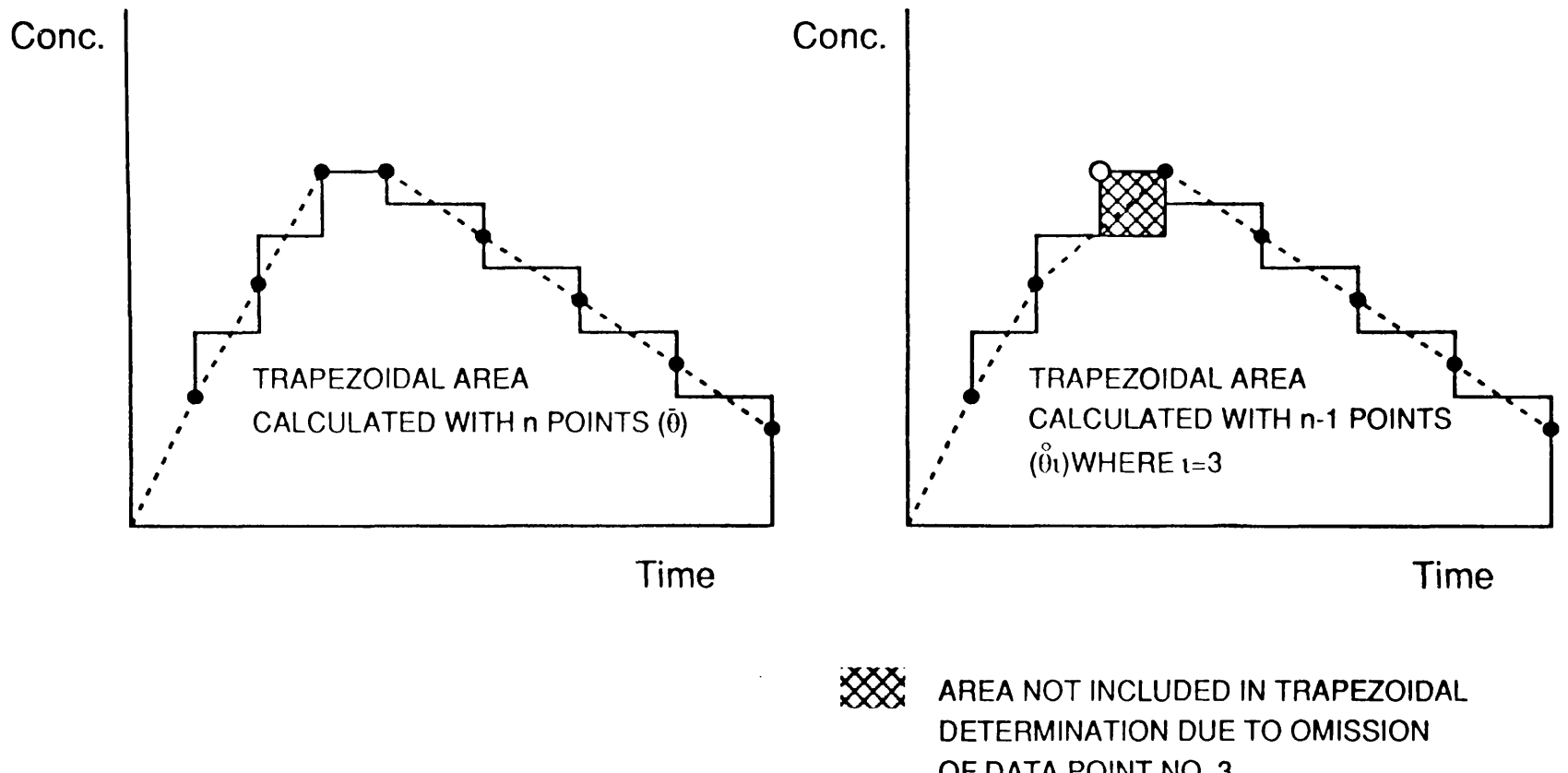
2. Starting from the first data point, each point is sequentially removed and replaced. With each point removed, a value for Θ is estimated, $\mathring{\Theta}_i$, using (n -1) points, where i= 1,...,n. Thus n estimates, $\mathring{\Theta}_i$, i= 1,...,n, are obtained.

3. A "pseudoparameter", Θ_i, is obtained from the areas calculated in steps 1 and 2, using the following equation:

$$\Theta_i = n\bar{\Theta} - (n-1)\mathring{\Theta}_i$$

4. The average value of the $n\Theta_i$ provides an estimate of $\bar{\Theta}$ and the standard error of the $n\Theta_i$ provides an estimate of the standard error associated with $\bar{\Theta}$. This error value is an estimate of the error associated

FIGURE 4.1 JACKKNIFE THEORY



with the full trapezoidal measurement. Once calculated, the standard error can be normalised by expressing it as a percentage of the "best estimate" of area, Θ. This is known as the "% Jackknife error". If a number of simulated concentration/time curves are obtained then a "mean % Jackknife error" value can be calculated. This will provide an estimate of the error in the AUC assessment.

The difference between each pseudo-area value and the average parameter (e.g. AUC) value will indicate the importance of that data point to the overall parameter (e.g.AUC) estimation. This may be important in determining:

(i) "design points" in the study protocol i.e. points which have a critical influence on the overall parameter estimate

(ii) which points are outliers.

4.3 CALCULATION OF PHARMACOKINETIC PARAMETERS

AUC's can be calculated by both parametric and non-parametric methods, the latter usually employing the trapezoidal rule (linear and logarithmic) the formulae of which are detailed in the Appendix.

This chapter examines the use of the Jackknife technique in both single dose and steady state simulated concentration/time profiles. Data from the single dose study described in Chapter 3 were used to provide *a priori* information. The steady-state simulations were performed using the pharmacokinetic parameter values obtained by fitting the appropriate pharmacokinetic model to the single dose data. This section describes the fitting of these pharmacokinetic models to the individual subjects' plasma piroxicam/concentration time data.

4.3.1 PATIENTS AND METHODS

The demographic details of the six healthy volunteers are given in Chapter 3.

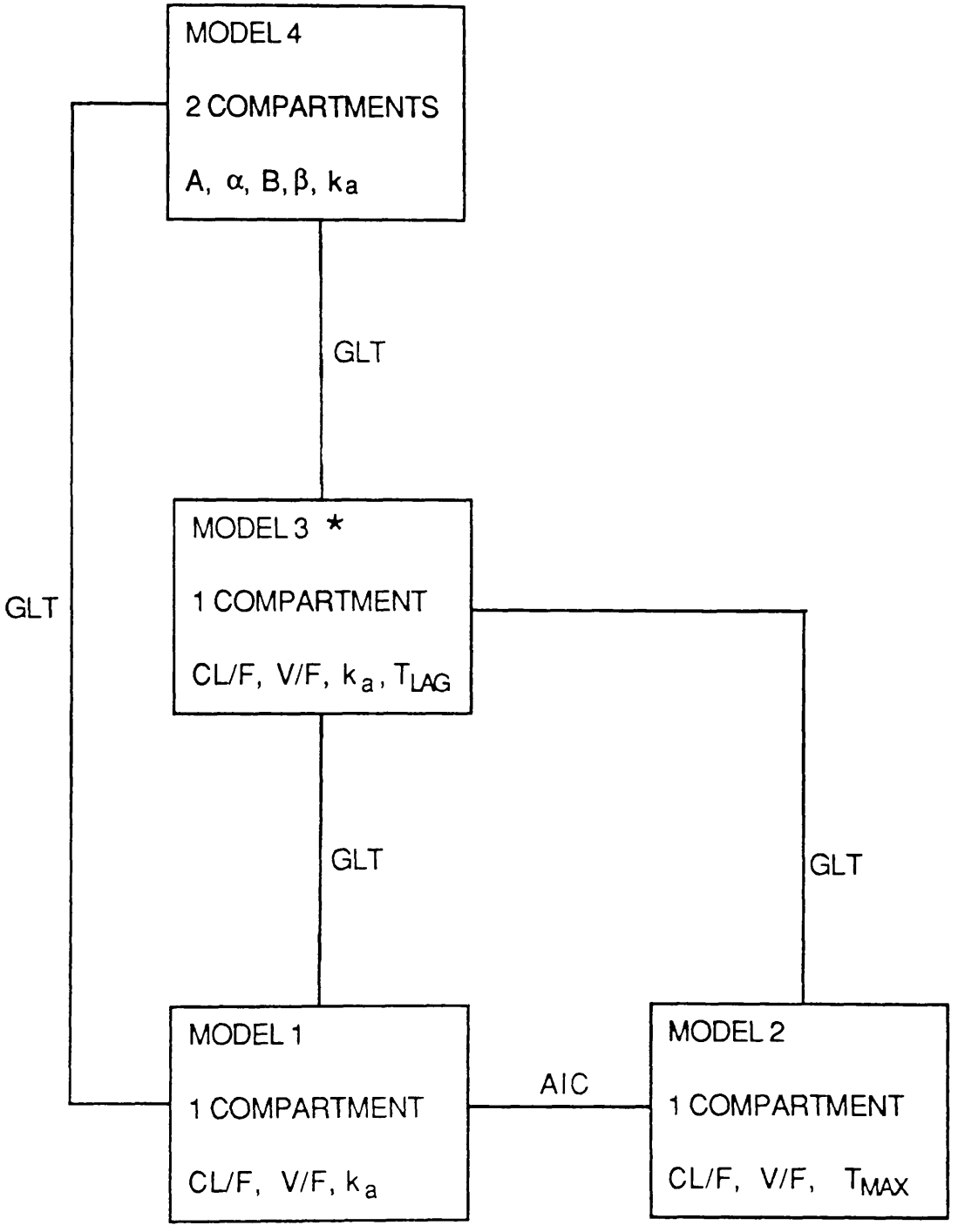
4.3.2 DATA ANALYSIS

The 4 pharmacokinetic models shown in Figure 4.2 were fitted to each plasma piroxicam concentration/time data set. The choice of "best model" was based on examination of the General Linear Test and the Akaike Information Criterion (Yamaoka et al 1978) as shown in Table 4.1 (formulae for which are given in the appendix).

4.3.3 RESULTS

A one compartment model with first order absorption (including lag time) and first order elimination (model 3) was found to be the "best model" using the criteria outlined above (Table 4.1). A summary of the pharmacokinetic parameters relevant to this model appears in Table 4.2.

FIGURE 4.2 : PHARMACOKINETIC MODELS : DESCRIPTION AND SELECTION



GLT - GENERAL LINEAR TEST

AIC - AKAIKE INFORMATION TEST

* - "BEST MODEL"

TABLE 4.1

PHARMACOKINETIC MODEL COMPARISON

SUBJECT		MODEL 1	MODEL 2	MODEL 3	MODEL 4	1 vs 2 (A.I.C.)	1 vs 3 (G.L.T.)	1 vs 4 (G.L.T.)	2 vs 3 (G.L.T.)	2 vs 4 (G.L.T.)	3 vs 4 (G.L.T.)
	(SSQ_{Res})	0.4735	0.3021	0.1414	0.3623						
1.	(D.F.)	35	35	34	33	2	3	4	3	n/s	n/s
	(A.I.C.)	-22.41	-39.48	-66.34	-28.59						
	(SSQ_{Res})	0.5644	0.4987	0.3935	0.5643						
2.	(D.F.)	35	35	34	33	2	3	n/s	3	n/s	n/s
	(A.I.C.)	-15.74	-20.44	-27.44	-11.74						
	(SSQ_{Res})	0.4492	0.2717	0.2840	0.4483						
4.	(D.F.)	36	36	35	34	2	3	n/s	n/s	n/s	n/s
	(A.I.C.)	-25.22	-44.82	-41.08	-21.24						
	(SSQ_{Res})	0.5964	0.5956	0.5955	0.5880						
4.	(D.F.)	34	34	33	32	n/s	n/s	n/s	n/s	n/s	n/s
	(A.I.C.)	-13.12	-13.17	-11.18	-9.65						
	(SSQ_{Res})	0.3505	0.2460	0.1676	0.3152						
5.	(D.F.)	35	35	34	33	2	3	n/s	3	n/s	n/s
	(A.I.C.)	-33.84	-47.29	-59.88	-33.88						
	(SSQ_{Res})	0.3691	0.3216	0.3002	0.3635						
6.	(D.F.)	34	34	53	32	2	3	n/s	n/s	n/s	n/s
	(A.I.C.)	-30.88	-35.97	-36.53	-27.45						

A.I.C. AKAIKE INFORMATION CRITERION
G.L.T. GENERAL LINEAR TEST
D.F. DEGREES OF FREEDOM
SSQ_{RES} RESIDUAL SUM OF SQUARES

TABLE 4.2

INDIVIDUAL MODEL PARAMETER ESTIMATIONS

SUBJECT	PARAMETER ESTIMATE ± S.D.			
	V/F (L)	CL/F(Lh^{-1})	T LAG (h)	Ka (h^{-1})
1	19.11±0.29	0.230±0.010	0.413±0.052	30.2±22.4
2	18.95±0.56	0.189±0.016	0.464±0.056	2.40±0.61
3	16.49±0.42	0.171±0.011	0.632±0.093	0.84±0.14
4	15.19±0.04	0.143±0.001	0.422±0.004	45.5±4.46
5	17.79±0.31	0.217±0.010	0.458±0.021	9.18±3.74
6	18.30±0.49	0.245±0.016	0.380±0.062	3.29±0.99
MEAN	17.64	0.199	0.464	15.24

SUBJECT NO. 1 Parameters for lowest plasma concentration time curve

SUBJECT NO. 4 Parameters for highest plasma concentration time curve

4.4 THE EFFECT OF VARYING THE NUMBER OF POINTS USED IN THE CALCULATION OF EXTRAPOLATED SINGLE DOSE AUC VALUES

Under normal circumstances, the number of data points used to calculate the slope of the terminal (log-linear) elimination phase of a concentration time curve is arbitrarily made. If the extrapolated AUC_0^∞ is made up only in small part by the AUC_0^t where, t, is the last time point, the potential for error in the overall AUC estimation is great.

This study examines the effect of varying the number of data points in the terminal elimination phase used to calculate the slope and, therefore, the eventual extrapolated AUC value. The individual AUC's obtained from the six volunteers in the single dose study described in Chapter 3 were used. A % Jackknife error value was obtained for the AUC_0^t value and comparisons were made between this error value and the % Jackknife error value obtained when the AUC was extrapolated to infinity using 3, 5, and 10 data points to define the slope of the terminal elimination portion of the individual concentration/time profiles. The results are shown in Table 4.3. Also shown, in parenthesis, is the regression coefficient obtained after linear regression analysis of the log concentration/time data was performed.

From the results obtained it can be seen that, as expected, reduction in the error associated with the extrapolated AUC estimation is made by increasing the number of data points used to define the slope. Reduction of the % Jackknife error is also associated with an increased regression coefficient value. When the number of data points used to define the slope was increased from 3 to 5 dramatic improvements were seen in subjects 2, 3, and 4. In all subjects increasing the number of data points to 10 resulted in % Jackknife error values approaching the values obtained during the interval AUC_0^t which on average contained 73% of the area once extrapolation was performed.

The potential therefore exists for errors in AUC estimation to be introduced when extrapolated areas are calculated using few data points. This is a consequence of one or

TABLE 4.3

EFFECT OF INCREASING NUMBER OF DATA POINTS IN EXTRAPOLATION ON AUC ERROR ESTIMATION

		% JACKKNIFE ERROR IN AUC ESTIMATION		
SUBJECT	$0 \rightarrow t$	$0 \rightarrow \infty$ (3 points)	$0 \rightarrow \infty$ (5 points)	$0 \rightarrow \infty$ (10 points)
1	1.4	4.0 (r=-0.9726)	5.8 (r=-0.9907)	2.1 (r=-0.9934)
2	3.5	30.7 (r=-0.5903)	12.1 (r=-0.8363)	4.5 (r=-0.9244)
3	2.9	134.5 (r=-0.7559)	30.5 (r=-0.8392)	7.3 (r=-0.9461)
4	3.3	102.1 (r=-0.6546)	32.0 (r=-0.7439)	7.2 (r=-0.9272)
5	2.2	9.0 (r=-0.9011)	7.5 (r=-0.9456)	3.1 (r=-0.9773)
6	2.6	9.3 (r=-0.7857)	15.5 (r=-0.7790)	5.0 (r=-0.9334)

more biased data points having a relatively greater influence on the overall extrapolated AUC estimation. Furthermore, if this extrapolated area is only made up in small part by the AUC_0^t the error in the AUC assessment will be increased.

4.5 EXAMINATION OF THE "LOG JACKKNIFE" VERSUS THE "LINEAR JACKKNIFE": A SIMULATION STUDY

The requirement to logarithmically transform the pseudoparameter in some instances has been described by Miller (1974). Using the pharmacokinetic parameters in Table 4.2, the two sets of parameters corresponding to the highest and lowest plasma concentration/time curves and the mean of the six individual parameter estimates were employed in a single dose simulation study. AUC's were obtained over the range 0-t and 0-∞ using 5, 10, or 15 data points to calculate the extrapolated area, with the addition of either 5, 10, or 15% random error to the simulated plasma concentration/time data points. This was repeated ten times in each individual and a mean % Jackknife error value was obtained for both linear and logarithmic transformations of the pseudoparameter. In total 540 simulated profiles were obtained.

Table 4.4 shows the results obtained over the interval 0-t. It can be seen that the results obtained were consistent across the range from high to low parameter sets. The mean % Jackknife error increased arithmetically as the random error increased. However, the log transformation of the pseudoparameters conferred no advantage to the mean % Jackknife error value obtained over this interval.

Table 4.5 shows the results obtained in the extrapolated AUC using 5, 10, or 15 data points. Once extrapolation occurs there is an increase in the mean % Jackknife error compared to the values obtained over the interval 0-t. In all instances, however, as the number of points used in the extrapolation is increased the mean % Jackknife error decreases. In most cases greater error is obtained when the high parameter set is used compared to the mean or the low parameter set. When many data points were used in the extrapolation, no difference was obtained between the mean % Jackknife errors after log transformation of the pseudoparameters. At low extrapolation numbers, however, smaller mean % Jackknife error values were obtained after log transformation of the pseudoparameters. The discrepancy in the results confirms Miller's observation

TABLE 4.4

ONE S.D. MEAN % JACKKNIFE ERROR FOR AUC_0^t ESTIMATION

	5%		10%		15%	
	NORMAL	LOG	NORMAL	LOG	NORMAL	LOG
Average	±8.59	-8.77 +9.23	±16.84	-16.25 +20.04	±25.63	-23.36 +28.91
High	±8.15	-7.82 +8.16	±16.36	-17.64 +20.43	±24.42	-25.35 +33.34
Low	±8.05	-8.69 +9.32	±17.79	-16.13 +17.84	±24.59	-23.58 +31.10

TABLE 4.5.

ONE S.D. MEAN % JACKKNIFE ERROR FOR AUC_0^{∞} ESTIMATIONS USING 5,10 OR 15 POINTS TO EXTRAPOLATE

RANDOM ERROR (%)	PARAMETER SET	5 POINTS		10 POINTS		15 POINTS	
		NORMAL	LOG	NORMAL	LOG	NORMAL	LOG
5%	(Average)	±52.05	-30.41 +42.98	±20.66	-23.52 +31.23	±17.34	-15.94 +18.09
5%	(High)	±100.19	-46.22 +85.88	±34.46	-25.73 +35.33	±22.18	-21.82 +28.90
5%	(Low)	±45.15	-26.37 +36.86	±26.13	-17.03 +21.31	±14.26	-14.91 +17.16
10%	(Average)	±123.28	-60.36 +154.64	±41.06	-31.83 +45.77	±31.02	-30.15 +43.51
10%	(High)	±95.74	-68.55 +220.03	±63.23	-49.42 +97.07	±44.98	-39.72 +92.49
10%	(Low)	±89.99	-57.48 +137.48	±33.02	-28.77 +40.60	±27.84	-25.29 +33.42
15%	(Average)	±145.32	-59.22 +146.69	±63.71	-46.51 +88.56	±61.39	-38.79 +63.07
15%	(High)	±220.95	-72.50 +262.92	±94.33	-50.11 +138.67	±58.74	-50.08 +98.42
15%	(Low)	±134.53	-50.74 +99.77	±61.32	-46.98 +86.93	±54.56	-34.69 +54.33

that the use of the log transformation of the pseudoparameters can reduce bias (Miller 1974).

As smaller mean % Jackknife error values (with less than 10 data points used to calculate the slope of the terminal elimination portion) were obtained after log transformation of the pseudoparameters, the use of this transformation realistically applies to most situations where AUC's are calculated.

4.6 PROSPECTIVE POWER CALCULATION

As described earlier, this study was initiated to investigate the theoretical inhibition of piroxicam metabolism as a result of H_2 receptor antagonist coadministration. As the results would depend largely on an AUC comparison, determination of the error in the AUC estimation has to be made in order to justify the eventual number of subjects included in the study. The straightforward geometric use of the trapezoidal rule (both linear and logarithmic) ignores the fact that the computed area will be subject to error. This is in contrast to pharmacokinetic model fitting using non-linear regression analysis, for example, where estimated errors in a particular parameter are studied closely. An assessment of the magnitude of this error is important in prospective power calculations as many interaction studies that rely on trapezoidal AUC assessment may fail to show any significant differences as a result of inadequate power to detect pre-set endpoints rather than the absence of an interaction *per se.*

4.6.1 METHODS AND RESULTS

Using the pharmacokinetic parameters in Table 4.2, the two sets of parameters which gave the highest and lowest plasma concentration profiles and the mean of the six individual parameter estimates were selected to provide the widest range of simulated profiles. Using these three sets of parameters, steady state simulations employing the proposed blood sampling schedule for the interaction study were generated as shown in Figure 4.3. 5, 10, or 15% error was introduced to mimic realistic experimental conditions as illustrated in Figure 4.4. Here, 15% random variability creates the erratic curve which defines the area subsequently processed by the Jackknife technique. This procedure was repeated 50 times for each of the three parameter sets at the three levels of added error. The total number of simulations performed was therefore 450.

On examination of Table 4.6 it can be seen that whatever value of error was

FIGURE 4.3 **SIMULATED STEADY STATE PROFILES FOR HIGH, MEAN AND LOW PARAMETER SETS WITH NO ADDED ERROR**

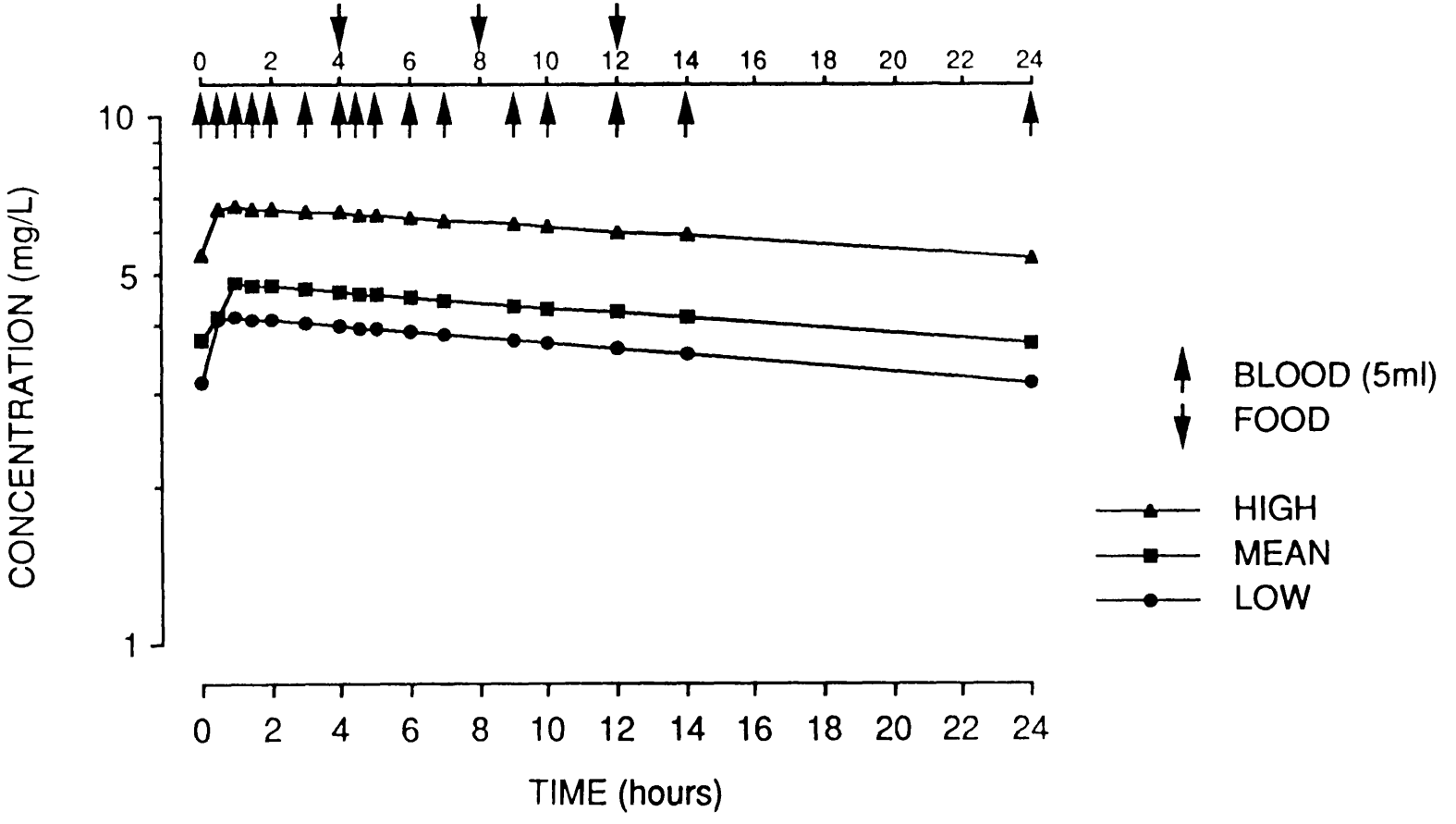


FIGURE 4.4 MEAN PARAMETER SET SIMULATION WITH 15% RANDOM ERROR ADDED

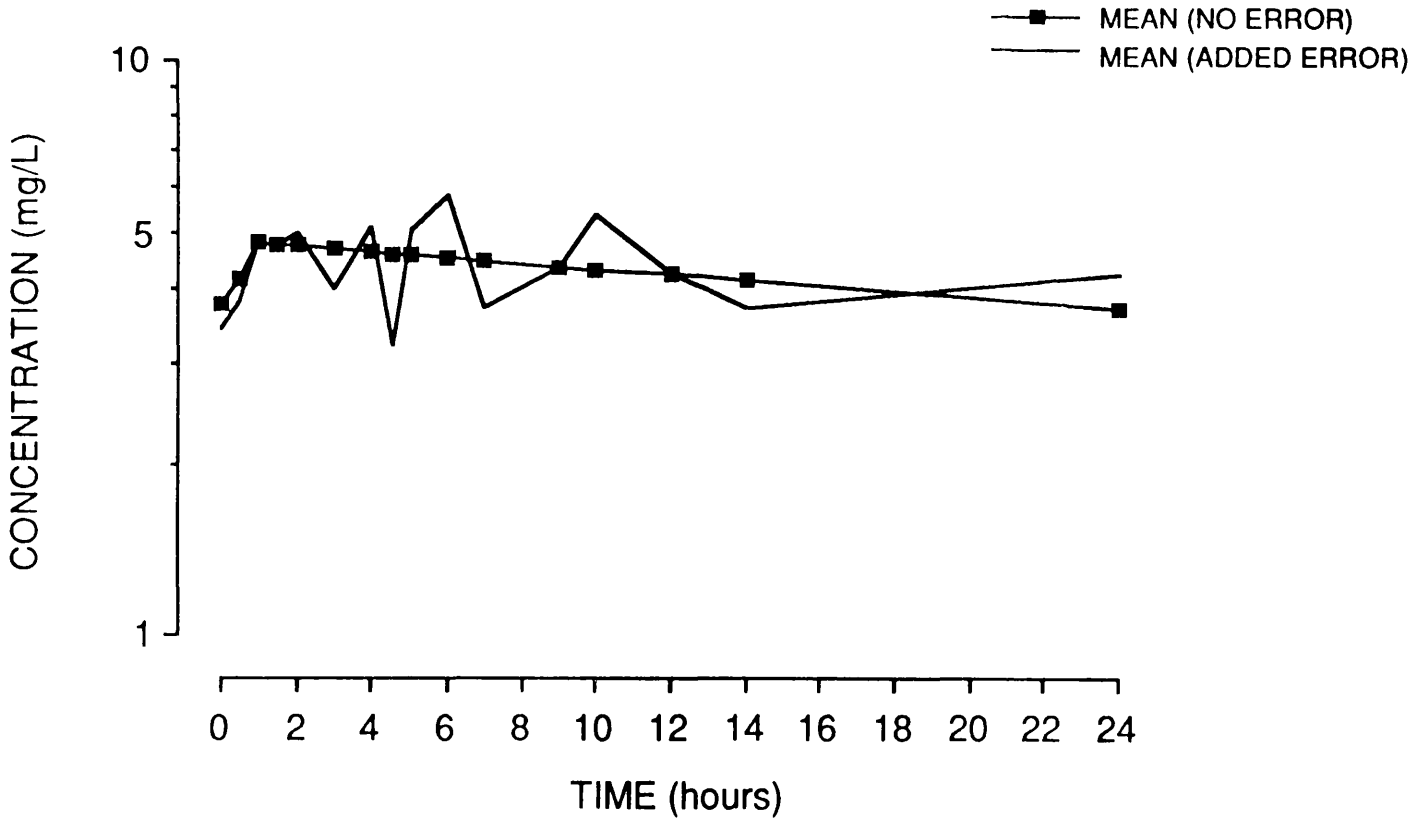


TABLE 4.6

ESTIMATED ERROR IN SIMULATED STEADY STATE AUC DETERMINATIONS

PROFILE DESCRIPTION	NUMBER OF SIMULATIONS AT EACH LEVEL OF ERROR	MEAN ± SD % JACKKNIFE ERROR AT EACH LEVEL OF ADDED ERROR		
		5%	10%	15%
High	50	1.86±0.66	3.66±1.73	5.52±2.58
Mean	50	1.88±0.88	3.51±1.64	5.08±2.26
Low	50	1.75±0.92	3.60±1.88	4.86±2.14

introduced, the mean % Jackknife error was uniform across the range of parameters. Furthermore as the level of random variability increased a corresponding increase in the mean % Jackknife error was obtained. It was considered appropriate to add 15% random variability as the average residual error obtained after the fitting procedure in the single dose pilot study was 14.6% (Table 4.7). With the addition of 15% random variability, a mean % Jackknife error of approximately 5% was obtained. This value, then, provides an estimate of the average error at steady state. It must be borne in mind, however, that as an interaction study usually examines the alteration in an AUC value due to the coadministration of another drug, the error in the ratio of the AUC's must incorporate the uncertainty generated by both AUC determinations. This can be estimated as follows:

$$R = AUC_1/AUC_2$$

$$(\delta R/R)^2 = (\delta AUC_1/AUC_1)^2 + (\delta AUC_2/AUC_2)^2$$

$$= 2.(\delta AUC/AUC)^2$$

therefore $$\delta R/R = \sqrt{2}.\,\delta AUC/AUC$$

and since $\delta AUC/AUC = 5\%$

$$\delta R/R = \underline{7\%}$$

where:

R is the ratio of the two areas, AUC_1:AUC_2

$\delta R/R$ is the average error in the ratio of the two AUCs

$\delta AUC_1/AUC_1$ is the average error in the determination of AUC_1

$\delta AUC_2/AUC_2$ is the average error in the determination of AUC_2

The value of 7% represents the minimum error that is ever attainable in the ratio

TABLE 4.7

RESIDUAL ERROR CALCULATION

SUBJECT	RESIDUAL SUM OF SQUARES	DEGREES OF FREEDOM	AVERAGE CONC.	RESIDUAL ERROR *
1	0.1414	34	0.55	11.64
2	0.3935	34	0.62	17.34
3	0.2843	35	0.70	13.00
4	0.5955	33	0.79	16.96
5	0.1676	34	0.60	11.70
6	0.3002	33	0.56	17.04
			MEAN + S.D.	14.61 ± 2.78%

* $\sqrt{\frac{SSQ_{Res}/DF}{conc}} \times \frac{100}{1}$

of AUC determinations with 15% random (experimental) error. If all subjects were identical this minimum value of 7% could be used in a power calculation to determine the number of subjects required to detect a preset difference in AUCs (e.g. a difference of 20% or more). More realistically, the $\delta R/R$ value should be increased to account for both inter and intra subject variability. Table 4.8 illustrates the effect on the study power by increasing the $\delta R/R$ values from 10 to 30%. The type I error value (α) has been set, for this instance, at 0.015 and not 0.05, the conventional level. This is to account for multiple comparisons being made between AUC's obtained in the same subject on three separate occasions i.e. the Bonferoni correction was applied. Clearly, as the variability in the AUC increases, the power to detect a 20% difference in AUC's decreases.

It was considered appropriate to propose that 15 subjects would be more than adequate to detect a 20% difference in AUC's in the piroxicam interaction study. Using 15 subjects in a paired design meant that even with a 23% $\delta R/R$ value the study would still have an 80% power to detect a 20% difference in AUC's.

TABLE 4.8

PROSPECTIVE POWER CALCULATION

TYPE I ERROR RATE (α)	0.015	0.015	0.015
TYPE II ERROR RATE (β)	0.000	0.096	0.477
DIFFERENCE TO BE DETECTED	20	20	20
VARIABILITY IN PARAMETER	10	20	30
POWER OF STUDY	1.000	0.904	0.523
NUMBER OF SUBJECTS	15	15	15

Subjects are paired and test was two-tailed

4.7 EFFECT OF INCREASING INTERVAL BETWEEN DATA POINTS

In the simulated steady state profile shown in Figure 4.3 there is a 10 hour gap between the last two data points. This interval, in many studies, can be even longer and the effect of altering this time interval on the error in the AUC assessment is considered in this section. Using the three sets of parameters listed before (high, mean, and low) steady state simulations were performed using the proposed sampling schedule (16 data points, interval 14-24 hours). The sampling schedule was altered: 15 data points, interval 12-24 hours; 14 data points, interval 10-24 hours; and 13 data points, interval 9-24 hours. 5, 10, or 15% random error was introduced as before. 50 simulations were performed in each instance, which in total amounted to 1800 simulations performed.

Again there was uniformity across the range of parameters (Table 4.9). At the 5% level of added error, little effect was found as a result of increasing the interval between the two data points. However, this was not the case when 10 or 15% random error was added to the simulations. This is a reflection of the very flat concentration/time profile shown by piroxicam, which, at steady state, shows little fluctuation over a 24 hour period.

TABLE 4.9

EFFECT OF INCREASING TIME INTERVAL BETWEEN PENULTIMATE AND LAST DATA POINTS ON AUC_0^t ESTIMATION ERROR

NO. OF DATA POINTS (PENULTIMATE TIME POINT)	% ERROR INTRODUCED 5	10	15	PARAMETER SET
16(14h)	1.88±0.88	3.51±1.64	5.08±2.26	(Average)
	1.86±0.66	3.66±1.73	5.52±2.58	(High)
	1.75±0.92	3.60±1.88	4.86±2.14	(Low)
15(12h)	2.12±1.13	3.58±1.79	5.51±2.85	(Average)
	1.97±0.92	3.72±1.92	5.74±3.66	(High)
	2.16±1.32	3.83±2.25	5.66±2.94	(Low)
14(10h)	2.26±1.22	3.82±2.07	6.90±3.58	(Average)
	1.97±1.15	3.87±2.35	6.26±3.38	(High)
	2.21±1.30	3.92±2.22	5.91±3.79	(Low)
13(9h)	1.88±1.36	4.86±2.57	6.58±4.47	(Average)
	2.02±1.48	4.86±3.38	6.79±4.14	(High)
	2.19±1.44	5.06±3.66	7.15±3.69	(Low)

4.8 DISCUSSION

This chapter has explored the relationship between the Jackknife technique and study design, with particular emphasis on AUC calculations. The AUC is a parameter that is easily calculated and is therefore very popular. Little regard, however, is paid to its limitations in relation to particular data sets because the confidence with which AUC's are calculated is never stated. It is unjustifiable to employ "model-independent" methods of analysis to present data that is too poor to be modelled by "model-dependent" methods: AUC estimation will also be compromised by poor quality data. However, the AUC estimation error is not obvious when the trapezoidal rule is applied. The Jackknife technique will, however, allow estimation of this error and will also allow certain protocol design features to be specified. This is of particular importance in the design of interaction studies (i.e. prospective analysis).

Furthermore as many interaction studies report a lack of effect as a result of coadministering two drugs, the Jackknife technique could also provide a retrospective assessment of a study's power to detect its particular endpoints (with the number of subjects employed).

CHAPTER 5

A STUDY OF THE CONSEQUENCES OF H_2 RECEPTOR ANTAGONIST COADMINISTRATION WITH CHRONIC PIROXICAM THERAPY IN PATIENTS WITH JOINT DISORDERS

5.1 INTRODUCTION TO CHAPTER 5

As outlined in Chapter 1, NANSAIDs are associated with a wide range of gastrointestinal side effects ranging from mild dyspepsia to frank ulceration and perforation, with the elderly being at the greatest risk from these toxic effects. It has been estimated that one third of all patients receiving these drugs are likely to exhibit some gastrointestinal symptom with up to 10% resulting in discontinuation of therapy. This obviously has important implications for patients with chronic conditions such as rheumatoid arthritis and osteoarthritis.

H_2 receptor antagonists or prostaglandin analogues can be co-prescribed with NANSAIDs to treat and/or prevent recurrence of drug induced gastropathy allowing patients to continue their NANSAID's with a reduced incidence of gastrointestinal side effects. The combination of H_2 receptor antagonists and NANSAIDs, in particular, has become common place in selected groups e.g. those with a previous history of dyspepsia or peptic ulceration, and the elderly.

Cimetidine, however, is implicated in a number of pharmacokinetic drug interactions. It can bind to hepatic cytochrome P450 leading to an inhibition of its metabolic function. This is of particular importance with drugs which have low therapeutic indices such as phenytoin (Bartle et al 1982), warfarin (Hetzel et al 1979) and theophylline (Roberts et al 1981). There is a real risk of overt toxicity.

There may also be a problem with drugs such as piroxicam. Increasing the steady state concentration may lead to increased risk of adverse toxic side effects. Daily doses in excess of 30mg do not confer any extra therapeutic benefit, but toxic effects are more prevalent (Data Sheet Compendium 1990). As the newer H_2 receptor antagonists (nizatidine, famotidine and ranitidine) have lower *in vitro* affinity for cytochrome P450 the same concern may not exist for these agents.

The object of this study was to find out whether the steady state pharmacokinetics of piroxicam or 5-hydroxypiroxicam would be altered by the concomitant

administration of H_2 receptor antagonists. On a theoretical basis, such a combination might lead to elevation of plasma levels of piroxicam, and an increased risk of piroxicam toxicity.

5.2 STUDY DESIGN

A randomised crossover study comparing the effects of the coadministration of either cimetidine or nizatidine on the steady state pharmacokinetics of piroxicam and its major metabolite 5-hydroxypiroxicam was carried out in an appropriate group of patients, namely patients with rheumatoid arthritis or other arthropathies requiring treatment with an NANSAID.

Inclusion Criteria

a) Patients with a joint disorder requiring treatment with an NANSAID

b) No concurrent treatment with an NANSAID

c) Normal renal and hepatic function as assessed by standard biochemical tests

Exclusion Criteria

a) Patients with a past history of peptic ulceration or alimentary blood loss

b) Patients who were receiving treatment with a H_2 receptor antagonist

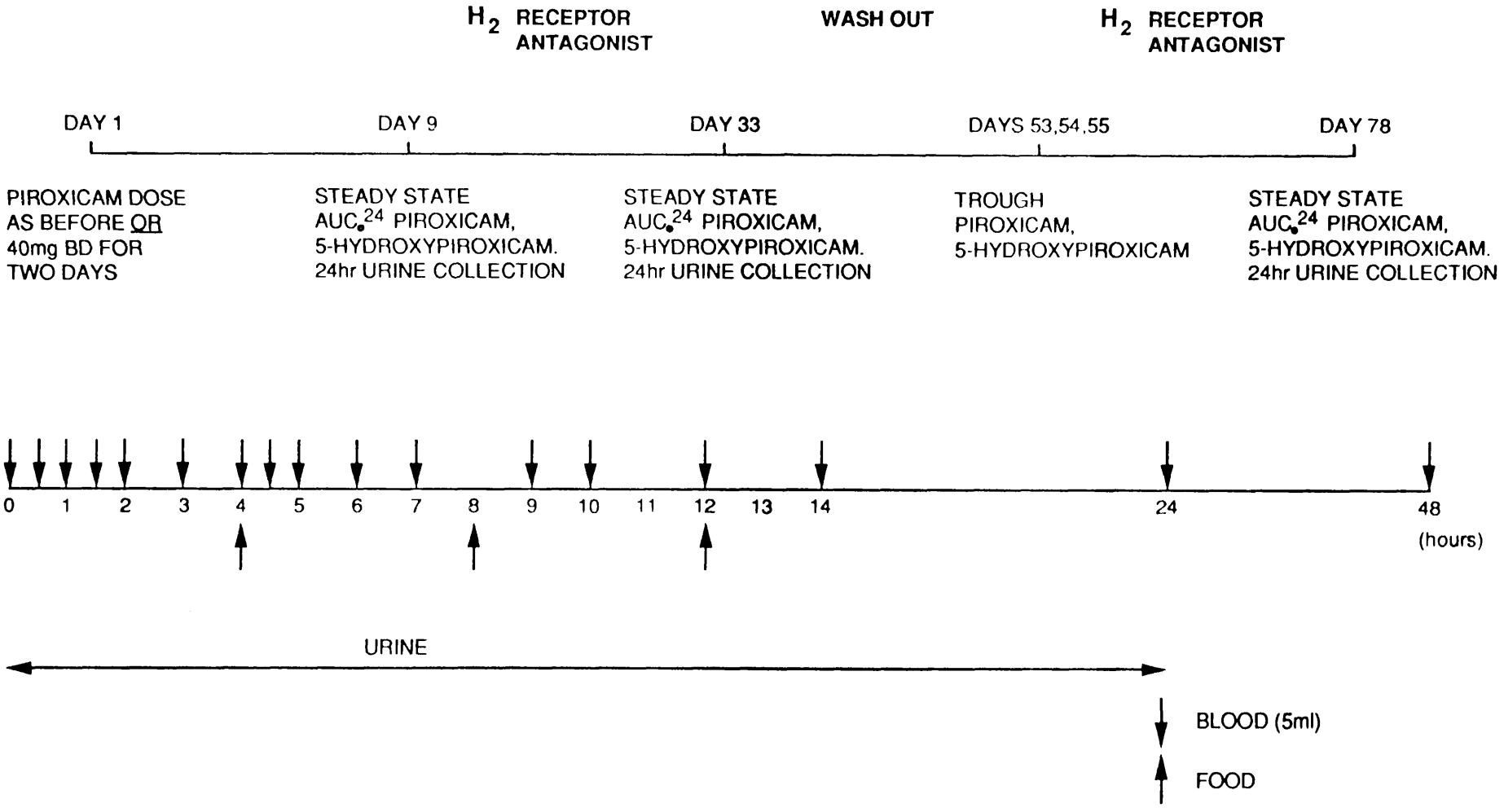
c) Women of childbearing age at risk of becoming pregnant

d) Patients who had donated blood within the previous 6 months

e) Any other significant medical history

Patients already receiving piroxicam were maintained at that maintenance dose throughout the study. If they were not receiving piroxicam, but were considered suitable candidates for inclusion in the study, piroxicam therapy was initiated with a loading dose of 40mg for two days, not less than eight days prior to the first study day. Figure 5.1 shows the study protocol in greater detail. There were three full study days i.e. days 8, 33 and 77. On each study day, after an overnight fast, the patients received piroxicam at time zero. Blood samples (5ml) were taken at 0, 0.5, 1, 1.5, 2, 3, 4, 4.5, 5, 6, 7, 9, 10, 12, 14, 24, and 48 hours. Urine was collected for 24 hours and the total volume noted. Meals were provided at 4, 8 and 12 hours. Their constituents are listed in Table 3.1. Patients were randomised for the order in which they received either cimetidine 400mg b.d., or nizatidine 150mg b.d. Each co-administration phase lasted

FIGURE : 5.1 STUDY DESIGN AND SAMPLING PROTOCOL



for 24 days because any alterations in the steady state concentrations of a drug with such a long elimination half life as piroxicam would be achieved slowly. Between days 35 and 55 there was a wash out period in which no H_2 receptor antagonist was taken. On days 53, 54, 55, trough blood samples were withdrawn allowing comparisons between these trough concentrations and those obtained on the first study day.

Plasma and urine were analysed for both piroxicam and 5-hydroxypiroxicam as described in Chapter 2.

Finally, it was considered crucial that compliance should be monitored. Success in this study depended on steady state levels being consistently maintained. After each treatment phase, the number of tablets or capsules remaining were compared with the expected number assuming 100% compliance. Any value outwith the range $80\% \leq x \leq 120\%$ was considered as non compliance.

5.3 PATIENT DEMOGRAPHIC DETAILS

In Chapter 4, it was shown that 15 patients were considered sufficient to detect any significant (>20%) alteration in either the piroxicam steady state AUC's. One patient who completed the study was excluded from the eventual analysis due to poor compliance (see Section 5.6). All patients had an arthropathy (11 rheumatoid arthritis, 3 osteoarthritis, 1 psoriatic arthritis) which was suitable for treatment by a single NANSAID, as assessed by a Consultant Rheumatologist (P.E.McGill). Twelve females and 2 males eventually completed the study. Table 5.1 shows the concomitant medications each patient received, a number of whom were also prescribed second line disease modifying agents. Five patients had mildly but consistently elevated alkaline phosphatase levels, and another two had consistently elevated gamma glutamyl transferase levels. These raised levels were in the older members of the group.

The mean age was 58 years (range 28-82), the mean weight was 61 kg (range 26-99) and the mean albumin was 40g/l (range 35-48, normal range for hospital 36-52 g/l). Two patients received 10mg piroxicam daily, eight 20mg, and four 30mg daily.

TABLE 5.1

PATIENT DEMOGRAPHIC DETAILS

PATIENT	MALE /FEMALE	AGE	WT. (kg)	DISEASE	ALBUMIN (g/L)	LAB. RESULTS	PIROXICAM DAILY DOSE (mg)	SECONDARY ANTI-RHEUMATIC AGENTS	CONCOMITTANT MEDICATION
1	F	63	67	O.A.	44	Normal	20	NO	TENORETIC O.D.
2	M	62	99	O.A.	48	↑ γ G.T.	20	NO	CAPTOPRIL 50mg B.D. BENDROFLUAZIDE 5mg B.D.
3	F	50	50	R.A.	35	Normal	30	PENICILLAMINE 250mg T.I.D. HYDROXYCHLOROQUINE SULPHATE 200mg O.D. GOLD I/M	MISOPROSTOL 0.2mg T.I.D. PREMPAK-C 0.625 O.D.
4	F	53	51	R.A.	42	Mild ↑ ALK. PHOS.	10	NO	NO
5	F	28	54	R.A.	39	Normal	20	SULPHASALAZINE E/C 500mg Q.I.D.	MARVELON O.D.
6	F	82	68	O.A.	45	↑ γ G.T.	20	NO	ATENOLOL 50mg O.D. ISOSORBIDE DINITRATE 10mg T.I.D. FRUSEMIDE 40mg 1-2 DAILY THYROXINE 0.05mg T.I.D. CYCLANDELATE 400mg T.I.D BETAHISTINE 8mg P.R.N. G.T.N. 0.5mg P.R.N. CO-CODAMOL 2 Q.I.D.

7	F	57	57	R.A.	38	Mild ↑ ALK. PHOS.	30	METHOTREXATE 2.5mg WEEKLY	FERROUS SULPHATE 200mg T.I.D. ASCORBIC ACID 10mg B.D. PARACETAMOL 1g B.D.
8	F	55	75	R.A.	42	Mild ↑ ALK. PHOS.	30	NO	ATENOLOL 50mg O.D. CALCIUM WITH VIT.D. B.D. EVENING PRIMROSE OIL B.D.
9	F	45	81	R.A.	38	Normal	20	SULPHASALAZINE 1g B.D. HYDROXYCHLOROQUINE SULPHATE 200mg O.D.	LISINOPRIL 10mg O.D. PREMPAK-C 0.625 O.D. DIAZEPAM 5mg NOCTE CO-CODAMOL 2 Q.I.D.
10	F	64	70	Psoriatic Arthritis	39	Mild ↑ ALK. PHOS.	20	NO	NO
11.	F	63	26	R.A.	37	Mild ↑ ALK. PHOS.	10	GOLD I/M	NO
12	M	68	67	R.A.	38	Mild ↑ UREA	20	SULPHASALAZINE 500mg Q.I.D.	TIMOLOL 10mg DAILY QUININE SULPHATE 200mg NOCTE
13	F	71	64	R.A.	38	Mild ↑ A.S.T. A.L.T.	20	NO	DIGOXIN 0.125mg DAILY THYROXINE 0.05mg DAILY
14	F	57	67	R.A.	39	Normal	20	SULPHASALAZINE 500mg Q.I.D.	PARACETAMOL 500mg P.R.N.
MEAN ± S.D.		58±13	64±17		40±4 (Normal range 36-52)				

5.4 DATA ANALYSIS

Area's under the plasma concentration time curves were estimated by a combination of both the logarithmic and linear trapezoidal rules (linear from 0 to 14 hours and logarithmic from 14 to 24 hours) for both piroxicam and 5-hydroxypiroxicam . Urinary concentrations of 5-hydroxypiroxicam were determined before and after incubation with glucuronidase enzyme and the percentage daily piroxicam dose excreted in the urine over 24 hours as 5-hydroxypiroxicam was estimated for both pre and post glucuronidase incubation.

Clearance estimates for piroxicam were obtained by dividing the daily dose by the AUC (within the dosage interval), i.e. D/AUC_0^{24}. This value was then divided by the patient's weight in order to standardise the results. Total 5-hydroxypiroxicam renal clearance was calculated using the relationship:

$$\text{renal clearance} = \frac{\text{urine flow x urine concentration}}{\text{average plasma concentration}}$$

During each treatment phase, and during the washout period, the 0, 24 and 48 hour samples corresponded to trough steady state levels. As these trough samples were obtained during two "piroxicam only" phases it was possible to determine whether the original steady state conditions had reformed during the wash out period prior to commencing the second H_2 receptor antagonist.

5.5 STATISTICAL ANALYSIS

Comparisons were made between the following parameters on the three study days:- piroxicam plasma AUC_0^{24}; plasma 5-hydroxypiroxicam AUC_0^{24}; the ratio of these two; the percentage daily dose excreted in the urine as 5-hydroxypiroxicam before and after glucuronidase incubation and the mean of the three trough steady state piroxicam and 5-hydroxypiroxicam concentrations on each study day and during the wash-out period. Logarithmic transformations were performed if considered appropriate to normalise data. These transformations gave rise to the asymmetry seen in some of the Figures. Repeated measures one way ANOVA with Bonferoni correction was used to detect any differences in the mean values.

5.6 RESULTS

The degree of compliance on each treatment phase is shown in Table 5.2 for all patients. Patient No. 15 was outside the limits of between 80 and 120% compliance on two occasions and was excluded from the subsequent statistical analysis.

Table 5.3 and Figure 5.2 show the effect of coadministering nizatidine and cimetidine on the steady state plasma piroxicam AUC's in the remaining 14 patients. No significant difference was found between the mean piroxicam AUC on the three study days. Table 5.4 and Figure 5.3 show the influence of the H_2 receptor antagonists on the steady state plasma 5-hydroxypiroxicam AUC's. Again no statistically significant differences were found between the mean AUC's on the three study days. When the ratio of these two parameters were considered, however, significant differences emerged between the piroxicam only study day and both the piroxicam/nizatidine and the piroxicam/cimetidine study days (Table 5.5, Figure 5.4). The ratio of metabolite to parent drug allowed detection of more subtle inhibitory effects than examination of the individual AUC's. For example, on the nizatidine study day the mean 5-hydroxypiroxicam AUC had decreased, but not to a significant level compared to the piroxicam only study day, while the mean piroxicam AUC had increased, but again not to a significant degree. However, when the ratio of these AUC's was considered, the reduced numerator value (5-hydroxypiroxicam) in addition to the increased denominator value (piroxicam) resulted in a ratio that was significantly lower than that ratio obtained on the piroxicam only study-day. When the cimetidine study day was considered, the decreased ratio appeared to be derived solely from an increased denominator value (piroxicam).

Urinary excretion of 5-hydroxypiroxicam was examined to investigate the influence of alterations in the renal excretion of metabolite on the plasma concentrations. As discussed in Chapter 1, piroxicam is extensively metabolised with 75% of a total dose being excreted in the urine and faeces as either 5-hydroxypiroxicam

TABLE 5.2

PATIENT % COMPLIANCE ON EACH TREATMENT PHASE

PATIENT	PIROXICAM ONLY	PIROXICAM NIZATIDINE		PIROXICAM ONLY	PIROXICAM CIMETIDINE	
	(PIROX)	(PIROX)	(NIZAT)	(PIROX)	(PIROX)	(CIMET)
1	100	100	100	95	100	96
2	100	100	100	100	100	100
3	100	103	100	100	100	100
4	112	100	100	*	100	98
5	100	100	100	100	108	98
6	100	100	100	100	100	100
7	81	97	105	98	100	100
8	96	100	100	100	95	105
9	95	100	82	100	100	100
10	82	100	100	98	100	100
11	100	100	100	110	100	100
12	100	100	102	100	100	98
13	100	100	98	100	100	100
14	100	100	100	100	100	96
15	64	84	88	79	120	104

* No assessment made

TABLE 5.3

PLASMA PIROXICAM AUC's ON THREE STUDY DAYS

PIROXICAM AUC_0^{24} (mg/L/h)

PATIENT	PIROXICAM ONLY	PIROXICAM / NIZATIDINE	PIROXICAM / CIMETIDINE
1	230.04	279.64	380.94
2	167.97	171.59	175.04
3	159.49	147.54	136.82
4	149.37	152.59	153.00
5	316.12	350.27	391.59
6	162.86	132.45	166.83
7	240.81	245.79	221.64
8	232.31	264.67	281.86
9	74.32	69.89	66.90
10	108.07	163.34	194.64
11	92.40	92.91	94.82
12	77.68	74.04	63.70
13	316.97	306.16	341.38
14	193.88	183.19	198.45
MEAN	162.39	167.34	177.68
± 1 S.D.	100.48-262.43	100.48-278.66	99.48-317.35

ANOVA $p = 0.208$

PIROXICAM VS PIROXICAM / NIZATIDINE	N/S
PIROXICAM VS PIROXICAM / CIMETIDINE	N/S
PIROXICAM / NIZATIDINE VS PIROXICAM / CIMETIDINE	N/S

FIGURE : 5.2 PLASMA PIROXICAM AUC'S ON THREE STUDY DAYS

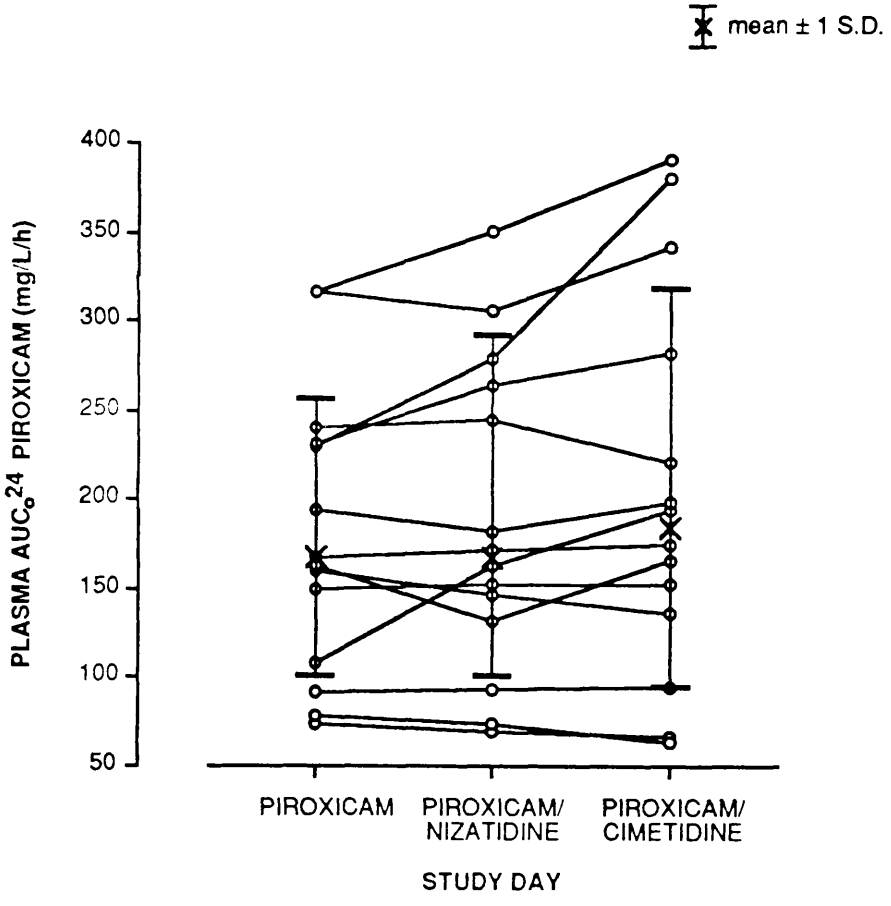


TABLE 5.4

PLASMA 5-HYDROXYPIROXICAM AUC's ON THREE STUDY DAYS

PATIENT	5-HYDROXYPIROXICAM AUC_0^{24} (mg /L/h) PIROXICAM ONLY	PIROXICAM / NIZATIDINE	PIROXICAM / CIMETIDINE
1	45.36	49.66	60.12
2	32.59	28.26	28.52
3	33.18	32.13	23.71
4	37.85	30.42	39.78
5	34.80	26.26	48.47
6	37.68	29.10	30.90
7	49.18	49.88	40.50
8	45.71	50.23	51.78
9	12.14	10.46	9.70
10	32.71	45.27	48.40
11	21.61	21.71	21.26
12	13.46	11.08	10.32
13	44.09	39.99	42.34
14	29.69	25.41	31.00
MEAN	31.19	29.08	30.88
± 1 S.D.	20.29-47.94	21.54-39.23	17.64-54.05

ANOVA p = 0.405

PIROXICAM VS PIROXICAM / NIZATIDINE N/S

PIROXICAM VS PIROXICAM / CIMETIDINE N/S

PIROXICAM / NIZATIDINE VS PIROXICAM / CIMETIDINE N/S

FIGURE : 5.3 PLASMA 5 - HYDROXYPIROXICAM AUC's ON THREE STUDY DAYS

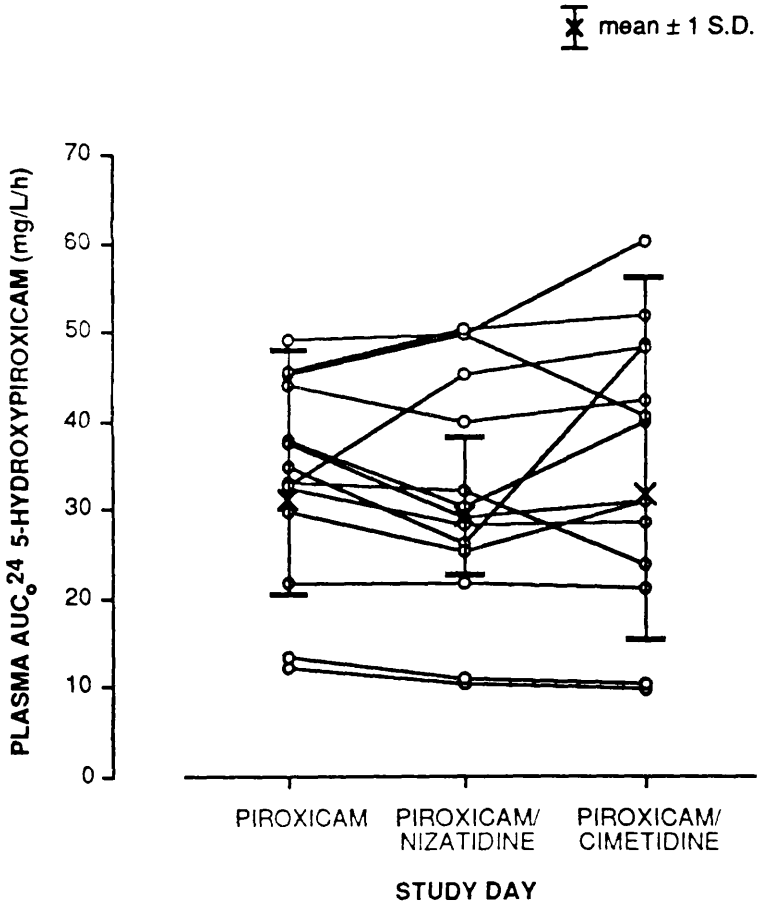


TABLE 5.5

RATIO OF 5-HYDROXYPIROXICAM : PIROXICAM AUC's ON THREE STUDY DAYS

PATIENT	PIROXICAM ONLY	PIROXICAM / NIZATIDINE	PIROXICAM / CIMETIDINE
1	0.197	0.178	0.158
2	0.194	0.165	0.163
3	0.208	0.218	0.173
4	0.253	0.199	0.260
5	0.110	0.075	0.124
6	0.231	0.219	0.185
7	0.204	0.203	0.183
8	0.197	0.189	0.184
9	0.163	0.149	0.145
10	0.303	0.277	0.249
11	0.234	0.234	0.224
12	0.173	0.150	0.162
13	0.142	0.131	0.124
14	0.153	0.139	0.156
MEAN	0.197	0.180	0.178
±1 S.D.	0.148-0.246	0.129-0.231	0.136-0.220

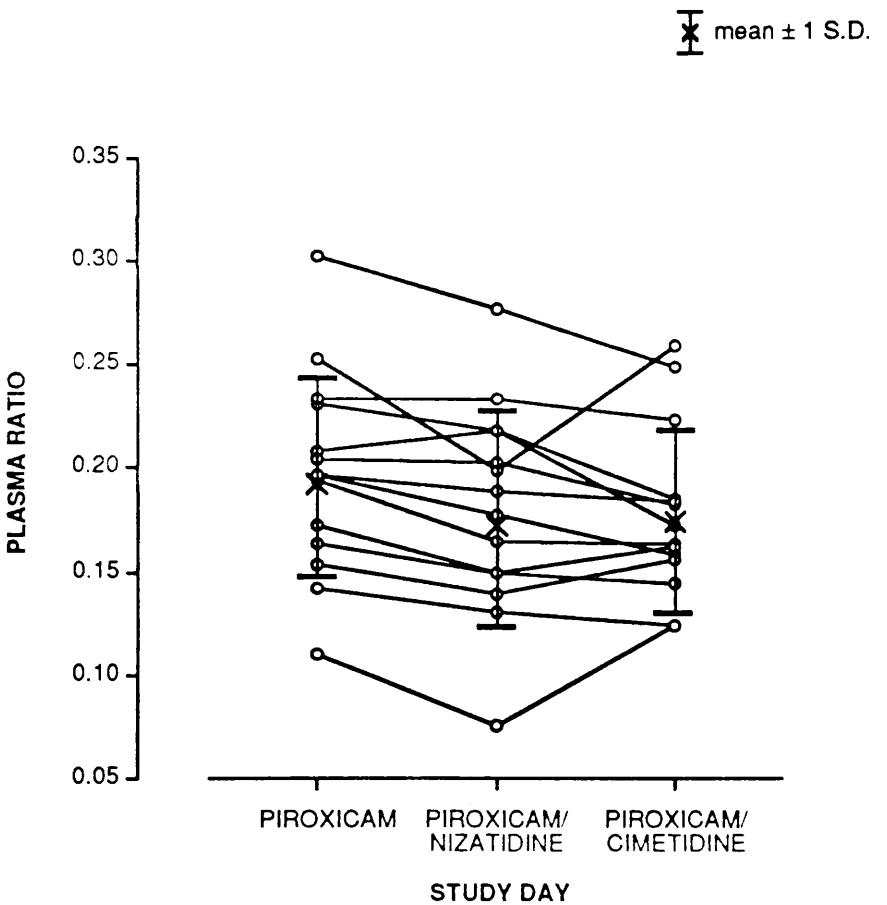
ANOVA $p = 0.007$

PIROXICAM VS PIROXICAM / NIZATIDINE SIGNIFICANT

PIROXICAM VS PIROXICAM / CIMETIDINE SIGNIFICANT

PIROXICAM / NIZATIDINE VS PIROXICAM / CIMETIDINE N/S

FIGURE : 5.4 RATIO OF PLASMA 5 - HYDROXYPIROXICAM : PIROXICAM AUC'S ON THREE STUDY DAYS



or 5-hydroxypiroxicam glucuronide. Since less than 5% is excreted as parent drug in the urine, urinary piroxicam concentrations were not quantified. Table 5.6 shows the results before and after the 5-hydroxypiroxicam glucuronide underwent *in vitro* reverse metabolism to reform 5-hydroxypiroxicam. No statistical differences were obtained in the amount of 5-hydroxypiroxicam excreted as either 5-hydroxypiroxicam or as 5-hydroxypiroxicam glucuronide on each study day. Alteration of glucuronide formation would not be expected due to H_2 antagonist coadministration (Patwardhan et al 1980). Glucuronidation is a type II metabolic pathway, i.e. conjugation, and is not mediated by microsomal enzymes such as cytochrome P450. Figure 5.5 illustrates the total 5-hydroxypiroxicam excreted in the urine on each study day expressed as a percentage of the piroxicam daily dose.

The AUC values shown in Figures 5.2 to 5.5 have not been corrected for dosage. Calculation of clearance will therefore show the true inter-subject variability in this group. Examination of the individual patient's clearance will also allow the determination of any age-related effects. Some authors have described correlations between increasing age and decreased piroxicam clearance (Chapter 1). Table 5.7 illustrates the piroxicam clearance on each study day and Figure 5.6 is a plot of clearance versus age. Figure 5.6 shows that in this group of patients, there is no relationship between age and clearance for this drug.

The renal clearance of 5-hydroxypiroxicam was calculated for each subject on each study day (Table 5.8) and this was plotted against age (Figure 5.7). Again no association existed between age and 5-hydroxypiroxicam renal clearance, consistent with the majority of studies which examined age related phenomena.

Finally when the mean of the three trough (0, 24, 48 hour) piroxicam or 5-hydroxypiroxicam concentrations were calculated on four separate occasions (three study days and the wash-out period) no statistically significant differences were obtained (Tables 5.9, 5.10).

TABLE 5.6.

5-HYDROXYPIROXICAM EXCRETED IN URINE EXPRESSED AS % PIROXICAM DAILY DOSE

PATIENT	PIROXICAM ONLY		PIROXICAM/NIZATIDINE		PIROXICAM/CIMETIDINE	
	5-OHP	5-OHP+ 5-OHPG	5OHP	5OHP+ 5OHPG	5OHP	5OHP+ 5OHPG
1	0.95	13.1	2.25	15.3	1.90	18.3
2	4.40	32.5	4.20	27.3	6.70	30.8
3	3.97	35.8	1.03	20.1	3.43	40.2
4	6.80	10.6	2.20	3.3	3.90	8.3
5	13.55	17.1	2.70	17.4	4.10	16.3
6	22.45	23.5	2.35	14.7	12.80	18.3
7	1.93	18.8	5.29	38.3	4.93	24.4
8	4.63	31.4	10.23	46.2	7.23	30.9
9	12.75	23.6	7.95	30.5	9.20	35.4
10	5.47	22.6	8.69	31.2	6.13	23.9
11	2.90	14.8	0.60	15.1	3.70	22.5
12	8.85	44.9	7.55	49.3	7.40	30.0
13	5.05	27.3	5.20	38.1	8.05	29.8
14	2.30	23.9	3.10	45.4	4.15	35.4
MEAN	5.0	22.6	3.5	23.6	5.4	24.3
± S.D.	2.2-11.6	15.2-33.8	1.5-7.9	11.5-48.4	3.3-8.8	16.1-36.6

	(5OHP)	(5OHP+5OHPG)
ANOVA	p=0.112	p=0.792
PIROXICAM vs PIROXICAM/NIZATIDINE	N/S	N/S
PIROXICAM vs PIROXICAM/CIMETIDINE	N/S	N/S
PIROXICAM/NIZATIDINE vs PIROXICAM/CIMETIDINE	N/S	N/S

FIGURE : 5.5 TOTAL 5 - HYDROXYPIROXICAM EXCRETED IN URINE EXPRESSED AS A % OF PIROXICAM DOSE

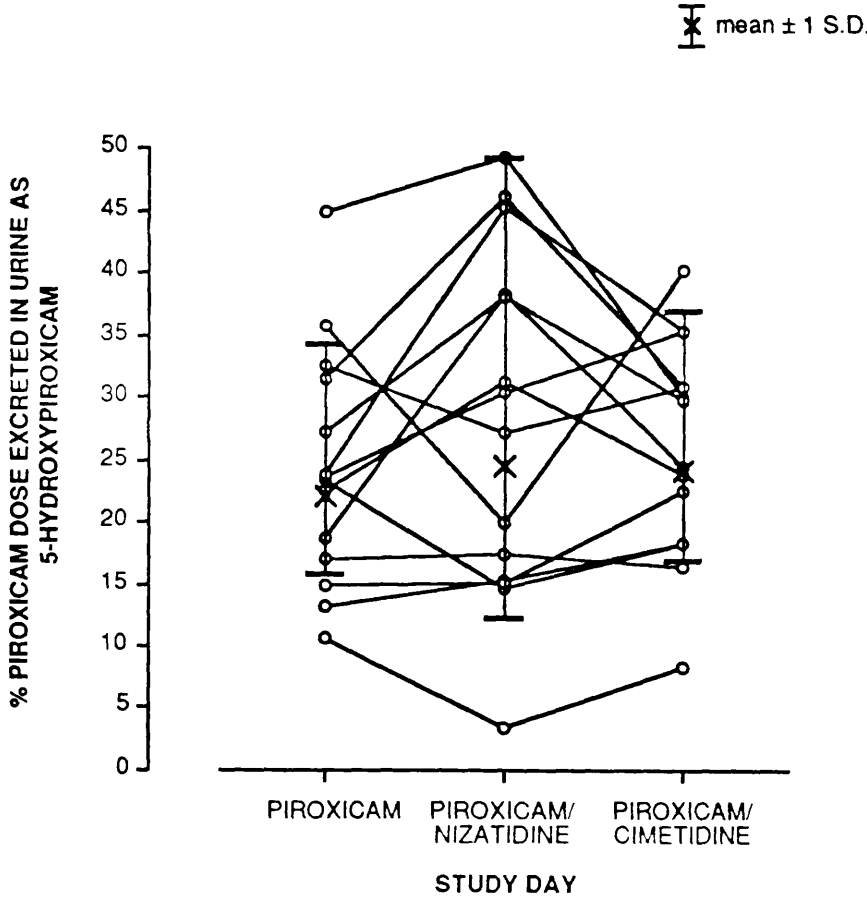


TABLE 5.7

PLASMA PIROXICAM CL/F FOR EACH STUDY DAY

PATIENT	AGE	WEIGHT (kg)	PIROXICAM ($L/h/kgx10^{-3}$)	PIROXICAM /NIZATIDINE ($L/h/kgx10^{-3}$)	PIROXICAM /CIMETIDINE ($L/h/kgx10^{-3}$)
1	63	67	1.30	1.07	0.78
2	62	99	1.26	1.17	1.15
3	50	50	3.76	4.05	4.38
4	53	51	1.31	1.28	1.28
5	28	54	1.17	1.06	0.95
6	82	68	1.81	2.23	1.76
7	57	57	2.18	2.14	2.37
8	55	75	1.72	1.51	1.42
9	45	81	3.34	3.53	3.68
10	64	70	3.97	2.63	2.20
11	63	26	4.19	4.14	4.05
12	68	67	3.83	4.03	4.66
13	71	64	0.99	1.02	0.92
14	57	67	1.54	1.63	1.51

FIGURE : 5.6 INDIVIDUAL PLASMA PIROXICAM CLEARANCES FOR EACH PATIENT ON EACH STUDY DAY

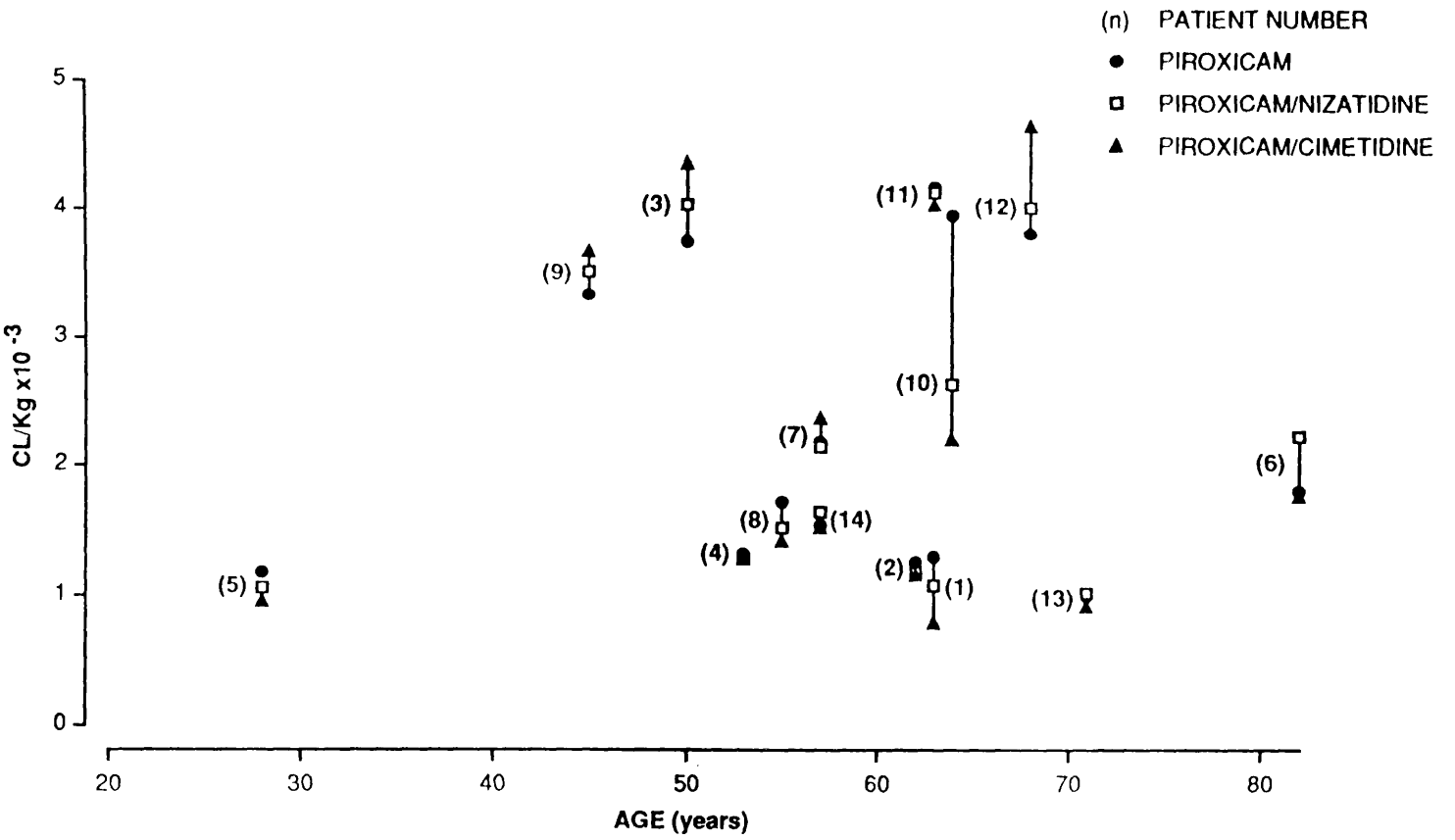


TABLE 5.8

5-HYDROXYPIROXICAM RENAL CLEARANCE FOR EACH STUDY DAY

			PIROXICAM ONLY				PIROXICAM / NIZATIDINE				PIROXICAM / CIMETIDINE			
PATIENT	AGE	WT.	URINE FLOW (l/h)	URINE CONC. (mg/L)	AVERAGE PLASMA CONC. (mg/L)	$CL_R X10^{-4}$ (L/h/kg)	URINE FLOW (L/h)	URINE CONC. (mg/L)	AVERAGE PLASMA CONC. (mg/L)	$CL_R X10^{-4}$ (L/h/kg)	URINE FLOW (L/h)	URINE CONC. (mg/L)	AVERAGE PLASMA CONC. (mg/L)	$CL_R X10^{-4}$ (L/h/kg)
1	63	67	0.050	2.17	1.89	8.5	0.068	1.86	2.07	9.1	0.054	2.82	2.50	9.1
2	62	99	0.038	7.05	1.36	19.9	0.037	6.12	1.18	19.4	0.058	4.39	1.19	21.6
3	50	50	0.036	12.37	1.38	64.5	0.040	6.23	1.34	37.2	0.031	16.20	0.99	101.4
4	53	51	0.016	2.79	1.58	5.5	0.009	1.50	1.27	2.1	0.020	1.73	1.66	4.1
5	28	54	0.036	3.96	1.45	18.2	0.041	3.56	1.09	24.8	0.037	3.68	2.02	12.5
6	82	68	0.067	2.93	1.57	18.4	0.033	3.71	1.21	14.9	0.040	3.79	1.29	17.3
7	57	57	0.044	5.33	2.05	20.1	0.047	10.27	2.08	40.7	0.069	4.44	1.69	31.8
8	55	75	0.046	8.55	1.90	27.6	0.061	9.52	2.09	37.0	0.073	5.29	2.16	23.8
9	45	81	0.085	2.31	0.51	47.5	0.102	2.50	0.44	71.5	0.114	2.58	0.40	90.1
10	64	70	0.066	4.29	1.36	29.7	0.050	7.73	1.89	29.2	0.078	3.81	2.02	21.0
11	63	26	0.047	1.32	0.90	26.5	0.018	3.57	0.90	27.5	0.035	2.65	0.89	40.1
12	68	67	0.042	8.97	0.56	100.4	0.038	10.96	0.46	135.1	0.054	4.34	0.43	81.3
13	71	64	0.036	6.34	1.84	19.4	0.027	11.91	1.67	30.1	0.026	9.54	1.76	22.0
14	57	67	0.047	4.77	1.24	26.9	0.025	15.02	1.06	52.9	0.045	6.56	1.29	34.2

FIGURE : 5.7 INDIVIDUAL RENAL 5 - HYDROXYPIROXICAM CLEARANCES FOR EACH PATIENT ON EACH STUDY DAY

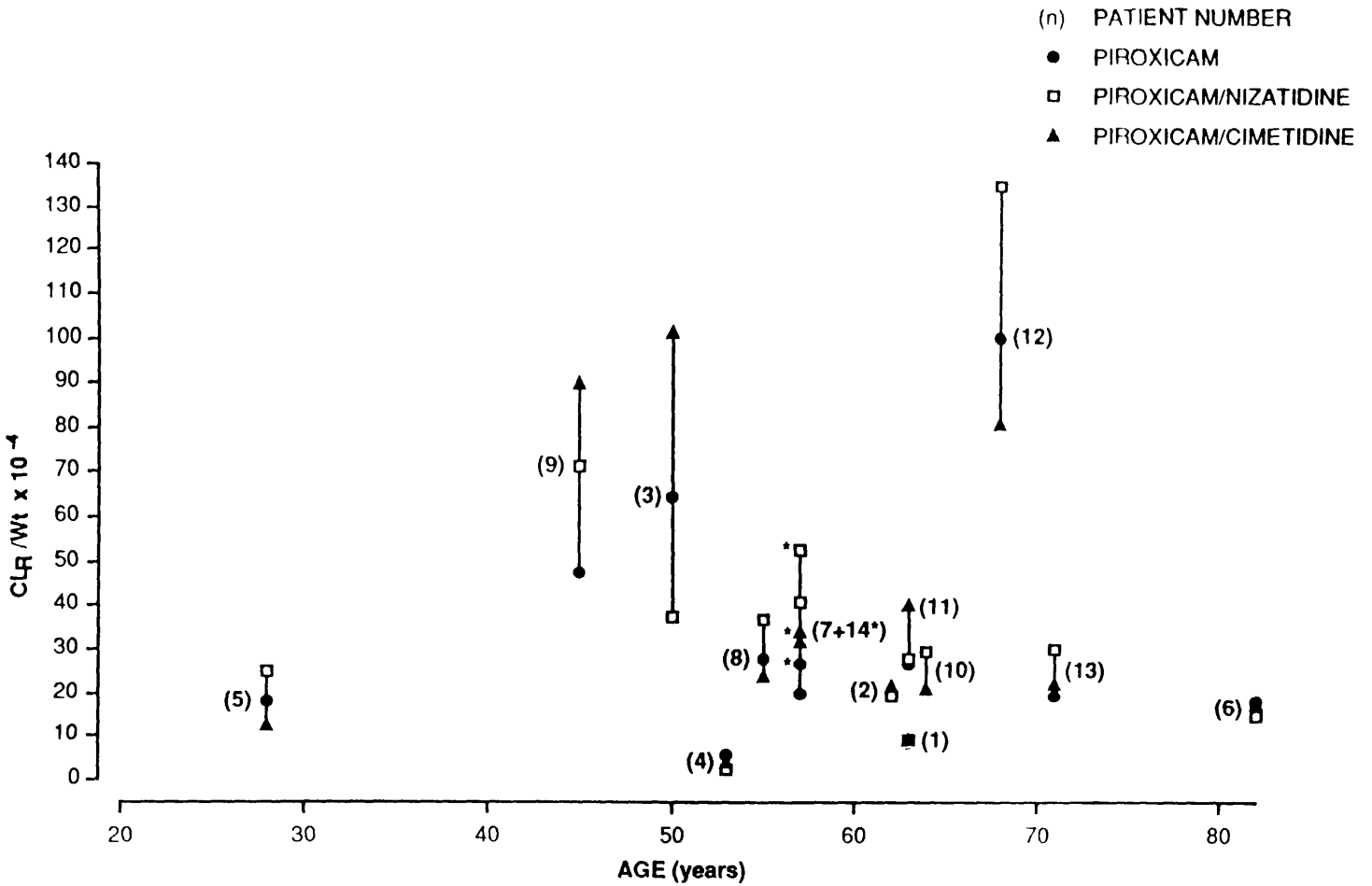


TABLE 5.9

MEAN (n=3) TROUGH PIROXICAM PLASMA CONCENTRATIONS (mg/L)

PATIENT	PIROXICAM / ONLY	PIROXICAM / NIZATIDINE	PIROXICAM / WASH OUT	PIROXICAM / CIMETIDINE
1	9.24	11.19	11.74	14.70
2	6.85	7.15	5.03	6.33
3	7.14	6.11	7.86	5.68
4	5.51	6.19	x	6.09
5	13.56	15.26	19.17	15.78
6	6.13	4.59	4.84	5.58
7	11.46*	10.84	9.42	11.09
8	10.16*	12.95	12.72	13.43
9	2.98*	2.99	3.23	2.70
10	5.20	6.72	6.82*	8.68
11	3.88	3.70	3.24*	3.86
12	2.36	2.21	2.32*	2.16*
13	13.54	13.70	12.68*	15.10
14	7.93	7.48	8.87*	7.77
MEAN	6.69	6.85	6.99	7.18
± 1 S.D.	3.92-11.46	3.79-12.35	3.72-13.17	3.80-13.56

ANOVA p = 0.543

PIROXICAM / ONLY	VS	PIROXICAM / NIZATIDINE	N/S
PIROXICAM / ONLY	VS	PIROXICAM / WASH OUT	N/S
PIROXICAM / ONLY	VS	PIROXICAM / CIMETIDINE	N/S
PIROXICAM / NIZATIDINE	VS	PIROXICAM / WASH OUT	N/S
PIROXICAM / NIZATIDINE	VS	PIROXICAM / CIMETIDINE	N/S
PIROXICAM / WASH OUT	VS	PIROXICAM / CIMETIDINE	N/S

* n = 2

x No assessment made

TABLE 5.10

MEAN (n=3) TROUGH 5-HYDROXYPIROXICAM PLASMA CONCENTRATIONS (mg/L)

PATIENT	PIROXICAM / ONLY	PIROXICAM / NIZATIDINE	PIROXICAM / WASHOUT	PIROXICAM / CIMETIDINE
1	1.94	2.31	2.09	2.48
2	1.45	1.23	1.38	1.29
3	1.39	1.43	1.91	0.93
4	1.59	1.38	x	1.77
5	1.54	1.04	1.84	2.05
6	1.56	1.27	1.29	1.31
7	2.16*	1.92	1.81	1.89
8	1.88*	2.39	2.46	2.47
9	0.46*	0.40	0.54	0.40
10	1.36	1.91	1.96*	2.18
11	0.92	0.88	0.66*	0.84
12	0.49	0.44	0.51*	0.42*
13	1.94	1.82	2.08*	1.98
14	1.11	1.10	1.16*	1.17
MEAN	1.41	1.39	1.52	1.51
± 1 S.D.	0.89-1.93	0.77-2.01	0.87-2.16	0.81-2.21

ANOVA p = 0.329

PIROXICAM / ONLY	VS	PIROXICAM / NIZATIDINE	N/S
PIROXICAM / ONLY	VS	PIROXICAM / WASH OUT	N/S
PIROXICAM / ONLY	VS	PIROXICAM / CIMETIDINE	N/S
PIROXICAM / NIZATIDINE	VS	PIROXICAM / WASH OUT	N/S
PIROXICAM / NIZATIDINE	VS	PIROXICAM / CIMETIDINE	N/S
PIROXICAM / WASH OUT	VS	PIROXICAM / CIMETIDINE	N/S

* n = 2

x No assessment made

5.7 RETROSPECTIVE POWER CALCULATION

In Chapter 4 it was shown that 15 patients were considered sufficient to detect a difference of greater than 20% in the steady state AUC's of piroxicam as a result of H_2 receptor antagonist coadministration. In this section examination will be made of the assumptions used in determining the power calculation, and as a consequence, the validity of the results obtained.

The mean % difference in the piroxicam steady state AUC as a result of combination with cimetidine was 14% for this group of patients. This was statistically a non-significant difference. However, the prospective power determination (Chapter 4) was calculated with respect to the number of subjects required to detect a greater than 20% difference in the steady-state AUC.

A retrospective assessment should be made, of the study's power, using the number of subjects who actually met the study requirements for inclusion in the eventual data analysis.

As no steady state AUC's were determined on two separate occasions an estimate of the variability in this parameter was obtained by examining the mean trough plasma concentrations (Table 5.9). There were two "piroxicam only" phases during the study i.e. the first study day and the wash-out period and these were used to estimate variability. When calculated, a value of 10% was obtained and this acted as a substitute for the intra-subject variability in the AUC.

Table 5.11 lists the retrospective power calculation and Figure 5.8 shows the power curve generated using the above data. With 14 subjects there was a 99% power to detect the 14% difference in piroxicam steady state AUC's. It is therefore possible to be confident that the findings of this study were valid.

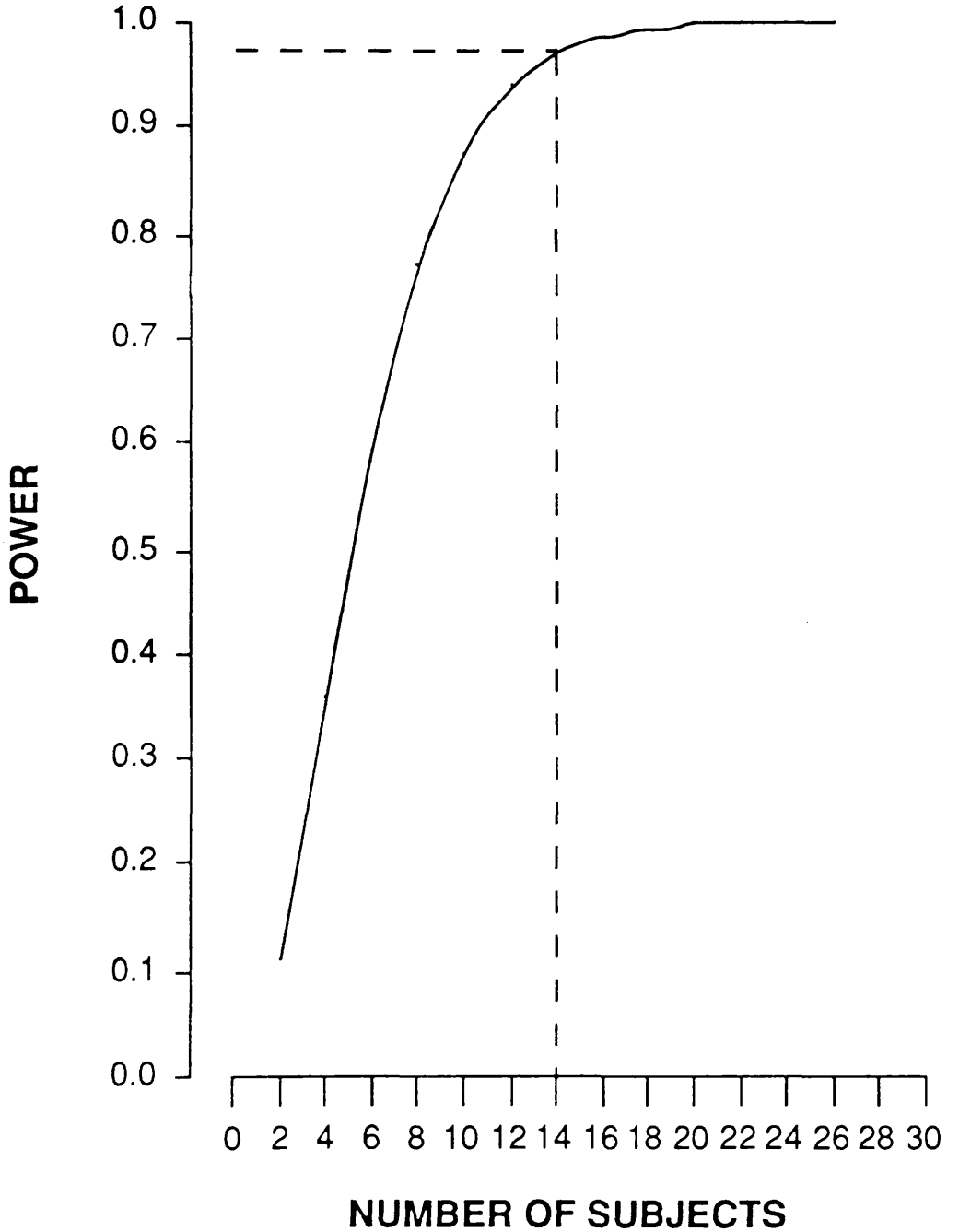
TABLE 5.11

RETROSPECTIVE POWER CALCULATION

TYPE I ERROR RATE (α)	0.015
TYPE II ERROR RATE (β)	0.005
DIFFERENCE TO BE DETECTED	14
ESTIMATED VARIABILITY IN PARAMETER	10
POWER OF STUDY	0.995
NUMBER OF SUBJECTS	14

Subjects are paired and test was two-tailed

FIGURE 5.8 RETROSPECTIVE POWER CURVE



5.8 DISCUSSION

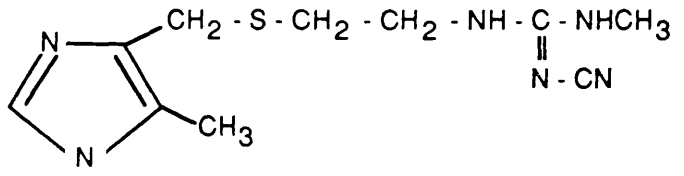
If there was a significant inhibition of piroxicam metabolism by the H_2 receptor antagonists, less metabolite would be formed and the levels of parent drug in the plasma would rise. However, if this was only a mild inhibition, parent and metabolite AUC's might well fail to show a statistically significant difference. The ratio of metabolite to parent drug would, however, will allow the detection of milder inhibitory effects.

It can be seen that, in terms of steady state piroxicam and 5-hydroxypiroxicam AUC's, there was no significant pharmacokinetic interaction, and therefore by implication, no clinically significant interaction between piroxicam and these two H_2 receptor antagonists in the patients studied. Theoretically this is an unexpected result. Piroxicam has been shown earlier to be extensively metabolised principally to 5-hydroxypiroxicam with subsequent conjugation with glucuronic acid. The hydroxylation step is cytochrome P450 dependent, and cimetidine can inhibit this enzyme's metabolic function. Inhibition by cimetidine is rapid and can occur after a single dose. Recovery is equally rapid, and no tolerance to inhibition is apparent on chronic therapy (Speed et al 1982).

Cytochrome P450 has been shown to exist in multiple molecular forms with different but overlapping substrate specificities (Lu 1979). Depending on the nature of the isoenzyme and substrate, the different forms of cytochrome P450 can catalyse the metabolism of a particular substrate at comparable rates but bio-transfer other substrates at significantly different rates (Lu 1979). This is likely to be an important factor when the results obtained in this study are considered.

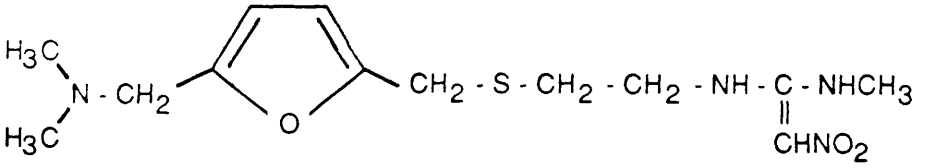
In order to attempt an explanation, examination has to be made of the mechanisms by which cimetidine, and other H_2 receptor antagonists bind to cytochrome P450. Figure 5.9 shows the chemical structure of the four H_2 receptor antagonists currently available in the U.K.. Cimetidine is the only molecule which

FIGURE: 5.9 CHEMICAL CLASSIFICATION OF H_2 RECEPTOR ANTAGONISTS



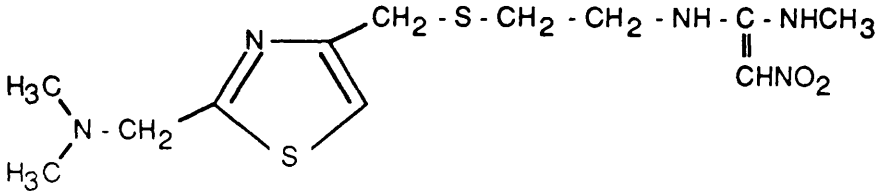
CIMETIDINE

(IMIDAZOLE)



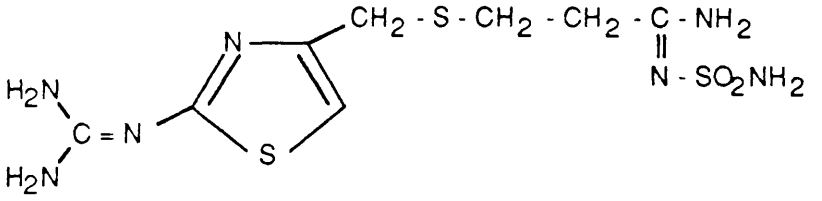
RANITIDINE

(AMINOMETHYL FURAN)



NIZATIDINE

(AMINOMETHYL THIAZOLE)



FAMOTIDINE

(GUANIDINOTHIAZOLE)

contains an imidazole ring and it appears that this moiety can form co-ordinate bonds with haem in the cytochrome P450 molecule resulting in an inhibition of the enzyme's metabolic function (Knodell et al 1982).

Ranitidine can also bind to cytochrome P450 groups but the enzyme-substrate complex formed is less stable than that obtained with cimetidine (Rendich et al 1982, Smith & Kendall 1988). Hoensch et al (1985) showed that both cimetidine and ranitidine could inhibit O-deethylation in human microsomes, but in the case of cimetidine the inhibition was five times more potent. The authors stated that cimetidine was a relatively non specific inhibitor of all cytochrome P450 isoenzymes, whereas ranitidine was more selective. This is probably due to all isoenzymes of cytochrome P450 containing a haem group.

Studies by Reilly et al (1983) have shown cimetidine combined to two distinct and independent classes of binding sites on cytochrome P450. These represent binding via the imidazole ring (which will have a lower affinity) and specific substrate binding via both the imidazole ring and the side chain of the molecule (which will have a higher affinity). Reilly et al (1983) suggested that cimetidine could bind differentially to the various isoenzymes of cytochrome P450.

The other H_2 receptor antagonists have also been shown to bind to cytochrome P450 *in vitro*, but with greatly varied affinities. When Klotz et al (1987) examined cimetidine, oxmetidine, ranitidine, famotidine, and nizatidine, with respect to their inhibitory effects on three human microsomal enzymes, aryl hydrocarbon hydroxylase, 7-ethoxycoumarin-O-deethylase, and 7-ethoxyresorifin-O-deethylase, oxmetidine was the most potent inhibitor, cimetidine was second, and nizatidine third, but ten times less potent than cimetidine. The other H_2 antagonists can therefore cause mild inhibition of cytochrome P450 metabolism due to competitive displacement. However, their binding affinity is much lower than that of cimetidine, and the potential for causing significant interactions is, therefore, reduced.

It is unknown whether the metabolism of piroxicam is specific to one or to a range of isoenzymes (Pfizer UK, Medical Information). It can be assumed, however, that if piroxicam was metabolised via an isoenzyme to which cimetidine bound with a high affinity, then inhibition of the drug's metabolism would be the result. We must conclude, therefore, that the isoenzyme(s) responsible for the metabolism of piroxicam undergo low affinity, non substrate-specific binding to cimetidine, resulting in either mild or no inhibition of piroxicam metabolism.

Piroxicam is not the only drug which, on theoretical grounds, is surprisingly unaffected by cimetidine coadministration. Selective inhibition of specific theophylline pathways has been shown by Grygiel et al (1984). In this study in humans, cimetidine inhibited the 3- and 7-demethylation pathways but not the 8-oxidation, all of which are cytochrome P450 dependent. No effect was seen on tolbutamide hydroxylation, (Dey et al 1983), ibuprofen hydroxylation (Conrad et al 1984), isoxicam hydroxylation (Farnham 1982), naproxen hydroxylation (Holford et al 1981), misonidazole demethylation (Begg et al 1983), mexiletine oxidation (Klien et al 1985), steroid hydroxylation and epoxidation (Somogyi & Muirhead 1987) in humans. Therefore it cannot be assumed that cimetidine is a universal inhibitor of phase I hepatic drug metabolic pathways, and the results obtained in this study would appear to support this contention.

In conclusion, this study showed no significant alteration in the steady state pharmacokinetics of either piroxicam or 5-hydroxypiroxicam as a result of H_2 receptor antagonist coadministration in patients receiving this drug for joint disorders. It must be considered unlikely, therefore, that any adverse events would occur in patients as a result of this coadministration.

CHAPTER 6

GENERAL DISCUSSION

When the general population is considered, the prevalence of peptic ulcer lies between 5 and 10%. Despite an overall decrease in the incidence of peptic ulcer in recent years, rising rates are evident in some groups. Coggon et al (1981) showed ulcer perforation, as determined by hospital admission rates between 1958 and 1977, to be less common in young adults (35 and under) with perforated gastric ulcers occurring at 20% of the previous frequency. However, in middle aged and elderly women there was an increase in the incidence of perforated duodenal ulcers. When Walt et al (1986) expanded the time scale studied (1958 to 1982), an increased duodenal perforation rate was again shown in elderly females. However, in the very elderly an upward trend was also shown in males and the question arose as to what role increased NANSAID use in these groups was having on the ulcer epidemiology. It has been shown in a number of case controlled studies, that the over 60's, in particular, carry an increased risk of ulcer complications (Langman 1989, Jick et al 1987). Collier & Pain (1985) documented a highly statistically significant correlation between the annual number of elderly patients with perforated peptic ulcer taking NANSAIDs and the annual number of prescriptions issued for these drugs. The true incidence rate, however, is difficult to ascertain as it is not known how many individual patients were contained in the total number of prescriptions dispensed, and also by the presence of asymptomatic gastrointestinal upsets which do not allow full quantitation of the extent of the problem with these drugs (Roth 1986).

Piroxicam was one of a whole series of NANSAIDs that came under close scrutiny in the 1980's with regard to their potential to cause gastrointestinal and other toxic side effects. A series of reports in the scientific literature implicated piroxicam as an unsafe drug and one that, if not withdrawn altogether, should have its licence significantly limited. On closer inspection of the data citing piroxicam as a dangerous drug, it appeared that many of the conclusions were not valid. Many papers were flawed due to the reliance on spontaneous adverse reaction reports. Adverse Drug

Reaction (A.D.R.) reporting schemes are biased by many factors, principally their inability to determine the number of patients exposed to a drug, making incidence rates impossible to calculate. Further bias is introduced when a drug is initially marketed, as reporting rates tend to be greater for newer rather than for established agents. The C.S.M. published data which showed that piroxicam could not be distinguished from the other NANSAIDs (apart from low dose ibuprofen) in terms of its overall toxicity profile.

The drug, therefore, has had a controversial history, although its undoubted clinical efficacy, and at the time, unique "once a day" administration, ensured its commercial success and continued use.

In terms of the toxicity controversy, the case against piroxicam was found "not proven". However, the question of whether the drug undergoes enterohepatic circulation, has received a lot of speculation but little experimentation. This question was aroused by the presence of "multiple peaks" in the plasma concentration/time profiles in a number of studies. Many authors felt that enterohepatic circulation would contribute to the drug's long elimination half life in man, although enterohepatic circulation *per se* does not guarantee a prolonged elimination half life. Indomethacin, for example, undergoes well documented enterohepatic circulation, but has an elimination half life in man of only 2-4 hours.

In this thesis, a close inspection has been made of these "multiple peaks" in an attempt to characterise their appearance in terms of physiological events altering the individual's pharmacokinetic disposition of the drug. It was shown that after high protein and fat containing meals, perturbations appeared in the plasma concentration/time profiles in both healthy volunteers and in patients receiving piroxicam. These perturbations were not consistent with the "classical" timing of enterohepatic circulation-related events, generated by bile release following food. The perturbations found in this study followed a consistent pattern, a rapid decline in the

plasma concentration followed by a more sustained rise- certainly not a "peak". When the excretion of the drug and its metabolites into the bile was examined, the results agreed with a previous study examining piroxicam and 5-hydroxypiroxicam concentrations in the bile. In that study (Verbeeck et al 1986) no piroxicam or 5-hydroxypiroxicam was detected 15 hours after a single 20mg dose of piroxicam in a healthy volunteer. However, no information was given regarding the assay technique employed and its limits of detection. Furthermore, no attempt was made to quantify the presence of 5-hydroxypiroxicam glucuronide. The study was also flawed in the respect that only a single 20mg dose of piroxicam was given making detection of small amounts of metabolite difficult.

The inaccessibility of the biliary system makes studies of biliary excretion inherently difficult. This was certainly the case in the current studies. It was considered experimentally desirable to study patients who had not suffered recent surgical trauma (e.g. those with T-tubes inserted after cholecystectomies). It was also considered desirable to employ a technique which enabled total biliary output to be collected (which is certainly not the case with T-tubes). An alternative to T-tubes, naso-biliary drains, inserted to relieve blockages in the biliary system, were thought to be ideal investigational tools but at a practical level, only one suitable patient was identified. Nevertheless, interesting results were obtained. Piroxicam did not enter the bile to any great extent, with the concentrations obtained being only 2% of those achieved in the plasma. When 5-hydroxypiroxicam was considered, biliary concentrations were approximately 33% and 25% of those found in the plasma and in the urine respectively. The most significant result, however, was the finding of 5-hydroxypiroxicam in the form of 5-hydroxypiroxicam glucuronide in the bile, at half the urine concentration. This has been previously unreported, and the biliary route must therefore be considered as a major route of excretion of the principle piroxicam metabolite in man.

When enterohepatic circulation is considered, however, it would appear that piroxicam is not present in the bile in large enough quantities to qualify as a candidate for significant enterohepatic circulation. However, the same cannot be said for 5-hydroxypiroxicam as it is excreted in the bile (as its glucuronide) to a significant extent. The consequences of any 5-hydroxypiroxicam enterohepatic circulation must be considered innocuous as 5-hydroxypiroxicam possesses little anti-inflammatory activity, and by implication, there will be little scope for gastrointestinal toxicity. Only if the metabolite undergoes reverse metabolism to reform parent drug, could this become significant.

The conclusion that the perturbations found in plasma piroxicam concentrations were independent of biliary involvement was substantiated by Guentert et al (1988). Although in this study the coadministration of cholestyramine reduced the elimination half life of the drug (which the authors contended was a result of increased biliary clearance) the "multiple peaks" were not abolished, casting doubt on the role of bile in their generation.

It therefore appears unlikely that bile is involved to any extent in the generation of these "multiple peaks". Investigation was therefore made of other events accompanying the consumption of a meal that may be important in their generation. Consideration was made, on both a physiological and a pharmacokinetic basis, of the alterations to various parameters that may occur after consumption of a meal, with a particular nutritional constituency. A possible role of fat in displacing albumin bound piroxicam was discussed and the consequences of such a displacement on the clearance and volume of distribution of the drug was investigated via simulation studies. From these studies it was predicted that any transient alterations in clearance or volume of distribution, as a result of increasing the unbound fraction of the drug in plasma, would have little or no effect on the total plasma piroxicam concentration, and therefore the consequences of a displacement reaction must be considered trivial. However,

alteration in the plasma volume, as a result of food consumption, may explain the results obtained, and simulations were shown to substantiate this contention. This proposed increase in plasma volume would result in a "dilution" of the total (bound and unbound) piroxicam and metabolite circulating in the plasma, whereas an increased clearance of piroxicam would cause an increased metabolite production. In all cases, the food induced fall in piroxicam concentration was mirrored by a fall in 5-hydroxypiroxicam concentration, suggesting that plasma volume dilution was occurring. It was speculated that the mechanisms involved in this altered volume were a consequence of osmotic events accompanying the consumption of a meal with a high protein content.

It was therefore decided, to standardise both the content and timing of food administration in all future studies.

In the 14 subjects who participated in the H_2 receptor antagonist study, consistent falls in plasma piroxicam and 5-hydroxypiroxicam were detected when the standard lunch was consumed with no alteration in plasma concentrations found after consumption of the standard evening meal. This study examined whether the effects on steady state AUCs of piroxicam due to the coadministration of H_2 receptor antagonists would achieve clinical importance (cimetidine is an established agent known to inhibit cytochrome P450 to a significant extent, and nizatidine is a newer agent with lower *in vitro* affinity for this enzyme). As already described, NANSAID induced gastropathy is a well recognised consequence of both acute and chronic administration of these drugs, and as a result, prophylactic treatments have become the focus of much attention. Only full doses of H_2 receptor antagonists have been shown to heal or prevent ulcer recurrence, with duodenal lesions found to be more likely to respond to treatment. However, it was important to consider the theoretical effects of cimetidine (and nizatidine) inhibition of cytochrome P450 metabolism in patients receiving drugs that are metabolised by this pathway (which includes most NANSAIDs).

Prior to commencing the study, careful consideration was made of the

experimental design. Many studies which examine theoretical interactions fail to show any differences as a result of inadequate power to detect the pre-set differences in the parameter under investigation (e.g. AUC). Furthermore AUC estimations are considered impervious to poor study designs, and as a result, are considered appropriate for any data set. It was important that these factors should be addressed in full prior to commencing the interaction study, so that the subsequent results could be obtained with a degree of statistical confidence. The Jackknife technique was introduced, and examples made of its application to study design optimisation. A prospective power calculation, incorporating an estimated uncertainty component for the steady state AUC determinations, was described. This allowed a conservative estimation of the number of patients required to detect the pre-set endpoints of a greater than 20% alteration in the steady state piroxicam AUC. Investigation was also made of the influence of the number of points used in extrapolation on an overall AUC estimation error. Not surprisingly, as the number of points used in the extrapolation increased, the AUC estimation error fell. Marked improvements were seen when the number was increased from 3 to 5 in many instances. Furthermore, the requirement to logarithmically transform the pseudoparameters was highlighted when few points were used in the extrapolation.

Once the study design requirements were addressed, the interaction study proceeded. Patients receiving piroxicam for a variety of arthropathies, had steady state piroxicam and 5-hydroxypiroxicam AUCs calculated on three separate occasions. One, while receiving piroxicam alone, and the other two while receiving one of the two H_2 receptor antagonists, in a randomised cross over manner. There was no significant alteration in the steady state AUCs of piroxicam and 5-hydroxypiroxicam found in this study, although the ratio of these two (5-hydroxypiroxicam:piroxicam) was significantly decreased in combination with either of the H_2 receptor antagonists. This would indicate a mild, but clinically non-significant interaction which on theoretical

grounds, may be a result of cimetidine and nizatidine binding to the isoenzyme responsible for piroxicam metabolism with low affinity. Certainly there is precedence in the literature to show that cimetidine cannot be considered as a universal inhibitor of phase I hepatic biotransformations.

This study has important implications with regard to the cost effective treatment of NANSAID induced gastropathy. Table 6.1 outlines the relative costs of one month's maintenance treatment with the four H_2 receptor antagonists currently available in the U.K.. The costs are detailed in terms of both in-patients and out-patients, but, the vast majority of patients receiving these drugs would obtain their treatment from their General Practitioners. From Table 6.1, it can be seen that the cost of one month's cimetidine treatment is approximately 33% less than the other H_2 receptor antagonists. There is, therefore, a potential for saving, if prescription habits can be altered. Ranitidine is commonly preferred as it does not to inhibit cytochrome P450 to the same extent as cimetidine. Cimetidine therapy also has been associated with reversible gynaecomastia (2 in 1000 men treated). Certainly if the patient is not receiving a drug that is known to interact with cimetidine to a clinically significant effect (warfarin, theophylline and phenytoin) there is no reason why cimetidine cannot be regarded as the drug of choice in the treatment and maintenance of remission of piroxicam induced gastropathy.

TABLE 6.1

ONE MONTHS MAINTENANCE THERAPY WITH THE H_2 RECEPTOR ANTAGONISTS

DRUG			COST (£) GENERAL PRACTICE	HOSPITAL
CIMETIDINE	400mg	NOCTE	9.34	4.72
RANITIDINE	150mg	NOCTE	14.88	5.86
FAMOTIDINE	20mg	NOCTE	15.00	6.90
NIZATIDINE	150mg	NOCTE	15.11	15.11

Prices - Exclude V.A.T., correct for 11/90

APPENDIX

FORMULAE

1) Linear Trapezoidal Rule

$$AUC = \Sigma\ 1/2\ (Y_1 + Y_2)\ \Delta X$$

2) Logarithmic Trapezoidal Rule

$$AUC = \sum \frac{(Y_1 - Y_2)\ \Delta X}{LnY_1 - lnY_2}$$

3) General Linear Test

$$G.L.T. = \frac{SSQ_{RES(Reduced)} - SSQ_{RES(Full)}}{D.F._{(Reduced)} - D.F._{(Full)}} + \frac{SSQ_{RES(Full)}}{D.F._{(Full)}}$$

4) Akaike Information Criterion

$$A.I.C. = N.Ln(WSSQ_{RES}) + 2.P$$

SSQ_{RES}	=	Residual Sum of Squares (unweighted)
$WSSO_{RES}$	=	Residual Sum of Squares (weighted)
D.F.	=	Degree's of Freedom
N	=	Number of Data Points
P	=	Number of Parameters

PROGRAMME FOR ΔCl, ΔV SIMULATIONS

```
10   DEFINT I-N
20   CL$=CHR$(27)+"E"
50   DT = .01
52   T = 0
100  GOSUB 1000:                                    REM get details
102  OPEN FSPEC$ FOR OUTPUT AS #2
103  GOSUB 4000:                                    REM output details
105  FOR K = 1 TO HOURS
110  FOR I=1 TO 10
120  FOR J=1 TO 10
125  T = T + DT
130  GOSUB 2000:                                    REM get clearance,ke
140  GOSUB 3000:                                    REM get conc
160  NEXT J
165  PRINT#2,USING "Time = ###.##,  Cl. = ##.##,  V = ##.##, Conc = ##.###,
     (Conc = ###.###)";T,CL,V,CONC,SCONC
166  IF ISPEC = 0 THEN 170
168  PRINT USING "Time = ###.##,  Cl. = ##.##,  V = ##.##,  Conc = ###.###,
     (Conc = ###.###)";T,CL,V,CONC,SCONC
170  NEXT I
180  NEXT K
998  CLOSE#2
999  END
1000 REM ************************************* study details
1005 PRINT CL$
1010 INPUT "steady clearance                          (l/hr)  ";CL0
1020 INPUT "delta clearance                           (l/hr)  ";DCL
1030 INPUT "rate constant for cl recovery             (/hr)   ";XCL
1040 INPUT "starting conc                             (mg/l)  ";CONC
1050 INPUT "steady volume                             (l)     ";V0
1052 INPUT "delta volume                              (l)     ";DV
1053 INPUT "rate constant for volume change           (/hr)   ";XVA
1054 INPUT "rate constant for volume recovery         (/hr)   ";XV
1060 INPUT "dose                                      (mg)    ";DOSE
1065 INPUT "absorption rate constant                  (/hr)   ";XKA
1070 INPUT "time (post dose) for food                 (hr)    ";TFOOD
1080 INPUT "time to monitor                           (hr)    ";HOURS
1120 INPUT "Output device.(S=Screen,P=Printer,F=File)[S]      ";A$
1130 ISPEC=0:FSPEC$="scrn:":
1140 IF A$="P" OR A$="p" THEN FSPEC$="lpt1:":ISPEC=1:GOTO 1200
1150 IF A$<>"F" AND A$<>"f" THEN 1200
1160 INPUT "Output file name (incl. drive & ext)              ";FSPEC$
1165 ISPEC=1
1200 GUT = DOSE
1210 SKE = CL0 /V0
1220 SC0 = CONC
1999 RETURN
```

(continued on next page)

```
2000 REM ************************************ get clearance & volume
2010 CL = CL0
2020 IF T < TFOOD THEN  v = v0:v1 = v : goto 2999
2030 CTIME = T - TFOOD
2040 CL = CL0 + DCL * EXP( - XCL * CTIME)
2050 v1 = v
2055 V = v0 + DV * (EXP( - XV * CTIME) - EXP( - XVA * CTIME))
2999 RETURN
3000 REM ************************************* get conc
3005 DGUT = XKA * GUT * DT
3010 DCONC = DGUT / V - (CL/V) * CONC * DT
3020 CONC = v1 * CONC / v + DCONC
3030 GUT = GUT - DGUT
3040 SCONC=SC0*EXP(-SKE*T) + DOSE*XKA*(EXP(-XKA*T)
    -EXP(-SKE*T))/(V0*(SKE-XKA))
3999 RETURN
4000 REM ************************************ output details
4010 PRINT#2,"steady clearance                    (l/hr)  ";CL0
4020 PRINT#2,"delta clearance                     (l/hr)  ";DCL
4030 PRINT#2,"rate constant for cl recovery       (/hr)   ";XCL
4032 PRINT#2,"steady volume                       (l/hr)  ";V0
4034 PRINT#2,"delta volume                        (l/hr)  ";DV
4035 PRINT#2,"rate constant for vl change         (/hr)   ";XVA
4036 PRINT#2,"rate constant for vl recovery       (/hr)   ";XV
4040 PRINT#2,"starting conc                       (mg/l)  ";CONC
4060 PRINT#2,"dose                                (mg)    ";DOSE
4065 PRINT#2,"absorption rate constant            (/hr)   ";XKA
4070 PRINT#2,"time (post dose) for food           (hr)    ";TFOOD
4080 PRINT#2,"time to monitor                     (hr)    ";HOURS
4100 PRINT#2," "
4110 PRINT#2," "
4999 RETURN
```

PRESENTATIONS

1) The Use Of The Jackknife Technique To Estimate Error In AUC Determinations And Study Design Optimisation

P.A.Milligan, A.W.Kelman, & B.Whiting

IV Word Conference On Clinical Pharmacology And Therapeutics
Mannheim-Heidelberg, July 28th, 1989.

2) Enterohepatic Recirculation And Piroxicam

P.A.Milligan, A.W.Kelman, & B.Whiting

IV European Congress of Biopharmaceutics and Pharmacokinetics
Geneva, April 18th, 1990.

3) The Use Of The Jackknife Technique To Determine Error In AUC Estimation

P.A.Milligan, A.W.Kelman, & B.Whiting

XVI Annual Open Meeting Drug Metabolism Discussion Group
University Of York, September 20th, 1990.

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