MECHANISMS FOR ANAESTHETIC MEDIATED CHANGES IN DRUG DISPOSITION

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CONTENTS

Chapter 1

Introduction

Page 17

1.1 Background

1.2 Potential interactions in anaesthesia

1.3 Metabolism

1.3.1 Enzyme function and biotransformation

1.3.2 Action of anaesthetic agents

a) Halothane

b) Enflurane

c) Isoflurane

d) Acute versus chronic exposure

e) Intravenous induction agents

f) Opioids

1.3.3 Summary

1.4 Hepatic blood flow

1.4.1 Anatomy and regulation

1.4.2 Effect of anaesthetic agents

a) Halothane

b) Enflurane

c) Isoflurane

d) Intravenous induction agents

e) Spinal anaesthesia

1.4.3 Summary

1.5 Distribution of drugs

1.5.1 Volume of distribution

1.5.2 Protein binding

1.5.3 Summary

Chapter 2

Hepatic blood flow

Page 50

2.1 Anatomy

2.1.1 Macroscopic anatomy

2.1.2 Microscopic anatomy

2.2 Hepatic blood flow

2.2.1 Total hepatic blood flow

2.2.2 Hepatic arterial flow

2.2.3 Portal venous flow

2.2.4 Reciprosity

2.3 Regulation of hepatic blood flow

2.3.1 Metabolic control

2.3.2 Neuronal control

2.3.3 Hormonal control

2.3.4 Other factors

2.4 Measurement of hepatic blood flow

2.4.1 Techniques

2.4.2 Indirect measurement

2.4.3 Clearance techniques

a) Bromsulphthalein

b) Indocyanine green

c) Other substances

d) Metabolic clearance

e) Dual route administration

2.4.4 Inert gas washout

2.4.5 Fractional distribution

2.4.6 Direct methods of measurement

a) Diversion of flow

b) Electromagnetic flow probe

c) Ultrasonic probe

d) Heat exchange

Chapter 3

Hepatic drug metabolising enzymes

3.1 Introduction

3.2 Drug metabolism reactions

3.2.1 Phase I reactions

3.2.2 Phase II reactions

3.2.3 P450 enzyme system

3.3 Models of hepatic drug metabolism

3.3.1 Venous equilibration model

3.3.2 Parallel tube model

3.3.3 Comparison of the models

3.4 Hepatic drug elimination

3.4.1 Hepatic blood flow

3.4.2 Drug binding

3.4.3 Intrinsic clearance

3.4.4 Elimination of the metabolite

3.5 Assessment of hepatic enzyme activity

3.5.1 Introduction

3.5.2 Antipyrine

3.5.3 Aminopyrine

3.5.4 Other drugs

3.5.5 Endogenous metabolites

3.6 Factors affecting drug metabolism

Chapter4

Studies

Page 111

4.1 Anaesthesia studies with the dog

4.1.1 Introduction

4.1.2 Hepatic extraction and route of administration

4.1.3 Dual route administration

4.1.4 Aim of studies

4.2 Human study

4.2.1 Cimetidine

4.2.2 Enprostil

4.2.3 Aim of study

Chapter 5

Methods

5.1 Dog studies

5.1.1 Outline

5.2 Preparation of study dogs

5.2.1 Surgery

5.2.2 Animal care

5.3 Anaesthesia

5.3.1 Induction

5.3.2 Halothane group

5.3.3 Isoflurane group

5.3.4 Enflurane group

5.3.5 Fentanyl - atracurium - Nitrous oxide group

5.3.6 Monitoring

5.4 Drug administration and sampling

5.4.1 Drug administration

5.4.2 Blood sampling

5.5 Human study

5.5.1 Outline

5.5 2 Subjects

5.5.3 Drugs

5.5.4 Indocyanine green study

5.5.5 Propranolol

5.5.6 Side effects

5.6 Drug analysis

5.6.1 Propranolol assay

5.6.2 HPLC system

5.6.3 Scintillation counting

5.6.4 Measurement of drug binding

5.7 Calculations

5.7.1 Dose of drug injected

5.7.2 Area under time-concentration curve

5.7.3 Clearance

5.7.4 Hepatic extraction ratio

5.7.5 Elimination constant and half-life

5.7.6 Volume of distribution

5.7.7 Hepatic plasma flow

5.8 Statistical analysis

5.9 Ethical considerations

Chapter 6

Thesis Results

Page 151

6.1 Control

6.2 Halothane

6.3 Isoflurane

6.4 Enflurane

6.5 Fentanyl

6.6 Comparison of anaesthesia groups

6.7 Enprostil and cimetidine study

Chapter 7

Discussion

Page 168

7.1 Dog studies

7.1.1 Action on enzymes

7.1.2 Action of anaesthesia

7.1.3 Hypoxia

7.1.4 Critique of methods

7.1.5 Recent studies

7.1.6 Summary

7.2 Human study

Appendices

Page 196

Appendix 1 Pharmacological Abbreviations used in text

Appendix 2 Consent form for Enprostil study

References

Page 201

Tables and Illustrations

Tables

I - Control study pharmacokinetic variables Day 1

II - Control study pharmacokinetic variables Day 2

III - Control study pharmacokinetic variables Day 3

IV - Control study mean values Days 1-3

V - Halothane study pharmacokinetic variables Day 1

VI - Halothane study pharmacokinetic variables Day 2

VII - Halothane study pharmacokinetic variables Day 3

VIII - Halothane study mean values Days 1-3

IX - Isoflurane study pharmacokinetic variables Day 1

X - Isoflurane study pharmacokinetic variables Day 2

XI - Isoflurane study pharmacokinetic variables Day 3

XII - Isoflurane study mean values Days 1-3

XIII - Enflurane study pharmacokinetic variables Day 1

XIV - Enflurane study pharmacokinetic variables Day 2

XV - Enflurane study pharmacokinetic variables Day 3

XVI - Enflurane study mean values Days 1-2

XVII - Fentanyl study pharmacokinetic variables Day 1

XVIII - Fentanyl study pharmacokinetic variables Day 2

XIX - Fentanyl study pharmacokinetic variables Day 3

XX - Fentanyl study mean values Days 1-3

XXI - Comparison of pharmacokinetic variables for all 5 groups

XXII - Placebo study day pharmacokinetic variables

a) subjects 1-5, b) subjects 6-9
XXIII - Cimetidine study day pharmacokinetic variables
a) subjects 1-5, b) subjects 6-9
XXIV - Enprostil study day pharmacokinetic variables
a) subjects 1-5, b) subjects 6-9
XXV - Enprostil study mean values
XXVI - Hepatic blood flow values calculated by the propranolol and ICG methods

Figures

1 - The catalytic cycle of cytochrome P450
2 - Hepatic blood flow, hepatic extraction and hepatic clearance
3 - Control group portal time-concentration curves
4 - Control group IV time-concentration curves
5 - Halothane group portal time-concentration curves
6 - Halthane group IV time-concentration curves
7 - Halothane study changes in Clint, Cls and HPF
8 - Isoflurane study portal time-concentration curves
9 - Isoflurane study IV time-concentration curves
10 - Isoflurane study changes in Clint, Cls and HPF
11 - Enflurane study portal time-concentration curves
12 - Enflurane study IV time-concentration curves
13 - Enflurane study changes in Clint, Cls and HPF
14 - Fentanyl study portal time-concentration curves

15 - Fentanyl study IV time-concentration curves
16 - Fentanyl study changes in Clint, Cls and HPF
17 - Intrinsic clearance for all 5 groups
18 - Change in intrinsic clearance from Day 1
19 - Systemic clearance for all 5 groups
20 - Change in systemic clearance from Day 1
21 - Hepatic plasma flow for all 5 groups
22 - Change in hepatic plasma flow from Day 1
23 - Half-life of intravenous propranolol
24 - Enprostil study oral time-concentration curves
25 - Enprostil study IV time concentration curves
26 - Enprostil study changes in Clint, Cls and HPF
27 - Comparison of HPF calculated by ICG and propranolol methods
28 - Relationship of Cls and HPF

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Summary

Anaesthesia may alter acutely the disposition of drugs in the body. This may be produced by changes in hepatic blood flow, by changes in hepatic drug-metabolising enzyme activity or by changes in the distribution of the drug in the body. The known effect of anaesthesia on these determinants is described. The control of and factors affecting hepatic blood flow are discussed, as are the methods of measuring it. Hepatic drug metabolising enzymes are described. Their activity, mathematical models,methods of assessment and factors altering activity are discussed.

The effect of four different anaesthetics was studied using a canine model with propranolol administered as the test drug. Five days after surgery to insert portal venous andfemoral arterial and venous cannulae, each animal was studied on three consecutive days using dual-route administration of propranolol. That is, simultaneous administration of radiolabelled intravenous propranolol and unlabelled intraportal propranolol. The dogs were studied while awake on the 1st and 3rd days and while anaesthetised on the 2nd day. The anaesthetic agents studied were halothane, isoflurane, enflurane and a fentanyl, atracurium and nitrous oxide technique. Blood samples were taken at regular intervals for

determination of plasma propranolol and plasma labelled propranolol concentrations. The pharmacokinetic variables for each dog were calculated from these concentrations.

All four anaesthetic regimens produced a decrease in the intrinsic clearance (enzyme dependent) of 50-60% reflecting a marked inhibition of the enzyme function. The systemic (intravenous) clearance decreased by some 30-40%. This was due in part to the fall in hepatic blood flow of 25-35% but also as a result of the decreased enzyme activity.

An additional study to evaluate this technique in man was undertaken. This involved a randomised double blind study of the effect on dual-route propranolol (oral and IV) of a known enzyme inhibitor, cimetidine, a prostaglandin analogue, enprostil, which may have altered hepatic blood flow and a placebo. Inhibition of propranolol metabolism was produced by cimetidine but no effect on hepatic blood flow was found with either enprostil or cimetidine.

Chapter 1

Introduction

1.1 Background

1.2 Potential interactions in anaesthesia

1.3 Metabolism

- 1.3.1 Enzyme function and biotransformation
- 1.3.2 Action of anaesthetic agents
  - a) Halothane
  - b) Enflurane
  - c) Isoflurane
  - d) Acute versus chronic exposure
  - e) Intravenous induction agents
  - f) Opioids
- 1.3.3 Summary

1.4 Hepatic blood flow

- 1.4.1 Anatomy and regulation
- 1.4.2 Effect of anaesthetic agents
  - a) Halothane

b) Enflurane

c) Isoflurane

d) Intravenous induction agents

e) Spinal anaesthesia

1.4.3 Summary

1.5 Distribution of drugs

1.5.1 Volume of distribution

1.5.2 Protein binding

1.5.3 Summary

1.1 Background

The practice of anaesthesia involves the acute administration, intravenously or by inhalation, of drugs to produce and maintain sleep. The development and clinical use of anaesthetic agents has demanded that the pharmacology and the physiological effects of each drug be known in great detail. Historically this has led to a close association between anaesthesia and the disciplines of pharmacology and physiology. Early examples of this may be found in the dose-response relationships described by Snow for Chloroform and Guedel's planes of anaesthesia for ether. In a review of the history of pharmacokinetics, Wagner (1) cites contributions from the anaesthetic literature at all stages of the development of this discipline. This included descriptions of the uptake, distribution and elimination of inhalational agents including ether (2,3) and halothane (4,5), and intravenous agents such as thiopentone (6).

Over the years the anaesthetists' knowledge of drugs has paralleled the advances in pharmacological methods. The development of chromatographic systems which are capable of measuring very small concentrations of drug in biological fluids has made the anaesthetist aware of the prolonged elimination time for some anaesthetic

agents and, therefore, the potential for drug interaction in the post-operative period. The ability to determine the changes in plasma concentration of a drug over a period of time has led to the development of mathematical models to describe the distribution and elimination of drugs. These pharmacokinetic principles were applied at an early stage to drugs used in anaesthesia, and, more recently, applied to the design of infusion regimens for intravenous anaesthesia.

The potential for two drugs interacting to produce an inhibition or potentiation of effect has been known for many years. In hypotensive anaesthesia, for example, halothane was used to potentiate the hypotension produced by ganglion blockade and, therefore to minimise the amount of ganglion blocking drug required. However, apart from drug interactions which produce a physiological response, such as changes in blood pressure, heart rate or respiratory rate, the acute action of anaesthesia on other drugs is not well described. This may arise from the assumption that the effect of anaesthesia and anaesthetic agents will be transient and that changes will not persist into the post-operative period. There is little evidence to support this assumption.

The aim of these studies is to determine the effect of anaesthetic agents on drug disposition during and after anaesthesia.

1.2 Potential interactions in anaesthesia

The area of study of this project is the effect of anaesthesia on the hepatic determinants of drug disposition. Accordingly, the effects on renal or pulmonary drug clearance will not be dealt with in detail.

There are three mechanisms affecting drug disposition which may be altered by anaesthesia :-

a) Drug metabolism by hepatic enzyme systems,

b) Hepatic blood flow, that is the rate of delivery of a drug to the liver

c) alteration of the distribution of a drug in the body by changes in volume of distribution or degree of protein binding.

These three determinants will be dealt with separately.

1.3 Metabolism

1.3.1 Enzyme function and Biotransformation of drugs

The biotransformation of drugs describes the process of alteration of the chemical structure and biological properties of a drug by metabolism. The end result of this process is usually a more polar compound which is easier to excrete. Most organic compounds are slowly eliminated from the body if they remain unchanged chemically. The physico-chemical properties of each compound will determine the extent of distribution throughout the body. A highly lipid soluble compound would be excreted slowly by the kidney unless rendered more water soluble and polar by metabolism. Biotransformation does not necessarily inactivate a drug as some drugs require metabolism to activate them and others have active metabolites.

The hepatic enzymes are the main mechanism for removal of drugs from the body. The basic reactions involved are oxidation, reduction, hydroxylation and conjugation. These are usually classified as Phase I (oxidation, reduction and hydroxylation) and Phase II reactions.

Phase I reactions : Oxidation reactions involve the

loss of electrons, usually by addition of an oxygen atom to the substrate, and are catalysed by hepatic microsomal enzymes known collectively as the cytochrome P-450 system or mixed function oxidases. This group of enzymes are discussed below. Reductive reactions involve the gain of electrons and are much less common. Examples of reductive reactions in anaesthesia include the metabolism of azo- and nitro- groups to their corresponding amines as in the breakdown of fazadinium and nitrazepam respectively. The dehalogenation of halothane to trifluoroethane is a reductive process which is normally a minor pathway of metabolism but may be of importance in the development of halothane hepatotoxicity. Hydrolysis of esters and amides by esterases and amidases occurs in many tissues including the liver. Examples in anaesthesia include the metabolism of ester local anaesthetic agents, pethidine and atropine.

Phase II reactions : Conjugation reactions are essentially synthetic. They involve the conjugation of a drug, or drug metabolite produced by a Phase I reaction, to form an inactive, polarised, water soluble compound for excretion. The substrates commonly involved include glucuronic acid, sulphates, acetyl groups, amino acids such as glycine and methyl groups. These compounds are then excreted in the bile or the urine.

P450 enzyme system : The P-450 system, or mixed function oxidase system, is able to metabolise a wide range of substances, ie has broad substrate specificity. The amount and activity of the system may also be increased or decreased by exposure to drugs. Increased activity and concentration of enzymes, induction, is associated classically with barbiturate administration but this response is produced by many drugs and chemicals. The range of enzymes induced varies. Barbiturates produce a generalised increase of enzyme activity but ethanol and cigarette smoke produce a narrower band of increased activity. The activity of the P-450 system is decreased by the administration of cimetidine and other drugs. A more comprehensive description of the P450 system is given in Chapter 3.

1.3.2 Actions of anaesthetic agents on drug metabolism

In vitro , animal and human studies

The effect of anaesthesia and surgery on metabolism of several model compounds has been described. The majority of human studies have been undertaken between 3 and 8 days after anaesthesia and this introduces a series of variables which make interpretation of the results very difficult. This is perhaps best illustrated

in the study by Pessayre and colleagues (7) who reported that short operations, less than 2 hours, caused an increase in antipyrine metabolism, whereas, long operations, greater than 4 hours, caused a decrease in antipyrine metabolism. However, this is only one aspect of their population of patients who had a variety of surgical procedures, different intra- and post-operative drug therapies, different durations of parenteral infusions and a variety of post-operative complications any of which may have an influence on drug metabolism. Nimmo and colleagues (8) demonstrated a decrease in antipyrine half life one week after halothane anaesthesia, suggesting microsomal enzyme induction. The antipyrine half life had returned to the pre-operative control value at four weeks. Duvaldestin and co-workers (9) found a similar decrease in antipyrine half life 4-8 days after halothane or neurolept anaesthesia. The physiological response to surgery results in changes in the haemodynamics and in the protein binding of drugs which would be a major factor in the altered drug disposition found in these studies. It is therefore difficult to attribute all or indeed any of these changes found at 3-8 days after surgery to the action of anaesthetic agents. The field of interest of this project, however is limited to the effects of anaesthesia in the immediate perioperative period and the acute effects of the various anaesthetic

agents are reviewed.

a) Halothane

The effect of halothane on hepatic function has been extensively investigated in relation to halothane hepatitis and has provided some insight to the mechanisms involved in this process. This in turn has established a basis from which to assess the effect of halothane on drug metabolism. In 1971 Brown described the effect of halothane on the ability of isolated rat hepatocytes to metabolize drugs (10). He demonstrated a marked difference in effect of halothane on Type 1 and on Type 2 oxidative reactions. Type 1 reactions, for example aminopyrine metabolism, were inhibited by the presence of halothane whereas some enhancement was shown on Type 2 reactions such as the breakdown of analine. This study concluded that halothane produced an effect on part of the cytochrome P450 enzyme system involved in drug metabolism. In a subsequent study, Brown showed a dose-related, non-competitive inhibition of rat microsomal glucuronyltransferase activity in the presence of halothane, methoxyflurane, chloroform or diethyl ether (11). Halothane has also been shown to selectively inhibit the N-dealkalation of bupivacaine by isolated rat hepatocytes (12). Aune and colleagues (13) in an *in vitro* study demonstrated a dose related inhibition of antipyrine oxidation and, to a lesser

extent, of paracetamol conjugation in the presence of halothane. An *in vitro* study has demonstrated that exposure to halothane produces an inhibition of P450 activity (14).

More recent work using animal models has confirmed these basic observations. Dale and colleagues (15) demonstrated a decrease in NADPH- cytochrome reductase activity in rats following exposure to 0.05 MAC halothane. Fish and Rice (16), again in rats, showed an inhibition of enflurane metabolism, as measured by serum inorganic fluoride production, during simultaneous exposure to halothane and enflurane. The pharmacokinetics of diazepam and its metabolism were altered by halothane anaesthesia in rats (17). In contrast, Berger and colleagues (18) found halothane anaesthesia had no effect on the pharmacokinetics of theophylline in dogs, but in this study sampling may not have been continued for long enough to accurately define the kinetic parameters (19). Wood and Wood (20) found a marked, dose related decrease in the rate of aminopyrine metabolism in rats at 2 and 24 hours after halothane anaesthesia suggesting a much longer effect than had been previously demonstrated. In a study comparing the effect of four different anaesthetic agents on post-operative hepatic function in cirrhotic and non-cirrhotic rats, Baden and colleagues (21) demonstrated a minimal degree of hepatic dysfunction 48

hours after anaesthesia in both groups and with each anaesthetic agent (halothane, enflurane, isoflurane or fentanyl). Halothane has been shown in studies using a dog model to affect the metabolism of several drugs including pethidine (22), lignocaine (23,24), and verapamil (25) and to decrease the extraction of indocyanine green (26). In a sheep model, halothane anaesthesia has been shown to cause a marked but short lasting decrease in the hepatic clearance of pethidine (27) and a reduction in the hepatic extraction and clearance of chlormethiazole (28).

There have been relatively few studies of human hepatic metabolism during anaesthesia. Two studies (29,30) found a decrease in the production of halothane metabolites during halothane anaesthesia, and proposed that halothane inhibited its own dehalogenation. Plasma fentanyl concentrations have been demonstrated in man to be markedly elevated during halothane anaesthesia when compared with a control group (31,32). Fish and Rice (33) demonstrated a similar degree of inhibition of enflurane metabolism in man during concurrent halothane administration as they had previously found in rats. Halothane anaesthesia has been shown to alter the pharmacokinetics of lignocaine in man (34) but not of thiopentone (35).

b) Enflurane

The effects of enflurane on hepatic drug metabolism have been studied less extensively than those of halothane. Aune and colleagues (13) found a similar degree of inhibition of antipyrine and paracetamol metabolism in rat hepatocytes exposed to enflurane or halothane. DaRocha-Reis (36) found no increase in rat microsomal proteins following exposure to enflurane at various concentrations for increasing lengths of time. Dale and colleagues (15) showed exposure to enflurane had little effect on rat microsomal enzyme activity. Wood and Wood (20) found enflurane anaesthesia had no effect on aminopyrine metabolism in the rat and Berger and colleagues (18) found no alteration in theophylline pharmacokinetics in dogs. However, Merin and colleagues found a reduction in the clearance of verapamil in dogs during enflurane anaesthesia.

There are few studies of the acute effects of enflurane in man. Berman and co-workers (37) found altered cortisol metabolism in a group of volunteers immediately following enflurane anaesthesia. They showed a shift in the ratio of the 6-OHF to 17-OHCS metabolites which they suggested was due to induction of hepatic microsomal enzymes. It is possible that this change was due, in part, to inhibition of hepatic clearance of metabolites. Ghoneim and van Hamme (38) found enflurane anaesthesia had no effect on the pharmacokinetics of

thiopentone. Schuttler and colleagues (39) demonstrated that, in contrast to fentanyl anaesthesia, enflurane anaesthesia had no effect on etomidate pharmacokinetics. Oikkonen and colleagues (40) did not find consistent changes in antipyrine metabolism immediately after enflurane or balanced anaesthesia. However, this study included patients who had factors which may have influenced hepatic enzyme activity, such as recent anaesthesia and concurrent administration of drugs known to induce enzymes. Antipyrine clearance and half-life were unchanged 4 and 7 days after enflurane anaesthesia (41).

c) Isoflurane

There are as yet relatively few studies of the effect of isoflurane on drug metabolism. Wood and Wood (20) demonstrated that isoflurane anaesthesia decreased the elimination rate of aminopyrine in rats but to a smaller and shorter lasting extent than halothane. Fiserova-Bergerova (42) demonstrated in rats exposed to sub-anaesthetic concentrations of isoflurane and halothane or nitrous oxide and halothane, that isoflurane inhibited the oxidative metabolism of halothane but enhanced the reductive metabolism. A subsequent study confirmed this effect but demonstrated that it was transient by showing that the metabolism of halothane administered 4 or 24 hours after the

isoflurane was unaffected (43). Nitrous oxide had no effect on halothane metabolism.

d) Acute versus chronic exposure to volatile agents

In a manner similar to the influence of time between exposure to volatile agents and time of testing drug metabolism described above, the duration and extent of exposure to these agents also appears to alter drug metabolism. *In vitro* studies with rat hepatocytes have demonstrated an inductive effect following prolonged exposure to low dose ether (44,45) but no effect following exposure to halothane (45) or methoxyflurane (44). Antipyrine metabolism has been shown to be increased in anaesthetists and theatre personnel regularly exposed to volatile anaesthetic agents (46,47,48). In one of these studies (47) the subjects were not exposed to volatile agents for three weeks and their antipyrine clearance decreased. The biotransformation of halothane by anaesthetists with regular exposure to anaesthetic agents has been shown not to be altered significantly (29,49).

e) Intravenous induction agents

As intravenous induction agents are relatively rarely used alone to provide anaesthesia there are few descriptions of their effect in isolation on drug disposition. The enzyme inducing effect of barbiturates has not been

demonstrated following single exposure to thiopentone or methohexitone during anaesthesia (50).

f) Opioids

Alteration of hepatic enzyme function by opioid drugs has not been demonstrated *in vivo* . An *in vitro* study using human liver microsomes has suggested a competitive inhibition of desipramine hydroxylation by both fentanyl and alfentanil (51).

1.3.3 Summary

Anaesthetic agents have been shown to produce an effect on hepatic microsomal enzyme activity *in vitro* and in animal and human studies. Halothane is the most extensively investigated agent. An inhibitory action on the oxidative metabolism of many drugs has been demonstrated in all three types of study during or following exposure to halothane. The effect of enflurane is defined less clearly. Some studies have suggested a similar effect to that found with halothane but the majority have found enflurane anaesthesia to have little effect on hepatic drug metabolism. There are few studies of the effect of isoflurane anaesthesia but it appears that isoflurane may have a similar inhibitory effect to that of halothane. Chronic exposure to a low dose of

volatile agent appears to cause hepatic enzyme induction. Intravenous anaesthetic agents and opioids have undergone only limited study but do not appear to have a major effect on hepatic enzymes.

1.4 Hepatic blood flow

1.4.1 Anatomy and regulation

The liver receives its blood supply from the hepatic artery and the portal vein. The hepatic artery arises from the coeliac artery and supplies 30-40% of the total hepatic blood flow. The portal vein is formed by the confluence of the superior mesenteric and splenic (hence inferior mesenteric) veins and thus carries blood from all areas of the alimentary tract within the abdomen as well as supplying 60-70% of hepatic blood flow. The hepatic artery and portal vein run in parallel and undergo serial branching throughout the liver. There is no vascular communication between the right and left halves of the liver. The terminal branches of the portal vein drain into the hepatic sinusoids.

Hepatic blood flow is determined principally by hepatic arterial resistance for arterial flow and for venous flow by splanchnic vascular and portal vascular resistance. The control of these determinants appears to be mainly extrinsic. There is, however, a relationship of reciprocity between the hepatic arterial and portal flows which has a hydrodynamic basis. This interaction results in an increased resistance in one circuit in response to an increased flow through the other. It is possible that hepatic haemodynamics are geared towards

maintenance of portal venous pressure rather than portal blood flow. Neural control of hepatic flow is exerted by both the sympathetic and vagal nerves but this is relatively weak when compared with other organs.

The extrinsic controls of hepatic blood flow are principally metabolic in origin and include the portal venous pH, pCO2 and pO2. These substances are all likely to be present after a meal and in part account for the increased blood flow found with increased hepatic metabolic activity.

The anatomy, regulation and measurement of hepatic blood flow are described more fully in Chapter 2.

1.4.2 Effect of anaesthetic agents on hepatic blood flow

Animal and human studies

a) Halothane

The effect of halothane on regional circulation was initially investigated as a possible explanation of hepatic damage following anaesthesia. Following the description of a decrease in splanchnic circulation in man during halothane anaesthesia by Epstein and colleagues in 1965 (52), the same group assessed the effect on hepatic blood flow using indocyanine green extraction (53). They found that halothane anaesthesia

caused a 25-30 % fall in hepatic blood flow from a pre-anaesthetic control value. Using a dog model to measure hepatic blood flow by the disappearance of radiolabelled Xenon injected into the portal vein, Ahlgren and colleagues (54) found portal blood flow decreased with increasing depth of halothane anaesthesia during spontaneous and assisted ventilation. Gelman (55) using a colloidal gold technique to measure hepatic blood flow in man, found halothane anaesthesia in the absence of surgery resulted in a decrease to 84% of control value. When these measurements were repeated during surgery it was noted that body surface surgery cause a further fall to 76% of control whereas upper abdominal surgery resulted in a fall to 42% of the control value of hepatic blood flow. Thulin and colleagues (56) using an electromagnetic flow probe in dogs described a decrease to 50-60% of control value in both hepatic artery and portal vein flow during halothane anaesthesia. They also noted a decrease in splanchnic oxygen flow and uptake of around 50%. Hughes and colleagues (57) also using electromagnetic flow probes in greyhounds found a dose related decrease in hepatic arterial, portal venous and total hepatic blood flows. They showed no change in hepatic arterial resistance or hepatic oxygen consumption. Tranquilli and co-workers (58) using a microsphere technique, demonstrated a dose related decrease in regional blood

flow, including splanchnic but not hepatic arterial flow, during halothane anaesthesia in swine. They noted, however, that the percentage of the cardiac output received by the liver was increased to 162% of the control value. Using a similar microsphere technique in dogs, Gelman and colleagues (59) found a dose related decrease in pre-portal flow during halothane anaesthesia, but that hepatic artery flow was not altered at 1 MAC and decreased at 2 MAC of halothane. Gelman and Dillard (60) found halothane anaesthesia resulted in a 40% rise in hepatic oxygen supply from a pentobarbitone control value. In a study of the reciprocity of hepatic blood flow, that is portal vein and hepatic artery flows, Seyde and Longnecker (61) demonstrated that, in contrast to isoflurane and enflurane, halothane anaesthesia in the rat impaired this mechanism, as assessed by the ratio of hepatic arterial to portal venous flow.

b) Enflurane

Irestedt and Andreen (62) studied the effect of 1 MAC enflurane anaesthesia on hepatic blood flow, electromagnetically measured, in dogs and found decreases in hepatic arterial and portal venous flows to 65-70% of control and no significant effect on hepatic oxygen uptake. They concluded that these changes were primarily due to cardiovascular depression and that

splanchnic blood flow was well preserved. Hughes and colleagues (57) in their study of greyhounds found dose related decreases in hepatic arterial and portal blood flows down to a value of 44% of control at 3% enflurane anaesthesia. In contrast to halothane anaesthesia, hepatic arterial resistance decreased significantly with enflurane. Tranquilli and colleagues (58) in a study of swine found no change in hepatic arterial flow but a marked fall in splanchnic flow during anaesthesia with 4% enflurane. The percentage of cardiac output increased to 133% of control during anaesthesia. Seyde and Longnecker (61) found preservation of the reciprocity of flow in the hepatic artery and portal system in rats during enflurane anaesthesia.

c) Isoflurane

There are fewer studies of the effect of isoflurane anaesthesia on hepatic blood flow than with the other agents. Gelman and colleagues (59) using a microsphere technique in dogs described a dose related decrease in pre-portal flow but a dose independant increase in hepatic arterial flow during isoflurane anaesthesia at 1 and 2 MAC. In a subsequent study, Gelman and Dillard (60) found 1 MAC isoflurane anaesthesia in the dog increased the hepatic oxygen supply by 70% when compared to pentobarbitone aneasthesia. As with enflurane,

isoflurane anaesthesia appears to preserve the reciprocity of hepatic blood flow in rats (61). Isoflurane anaesthesia produced no change in hepatic arterial flow in pigs (63). Lam and colleagues (64) demonstrated preservation of hepatic blood flow, as estimated from indocyanine green clearance, in man during isoflurane induced hypotension. They also found that on recovery of normal blood pressure, but still during anaesthesia there was a significant fall in estimated hepatic blood flow.

d) Intravenous induction agents

The use of intravenous induction agents as the sole method of anaesthesia has not been studied but there are several studies where a combination of nitrous oxide and incremental doses of an induction agent have been used. The effect of nitrous oxide on hepatic blood flow has been studied in dogs by Thomson and colleagues (65), who found a dose related decrease in hepatic arterial and portal venous blood flow, reaching a maximum decrease of 20% with 70% nitrous oxide. Levy and colleagues (66), using disappearance of radioactive colloid to estimate hepatic blood flow, found a decrease of nearly 40% during thiopentone infusion- nitrous oxide anaesthesia in spontaneously breathing patients. In the same study, a mean decrease of 15% was found during thiopentone-nitrous oxide- suxamethonium anaesthesia. Epstein and

co-workers (67) found little change in estimated hepatic blood flow during nitrous oxide anaesthesia following thiopentone induction.

Using a dog model, Irestedt and Andreen (68) demonstrated a 25% fall in total hepatic blood flow during neurolept anaesthesia with droperidol,fentanyl and nitrous oxide.

e) Spinal anaesthesia

Mueller and colleagues (69), in a study of spinal anaesthesia using procaine, described a mean fall in hepatic blood flow of about 20% during low (T10) and a fall of 30% during high (T2-3) spinal anaesthesia. Kennedy and colleagues (70) found that lignocaine spinal anaesthesia to T5 level caused a 23% decrease in hepatic blood flow at 1/2 hour which had recovered to 90% of control at 1 hour.

1.4.3 Summary

Anaesthesia produces a decrease in hepatic blood flow. With the inhalational agents this change appears to be dose related. Halothane anaesthesia causes a dose related decrease in both hepatic arterial and portal venous flow and impairment of the reciprocity of the two flows. Enflurane anaesthesia appears to have less effect on arterial blood flow but at higher concentrations the total flow is lower than with halothane due primarily to

the cardiovascular depressive effect of enflurane. There are fewer studies during isoflurane anaesthesia but it appears that reciprocity is maintained with an overall decrease in total hepatic blood flow but a relative increase in hepatic arterial flow. Decreases of 20-25% in total hepatic blood flow have been demonstrated during intravenous anaesthesia supplimented by nitrous oxide and during subarachnoid anaesthesia.

1.5 Distribution of drugs in the body

The final mechanism by which the disposition of a drug may be altered is by change in its distribution within the body. This may occur by changes in the volume of distribution of the drug or by changes in the extent of protein binding, hence amount of free drug in the plasma.

1.5.1 Volume of distribution

The volume of distribution of drugs has been shown to be altered in disease states. A decrease in the volume of distribution of lignocaine has been demonstrated in patients with congestive cardiac failure, perhaps arising from poor tissue perfusion (71). The effect of renal or hepatic disease on the volume of distribution of neuromuscular blocking agents has been studied. In renal disease, no significant effect on the volume of distribution of d-tubocurarine (72), pancuronium (73,74), atracurium (75,76) or vecuronium (77) has been demonstrated but Ramzan (78) found a significant increase with gallamine. In hepatic disease a less clear picture is found. With pancuronium Duvaldestin (79) and Westra (80) demonstrated a significant increase in volume of distribution in hepatic disease (cirrhosis,

biliary obstruction) but Somogyi (81) found no change. Westra (80) found no change in the distribution volume of gallamine but Ramzan (82) found a significant increase in patients with biliary obstruction. No significant changes have been demonstrated for atracurium (75) or vecuronium (83,84) in patients with hepatic disease.

The acute effect of inhalational or intravenous anaesthetic agents on the distribution of drugs has not been studied extensively (85,86). The apparent volume of distribution of antipyrine was shown to be unchanged in the postoperative period (87). The plasma volume and extracellular fluid volume are decreased after operation and this may influence drug distribution during this period. The majority of studies of drug distribution in the presence of anaesthesia have found no significant or consistant change in the apparent volume of distribution.

1.5.2 Protein- binding

Many drugs are transported in the blood to their site of action bound to plasma proteins. An equilibrium is formed between unbound drug and a reversible drug-protein complex and this relationship obeys the law of mass action. Therefore, a fall in the concentration of unbound drug results in dissociation of the

drug-protein complex to maintain the unbound drug concentration. The pharmacological effects of a drug are produced by the unbound fraction which can cross biological membranes to reach the target organ or receptor much more readily and is also eliminated from the body more easily. Changes in the amount of unbound drug, the free fraction, can result in changes in the pharmacokinetics and in the pharmacological action of a drug. The majority of drugs are bound to plasma albumin, alpha-1 acid glycoprotein (AAG) or lipoproteins. Drugs may bind to more than one protein eg prednisolone which binds to albumin, AAG and transcortin (a lipoprotein). Changes in the plasma concentration of these proteins, by disease or physiological response, will alter the free fraction of a drug. This is discussed below. Anaesthesia may alter the protein binding of a drug by two mechanisms. Drugs used for anaesthesia, or their metabolites, may compete for protein binding sites and displace other drugs. Anaesthesia may influence the production of proteins, especially AAG, and thus alter the total drug binding capacity of the blood.

a) Competition

The displacement of one drug by another competing for the same binding site is characterised by the interaction of phenytoin and warfarin. The introduction of phenytoin therapy to a patient already taking

warfarin results in increased effect of the warfarin because of displacement from the binding site on plasma albumin (88). Albumin has at least three binding sites which have been identified with marker drugs. These sites are characterised by discrete binding of diazepam, digitoxin and warfarin. Some drugs are able to bind to more than one site, eg sodium valproate binds to both the diazepam and the warfarin site (89). Diazepam can be displaced from its binding site on albumin by other benzodiazepines, penicillin derivatives and oral hypoglycaemics (90).

The volatile anaesthetic agents can, at high concentration, cause structural changes in plasma proteins (91). This may alter the affinity of the protein - drug binding. In vitro studies have shown that enflurane, halothane and trifluoroacetic acid (a halothane metabolite) displace diazepam from albumin but do not affect the binding of propranolol or prazocin (92). There is little information on the in vivo effect of anaesthetic agents on drug-protein binding. Sulphaphenazole may displace thiopentone from protein binding sites leading to an enhancement of effect (93). In vitro studies suggest that bupivacaine which is highly protein bound may be displaced from its binding site by diphenylhydantoin, quinidine, pethidine and desipramine (94). If this were to apply in vivo toxic effects may be noted at normal dose ranges as

bupivacaine has a low therapeutic ratio (95). Bupivacaine displaces mepivicaine *in vitro* (96) suggesting an interaction between amide local anaesthetics.

An *in vivo* effect of competitive binding is a possible explanation of the increased induction dose required with the emulsion formulation of propofol when compared with that using the Cremophor formulation (97). An increase in drug binding to liposomes was postulated.

b) Altered binding capacity

The effects of the altered drug binding capacity resulting from the decreased or abnormal plasma protein concentrations in patients with renal or hepatic disease are well known in anaesthesia and are of clinical significance. Significant elevations in free drug have been demonstrated in patients with renal disease for thiopentone (98), etomidate (99), diazepam (100), midazolam (101) and alfentanil (102), and in patients with liver disease for morphine, thiopentone (103,104) and diazepam (105). The marked elevation of AAG concentration seen in patients following trauma and thermal injury leads to increased d-tubocurarine binding and hence to an increased dose requirement in these patients (106).

The effects of anaesthesia on drug binding capacity

have been described following acute but not chronic exposure. Acute exposure studies describe the changes found following anaesthesia and surgery and imply a single exposure to anaesthetic agents. Chronic exposure studies of drug metabolism have been conducted using anaesthetists and operating theatre personnel who are regularly exposed to low doses of anaesthetic agents in their working environment. However, these studies have used antipyrine, which undergoes minimal protein binding, as the model drug and binding changes were not studied.

The effect of surgery and anaesthesia on drug binding has been demonstrated in several studies. Fremstad and colleagues (107) found an increase in the percentage binding of quinidine immediately following surgery which persisted for several days. Feely (108) reported a similar increase in propranolol binding following thyroid surgery. These changes closely paralleled changes in the acute phase reactant proteins, in particular in alpha-1 acid glycoprotein which avidly binds both of these drugs. Alpha-1 acid glycoprotein levels have been shown to be elevated, with resultant alteration in drug binding, during chronic cannulation (109) and in chronic inflammatory disease (110,111).

A more rapid effect on drug binding has been demonstrated during the administration of heparin. Wood and colleagues (112) found an altered binding of

propranolol during heparinisation for cardiopulmonary bypass. Alteration in the extent of binding of several benzodiazepines has been demonstrated in volunteers given heparin (113).

A human study which measured the plasma binding of propranolol and verapamil in a group of patients undergoing cardiac surgery found this was not altered by anaesthesia. The extent of plasma binding of both drugs was not altered by administration of diazepam, fentanyl or halothane but was altered by heparin and cardio-pulmonary bypass (114).

An in vitro study by Dale and Nilsen (92) found that at clinical concentrations enflurane increased the free fraction of diazepam by 60%. The halothane metabolite tri-fluroacetic acid (TFA) at a concentration found post-operatively increased the free fraction of diazepam by up to 90%. Halothane, TFA or enflurane did not significantly alter the binding of propranolol or prazocin.

1.5.3 Summary

There is little evidence that anaesthetic agents produce an acute change in the volume of distribution of other drugs. Competition with other drugs for drug binding sites on plasma proteins has been demonstrated for inhalational, intravenous and local analgesic

agents. The unbound fraction of many drugs including anaesthetic agents is increased in patients with liver or renal disease. In the postoperative period the increase in phase reactant proteins may alter the extent of drug binding.

Chapter 2

HEPATIC BLOOD FLOW

2.1 Anatomy

2.1.1 Macroscopic anatomy

2.1.2 Microscopic anatomy

2.2 Hepatic blood flow

2.2.1 Total hepatic blood flow

2.2.2 Hepatic arterial flow

2.2.3 Portal venous flow

2.2.4 Reciprocity

2.3 Regulation of hepatic blood flow

2.3.1 Metabolic control

2.3.2 Neuronal control

2.3.3 Hormonal control

2.3.4 Other factors

2.4 Measurement of hepatic blood flow

2.4.1 Techniques

2.4.2 Indirect measurement

2.4.3 Clearance techniques

a) Bromsulphthalein

b) Indocyanine green

c) Other substances

d) Metabolic clearance

e) Dual route administration

2.4.4 Inert gas washout

2.4.5 Fractional distribution

2.4.6 Direct methods of measurement

a) Diversion of flow

b) Electromagnetic flow probe

c) Ultrasonic probe

d) Heat exchange

2.1 Anatomy

2.1.1 Gross anatomy

The liver receives its blood supply from the hepatic artery and the portal vein. The hepatic artery arises from the coeliac artery and supplies 30 - 40% of the total hepatic blood flow. The portal vein is formed from the superior mesenteric and splenic (hence inferior mesenteric) veins. The hepatic artery and portal vein run together, undergoing serial branching accompanied by tributaries of the bile duct, throughout the liver. There is no communication between the right and left halves of the liver. The venous drainage is through the three (central, right and left) hepatic veins which join the inferior vena cava separately on the upper surface of the liver just below the diaphragm.

The biliary system is formed within the liver by the confluence of bile canaliculi into ductules which then form the bile ducts. These emerge from the liver as the right and left bile ducts and join to form the common hepatic duct. The cystic duct from the gallbladder joins the common hepatic duct to form the common bile duct which runs behind the duodenum and pancreas and opens

into the second part of the duodenum, about 10cm from the pylorus, at the ampulla of Vater, usually in common with the pancreatic duct.

2.1.2 Microscopic anatomy

The microscopic anatomy of the liver is described classically using the hexagonal, lobular model. Modern descriptions are derived from the structure of the hepatic microcirculation and define the hepatic acinus as the basic unit. An acinus is an irregular parenchymal mass surrounding a terminal portal vein and its accompanying hepatic arteriole and bile ductule. The portal vein is the key structure of the hepatic microcirculation. The portal vein undergoes successive subdivisions, accompanied by hepatic arterial branches, to terminal divisions 20 - 30μm in diameter which have very short side branches opening into the hepatic sinusoids. The sinusoids are lined by endothelial and Kupffer cells and, from them, the blood perfuses the liver parenchyma and drains directly into a branch of the hepatic vein. Kupffer cells (phagocytic endocytes) are part of the reticulo-endothelial system. They play a role in bilirubin production and act as scavengers of the hepatic circulation, phagocytosing particulate matter such as damaged erythrocytes. Hepatocytes are

polygonal epithelial cells, approximately 30um in diameter, which form a single cell plate with one surface facing the sinusoidal space and the other facing the bile canaliculus. The sinusoidal surface is covered in microvilli related to the cell's secretory and absorptive activity. The canalicular surface has an uneven covering of microvilli.

2.2 Hepatic blood flow

2.2.1 Total hepatic blood flow

The liver receives approximately one quarter of the cardiac output, resulting in a total hepatic blood flow of about 1500ml.min^{-1}, that is over 1ml per gram of liver tissue per minute. The intrahepatic distribution of the hepatic artery and portal vein, and the hepatic microcirculation are described above. The hepatic artery normally contributes 30-40% of the total hepatic blood flow.

The total hepatic blood flow is determined by the perfusing pressure (arterial and portal venous pressure) and the resistance. The resistance to flow is determined by three factors : the hepatic arterial resistance, the vascular resistance of the intestinal vessels, and the intrahepatic portal vascular resistance.

2.2.2 Hepatic arterial flow

The mean hepatic artery pressure is about 100mmHg and the arterial bed demonstrates pressure-induced autoregulation. The hepatic arterial flow is subject to some degree of pressure-induced autoregulation

(115-120). The degree of autoregulation is not of major physiological significance and the classic pressure-flow relationship of, for example, the coronary artery has not been demonstrated consistantly (115,117). It has been suggested that hepatic arterial autoregulation may be of greater importance when the liver is active metabolically than in the denervated preparation used for autoregulation studies (121,122).

2.2.3 Portal venous flow

The portal flow does not appear to have direct autoregulation, but is subject to an inflow control from the splanchnic circulation. Portal venous flow accounts for 60-70% of total hepatic flow. The pressure within the portal vein is about 200 mmH2O, falling to 50 mmH2O in the terminal portal veins. Most studies have demonstrated a linear relationship between pressure and flow for the portal system (123-128). The portal vascular resistance increases in the presence of low portal blood flow and decreases at very high flows. This supports the theory that the regulatory mechanism governing portal flow is the maintenance of portal venous pressure. This theory is attractive in that the input into the portal vein can be varied by alterations in the splanchnic blood flow and that the portal vein serves to provide a constant perfusing pressure for the

sinusoids.

2.2.4 Reciprocity

A reciprocity between hepatic arterial and portal venous blood flow has been demonstrated in man (129-133). For example, the reduction of portal flow during haemorrhage is compensated for, in part, by an increase in hepatic arterial flow. This inter-relationship is inadequate to compensate for complete obstruction of one of the flows. It is possible that the portal flow may be governed by changes occurring in the hepatic arterial flow in response to the metabolic oxygen demands of the liver.

2.3 Regulation of hepatic blood flow

The regulation of flow through the liver is subject to metabolic, neuronal and hormonal factors.

2.3.1 Metabolic control

The increase in the metabolic activity of the liver which occurs after eating results in an increase in the total hepatic blood flow (134,135). This is due to, in part, the increase in splanchnic blood flow producing an increase in portal flow (135,136), and in part to an increase in hepatic arterial flow (134). A similar pattern is seen during enzyme induction (137,138). Both of these changes are accompanied by an increase in both portal and arterial oxygen extraction. The stimulus to the increase in flow may be a local metabolic change resulting from the increased oxygen demand. The effect of alterations in arterial and portal oxygen and carbon dioxide tensions has been described in animal studies. In the dog an arterial pO2 of 5.8kPa had no detectable effect on arterial or portal flow, but severe hypoxia (paO2 3.3kPa) produced a decrease in hepatic arterial flow and an increase in hepatic arterial resistance (139,140). An increase in portal venous oxygen content caused an increase in hepatic arterial resistance (130)

but an arterial pO2 of 53kPa had no effect on hepatic blood flow (140). Also in the dog, a moderate increase in arterial pCO2 (8.6kPa) produced a small rise in arterial and larger rise in portal venous blood flow (139). A similar effect on flow was seen with hypercapnia and acidosis (pH 6.8) (129). Hypocapnia and alkalosis cause a fall in arterial and portal flow (129,139). Decreasing the pH of portal blood produced a marked increase in portal resistance and a smaller decrease in arterial resistance (130). It is likely that changes in the portal rather than systemic pO2, pCO2 and pH are a more important influence on hepatic blood flow.

2.3.2 Neuronal control

The hepatic vasculature is known to receive innervation from both the sympathetic and parasympathetic systems. It seems likely that stimulation of the vagus nerve has little effect on hepatic blood flow in the dog. It may be that vagal stimulation alters the microcirculation without affecting the overall flow. An increase in sinusoidal diameter during vagal stimulation has been demonstrated in the rat (141). Stimulation of the sympathetic peri-arterial nerves, in the cat (142) or dog (143-145), causes arterial constriction, suggesting that this is an important regulator of hepatic vascular tone. The same

frequencies of stimulation also cause an increase in portal pressure and portal vascular resistance in both the cat (142) and dog (144,145). Sympathetic stimulation also causes a slower, sustained contraction of the capacitance vessels within the liver with resultant decrease in hepatic volume (146). In this way the liver acts as a reservoir for blood which can be released into the ciculation in a controlled manner during periods of sympathetic stimulation. In the dog a maximum of 60% of the hepatic blood volume can be released (147).

2.3.3 Hormonal control

The hepatic and splanchnic vascular bed contains both alpha- and beta- adrenergic receptors. Alpha-adrenoreceptor stimulation resulting from an infusion of noradrenaline reduces total hepatic blood flow by hepatic arterial and portal venous constriction in both the awake and anaesthetised dog (148-150). This effect is present during infusion of noradrenaline to therapeutic concentrations but such concentrations will not be reached by endogenous noradrenaline release. The effect can be blocked by alpha-adrenoreceptor antagonists (133). Beta-adrenoreceptor stimulation produces a hepatic arterial vasodilation but has no effect on portal vascular resistance which indicates an absence of beta-adenoreceptors in the portal vein

(123,151,152,153). The effect of adrenaline at basal physiological concentrations is to cause hepatic arterial vasodilation and increase splanchnic flow and, therefore, portal flow (154). An increase in adrenaline concentration would tend to increase hepatic blood flow, but this may well be accompanied by an increase in sympathetic nervous activity which has an arterial and venous constrictor effect (155). The resultant total effect will depend on the balance of these two mechanisms. The beta-adrenergic agonist isoprenaline has been shown to cause hepatic arterial vasodilation (120,123). This can be attenuated by beta-adrenergic blockade with propranolol (120,123). Propranolol does not affect the portal venous constrictor effect of noradrenaline (152) and intensifies the arterial constrictor effect of mixed alpha- and beta- adrenergic agonists such as adrenaline (133,156). Beta-adrenergic blockade with propranolol does not alter either hepatic arterial or portal venous resistance, but a transient increase in hepatic arterial resistance following 0.1mg/kg IV in the dog has been described (151). An overall reduction in hepatic blood flow of 10-20% has been described during beta-adrenergic blockade, due mainly to the decrease in cardiac output.

2.3.4 Other factors

Hepatic blood flow is increased after eating and this is due to increased flow in both the hepatic arterial and the splanchnic (portal) systems. Several mechanisms appear to be responsible for this postprandial hyperaemia, including : increased osmolarity of the portal venous blood (157), bile acids and salts (158) and a number of gastrointestinal hormones (159). None of the hormones administered alone exerts sufficient effect on hepatic blood flow to be considered a regulator of flow but in combination they probably produce the hepatic arterial vasodilation seen after eating (160). Glucagon causes hepatic arterial dilation and antagonises hepatic arterial vasoconstriction (161-163). It is likely that these properties are of importance when glucagon is released during stress in that they will maintain hepatic arterial flow to meet the increased oxygen demands of glycogenolysis (164).

The splanchnic circulation, hence the portal flow, is influenced by respiration, intra-abdominal pressure, gravity and by gut wall activity. During exercise there is a marked decrease in portal flow due to splanchnic vasoconstriction.

2.4 Measurement of hepatic blood flow

2.4.1 Techniques

The techniques for measurement of hepatic blood flow can be classified broadly into direct and indirect methods.

The direct methods are, in general, more invasive and include the application of electromagnetic flow probes and heat exchange thermocouples. They have been implanted both acutely and chronically in animal preparations to measure separately both portal and arterial flow. The use of such probes in humans is limited to the acute intra-operative situation, such as during abdominal surgery. The indirect methods have been applied to clinical research more widely and a fuller description of the indirect methods will be given.

2.4.2 Indirect measurement

The indirect methods of measuring hepatic blood flow have the advantage of being less invasive but the majority produce only an estimate of total hepatic blood flow. A further disadvantage of these methods is the inability to differentiate between the portal and

arterial flows. These techniques are dependent on removal of a marker substance from the blood by the liver. The most commonly used is the clearance method which is based on the Fick principle and hepatic blood flow is taken as the inverse of the arterio-venous difference in concentration across the liver.

2.4.3 Clearance and hepatic blood flow

Estimation of hepatic blood flow has been made by relating the rate of extraction or clearance of a substance from the blood by the liver to the blood flow through the liver. Such calculations are based on the Fick principle which states that, at steady state, the amount of a substance taken up by an organ is equal to the product of the arterio-venous difference in concentration of the substance and the blood flow through the organ.

$$\text{blood flow rate} = \frac{\text{amount removed/unit time}}{\text{arterio-venous difference}}$$

a) Bromsulphthalein

The use of this principle to estimate hepatic blood flow was described in 1945 by Bradley and colleagues (165). They measured the removal of bromsulphthalein (BSP) by the liver during a constant rate infusion. Samples of arterial and hepatic vein blood were taken to

measure a-v difference. It was assumed that the arterial concentration of BSP was constant and therefore that the rate of removal of BSP by the liver was equal to the rate of infusion. Thus ;

$$\text{H.B.F.} = \frac{\text{rate of infusion of BSP}}{\text{arterial - venous BSP conc.}} \times \frac{1}{\text{Haematocrit}}$$

As BSP is cleared only from the plasma this method actually measures hepatic plasma flow and correction using the haematocrit must be made to estimate hepatic blood flow.

This method makes several assumptions including : that the arterial concentration is constant, that only the liver removes BSP from the blood and that there is no enterohepatic circulation. In certain circumstances each of these may not hold true and may introduce error into the estimation.

The properties which are desirable in an indicator substance used for estimation of hepatic blood flow with this method include :

1. Rapid and complete mixing with the blood. This is of particular importance when the single injection technique is used as described below.
2. Removal from the blood only by the liver
3. No entero-hepatic circulation
4. A high rate of extraction from blood by the liver

which follows a first-order, concentration independent process.

5. No systemic or toxic effects.
6. Small volume of injection relative to blood volume

Bromsulphthalein does not fulfill all of these criteria. A small amount (<10%) of BSP undergoes extrahepatic extraction and a small amount of enterohepatic circulation may occur (166). The extraction of BSP can vary and may be less than 10% in the presence of a raised serum bilirubin (167).

b) Indocyanine green

The dye indocyanine green (ICG) is used more commonly for estimation of hepatic blood flow. ICG has a high hepatic extraction ratio and is removed almost exclusively by the liver. There is minimal enterohepatic circulation (168). Two different methods of administration have been used for ICG to determine hepatic blood flow (169). The method of continuous infusion described for BSP has been used, but as this requires cannulation of the hepatic vein, a simpler, less invasive method has been devised. This involves bolus injection into a central or peripheral vein and sampling from the systemic circulation. This technique has been used to measure cardiac output and arterial sampling is required. However, for measurement of

hepatic blood flow peripheral venous sampling is used usually (170).

Hepatic blood flow can then be estimated by calculating the total blood volume and the exponential rate of elimination (k) of ICG from the blood :

$$HBF = k \times \text{blood volume}$$

An alternative method calculates the slope (K) of the plot of the log of plasma ICG concentration against time and the extrapolated zero time concentration (Cp0) from this plot :

$$HBF = \frac{K.\ \text{Dose of ICG}}{Cp0} \quad X \quad \frac{1}{\text{Haematocrit}}$$

This method which essentially calculates the plasma clearance of ICG which is considered to be equivalent to hepatic plasma flow. This value must then be corrected by the haematocrit to obtain blood flow. This method is described more fully in the Methods section. A disadvantage of ICG is that is must be measured immediately as it is not stable at room temperature and deteriorates with time even when stored frozen (168).

The term clearance used in association with bromsulphthalein and indocyanine green is taken to describe the efficacy with which the liver removes the substance from the blood and does not imply any metabolic process. Both substances undergo two

processes, uptake from plasma and excretion in the bile, which require normal hepatic function. BSP also undergoes conjugation before excretion. In patients with hepatocellular damage or biliary obstruction the clearance of both ICG and BSP may be impaired.

c) Other substances

A similar form of clearance measurement can be made using colloids which are taken up avidly by the Kupffer cells. This method, described originally in 1952 (171,172), is based on the ability of Kupffer cells to remove colloidal particles of a certain size on first-pass. Therefore, with an extraction ratio of virtually 100% the need for hepatic vein sampling is avoided and the single injection technique described for ICG can be used. A number of radio-labelled substances such as P32 labelled chromic phosphate, I131 labelled albumin and colloidal gold (Au198) have been used to measure hepatic blood flow (172,173). The particle size is critical and false values may be obtained due to recirculation of small particles. This technique still requires blood sampling for counting the disappearance of the radioactivity. An alternative version of this technique involves external counting over the forehead or calf (174,175). This has been found to have a similar rate of disappearance (k) of radioactivity. However as

hepatic blood flow is equal to k times blood volume, blood volume must be calculated. The disadvantages of these methods include the expense and limitations on repeated measurement. The phagoctosing activity of Kupffer cells may be less affected by liver disease than hepatocyte activity and, therefore, a better estimate of flow may be obtained with this method in the presence of hepatic disease.

d) Metabolic clearance

The clearance of substances which require an hepatic enzymatic process for their elimination have been used to estimate hepatic blood flow. The use of this technique (176) is based on the venous equilibration model of hepatic drug clearance, which is described in Chapter 3. The clearance of a substance metabolised by the liver can be either enzyme or perfusion limited. An enzyme limited clearance occurs when a drug is of low hepatic extraction, that is, only a small amount of that presented to the liver is removed on each pass, and clearance is therefore independant of hepatic blood flow. Perfusion limited clearance occurs when the drug is removed in the liver by a process with a large reserve of enzyme of high affinity for the drug. This results in most of the drug being removed on a single pass through the liver and the rate of clearance being

determined by the amount presented to the liver, that is, the hepatic blood flow.

The measurement of hepatic blood flow based on this concept compares the oral and intravenous administration of the same dose of a drug. The drug must be absorbed completely from the gut and metabolised only in the liver. The hepatic blood flow can be calculated from the dose of drug and the difference in area under the time-concentration curves for the oral and intravenous administration.

$$\text{Hepatic blood flow} = \frac{\text{Dose}}{\text{AUCiv} - \text{AUCo}}$$

The drug is given on two separate occasions, once orally and once intravenously. This technique has been described in man (177,178). However, its use has been limited by the assumptions required to apply this method which influence its accuracy. The most important of these are that all the drug is absorbed when given orally and that the basal conditions of hepatic blood flow and enzyme activity are the same on the two study periods.

e) Dual route administration

A more accurate development of this is the dual route technique which is described in the Methods. This technique applies the the same model described above but

the drugs are administered simultaneously orally and intravenously (179). To differentiate between the two routes one dose is radiolabelled. This allows a smaller dose to be given by the oral route than by the intravenous which is appropriate for a drug of high first pass metabolism. Hepatic blood flow is then calculated from the formula

$$HBF = \frac{DOSEo.DOSEiv}{AUCiv.Do - AUCo.Div}$$

The derivation of this formula is given in the Methods.

2.4.4 Inert gas washout

As the name implies this technique measures the elimination of an inert gas injected into the portal system. Radio-labelled gas,such as 85Krypton (180) or 133Xenon (181), is injected into a tributary of the portal vein or into the hepatic artery and its passage through the liver (washout) recorded by a gamma camera placed externally over the liver (182). This method requires an operative procedure which limits its use in patients to those undergoing laparotomy or to animal studies. A less invasive form of this technique has been developed which involves inhalation of a trace dose of 133Xenon in oxygen in a closed system to equilibration (183,184). This occurs rapidly (<5min) due to the relative insolubility of xenon. A scintillation counter

measures the radioactivity over the liver at equilibration and over a 15min washout period after the breathing system is removed. The calculation of blood flow from external scintillation counting is complex but values similar to those found with other methods have been produced.

2.4.5 Fractional distribution

This method relies on the distribution of a centrally injected substance by the regional blood flow into organs where it is taken up by the cells. Radiolabelled compounds such as I131 antipyrine, 42KCl and 86RbCl, have been used (185). This technique is of use only in animal studies as the tissue has to be removed for scintillation counting. A similar limitation is found with the use of radio-labelled microspheres (5-50um in diameter) which are injected centrally and become wedged in the capillaries of the target organ. This technique can be used for serial measurements by using spheres of different sizes and labelled with different isotopes (59,186).

2.4.6 Direct methods of measuring hepatic blood flow

Several methods of measuring flow have been described which are now only of historical interest. As implied by

the title all these methods are invasive. The older techniques involve diversion of the blood flow through the measuring instrument, whereas the more modern techniques are applied to the vessel without interupting its continuity.

a) Diversion of flow

The simplest method was to divert the flow through a vessel into a graduated cylinder for a fixed time period. The flow, however, would be influenced by the change in circulating volume. More sophisticated instruments, such as the Ludwig stromuhr or liquid rotameters, returned the measured blood flow to the circulation. The Ludwig stromuhr involves a system of four tubes diverting blood from a vessel to a measuring chamber and returning it to the vessel. By clamping two of the tubes blood can be diverted in either direction through an oil filled calibrated chamber and flow calculated from volume per unit time. The liquid rotameter works on the same principle as the gas rotameters on anaesthetic machines with displacement of a bobbin in a tube which is compensated for variations in flow and viscosity. The use of a rotating vane inside a tube inserted into a blood vessel has been described.

b) Electromagnetic flow probe

The technique most commonly used for direct measurement of hepatic blood flow is an electromagnetic flow meter. These instruments are based on the principle of measuring the induced electromagnetic field created when blood flows at right-angles to a magnetic field (187). The voltage induced by the blood flow is proportional to its average cross-sectional velocity. The probe used to measure flow through larger vessels, such as the hepatic artery or portal vein, is C-shaped. This design allows the probe to wrap round the vessel creating a uniform magnetic field of constant cross-sectional area and maintains the vessel diameter. This instrument has been used in man most frequently during porto-caval shunt procedures to assess the extent and direction of flow (188). In animal studies such probes have been used both acutely and chronically implanted (189,190). The problems encountered include stability of baseline values and calibration.

c) Ultrasonic probes

Ultrasonic blood flowmeters utilise the distorting effect of a moving liquid on soundwaves of high frequency and very short wavelength. Two basic designs are used. The first uses two transducers placed on either side of

the vessel such that the line connecting them forms a small acute angle with the direction of blood flow. Both transducers act alterately as transmitter and receiver the direction changing several hundred times a second. The velocity of the soundwaves travelling with the direction of blood flow will be higher than that of those travelling upstream. The difference between the two can be used to measure the velocity of the blood flow. The second method uses the principle of the Doppler phase shift. A transmitter and receiver are placed beside each other outside a vessel angled against the direction of flow. The small change in frequency from the transmitted to the received sound caused by reflection by the moving blood is detected by the receiver. This change is proportional to the velocity of the blood flow. It can be seen that both these methods measure velocity of flow rather than flow but flow can be calculated if the cross-sectional area and velocity profile are known. Despite this disadvantage these instruments have proved useful and can be made small enough to fit in a catheter tip.

d) Heat exchange

Heat exchange methods relate the loss of heat of a heated element to the rate of blood flow (191). The simplest method involves a heated thermocouple placed in

the vessel and the rate of heat loss measured. This, however, only measures change in flow rather than quantitative flow. A more sophisticated development places two thermistors in a catheter tip, one heated, one not and the difference in temperature over a time period gives a measure of heat clearance which is related to blood flow (192). These techniques have been used acutely in man (193) and also as chronic implants in animal studies (194).

Chapter 3

Hepatic drug metabolising enzymes

3.1 Introduction

3.2 Drug metabolism reactions

3.2.1 Phase I reactions

3.2.2 Phase II reactions

3.2.3 P450 enzyme system

3.3 Models of hepatic drug metabolism

3.3.1 Venous equilibration model

3.3.2 Parallel tube model

3.3.3 Comparison of the models

3.4 Hepatic drug elimination

3.4.1 Hepatic blood flow

3.4.2 Drug binding

3.4.3 Intrinsic clearance

3.4.4 Elimination of the metabolite

3.5 Assessment of hepatic enzyme activity

3.5.1 Introduction

3.5.2 Antipyrine

3.5.3 Aminopyrine

3.5.4 Other drugs

3.5.5 Endogenous metabolites

3.6 Factors affecting drug metabolism

3.1 Introduction

The liver is the principal site of drug metabolism in the body. The primary mechanism is the biotransformation of lipid soluble compounds into more water soluble ones which are more easily excreted in the bile or by the kidneys. The resultant compound may be a smaller fragment of the original molecule or a new larger molecular weight compound is formed by linkage (conjugation) of all or part of the original molecule with another produced in the liver. In general, metabolites with a molecular weight of 500-1000 are predominantly excreted by the liver and the smaller ones are excreted renally. The hepatic drug metabolising enzymes are found mainly in the smooth endoplasmic reticulum of the hepatocyte, but are present also in the mitochondria and the cytoplasm.

The basic reactions involved in the hepatic metabolism of drugs are oxidation (loss of electrons), reduction (gain of electrons), hydrolysis (addition of an -OH group)and conjugation (linkage with another compound). These reactions can be divided into Phase I (oxidation, reduction and hydrolysis) and Phase II (conjugation) reactions. For many drugs the metabolite of an initial Phase I reaction undergoes a subsequent Phase II reaction before excretion.

3.2 Drug metabolism reactions

3.2.1 Phase I reactions

Oxidation reactions

Oxidation of drugs by the liver is carried out by a series of enzymes found in hepatic microsomes known as the mixed function oxidases or cytochrome P-450 system. These enzymes are haemoproteins and contain an iron molecule which is able to accept and donate electrons to catalyse reactions. Oxidation reactions add an oxygen atom to the substrate and this in some cases results in cleavage of the molecule. A variety of reactions involving the P-450 system come under the general heading of oxidations and these are summarised below, with examples relevant to anaesthesia (195). These reactions include ; aliphatic hydroxylation (thiopentone), aromatic hydroxylation (fentanyl), O-dealkylation (pancuronium), N-dealkylation (morphine), N-oxidation (pethidine), desulphuration (thiopentone) and dehalogenation (halothane).

Reductive reactions

Reductive reactions are much less common and include the metabolism of azo- (fazadinium) and nitro- (nitrazepam) groups to their corresponding amines. The

dehalogenation of halothane to trifluoroethane is a reductive process which is normally a minor pathway of metabolism but may important in the development of halothane hepatotoxicity.

Hydrolysis

Hydrolysis of esters and amides occurs in most tissues including the liver. Esterases and amidases are involved in the metabolism of an number of drugs used in anaesthesia, including amide and ester local anaesthetics, atropine and pethidine.

3.2.2 Phase II reactions

Phase II reactions are essentially synthetic and involve the conjugation of a drug, or drug metabolite produced by a Phase I reaction, to form an inactive, polarised, water soluble compound for excretion. The substrates commonly involved include glucuronic acid, sulphates, acetyl groups (acetylation), amino acids (eg. glycine) and methyl groups (N-methylation). The oxidative metabolite of thiopentone, thiopentone carboxylic acid, is conjugated with glucuronide and excreted in the urine.

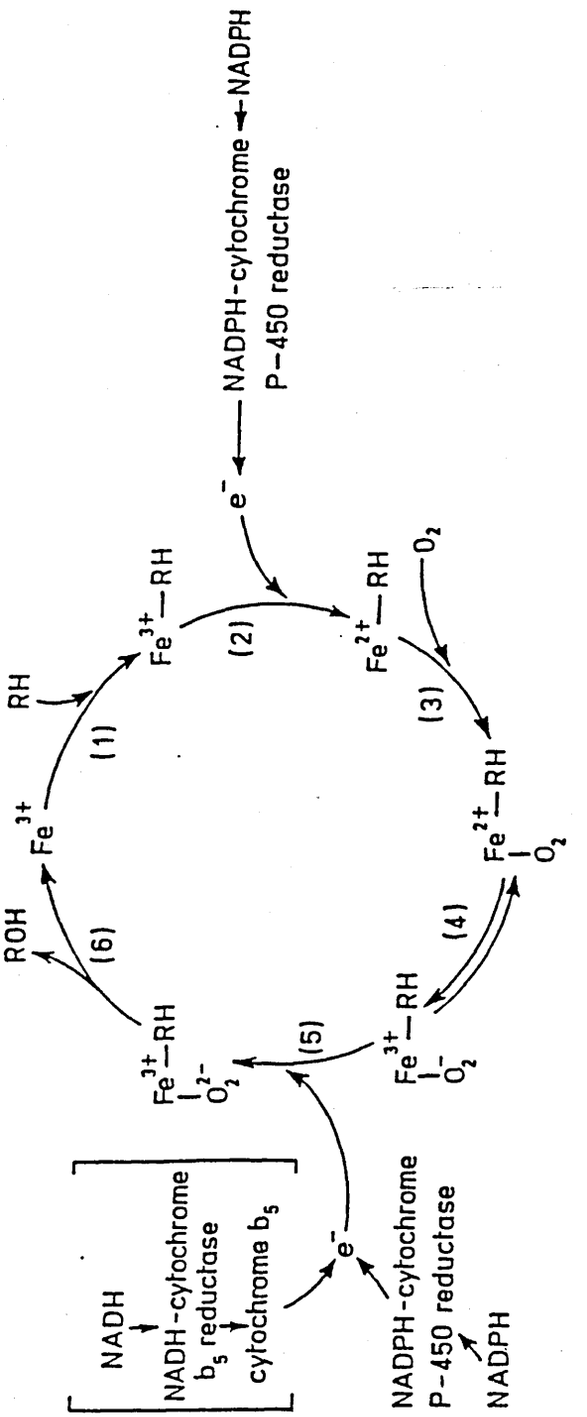
3.2.3 P450 enzyme system

The cytochrome P-450 system or mixed function

oxidases are located in the hepatic smooth endoplasmic reticulum. They are pigmented haemoproteins which are involved in the metabolism of numerous endogenous and exogenous substances. The name P-450 derives from the observation that in a reducing environment (carbon monoxide) a microsomal suspension of the pigmented haemoproteins bound the carbon monoxide reversibly to exhibit a broad light absorption band with maximal intensity at the wavelength 450nm (196,197). This pigment (cytochrome) is the final stage in a series of catalytic steps and is involved directly in binding of the drug substrate and oxygen to microsomes. The mixed function oxidase system comprises of Cytochrome P450, the enzyme NADPH- cytochrome P450 reductase and a lipid component. The system is based on the ability of the iron in the haem molecule of cytochrome P450 to undergo cyclical oxidation-reduction reactions in conjugation with substrate binding and oxygen activation (198,199). The NADPH- cytochrome P450 reductase acts as the link between the NADPH-dependant electron transfer system, which involves several flavoproteins, and cytochrome P-450 - substrate complex (Figure 1). The enzyme acts as a carrier of an electron which it donates to the cytochrome-substrate allowing it to combine with an oxygen molecule (200). It is likely that a complex involving cytochrome P-450, oxygen and drug substrate is formed before one atom of the oxygen is incorporated

Figure 1

The catalytic cycle of cytochrome P450



RH - drug substrate ROH - drug metabolite

into the substrate and the other reduced to water with the addition of a further electron from the NADPH system. The product of the substrate-oxygen reaction, the drug metabolite, is then released and the reduced cytochrome P450 combines with another substrate molecule. Using radiolabelled oxygen, it has been shown that the oxygen used in this cycle is derived from air and not from water (201). The function of the lipid component is not fully known but it has been shown to be essential for the system's activity. It may have a role in substrate binding, facilitation of electron transfer or act as a template for the complex formation of the other molecules.

3.3 Models of hepatic drug metabolism

The pharmacokinetics of hepatic drug elimination has been studied widely and mathematical models developed to describe the limiting factors involved in hepatic clearance (202,203). The models make a number of assumptions regarding the extent of binding of the drug and the characteristics of the enzymes involved in the elimination of the drug including the Vmax (the maximum rate of the enzymatic process) and Km (the enzyme-substrate dissociation constant). They also assume that there is complete mixing between the hepatic arterial and portal venous blood before it reaches the sinusoids (204,205), that only unbound drug crosses membranes and that there is no barrier to diffusion across the membrane (206). The two basic models developed are the venous equilibration model and the parallel tube model.

3.3.1 Venous equilibration model

The venous equilibration model is described also as the "well-stirred" model (176,202). This arises from the assumption by this model that the liver is a single compartment with uniform drug concentration. The model also makes the assumption that the concentration of

unbound drug in the venous blood leaving the liver is the same as that to be found within the hepatocytes. That is, that during transition of the liver a state of equilibrium forms between the blood and the tissue. It assumes also that the rate of elimination of a drug by the liver is the product of the capacity of the liver to metabolise the drug times the unbound concentration of the drug in the liver.

The mathematic expression of this concept relates the hepatic blood flow (Q), the fraction of drug unbound in the blood (fu), the steady-state extraction ratio (E), the intrinsic clearance of the unbound drug by the liver (Clint) and the total drug clearance by the liver (Clh) :

$$Clh = QE = \frac{Q.fu.Clint}{Q + fu.Clint}$$

Thus, when Clint has a large value in comparison to Q, hepatic clearance will be proportional to Q. However, if Clint is small in comparison to Q, hepatic clearance becomes independent of Q. This can be illustrated by substitution of arbitrary values into the equation :

eg Q= 1000, Clint = 5000 Clh = 833

Q= 500, Clint = 5000 Clh = 455

Q= 1000, Clint = 2500 Clh = 714

where halving the blood flow almost halves the hepatic clearance but halving the Clint only produces a 15% reduction. In contrast when Clint is small :

Q = 1000, Clint = 100 Clh = 91

Q = 500, Clint = 100 Clh = 83

Q = 1000, Clint = 50 Clh = 47

the effect is reversed.

It follows from this that when the liver has a large capacity to metabolise a drug (ie the drug is of high hepatic extraction) the rate of elimination will be determined by the rate of presentation of the drug to the liver (ie the hepatic blood flow). The clearance of such a drug is described as perfusion limited. If the liver has a low metabolic capacity for a drug, low hepatic extraction, the rate of presentation of the drug will be less important and the determining factor will be enzymatic metabolic clearance rate. In this case the clearance of the drug is capacity limited.

3.3.2 Parallel tube model

The parallel tube model makes the same basic assumptions as the venous equilibration model but incorperates two additional ones (203). Firstly that the liver can be regarded as a group of parallel cylinders of the same length which have enzymes evenly distributed in all the cells surrounding each tube and that flow is in only one direction. The second assumption is that at any point along the length of the cylinder an equilibrium exists between drug in the cylinder and drug

at the enzymatic site. If the parallel tubes can be equated to hepatic sinusoids this model would appear to be more accurate physiologically, as it takes account of a concentration gradient of the drug along the length of the tube. This introduces a non-linearity to the solution of rate of removal of drug and therefore specific solution of velocity of removal, extraction ratio and clearance are not possible unless certain conditions are observed. If the concentration of unbound drug is less than the Km of any enzyme system and binding is not saturated by high drug concentrations a solution for the concentration of drug leaving the liver (Cout) can be derived ;

$$Cout = Cin.e^{-fbout.Clint,l/Q}$$

where Cin is concentration of drug entering the liver, fbout is the unbound fraction of drug leaving the liver and Clint,l is the maximal intrinsic clearance for the entire liver. Therefore :

$$\text{Extraction ratio } (E) = 1 - e^{-fbout.Clint,l/Q}$$

and as Cl =QE :

$$\text{Hepatic clearance} = Q.(1 - e^{-fbout.Clint,l/Q})$$

3.3.3 Comparison of the models

The two models will produce very similar values for extraction ratio and clearance when describing the behaviour of drugs at the extremes of the range of extraction. That is a drug of high extraction clearance will be limited by hepatic blood flow and for a drug of very low extraction clearance will be limited by enzyme function (intrinsic clearance). The differences between the models are evident when describing drugs of intermediate clearance using the determinants of hepatic blood flow, drug binding and intrinsic clearance. Prediction of the effect of changes in hepatic blood flow on a range of hepatic extraction and clearance values provides a reasonable correlation betwen the models with a maximal difference of 30% when the extraction ratio was between 0.7 and 0.8 (205). This agrees with an analysis of experimental data from a study of isolated perfused rat livers (207,208). There is reasonable agreement in predictions of bioavailability for most extraction ratios but when approaching unity (E=1.0) marked discrepancy occurs. There is very little difference in the predicted values for extraction or clearance with changes in unbound fraction for the entire range of extraction ratios. Changes in the intrinsic clearance produce a higher clearance using the venous equilibration model over the

full range of extraction ratios and this effect is most evident at high extraction ratios.

Validation of these models under experimental conditions using isolated perfused livers has proved difficult. The venous equilibration model appears to be the more consistant with these studies when describing the behaviour of high extraction drugs, such as lignocaine (209).

3.4 Hepatic drug elimination

The hepatic elimination of a drug can be influenced, theoretically, at a series of determinant steps in the process. These steps include ;

a) hepatic blood flow

b) the extent of binding of the drug to plasma proteins and blood cells

c) the uptake of the drug into the hepatocyte

d) the intracellular drug binding capacity

e) the rate and capacity of the enzymic reaction

f) the effect of the metabolite on the enzyme

g) the elimination of the metabolite into the bile or blood

Steps c - f can be considered under the general heading of intrinsic clearance. The contribution of, and the effect of changes in, these four factors, hepatic blood flow, drug binding, intrinsic clearance and metabolites, on the elimination of drugs is discussed below.

3.4.1 Hepatic blood flow

The effect of changes in hepatic blood flow on drug elimination have been outlined above in the description of the venous equilibration model. The importance will

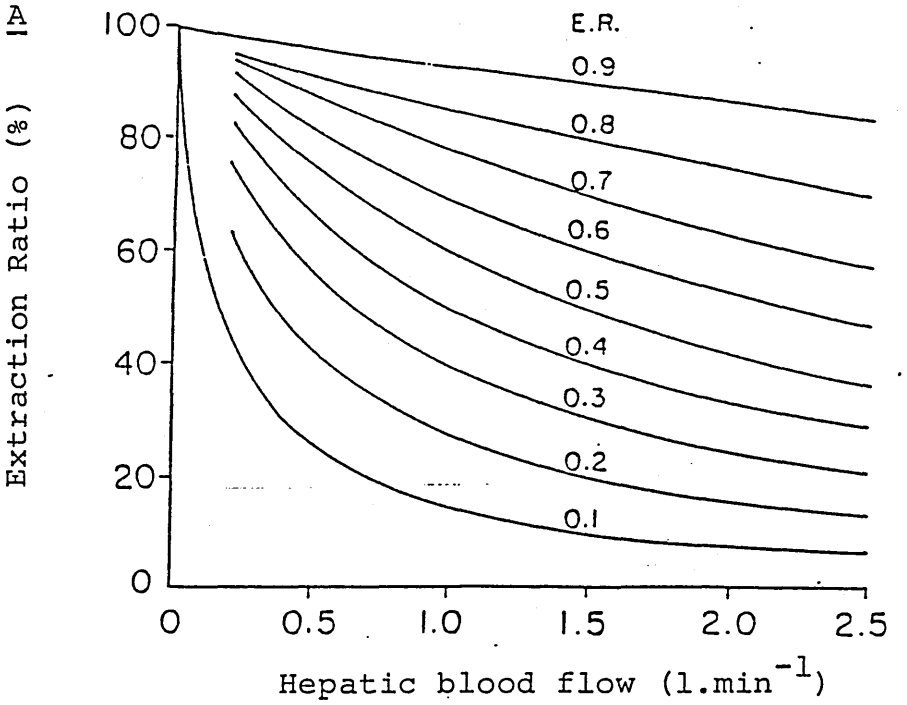
depend on the route of administration of the drug and the extent of hepatic extraction. The clearance of a drug of high hepatic extraction given intravenously is proportional to hepatic blood flow (ie it is perfusion limited) and therefore, alterations in flow will produce a change in clearance (210). There would be no change in the clearance of an orally administered high extraction drug but the bioavailability would alter (Cliv = Clo x F). For drugs of low hepatic extraction the hepatic blood flow has little influence on the rate of elimination after either oral or intravenous administration (176). The inter-relationship between these variables can be illustrated by computer simulations which predict the effect on clearance or extraction ratio of changes in hepatic blood flow (Figure 2).

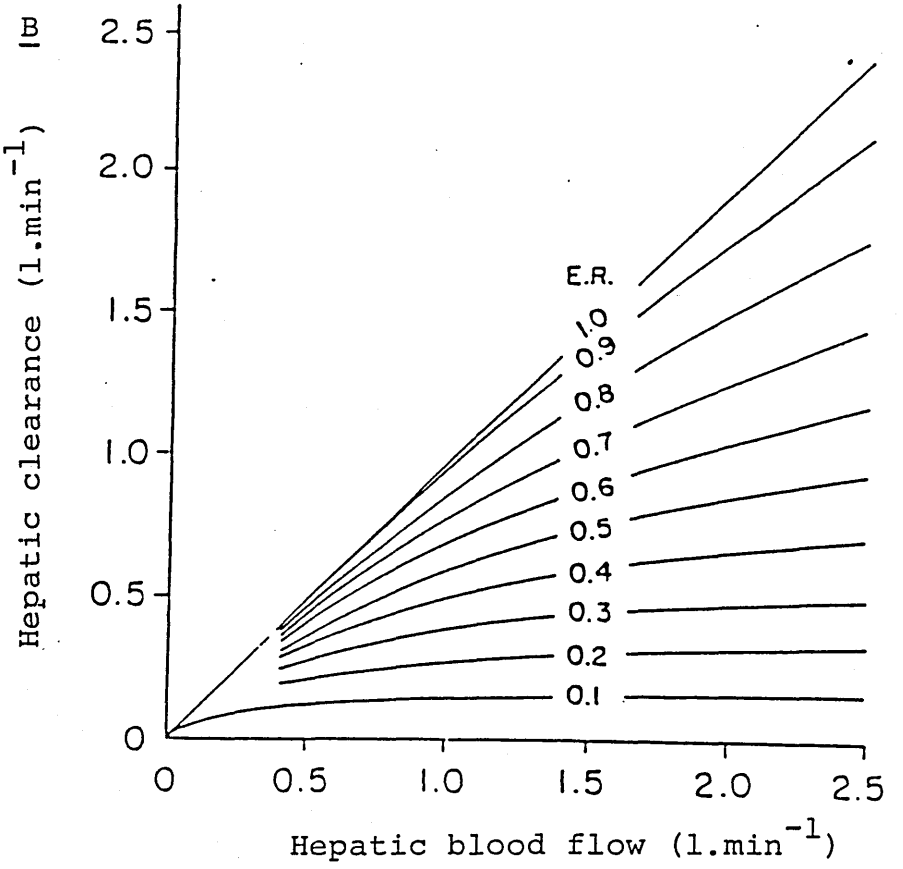
3.4.2 Drug binding

Within the blood a drug exists in two forms, unbound and bound. The drug is bound reversibly to plasma proteins and lipoproteins and to blood cells. The amount of unbound drug (free drug) is the principal determinant of drug efficacy. The free drug is available to cross membranes and reach receptor sites. An equilibrium forms across a membrane between the free drug concentrations on either side of the membrane and is independent of the

Figure 2

Effect of change hepatic blood flow on
A) extraction ratio and B) hepatic clearance





Adapted from Wilkinson & Shand (176)

bound concentration. That is, if there is a difference in extent of binding on either side of a membrane the total amount of drug on each side will be different but the free concentration will be the same. If a drug is highly bound (>90%) small changes in the extent of binding will have a marked effect on the amount of free drug, and therefore, on its biological effect. However, a drug which has a low extent of binding will show only a small change in free drug concentration in response to a small change in binding.

The effect of drug binding on hepatic elimination depends on the extent of hepatic extraction of the drug. A high hepatic extraction drug, such as propranolol (E=0.8), may be highly protein bound (propranolol 10% free). This suggests that on a passage through the liver both free and bound propranolol are taken up from the blood. The affinity for binding the drug for the proteins in the hepatocyte must be greater than its affinity for the plasma proteins. The clearance of propranolol is not limited by protein binding (211). A decrease in plasma protein binding will tend to increase the volume of distribution and increase the half-life of a high extraction drug which is also lipid soluble (ie has a large volume of distribution) (176,212). Little effect is noted on either volume of distribution or half-life of a high extraction drug with a small volume of distribution. For a drug of low hepatic extraction

such as warfarin, the free fraction will be larger than the extraction ratio. Therefore the rate of clearance will be limited by the extent of protein binding, ie the clearance will increase as the free fraction increases (213). A low extraction drug of small volume of distribution will not change volume but may decrease its half-life with a decrease in protein binding. If the volume of distribution is large it will tend to increase but no change will be seen in half-life.

3.4.3 Intrinsic clearance

The intrinsic clearance has been defined as the maximal ability of the liver to remove drug irreversibly by all pathways in the absence of any flow limitations. This term covers the uptake of a drug into the hepatocyte, its transport in the cell, the enzymatic metabolic process and the effect of metabolites. One of these steps will be the rate limiting step of intrinsic clearance. Uptake of a drug into the cell may be an active process as for many organic compounds (214) or a passive process with the drug moving down a concentration gradient across the cell membrane. Lipid soluble drugs will traverse the membrane more easily.

The gradient across the membrane is maintained by the efficient and rapid binding of the drug by intracellular proteins, and also by the rapid formation of

enzyme-substrate complexes. The intracellular binding of a drug could be the rate limiting step in the process if there was a higher affinity for the substrate with the intravascular proteins than with the intracellular ones, or if the capacity of the intracellular proteins was saturated easily (214).

The kinetics of the drug metabolising enzymes will be the major influence on drug elimination. The velocity of an enzymic reaction will be determined by the amount of enzyme available, the amount of substrate present and the action of any inhibitory or acceleratory mechanisms (215). The general formula for an enzyme substrate reaction is ;

$$E + S \Rightarrow ES \Rightarrow E + M$$

where E is the concentration of enzyme present, S is the concentrtion of substrate, ES is the enzyme-substrate complex and M is the metabolic product of the reaction. The formation of ES is a rapid process but its breakdown is much slower and an equilibrium is formed between ES and E and S. The relationship between the velocity of reaction (v) and substrate concentration describes a hyperbolic curve. This is the basis of the Michaelis-Menten equation;

$$v = Vmax.Km$$

where v is the velocity of the reaction, Vmax is the maximum velocity of reaction when the enzyme is saturated with substrate and Km is the enzyme-substrate dissociation constant. Km describes the relationship between enzyme, substrate and enzyme-substrate complex concentrations ;

$$Km = \frac{(E - ES).S}{ES}$$

Km can be determined using a Lineweaver-Burk plot which plots the reciprocal of v against the the reciprocal of S. The intersection of this line with the abscissa is -1/Km and the intercept with the ordinate is -1/Vmax.

It is likely that some drugs may be metabolised by more than one enzyme or that two drugs may compete for the same enzyme. In these situations the determination of Km is more complicated. The former case is likely to occur with drugs which undergo a simple oxidation with the P450 system.

The velocity of enzyme reaction can be enhanced or inhibited. This is different from enzyme induction or inhibition where the main change is in the concentration of enzyme available rather than a change in activity. The changes in velocity are usually related to the substrate concentration (215). High levels of substrate

may inhibit the reaction by several mechanisms including binding of co-factors of the reaction, binding to inactive sites on the enzyme which interfere with binding to the active site or by outstripping the supply of other reactants required for the reaction. The velocity of reaction may be enhanced by binding at sites on the enzyme other than the active one. Such binding may cause a steric change which increases the binding capacity of the enzyme.

The reactions involving the hepatic drug metabolising enzymes proceed at a slow rate relative to many biological enzymatic processes eg. cholinesterases. To compensate for this the enzyme systems are able to speed up turnover by increasing their concentration, that is by induction. There are a large number of substances, drugs and chemicals, which are known to produce enzyme induction including barbiturates (216,217), ethanol (218,219) and cigarette smoke (220). Increase in enzyme activity will be noticed within 3-4 days of starting an inducing drug, reaching a maximum at 2 weeks, and will still be evident 2 weeks after discontinuation of the drug. The range of induced P450 enzymes will vary depending on the inducing drug. For example, a generalised inducion of many P450 forms will occur with phenobarbitone treatment (217), but a smaller range will be found with ethanol (219). Enzyme induction will result in a more rapid metabolism of drugs. Inhibition

of P450 enzymes has also been described and results in a slowing of elimination and prolongation of effect of a drug. Drugs shown to cause inhibition include cimetidine (221), chloramphenicol (222) and allopurinol (223). The effect of anaesthetic agents on enzymes has been discussed in Chapter 1. The effects of hepatic drug metabolising enzyme inhibition and induction will be most marked on drugs of low hepatic extraction.

Assessment of the activity of the hepatic drug metabolising enzymes is discussed later in the chapter (3.5).

It is possible for a metabolite of an enzymic process to interfere with the ongoing reaction which produces the metabolite. This may occur due to the metabolite binding on or near the active site of the enzyme, or as a chemical effect of the metabolite altering the environment of the reaction.

3.4.4 Elimination of the metabolite

The end product of drug metabolism is a more polar water-soluble molecule. This molecule or conjugate is excreted from the hepatocyte into the biliary or hepatic venous system. The metabolites released into the systemic circulation are excreted usually by the kidney. The excretion of metabolites from the hepatocyte may be a passive process but active transport systems have been

identified (224). It is not normally a rate-limiting step in the elimination of a drug. Obstruction of the biliary outflow could, in a manner parallel to that seen in bilirubin metabolism, lead to an intracellular accumulation of metabolite or spillage of a metabolite normally excreted in bile into the the systemic circulation. Defective uptake of drugs by hepatocytes has been demonstrated in experimentally produced cholestasis (225).

3.5 Assessment of hepatic enzyme activity

3.5.1 Introduction

The central role of the liver in drug metabolism makes it desirable to know the influence of hepatic enzymes on the elimination of a drug. This can be achieved by pharmacokinetic studies of the drug's elimination. In addition, it is desirable to evaluate the effect of a drug on the hepatic enzymes. This is achieved by measuring the effect of the drug on the pharmacokinetics of known substrates. The use of such model substrates can be extended usefully to measure the capacity and capabilities of the hepatic drug metabolising enzyme system. Such an approach has been used in the study of drugs which produce enzyme inhibition or induction and in the study of the genetic polymorphism of drug metabolism.

The properties of an ideal drug for use as a model for assessing hepatic enzyme activity would include (226) ;

a) single compartment kinetics allowing calculation of clearance from the elimination half-life.

b) elimination exclusively by hepatic metabolism

c) hepatic metabolism should be independent of hepatic blood flow and protein binding.

d) if given orally the drug should be rapidly and completely absorbed

Additional information about the drug metabolising enzymes can be obtained if the metabolite or metabolites of the drug can be measured in plasma or urine. This may allow the differentiation of effects on alternative pathways of metabolism to be evaluated. However, the rate of production of the metabolite and its rate of excretion may differ (227).

A number of drugs have been used to evaluate hepatic drug metabolising activity, but none has been shown to fulfill the criteria set out above completely. The drugs used include those of low hepatic extraction such as antipyrine, aminopyrine and diazepam, and high hepatic extraction drugs such as propranolol, galactose, pentazocine and lignocaine. The use of low extraction drugs has the advantage that their elimination is independent of hepatic blood flow. However, simultaneous administration of a low and a high extraction drug has shown a correlation between the clearance of both drugs in patients with hepatic disease. The most commonly used drugs, antipyrine and aminopyrine will be described. Their use and the advantages and disadvantages will be discussed.

3.5.2 Antipyrine

Antipyrine is a phenylpyrazolone derivative which has analgesic and antipyretic properties. Its mode of action is similar to that of the salicylates. Antipyrine has been used widely as a measure of hepatic enzyme activity because its metabolic profile is well suited to this task : it is well absorbed from the intestine after oral administration, its binding to tissues and proteins is limited, it has a low hepatic extraction ratio and therefore its elimination is independent of hepatic blood flow, almost all metabolism of the drug occurs in the liver and there is minimal (<5%) renal excretion (228). As it has single compartment kinetics both the plasma half-life and the clearance give a measure of hepatic metabolism (229). It has a relatively long half-life in man (8-14 hours) and, as the salivary concentration and plasma concentration are very similar, the former method of sampling has been found convenient. The long half-life is also a disadvantange which precludes its use in sequential studies, as several days washout is required.

The metabolism of antipyrine involves the production, by the P450 system, of three oxidative metabolites. Its metabolism has been used, therefore, to assess the effect of liver disease on drug oxidation reactions (230) and to assess the enzyme inhibitory or inductive

effects of a wide range of drugs, including anticonvulsant (231) and antitubercular drugs (232) (inducers) and beta-adrenergic (233) and H2 blockers (234) (inhibitors) and factors such as smoking and eating (235,236). Attempts have been made to use antipyrine metabolism as a predictor of the ability of the liver to metabolise other drugs but the correlation has been variable (228). This may due to the relatively non-specific metabolic profile of antipyrine which involves at least three discrete mixed function oxidases. Some studies have attempted to parallel the production of an individual metabolite with a test drug. The half-life of antipyrine is increased in liver disease (237), hypothyroidism (238) and with age (239). Antipyrine may cause an induction of its own metabolism (240) and this, also, prevents its use in serial studies over a short time period.

3.5.3 Aminopyrine

Aminopyrine is also a pyrazolone derivative and is related to antipyrine. It has a shorter half-life than antipyrine and undergoes de-methylation by the hepatic microsomal enzymes. The products of its metabolism include formaldehyde, formic acid and CO2. The last of these products has been used as an indirect measure of mixed function oxidase function. Administration of

aminopyrine radio-labelled with 14C allows measurement of its metabolism by detection of 14CO2 in expired breath (241). The expired CO2 is adsorbed in collection vials for subsequent scintillation counting. The aminopyrine breath test has been used in animal and human studies to evaluate the effect of drugs (242) and environment on drug metabolism. This test also proved to be a poor predictor of the metabolism of other drugs such as diazepam (243). The main advantage of the aminopyrine breath test is that it is relatively simple to perform. A similar principle, measurement of the 14CO2 metabolite, has been applied to other radio-labelled substances such as galactose (244), diazepam (222), mephenytoin (245) and caffeine (246).

3.5.4 Other drugs

Other drugs used as models of metabolising capability include theophylline which shows a variable response to known enzyme inducers (247) and hexobarbitone (248) which has been used in many animal studies and has the advantage over amylobarbitone (249) of being metabolised almost entirely by hepatic microsomal enzymes.

3.5.5 Endogenous metabolites

The measurement of endogenous metabolic products has

been advocated as a measure of hepatic enzyme function. D-glucaric acid is the end-product of the glucuronic acid carbohydrate metabolic pathway and is excreted in the urine. An increase in the production of D-glucaric acid has been noted following known enzyme inducing drugs (250,251). However, the mechanism of this change is not fully understood and it may not represent enzyme induction. The urinary excretion of 6b-hydroxycortisol, a minor metabolite of cortisol produced by the hepatic mixed function oxidases, has been proposed as a measure of enzyme induction (252). It appears to be a reasonable measure of enzyme induction (240,253) but not of enzyme inhibition (253). As cortisol production is subject to many influences the production of 6b-hydroxycortisol does not correlate well with the clearance of test drugs and therefore, cannot be used as a predictor.

3.6 Factors affecting hepatic drug metabolism

A number of physiological and environmental factors have been shown to influence the activity of hepatic drug metabolising enzymes.

Age

A large number of animal studies and human studies have demonstrated that with increasing age there is a decrease in the ability of the liver microsomes to metabolise drugs. Alterations in clearance and/or half-life have been demonstrated for many drugs in the elderly. These changes may be difficult to interpret as they are often accompanied by changes in drug binding and volumne of distribution. For example, diazepam half-life is prolonged in the elderly and the volume of distribution is increased (105). Antipyrine half-life is increased and clearance decreased suggesting that the metabolic activity of the liver decreases with age (239,254).

Sex

It might be expected from the difference in total body water and fat that the distribution of a drug would differ between male and females. This, however, does not appear to be manifest in different rates of elimination of drugs. A higher rate of drug metabolism has been noted in male than in female rats, but this is

the only species that this has been observed in. More effect might be expected with drugs dependent on renal elimination because of a lower glomerular filtration rate in females (255).

Species

Many studies of drug metabolism are done using an animal model initially. It is not possible to then extrapolate these results to any other species as the rate of of metabolism or pathway of metabolism may differ. There are many examples of these differences which can be illustrated by the different half-life of propranolol in dogs (1hr) and man (4hr) and the metabolism of sulphadimethoxine in rats (acetylation) and man (glucuronidation) (256). If the same metabolic pathway is present correction for body weight and circulation time (257) has demonstrated some correlation between body weight and oxidase activity in mammals (258).

Genetic

The inter-individual differences in response to a drug may be partly explained by the genetic variation within a study population. For many drugs the individuals' rate of drug metabolism would exhibit a normal distribution around a mean. For some drugs, however, a bimodal distribution may be found. The classic example of this is the acetylation of sulphadimadine which can discriminate between slow and

fast "acetylators". The percentage of acetylated drug in the urine at 6 hours was used to determine the dose of isoniazid for antitubercular treatment (259). More recent studies have shown that there are genetic variations in drug oxidation capability. This has led to "oxidation phenotyping" using the hydroxylation of the antihypertensive debrisoquine (260,261) or the oxidation of alkaloid sparteine (262) as discriminants. An autosomal recessive characteristic of poor ability to hydroxylate debrisoquine has been demonstrated (260). This characteristic does not appear to be associated with changes in antipyrine kinetics (263,264) but several reports have linked poor metabolisers with an increase in adverse effects of drugs undergoing oxidative elimination such as beta-adrenergic blocking drugs (265,266).

Liver disease

It would be expected that the changes produced by liver disease would include a decrease in the ability of the hepatocytes to metabolise drugs. These changes may be difficult to demonstrate in isolation as concurrent changes in hepatic blood flow and drug binding capacity are likely to be present. The nature and severity of the disease and the pharmacology of the drug will be important. A review of 30 studies comparing the disposition of a number of drugs in patients with or without liver disease found altered kinetics in only 60%

of the studies (267). Antipyrine elimination has been shown to be impaired more in patients with chronic liver disease than in patients with acute reversible liver disease (237). Studies with other drugs in various hepatic diseases such as hexobarbitone in acute hepatitis (268), pethidine in cirrhosis (269) and propranolol in chronic liver disease (270) have concluded that impairment of elimination of a drug increases with the severity of the disease but correlates poorly with biochemical measures of severity.

Renal disease

The presence of renal disease should not affect directly the hepatic metabolism of drugs. There may be an alteration in the disposition of the drug due to changes in total body water and altered drug-protein binding. An increase in the adverse effects due to accumulation of an active metabolite of an extensively metabolised drug, which is excreted by the kidney normally, might be expected.

Drug interactions

The ability of some drugs to influence the metabolism of other drugs has been recognised for many years. This interaction may accelerate or inhibit the rate of elimination. The increase in rate of metabolism of warfarin caused by concurrent barbiturate administration, with a resultant decrease in anticoagulant action, has been well documented. Drugs

associated with an inductive effect on the metabolism of other drugs include the barbiturates (217), phenytoin (271), phenylbutazone (272), carbamazipine (273) and rifampicin (274). Ethanol ingestion has an acute effect of slowing drug metabolism but chronic ingestion has an inductive effect (219). Cimetidine is known to inhibit the oxidative metabolism of many drugs (221). Elevation of plasma concentration of drug and increased therapeutic effect following co-administration of cimetidine has been demonstrated with beta-adrenergic blockers (275,276), theophylline (277), benzodiazepines (278), warfarin (234) and phenytoin (279). Other drugs associated with enzyme inhibition and therefore prolongation of drug effect are chloramphenicol (222), sulphaphenazole (280), monoamine oxidase inhibitors (281) and allopurinol (223).

Smoking

Tobacco smoke contains several thousand chemicals some of which are known to affect drug metabolism. The importance of taking a full history of smoking habits in subjects taking part in pharmacokinetic studies has been highlighted (220). Smoking has been shown to increase the rate of elimination of several drugs including antipyrine (239), theophylline (282) and pentazocine (220) but not to affect the metabolism of others such as diazepam (283), pethidine (284), phenytoin (285) and warfarin (286).

Environmental chemicals

The effect of environmental exposure, usually industrial, to chemicals has been shown to alter drug metabolism. Occupational exposure to chlorinated organic insecticides such as DDT (287) and to polychlorinated biphenyls (PCBs) (288) has been shown to produce enzyme induction as shown by a decrease in the half-life of antipyrine. Ingestion of dietary chemicals can also alter drug metabolism. This has been demonstrated by the increased rate of phenacetin metabolism after a diet of charcoal broiled beef, which has a high content of polycyclic hydrocarbons, when compared with a normal diet (289). A high protein - low carbohydrate diet caused a decrease in theophylline half-life and a low protein - high carbohydrate diet caused an increased half-life (290).

Chapter4

Studies

4.1 Anaesthesia studies with a dog model

4.1.1 Introduction

4.1.2 Hepatic extraction and route of administration

4.1.3 Dual route administration

4.1.4 Aim of studies

4.2 Human study

4.2.1 Cimetidine

4.2.2 Enprostil

4.2.3 Aim of study

4.1 Anaesthesia studies with a dog model

4.1.1 Introduction

The pharmacokinetics of many drugs have been noted to be altered in the peri-operative period. There are many interacting factors involved in these changes including the stress response to surgery, alterations in regional blood flow and the effect of anaesthetic agents.

Anaesthetic agents, both inhalational and intravenous, may alter drug pharmacokinetics by effects on any of the principle determinants of drug disposition. As discussed in the introduction, these are the distribution of a drug in the body, the hepatic blood flow and the drug metabolising enzyme activity. There is a growing amount of evidence that the inhalational anaesthetic agents, especially halothane, cause alterations in the peri-operative pharmacokinetics of several drugs but at present there is little data on the effect of intravenous anaesthetic agents.

4.1.2 Hepatic extraction and route of administration

As most drugs are metabolised by the liver, any effect on the disposition of a model drug by an anaesthetic agent is likely to be due to changes in

hepatic drug clearance which will result from alterations in hepatic blood flow and hepatic drug-metabolising enzyme activity. The extent of this effect will depend on the degree of hepatic extraction of the model drug. The clearance of a drug of high hepatic extraction when given intravenously will be determined by its rate of delivery to the liver ie. the hepatic blood flow, whereas the intravenous clearance of a drug of low hepatic extraction will be determined by hepatic enzyme activity. The clearance of both high and low hepatic extraction drugs when administered orally, the oral or intrinsic clearance, reflects hepatic enzyme activity and is unaffected by changes in hepatic blood flow.

4.1.3 Dual-route administration

It can bee seen that there is a different prime determinant of hepatic clearance for a drug of high hepatic extraction depending on its route of administration. When the drug is given orally, changes in clearance will reflect changes in metabolic enzyme activity and when given intravenously it will reflect changes in hepatic blood flow. Thus, by determining the disposition of a high extraction drug given both orally and intravenously it is possible to measure the intrinsic clearance (hepatic enzyme activity) and

estimate the hepatic blood flow (see Chapter 2). The model drug must be metabolised only by the liver and absorbed fully from the intestinal tract when given orally. This technique can be improved considerably by administering the drugs simultaneously. This removes the major problem of assuming that basal conditions are the same on two study days. The oral and intravenous doses of the drug are differentiated by the radio-labelling of one of the doses, usually the intravenous one. This technique has been developed using propranolol as the model drug (291,292). Propranolol is a beta-adrenergic blocking drug which has a high hepatic extraction ratio (>0.7) and is rapidly and completely absorbed when given orally (293).

This technique of dual-route administration of propranolol, simultaneous labelled intravenous and unlabelled oral administration, has been used previously to measure effects on drug disposition (294-296).

4.1.4 Aim of studies

The aim of these studies was to assess the effect of different anaesthetic techniques on drug disposition, in the dog, in the absence of other influences such as surgery. The technique of dual-route administration, with propranolol as the model drug, was used to determine the relative contributions of changes in

hepatic blood flow and hepatic enzyme activity to any alteration in drug disposition during anesthesia.

4.2 Human study

4.2.1 Cimetidine

Cimetidine, a histamine H2 receptor antagonist, is used widely for the treatment and prophylaxis of gastric ulcers. The concurrent administration of cimetidine has been shown to alter the pharmacokinetics of a large number of drugs (221). This usually has resulted in increased plasma concentrations of these drugs with the increased risk of unwanted side effects (234,297,298). This change has been attributed to a decrease in hepatic blood flow and an inhibition of hepatic enzymes (275). The decreased enzyme activity has been demonstrated in many studies and it is likely that cimetidine is an inhibitor of the cytochrome P450 enzymes (299,300). However, the effect of cimetidine on hepatic blood flow has not been demonstrated in all studies (300-303). The apparent decrease in hepatic blood flow demonstrated in some studies may be due an alteration in the extraction of the indicator drug used to estimate blood flow produced by cimetidine.

4.2.2 Enprostil

Enprostil (RS-84135, 11a,15a-dihydroxy- 9-keto

-16-phenoxy- 17,18,19,20-tetranorprosta- 4,5,13(t)-trienoic acid, methyl ester) , a research drug produced by Syntex, is a synthetic dehydro- prostaglandin E2. It has been shown to have effective anti-secretory and cytoprotective properties in animals. It is under investigation currently for the treatment of gastric and duodenal ulcers (304). The decrease in prostaglandin production caused by cyclo-oxygenase inhibitors such as aspirin or indomethacin leads to a decrease in basal gastric and intestinal blood flow (305-307). This suggests that splanchnic and thus hepatic blood flow is controlled at least partially by prostaglandins (308). It is thought that enprostil, therefore, may cause an increase in hepatic blood flow. It has been shown that, in vitro , prostaglandins affect the hepatic metabolism of several drugs including aminopyrine, benzopyrene and hexobarbitone (309,310).

4.2.3 Aim of study

The aim of this study was to compare the effects of cimetidine and the synthetic prostaglandin E2, enprostil, on hepatic blood flow and hepatic enzyme activity in man. Within the context of this series of studies it provides three further elements ;

1) the extension of the dual route technique to human subjects,

2) a validation of this technique with a known enzyme inhibitor and

3) a comparison of the hepatic blood flow measurement from this method with that of the more widely used indocyanine green method.

Chapter 5

METHODS

5.1 Dog studies

5.1.1 Outline

5.2 Preparation of study dogs

5.2.1 Surgery

5.2.2 Animal care

5.3 Anaesthesia

5.3.1 Induction

5.3.2 Halothane group

5.3.3 Isoflurane group

5.3.4 Enflurane group

5.3.5 Fentanyl - atracurium - Nitrous oxide group

5.3.6 Monitoring

5.4 Drug administration and sampling

5.4.1 Drug administration

5.4.2 Blood sampling

5.5 Human study

5.5.1 Outline

5.5 2 Subjects

5.5.3 Drugs

5.5.4 Indocyanine green study

5.5.5 Propranolol

5.5.6 Side effects

5.6 Drug analysis

5.6.1 Propranolol assay

5.6.2 HPLC system

5.6.3 Scintillation counting

5.6.4 Measurement of drug binding

5.7 Calculations

5.7.1 Dose of drug injected

5.7.2 Area under time-concentration curve

5.7.3 Clearance

5.7.4 Hepatic extraction ratio

5.7.5 Elimination constant and half-life

5.7.6 Volume of distribution

5.7.7 Hepatic plasma flow

5.8 Statistical analysis

5.9 Ethical considerations

Dog studies

5.1.1 Outline

This series of studies involved dual route administration of propranolol during anaesthesia to five groups of dogs. The groups studied were ;

Group 1 - control (no anaesthesia)
Group 2 - halothane anaesthesia
Group 3 - isoflurane anaesthesia
Group 4 - enflurane anaesthesia
Group 5 - fentanyl-atracurium anaesthesia

Cannulae were implanted in the portal vein, femoral vein and femoral artery of each dog five days before the study period.

In each group each dog was studied on three consecutive days ;

Day 1 - awake, 24 hours before anaesthesia
Day 2 - during anaesthesia (Group 1 awake)

Day 3 - awake, 24 hours after anaesthesia

The dual route administration involved simultaneous injection of unlabelled propranolol into the portal vein and tritium-labelled propranolol into the femoral vein.

Blood samples were taken over the 4 hour study period for assay of the plasma propranolol and tritiated propranolol concentrations.

5.2 Preparation of study dogs

5.2.1 Surgery

Five days before the first study day each dog had cannulae placed in the femoral vein, femoral artery and portal vein. The dogs were anaesthetised with pentobarbitone (30mg/kg), the trachea intubated and the lungs ventilated with air. The dog was placed supine and the abdomen and left side of the chest shaved and prepared with chlorhexidine solution. A short mid-abdominal, midline incision was made and the spleen was brought forward. A silastic catheter was then introduced into a small branch of the splenic vein and advanced into the portal vein. The position of the catheter was checked manually before it was secured with a suture. The ideal position was taken as being a few centimetres past the junction of the splenic and mesenteric veins. The proximal end of the catheter was then brought subcutaneously to the left chest wall, going through the peritoneum separate from the incision.

Initially, the arterial and venous cannulae were placed in the internal iliac vessels within the peritoneal cavity and brought trans-peritoneally then subcutaneously with the portal cannula to the left chest

wall. However, two dogs prepared early in the series developed intraperitoneal haematoma and could not be studied. It was decided that it would be easier to detect any post-operative bleeding if the cannulae were placed in the femoral vessels through a groin incision. This would also minimise the intra-abdominal surgery and decrease the length of the incision. In all other dogs the femoral vessels were mobilised and cannulated through an incision in the right groin. The cannulae were advanced some 15 centimetres into the vessels and secured with a suture. The proximal end was brought sub-cutaneously, without entering the peritoneum, to the left chest wall.

The three cannulae were brought out on the left chest wall and secured separately to the skin. The cannulae were flushed with heparinised saline and secured in a pouch on a canvas "jacket" placed round the chest over the forelimbs. This arrangement worked well during the first two studies but one dog bit through the pouch and damaged the cannulae. The cannulae were then put into a small metal box in the pouch. This was satisfactory for the next two studies but a dog again got access to the cannulae. For the final studies the cannulae were left in a loop subcutaneously until the first study day when they were brought out and placed in the box in the pouch. At the end of surgery the dog was allowed to breathe spontaneously and the trachea was extubated.

5.2.2 Animal care

The dogs were housed in the Animal Care Center, Vanderbilt University Medical School, in individual kennels. All the dogs were fed daily with the same brand of tinned dog meat and biscuits. The dogs were fasted overnight before the first study day, but had free access to water. They were fed on completion of the study period on days 1 and 2 and again fasted overnight.

During the study period on Days 1 and 3 of the first two studies the dogs were in a restraining sling. For the subsequent studies they were in a small cage which allowed them to lie down.

5.3 Anaesthesia

5.3.1 Induction

A standard induction routine was used in all the groups receiving anaesthesia. Anaesthesia was induced with thiopentone (6-8 mg/kg), the trachea intubated with an 8.0 cuffed endotracheal tube and the lungs ventilated using an Ohio 800 Ventilator. Ventilation was initially set at 10 ml/kg and adjusted in accordance with the end-tidal CO2 as described below. Anaesthesia was maintained with the volatile agent in oxygen. The concentration of inhalational agent was adjusted to twice minimum alveolar concentration (2 MAC). The MAC value for dogs for each agent was taken from published comparative data (311). In group 5, in addition to the fentanyl, anaesthesia was maintained with 66% nitrous oxide in oxygen.

Comparative MAC concentrations (%) in dogs and man (311)

	dog	man
Halothane	0.86	0.77
Isoflurane	1.28	1.15
Enflurane	2.1	1.68

Maintenance

5.3.2 Group 2 Halothane

Following induction of anaesthesia the lungs were ventilated with halothane 2.0 MAC (1.72% for dogs) in oxygen. End tidal halothane concentrations were measured by gas chromatography. The chromatogram was calibrated on the morning of each study by injection of known prepared concentrations of halothane in the range 0-5%. End-tidal gas samples were aspirated into a 30ml glass syringe from a sealed port on the endotracheal tube. This was done at 15 min intervals until a stable 2 MAC concentration was obtained (approximately 1 hour) and then at hourly intervals until the end of anaesthesia. The total time of halothane anaesthesia was calculated in terms of MAC-hours. An Engstrom Emma analyser was used in addition to the gas chromatography in the first three dogs studied in this group but it was found to be not as accurate as the gas chromatography. Anaesthesia was maintained for two hours before the administration of propranolol to allow stable haemodynamic conditions and continued throughout the four hour study period.

5.3.3 Group 3 Isoflurane

Anaesthesia was maintained in this study with isoflurane 2.0 MAC (2.55%) in oxygen. End tidal isoflurane concentration was measured by gas chromatography as described above. Two hours of anaesthesia were administered before the propranolol was given and anaesthesia maintained until the end of the study. The total MAC-hours of anaesthesia was measured.

5.3.4 Group 4 Enflurane

In this group maintenance of anaesthesia with enflurane 2.0 MAC (4.2%) was attempted initially. However, it proved difficult to maintain cardiovascular stability at this concentration and extreme hypotension resulting in death occurred. It was decided to maintain anaesthesia with enflurane 1.5 MAC (3.15%) in oxygen in this group. End tidal concentration was measured by gas chromatography. Two hours of stable anaesthesia was administered before the propranolol was given and anaesthesia was maintained until the end of the study period.

5.3.5 Group 5 Fentanyl - Atracurium-Nitrous oxide

In this group, following endotracheal intubation, the lungs were ventilated with nitrous oxide (66%) in oxygen. Muscle paralysis was achieved with atracurium using an initial dose of 0.3 mg/kg and subsequent bolus doses of 0.1 mg/kg every 20 min. Immediately after tracheal intubation an infusion of fentanyl was started. An initial short pilot study of infusions of fentanyl based on the regimens described by Murphy and Hug (312) was used to establish a suitable dosage. It was established that a two rate infusion, 0.75 µg/kg/min for 20 min then 0.22 µg/kg/min for the remaining study period, produced a suitable anaesthetic state. That is, there was no response to an external stimulus of paw pressure in a dog which had received no muscle relaxant. This regimen was used for the study. Anaesthesia was maintained for one hour before propranolol administration and continued until the end of the four hour study period. At the end of anaesthesia the fentanyl infusion was stopped and no further atracurium given. The dog was then allowed to recover consciousness and resume spontaneous ventilation before extubation of the trachea. The total dose of fentanyl and of atracurium was recorded.

5.3.6 Monitoring

In all the study groups ventilation was initially set to a tidal volume of 10 ml/kg. This was adjusted to maintain an arterial pCO2 within the range of 4.6 - 5.5 kPa on the basis of regular arterial blood gas measurements. Blood gas measurement was performed using a Corning automatic blood gas analyser which was calibrated each morning before the start of the study.

Arterial blood pressure was monitored continuously throughout anaesthesia by a pressure transducer and monitor attached to the femoral arterial cannula.

5.4 Drug administration and blood sampling

5.4.1 Drug administration

The administration of the unlabelled and labelled propranolol was performed in an identical manner on each study day. On days 1 and 3 the dog was allowed to settle in the cage for 20 - 30 min before drug administration. On day 2, two hours of anaesthesia with a volatile agent or one hour of fentanyl anaesthesia was completed before the drugs were given.

The unlabelled propranolol was given into the portal vein over a 10min period using a constant rate infusion pump. In all the studies 40mg of propranolol was used, with the exception of the enflurane study where 20mg were given. The smaller dose was used in the enflurane study to minimise the potentiation of the cardiovascular effects of the two drugs. At the start of the portal infusion, the labelled propranolol was given by bolus injection into the femoral vein. In all studies a dose of 200µCi of H3 -propranolol was used. The specific activity of this drug was 67 mCi/mg, only some 3ug of propranolol was given intravenously. Both cannulae (portal and femoral vein) were flushed with 20ml saline at the end of injection.

5.4.2 Blood sampling

An identical protocol for blood sampling was followed on each day in all of the studies. All blood samples were taken from the femoral arterial cannula. The samples were taken over a four hour period. A blank control sample was taken each day before the propranolol administration as was a sample for measurement of plasma binding of propranolol. Following injection of the propranolol, 8ml of arterial blood was withdrawn every 5min for the first hour and then every 15min over the next three hours. The blood was centrifuged for 10 min at 3000rpm and the plasma separated off and stored frozen at -20°C for subsequent measurement of plasma propranolol and H3-propranolol concentrations. Each blood sample was replaced by 20ml Hartman's solution to maintain blood volume. The analytical methods are described separately.

5.5 Human study

5.5.1 Outline

This study compared the effect on hepatic blood flow and hepatic enzyme activity of cimetidine and enprostil. Nine volunteers were enrolled into a Latin Square design study involving three treatments, that is the administration of cimetidine, enprostil or placebo each for six days. The drugs were administered in a double-blind randomised fashion. There was a seven day washout period between each treatment. On the fifth day of each treatment hepatic blood flow was measured by the indocyanine green extraction method. On the sixth day hepatic blood flow and hepatic enzyme activity were measured by the dual route propranolol method.

5.5.2 Subjects

Nine male subjects of mean age 23.2 +/- 3.3 years (+/- SD) and mean weight of 73.8 +/- 9.3 kg were enrolled into this study. All subjects had an essentially negative past medical history, a normal physical examination and normal haematological and biochemical profile. No other drugs, alcohol or tobacco

were taken throughout the study period. Written informed consent was obtained from all subjects.

5.5.3 Drugs

Each subject received, in a random order, six days treatment with each of the study drugs. A washout period of at least 8 days was observed between each treatment. The study drugs were enprostil 70µg (two 35µg capsules), cimetidine 600mg (two 300mg capsules) and placebo capsules. Enprostil is presented as a soft gelatin capsule and cimetidine in hard capsule form. Therefore, in order to maintain the blinding of the study, each dose consisted of two gelatin and two hard capsules. That is 2 enprostil / placebo soft capsules plus 2 cimetidine / placebo hard capsules.

The dose schedules were ;

enprostil - 2 35µg enprostil soft capsules plus 2 placebo hard capsules b.d. for six days,

cimetidine - 2 300mg cimetidine hard capsules plus 2 placebo soft capsules b.d. for six days and

placebo - 2 placebo hard capsules plus 2 placebo soft capsules b.d. for six days.

The drugs were administered at 8am and 8pm on the six study days.

5.5.4 Indocyanine green study

Hepatic blood flow was calculated for each subject using the indocyanine green method on the morning of the fifth day of each study period. Following an overnight fast, the subjects took their morning medication and reported to the study centre. After one hour lying supine to achieve basal conditions, they were given a dose of 0.5mg/kg indocyanine green by rapid (10 sec) bolus injection into an antecubital vein. Blood samples were taken from a vein in the contralateral arm at -1, 1,2,3,4,5,6,7,8,10,12,15 and 20 minutes following injection. These samples were heparinised and centrifuged at 3000 rpm for 30 min.

The plasma ICG concentration was then measured immediately using a Beckman spectrophotometer with absorbance set at 810nm. On each occasion a calibration curve was constructed using the subjects own plasma (-1 min sample). The clearance of ICG from the plasma was estimated from the slope (K) of the plot of the logarithm of the plasma ICG concentration against time, extrapolated to zero time concentration (Cp0). This was then applied to the equation :

$$\text{Clearance} = \frac{K.\ \text{Dose}}{Cp0}$$

The blood clearance, which was considered equivalent

to the hepatic blood flow, was obtained by correcting the plasma clearance for the haematocrit, which was measured at the time of study.

5.5.5 Propranolol

Hepatic blood flow was calculated using the dual route propranolol method on the sixth day of each study period. The subject took the final (morning) dose of the drug, again after an overnight fast, and reported to the Clinical Research Center. After a 30min rest period 80mg of propranolol was taken orally and simultaneously 50μCi H3- propranolol was injected intravenously. Blood samples were taken before the propranolol and at 5,15 and 30min and 1,2,3,4,5,6,7 and 8 hours after the propranolol. The samples were centrifuged and the plasma separated off and stored for analysis of propranolol and H3- propranolol concentrations as described below. A sample was also taken before the propranolol administration for determination of the plasma binding of propranolol by equilibrium dialysis. The subjects were allowed to eat after the 4 hour sample had been taken. Blood pressure and heart rate were measured at hourly intervals during the study.

5.5.6 Side effects

All subjects were questioned at the end of each phase as to any side effect they had noticed which may have been attributed to the study drug. An haematology screen, consisting of full blood count and differential white cell count, and a biochemical screen, consisting of urea, electrolytes and liver function tests (SMAC 18), were taken before starting and at the end of each phase.

5.5.7 Summary

Day 1 - 6	Phase 1 -	ICG study on day 5, propranolol study on day 6
Day 7 - 14	Washout period	
Day 15 - 20	Phase 2 -	ICG study on day 19, propranolol study on day 20
Day 21 - 28	Washout period	
Day 29 - 34	Phase 3 -	ICG study on day 33, propranolol study on day 34

5.6 Drug Analysis

5.6.1 Propranolol Assay

Propranolol concentrations were measured in plasma by a high pressure liquid chromatography (HPLC) method. Each plasma sample was prepared and analysed in duplicate. A mean value of the two samples was used for all calculations. The mobile phase for the HPLC was prepared daily and consisted of : 450ml acetonitrile, 600ml distilled water, 10ml glacial acetic acid and 0.15ml triethylamine. The mixture was de-aerated for 40min using a sonicator. These reagents and all others used in this method were purchased from Burdick and Jackson Inc and had been prepared by distillation in glass. The internal standard was N-ethyl-propranolol. A standard propranolol solution containing 4µg/ml propranolol hydrochloride was prepared for calibration of the system.

A standard calibration curve was run at the start and finish of each batch of samples. This was prepared as follows ;

Propranolol conc. (ng/ml)	0	25	50	100	200
plasma (ml)	1	1	1	1	1
standard propranolol (µl)	0	6.25	12.5	25	50
Internal standard (µl)	0	100	100	100	100
Distilled water (µl)	500	175	150	100	0

The samples for analysis were initially defrosted to room temperature. 1ml of the plasma was transfered to a 17ml culture tube and 100µl of internal standard and 1ml 1N sodium hydroxide added and the tube vortexed. 8ml of 1.5% isoamyl alcohol in heptane was then added and the mixture shaken for 5min then centrifuged at 1400rpm for 10min. 7ml of the organic layer was then transfered to another tube and blown down with nitrogen to dryness in a 50°C water bath. The solute was then reconstituted by addition of 200ul methanol for injection into the HPLC system (313). A volume of 50µl was injected onto the HPLC for analysis.

This method showed excellent reproducability with a 1-3% within day variability and serial analysis over the duration of all the analysis for the studies using the same plasma produced a varaibility of 3-7%. The pooled calibration samples for the human study demonstrated reproducability and linearity with a correlation coefficient of 0.999, slope of 0.352 and intercept of

5.6.2 HPLC system

The HPLC system was based on a Waters WISP 710A programmable autoinjector and pump which was linked to a Waters Data Module cosisting of an analyser and printer. The column was a Waters Bondapak C18 (250 x 4.6mm, 10µ). The fluorometric detection used a Schoeffel FS970 Fluoromonitor set at 228nm with a 295nm interference filter. The flow rate through the system was 2ml/min. The retention time for propranolol was around 6min and 8min for the internal standard. The output of the detector was recorded on a dual channel recorder (Linear Instruments Corp.). The propranolol concentration was calculated, by the analyser, from the peak height ratio of the propranolol and internal standard. A linear regression was calculated between the height of the propranolol peak for the known concentrations in the calibration curves and the corresponding internal standard peaks. The unknown concentrations of propranolol were calculated by dividing the peak height of the propranolol trace by the slope of the linear regression.

5.6.3 Scintillation counting

The 3H-propranolol concentrations were calculated by collecting the effluent of the HPLC throughout the period of each individual sample run during which the propranolol would appear in it. The retention time of the tritiated propranolol was similar to that of unlabelled propranolol. The collection of the effluent was done manually from the 5th to 7th minute of each sample run, as the retention time of propranolol was 6 minutes. The 4ml of effluent was then made up to 10ml with Unogel liquid scintillation counting fluid (Schwarz Chemicals). The radioactivity in each sample was then counted for 10min in an Isocap 300 scintillation spectrometer. This produced a measure of the 3H-propranolol concentration in dpm/ml (dpm = disintegrations per minute) (292).

5.6.4 Measurement of drug binding

The unbound fraction of propranolol in plasma was measured by equilibrium dialysis. This technique utilises the equilibration across a semi-permeable membrane of a protein-free phosphate buffer and a plasma sample which has a known amount of radio-labelled drug added to it. The free fraction is equal to the

concentration in the buffer divided by the concentration in the plasma.

The buffer was prepared by adding 197ml 0.066M KH_2PO_4 and 803ml 0.066M $Na_2HPO_4.7H_2O$. The pH of the solution was adjusted to 7.4. The equilibration cells were prepared by placing the moistened semi-permeable membrane between the two ports. 1ml of buffer was placed in one port and 1ml of plasma which had been mixed with 100ul of 3H-propranolol was placed on the other. The sealed cells were allowed to equilibrate for 4hours in a water bath at 37°C. After equilibration 100µl of each solution was added to separate scintillation vials with 10ml of Unogel scintillation fluid (Schwarz). Each vial was scintillation counted for 10min and the dpm calculated (314,315).

$$\% \text{ free propranolol} = \frac{\text{dpm of buffer}}{\text{dpm of plasma}}$$

5.7 Calculations

5.7.1 Dose of drug injected

The dose of propranolol administered was calculated by weighing the syringe before and after injection. The propranolol was supplied at a concentration of 1mg per 1ml. The dose given (mg) was therefore equal to volume (ml) which in turn was equal to the difference in weight between the full and empty syringe.

The dose of H3-propranolol was calculated from the concentration of and volume of drug injected. The concentration was calculated by storing a small aliquot of the fluid injected until the propranolol concentrations for that specific study were being run on the HPLC. A known quantity of this fluid (50µl) was then added to each of the calibration samples for that run. This involves eight samples, four at the start of the run and four at the end. The eluent from these samples was collected and scintillation counted as described above. The mean count (dpm) of the eight samples of known volume was used to calculate the total amount of drug administered by measuring the volume injected as described above. This method of calculation accounts for any decay that may occur while stored between injection

and measurement and, as the aliquot is put through the same extraction procedure as the samples, any contaminants, such as tritiated water, will be removed.

5.7.2 Area under the time-concentration curve

The area under the time concentration curve (AUC) was calculated for the propranolol and H3- propranolol for each subject on each day. This was calculated using the log-trapezoidal method on the Clinfo data handling package. This programme required an elimination constant to extrapolate the curve to infinity and therefore give an AUC to infinity. This was done with a curve fitting programme on the University DEC-10 mainframe computer. Each time-concentration curve was drawn out by hand on log graph paper. This allowed exclusion of any obvious outlying data points from the curve fit. The programme derived the elimination constant by linear regression analysis.

The calculation of these four basic values ; the intravenous dose of propranolol, the portal dose of propranolol and the areas under the time- concentration curves for the two routes of administration, allowed calculation of all the pharmacokinetic parameters. This included the systemic and portal clearance, the hepatic

extraction ratio, bioavailability, the elimination half-life, the volume of distribution and the hepatic plasma flow.

5.7.3 Clearance

Propranolol clearance following intravenous or systemic administration (Cls) was calculated as :

$$Cls = \frac{Xdiv}{AUCiv} \qquad (1)$$

where Xdiv is the dose of labelled propranolol administered intravenously and AUCiv is the area under the time concentration curve calculated by the trapezoidal rule for the intravenous labelled propranolol.

The clearance of the unlabelled propranolol administered into the portal vein (Clp) was calculated as :

$$Clp = \frac{Xdp}{AUCp} \qquad (2)$$

where Xdp is the dose of unlabelled drug administered into the portal vein and AUCp is the area under the time concentration curve for the unlabelled drug.

As propranolol is only metabolised in the liver and was injected into the portal vein, which is equivalent to 100% absorption following oral administration, the

apparent clearance of the portally administered propranolol (Clp) is numerically equal to the total intrinsic clearance (Clint).

Thus Intrinsic clearance (Clint) = Clp = $\frac{Xdp}{AUCp}$

5.7.4 Hepatic extraction ratio

The hepatic extraction ratio (E) and bioavailability (F) were calculated from the formulae ;

$$F = \frac{Cliv}{Clp}$$

and

$$F = (1-E)$$

5.7.5 Elimination constant and half life

The elimination rate constant (k) was calculated by linear regression analysis as described above and T1/2iv as :

$$T1/2iv = \frac{0.693}{k}$$

5.7.6 Volume of distribution

The apparent volume of distribution (Vb) was calculated as :

$$Vb = \frac{Cls}{k}$$

5.6.7 Hepatic plasma flow

The hepatic plasma flow (Q) was calculated as follows :

By definition, following intraportal administration of a drug of dose (Xdp), the amount of the drug entering the systemic circulation is equal to XdpF where F is the fractional systemic availability.

Thus :

$$Cls = \frac{XdpF}{AUCp} \qquad (3)$$

But as Xdp/AUCp = Clp (equation 2) , and by definition F=1-E where E is the hepatic extraction ratio :

$$Cls = (1-E)Clp \qquad (4)$$

Also by Fick's principle : Cls = QE , where Q = apparent hepatic plasma flow.

Thus substituting for E in equation 4 :

$$Q = \frac{Cls.Clp}{Clp - Cls}$$

Therefore by substituting for Clp and Cls (Equations 1 and 2):

$$\text{Hepatic plasma flow} = \frac{Xdiv.Xdp}{AUCivXdp - AUCpXdiv}$$

5.8 Statistical analysis

All data were stored on file on the Clinfo computer and in hard copy. The Clinfo system is an IBM based data handling system designed for medical and biological sciences. The package allows the construction of large spreadsheets for data storage and handling. It also provided basic mathematic functions and descriptive statistics as well as a wide range of parametric and non-parametric statistics.

In all the dog studies, for each group statistical comparisons between the 3 study days were performed using analysis of variance. Where this was significantly different ($p<0.05$) Student's t-test for paired data was carried out.

In all tests the level of significance was taken as $p<0.05$.

Comparison between the Groups was also by ANOVA and any significant differences were then subjected to a Mann-Whitney Test.

The demographic values for each study are presented as means and standard deviations. The calculated results for each study are presented in full, and as mean values with standard error of the mean.

In the human study comparison of the three drugs was

by ANOVA. Where differences were found Student's t-test for pair data was used to compare the groups.

5.9 Ethical considerations

All the dog studies had received the approval of the Vanderbilt University Medical School Research Ethical Committee. The care and kenneling of the dogs was supervised by the Vanderbilt Animal Care Center, which complies with national animal care standards and is inspected annually for compliance with regulations.

The enprostil study had been approved by the Research Ethical Committee and was run, in conjunction with Syntex Research, in accordance with the regulations of the United States Food and Drug Administration for new drugs. All the participants in the study had received a full verbal and written explanation of the study and had given written consent (Appendix).

Chapter 6

Thesis Results

6.1 Control

6.2 Halothane

6.3 Isoflurane

6.4 Enflurane

6.5 Fentanyl

6.6 Comparison of anaesthesia groups

6.7 Enprostil and cimetidine study

6.1 Control group

Three male mongrel dogs, of mean weight 22.3 +/- 3.6kg, were studied on three consecutive days without anaesthesia. The mean plasma concentrations for the portally administered propranolol are shown against time in Figure 3 and that for the intravenous labelled propranolol in Figure 4.

There were no significant differences in systemic clearance, hepatic plasma flow or plasma halflife on the three days. The intrinsic clearance, however, fell on Day 3 for two of the dogs. The individual values for the drug doses and derived data are shown for each day in Tables I-III , and the mean values (+/-SD) in Table IV.

Figure 3

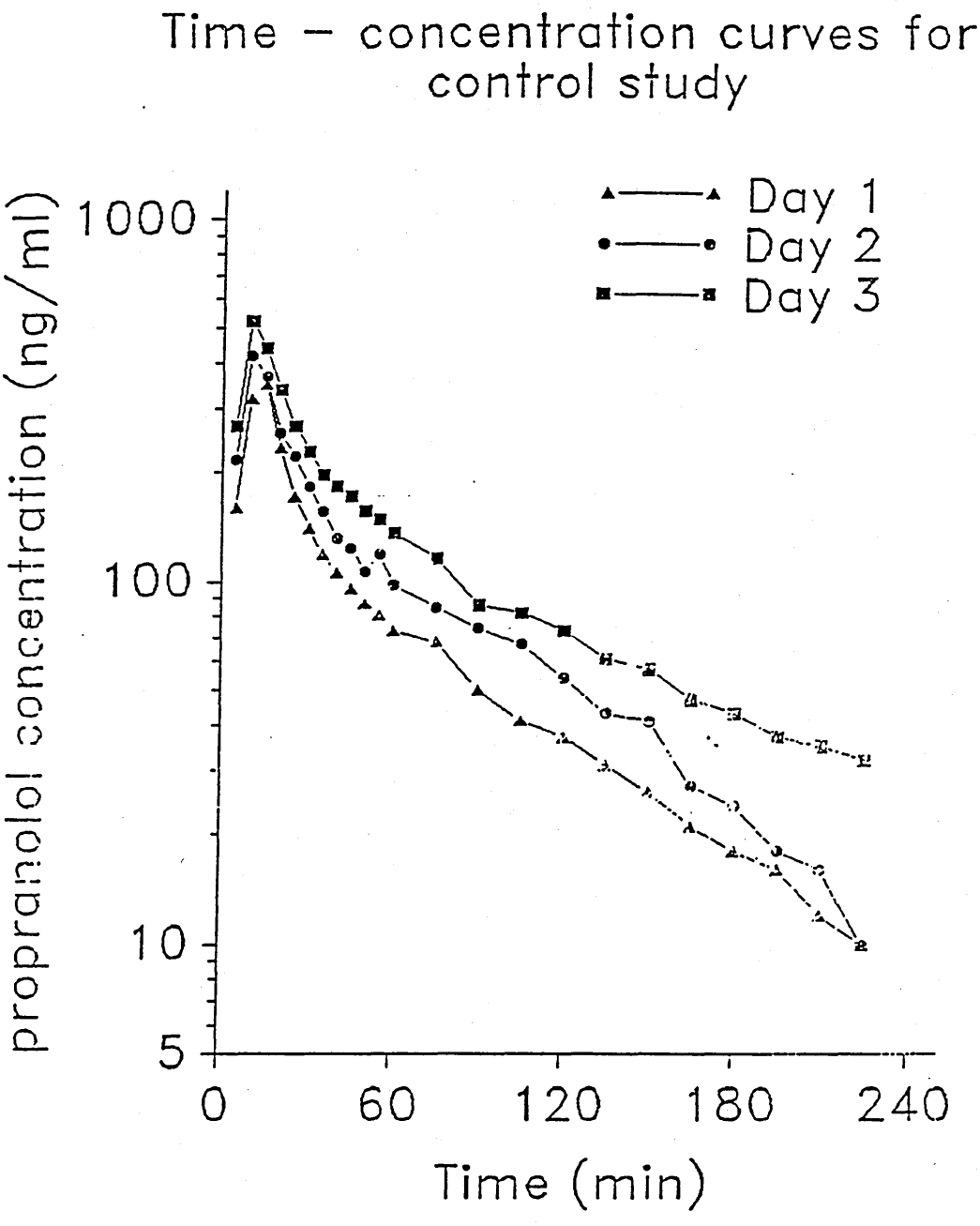
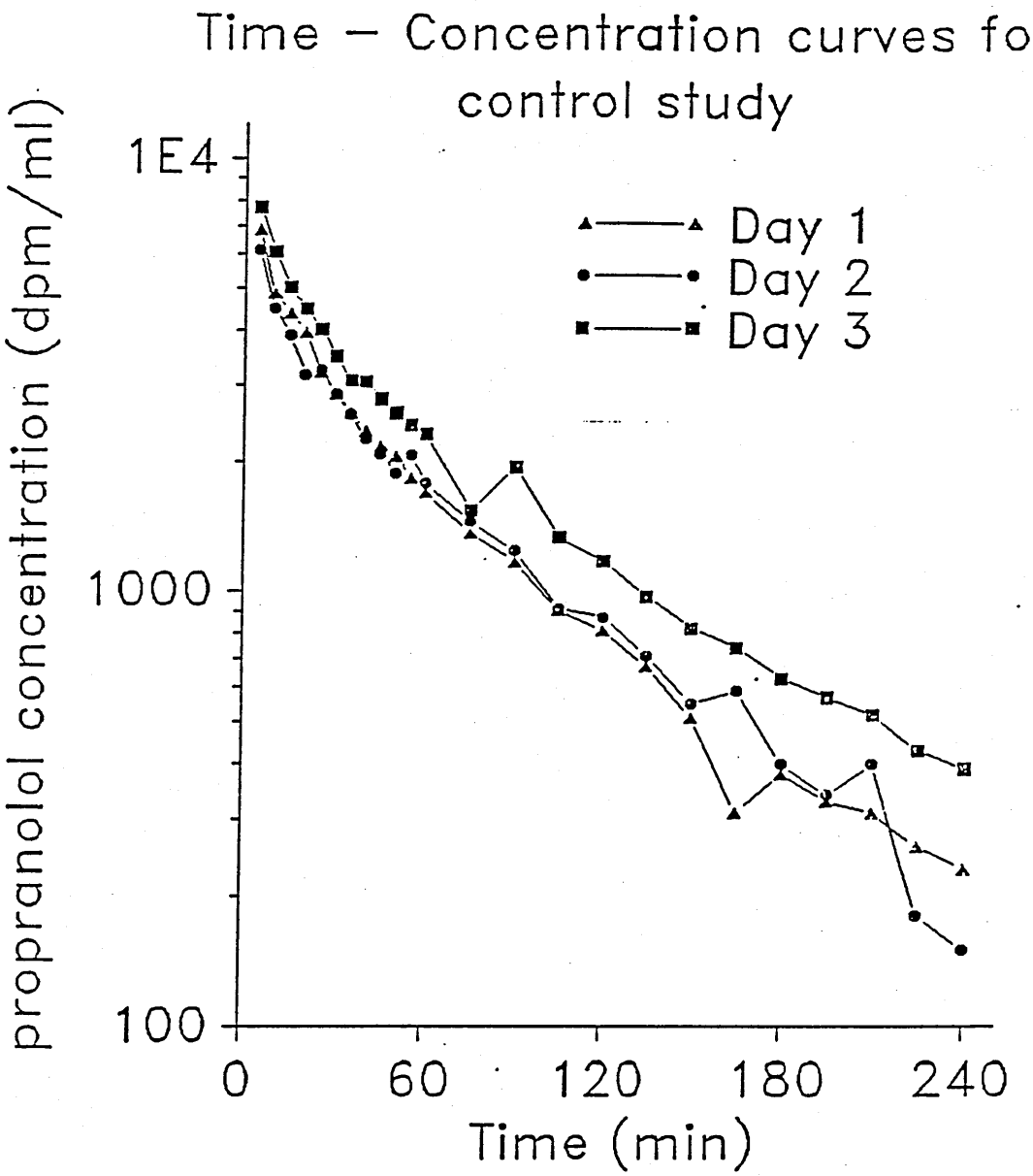

Time – concentration curves for control study
Day 1
Day 2
Day 3
propranolol concentration (ng/ml)
1000
100
10
5
0
60
120
180
240
Time (min)

Figure 4


Time – Concentration curves for control study
propranolol concentration (dpm/ml)
1E4
1000
100
Day 1
Day 2
Day 3
0
60
120
180
240
Time (min)

Symbols used in Tables

$Dose_p$	Dose of unlabelled propranolol given into portal vein
AUC_p	Area under time-concentration curve to infinity for portally administered drug
$Dose_{iv}$	Dose of ^3H-labelled propranolol given intravenously
AUC_{iv}	Area under time concentration curve to infinity for intravenously administered drug
Cl_{int}	Intrinsic clearance of propranolol
Cl_s	Systemic clearance of propranolol
H.P.F.	Hepatic plasma flow calculated by dual-route method
$T1\backslash 2_{iv}$	Half life of intravenously administered propranolol
Vb	Apparent volume of distribution
ER	Hepatic extraction ratio
% free	Percentage of unbound propranolol

Table I

Control Study Day 1

DOG NO.	1	2	3
$Dose_p$ (ng 10^7)	3.93	3.95	3.94
AUC_p (ng.min.10^3)	1.93	1.55	1.82
$Dose_{iv}$ (dpm.10^8)	1.89	2.04	2.00
AUC_{iv} (dpm.min.10^5)	3.16	3.11	3.49
Cl_{int} (ml.min^{-1})	2040	2550	2150
Cl_s (ml.min^{-1})	600	660	570
H.P.F. (ml.min^{-1})	850	880	780
$T1/2_{iv}$ (min)	54	38	119
V_b (litre)	47	36	98
E.R.	0.70	0.74	0.73
% free	6.8	4.8	10.6

Table II

Control Study Day 2

DOG NO.	1	2	3
$Dose_p$ (ng 10^7)	3.92	3.93	3.94
AUC_p (ng.min.10^3)	2.62	1.86	1.69
$Dose_{iv}$ (dpm.10^8)	1.86	2.20	2.05
AUC_{iv} (dpm.min.10^5)	3.04	2.60	3.70
Cl_{int} ($ml.min^{-1}$)	1490	2110	2320
Cl_s ($ml.min^{-1}$)	610	850	550
H.P.F. ($ml.min^{-1}$)	1030	1410	730
$T1/2_{iv}$ (min)	58	42	72
V_b (litre)	51	53	57
E.R.	0.60	0.64	0.74
% free	7.8	5.0	7.2

Table III

Control Study Day 3

DOG NO.	1	2	3
$Dose_p$ (ng 10^7)	3.94	3.91	3.96
AUC_p (ng.min.10^3)	2.86	2.42	2.91
$Dose_{iv}$ (dpm.10^8)	2.07	2.07	2.07
AUC_{iv} (dpm.min.10^5)	3.15	3.78	3.05
Cl_{int} (ml.min^{-1})	1380	1610	1360
Cl_s (ml.min^{-1})	650	550	580
H.P.F. (ml.min^{-1})	1250	830	1340
$T1/2_{iv}$ (min)	55	47	94
V_b (litre)	52	38	92
E.R.	0.53	0.66	0.58
% free	7.6	5.2	5.5

Table IV

Control study
Mean values (SD)

DAY	1	2	3
Cl_{int} ($ml.min^{-1}$)	2250 (270)	1980 (420)	1450 (140)
Cl_s ($ml.min^{-1}$)	610 (40)	670 (150)	590 (55)
H.P.F. ($ml.min^{-1}$)	840 (50)	1060 (340)	1140 (270)
$T1/2_{iv}$ (min)	70 (24)	57 (18)	65 (13)
V_b (litre)	60 (30)	54 (3)	61 (25)
E.R.	0.73 (.02)	0.65 (.08)	0.60 (.07)
% free	7.4 (2.9)	6.7 (1.4)	6.1 (1.3)

6.2 Halothane Anaesthesia

Six male mongrel dogs of mean weight 22.9 +/- 4.5 kg were studied.

The mean plasma concentrations of the portally administered propranolol at each time point on the three study days are shown in Figure 5. This shows an increase in the propranolol concentration and a slowing of the rate of elimination during anaesthesia when compared to the pre-anaesthetic values. These changes are still evident at 24 hours after anaesthesia.

The mean plasma concentrations of the H3- labelled propranolol on the three study days are plotted against time in Figure 6. This also demonstrates an increase in plasma propranolol concentration and decreased elimination rate during halothane anaesthesia which has not returned to pre-anaesthesia values 24 hours later.

The individual values for each study day are shown in Tables V-VII and the mean values for each day in Table VIII. The changes in the mean values for intrinsic clearance, systemic clearance and hepatic plasma flow are shown in Figure 7.

The intrinsic clearance of propranolol, that is the clearance of the portally administered drug, had a mean

value of 2110 +/- 298 $ml.min^{-1}$ on Day 1. This decreased by 62% to 799 +/- 233 $ml.min^{-1}$ during halothane anaesthesia and was still decreased by almost 50% to 1095 +/- 331 $ml.min^{-1}$ on Day 3.

The systemic clearance of propranolol, the clearance of the labelled intravenous drug, had a mean value of 470 +/- 33 $ml.min^{-1}$ on Day 1. This decreased by 40% to 280 +/- 38 $ml.min^{-1}$ during anaesthesia and had recovered to 333 +/- 57 $ml.min^{-1}$ on Day 3.

The mean elimination half-life of the intravenously administered propranolol was increased from 87 +/- 12 min on Day 1 to 155 +/- 23 min during anaesthesia and returned to 94 +/- 12 min on Day 3.

The hepatic extraction (E) of propranolol was decreased from a mean value of 0.75 +/- 0.04 on day 1 to 0.59 +/- 0.05 on Day 2 and 0.60 +/- 0.07 on Day 3. This resulted in an increased bioavailability (1 - E) of propranolol.

The volume of distribution did not change significantly throughout the study period.

The plasma binding of propranolol was reduced significantly during anaesthesia resulting in an increase in the free fraction from a mean of 7.1 +/- 1.0% on Day 1 to 9.6 +/- 1.4% on Day 2 and returning to 8.1 +/- 0.9% on Day 3.

The calculated hepatic plasma flow demonstrated a non-significant decrease from a mean value of 642 +/- 80

$ml.min^{-1}$ on Day 1 to 473 +/- 47 $ml.min^{-1}$ on Day 2 and returning to 583 +/- 83 $ml.min^{-1}$ on Day 3.

The mean duration of halothane anaesthesia was 11.7 +/- 0.4 MAC hours.

Figure 5 HALOTHANE

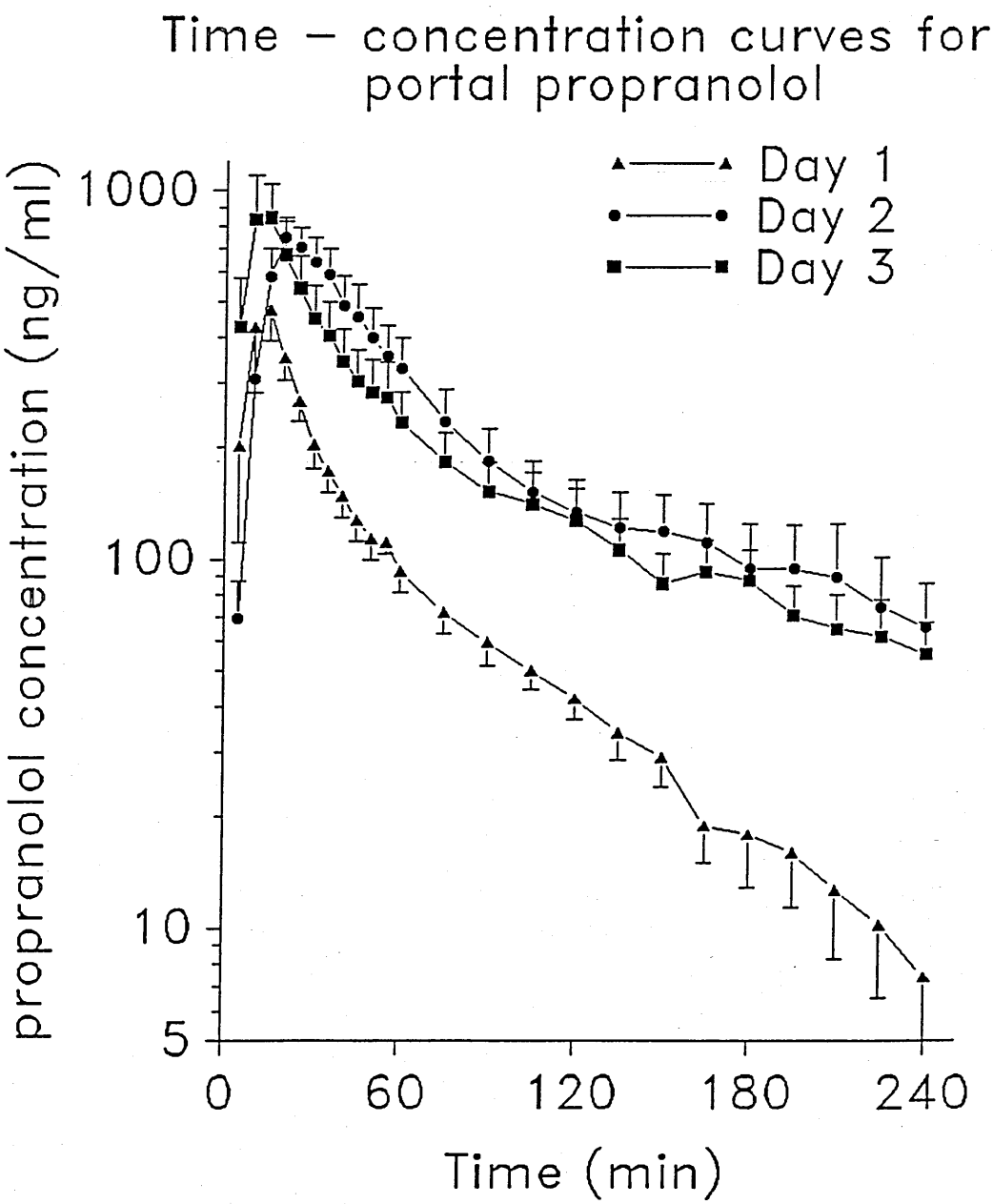


Figure 6 HALOTHANE

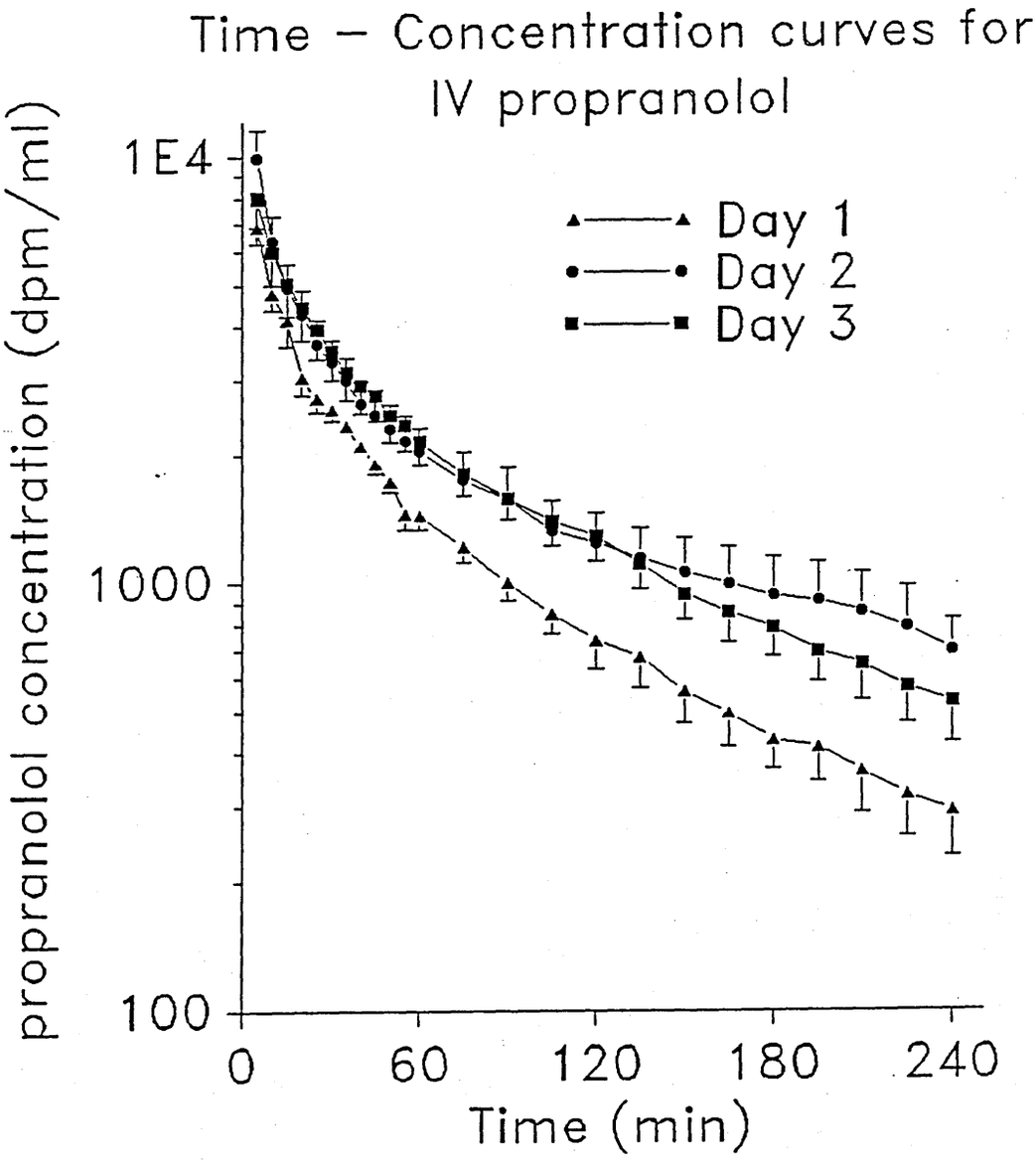


TABLE V

Halothane Study day 1

DOG NO.	1	2	3	4	5	6
$Dose_p$ (ng 10^7)	3.80	4.06	4.03	3.97	4.00	3.98
AUC_p (ng.min.10^4)	1.78	2.23	2.75	2.61	1.75	1.16
$Dose_{iv}$ (dpm.10^8)	1.28	1.28	1.67	1.73	1.42	1.77
AUC_{iv} (dpm.min.10^5)	2.40	3.59	3.52	2.88	3.13	4.09
Cl_{int} ($ml.min^{-1}$)	2130	1820	1460	1520	2290	3440
Cl_s ($ml.min^{-1}$)	510	360	470	600	450	430
H.P.F. ($ml.min^{-1}$)	660	440	690	990	570	490
$T1/2_{iv}$ (min)	58	128	70	61	97	110
V_b (litre)	44	66	47	53	63	68
E.R.	0.76	0.80	0.68	0.61	0.80	0.87
% free	5.9	7.1	5.1	4.5	9.4	10.8

TABLE VI

Halothane Study Day 2

DOG NO.	1	2	3	4	5	6
$Dose_p$ (ng 10^7)	4.05	3.98	3.99	4.08	3.97	3.96
AUC_p (ng.min.10^4)	2.09	7.83	1.37	4.73	5.92	7.41
$Dose_{iv}$ (dpm.10^8)	1.10	1.36	1.52	1.72	1.71	1.67
AUC_{iv} (dpm.min.10^5)	2.21	4.87	1.02	5.72	5.74	7.54
Cl_{int} ($ml.min^{-1}$)	1900	510	290	860	700	530
Cl_s ($ml.min^{-1}$)	430	280	150	300	300	220
H.P.F. ($ml.min^{-1}$)	560	620	310	460	580	380
$T1/2_{iv}$ (min)	73	209	182	83	135	218
V_b (litre)	73	84	39	36	58	70
E.R.	0.77	0.45	0.49	0.65	0.58	0.59
% free	8.8	7.8	6.3	7.9	11.7	15.7

TABLE VII

Halothane Study Day 3

DOG NO.	1	2	3	4	5	6
$Dose_p$ (ng 10^7)	4.19	4.02	3.87	3.97	3.91	3.99
AUC_p (ng.min.10^4)	1.76	6.60	5.54	7.38	2.06	8.27
$Dose_{iv}$ (dpm.10^8)	1.33	1.43	1.81	1.42	1.03	2.05
AUC_{iv} (dpm.min.10^5)	2.19	5.55	5.36	4.90	4.61	7.12
Cl_{int} (ml.min^{-1})	2340	610	700	540	1900	480
Cl_s (ml.min^{-1})	610	260	340	290	220	290
H.P.F. (ml.min^{-1})	820	440	650	610	250	720
$T1/2_{iv}$ (min)	73	146	70	77	91	107
V_b (litre)	64	54	33	32	29	44
E.R.	0.74	0.58	0.52	0.47	0.88	0.40
% free	8.0	7.9	6.0	5.6	10.8	10.6

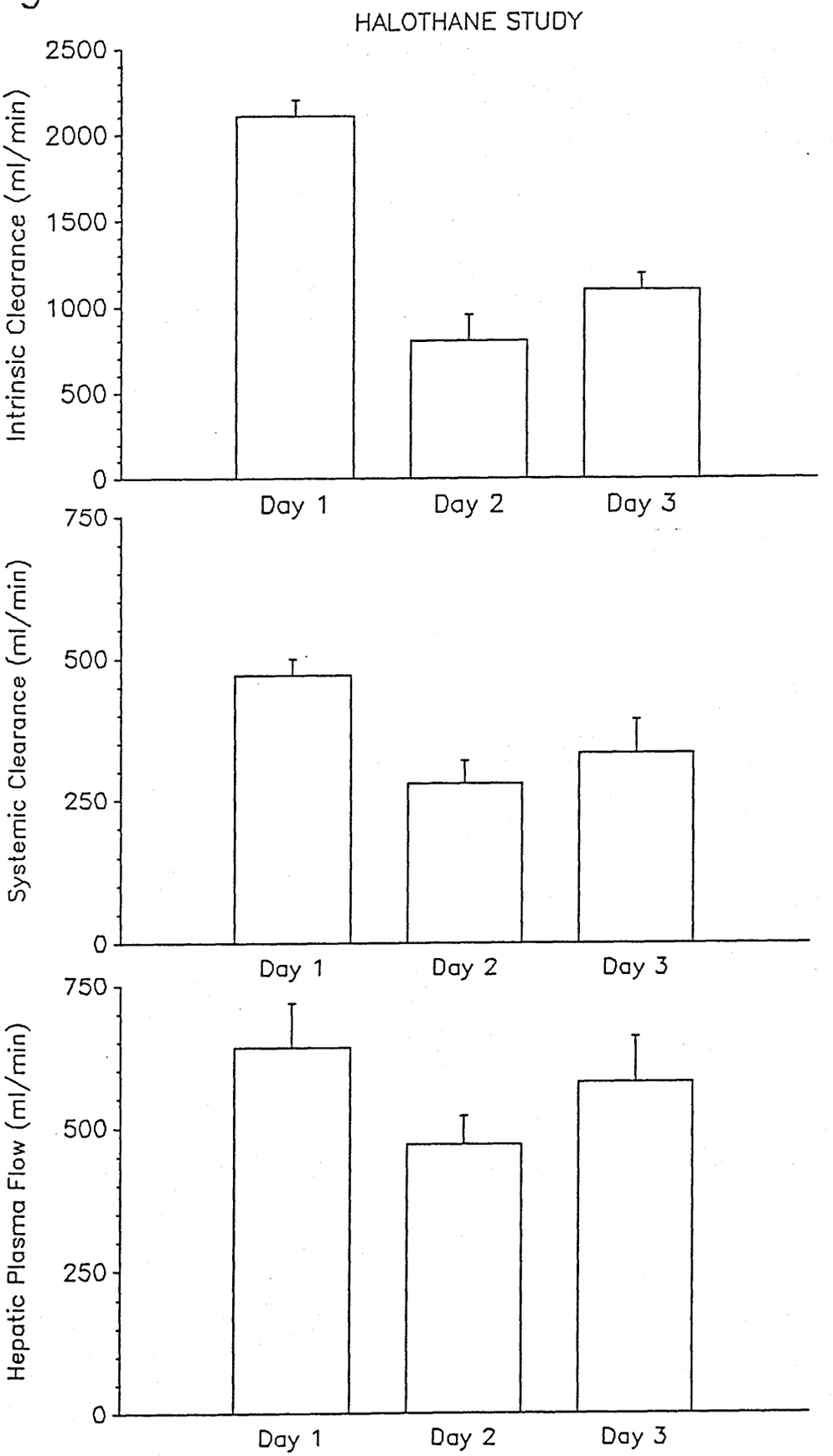
Table VIII

Halothane study
Mean values (SEM)

DAY	1	2	3
Cl_{int} ($ml.min^{-1}$)	2110 (300)	800* (230)	1100 (330)
Cl_s ($ml.min^{-1}$)	470 (30)	280* (40)	330 (60)
H.P.F. ($ml.min^{-1}$)	640 (80)	470 (50)	580 (80)
$T1/2_{iv}$ (min)	87 (12)	155* (23)	94 (12)
V_b (litre)	57 (4)	60 (8)	43 (6)
E.R.	0.75 (.04)	0.59* (.05)	0.60* (.07)
% free	7.1 (1)	9.6* (1.4)	8.1 (.9)

* $p<0.05$ compared with Day 1

Figure 7


HALOTHANE STUDY
Intrinsic Clearance (ml/min)
2500
2000
1500
1000
500
0
Day 1
Day 2
Day 3
Systemic Clearance (ml/min)
750
500
250
0
Day 1
Day 2
Day 3
Hepatic Plasma Flow (ml/min)
750
500
250
0
Day 1
Day 2
Day 3

6.3 Isoflurane anaesthesia

Six male mongrel dogs of mean weight 19.2 +/- 3.7 kg were studied. The duration of isoflurane anaesthesia was 11.2 +/-0.8 MAC hours.

The mean plasma concentrations of the intraportally administered propranolol are shown plotted against time in Figure 8. This demonstrates an increase in plasma concentration of propranolol during anaesthesia which has returned to the pre-anaesthesia level 24 hours later.

The mean plasma concentrations of the intravenously administered H3- labelled propranolol are shown in Figure 9. This shows a similar pattern of change to that of the intraportal route.

The individual values for each study day are shown in Tables IX-XI , and the mean values for each day in Table XII. The changes in the mean values for intrinsic clearance, systemic clearance and hepatic plasma flow are shown in Figure 10.

The intrinsic clearance of propranolol fell by over 50% during anaesthesia from a mean pre-anaesthetic value of 1552 +/- 210 $ml.min^{-1}$ to 723 +/- 50 $ml.min^{-1}$ ($p<0.05$). On Day 3, this had returned to 1853 +/- 556 $ml.min^{-1}$

(NS).

The systemic clearance of propranolol decreased significantly from a mean value on Day 1 of 479 +/- 56 $ml.min^{-1}$ to 286 +/- 41 $ml.min^{-1}$ during anaesthesia ($p<0.05$). This fall of 40% during anaesthesia was not evident 24 hours later when the mean value was 454 +/- 51 $ml.min^{-1}$.

The mean elimination half-life of the intravenously administered propranolol was significantly increased from the Day 1 value of 68.5 +/- 6.7 min to 171.6 +/- 42.5 min during anaesthesia ($p<0.05$). This had fallen to 84.6 +/- 8.9 min on Day 3.

The hepatic extraction of propranolol fell from 0.66 on Day 1 to 0.61 on Day 2. These changes are reflected in the bioavailability.

There was no significant change in the volume of distribution throughout the study. There was no significant change in the free fraction of propranolol during the study.

There was a non-significant decrease in the calculated hepatic plasma flow from a mean value on day 1 of 884 +/- 304 $ml.min^{-1}$ to 506 +/- 96 $ml.min^{-1}$ during anaesthesia and a return to 721 +/- 79 $ml.min^{-1}$ on Day 3.

Figure 8 ISOFLURANE

Time – concentration curves for portal propranolol

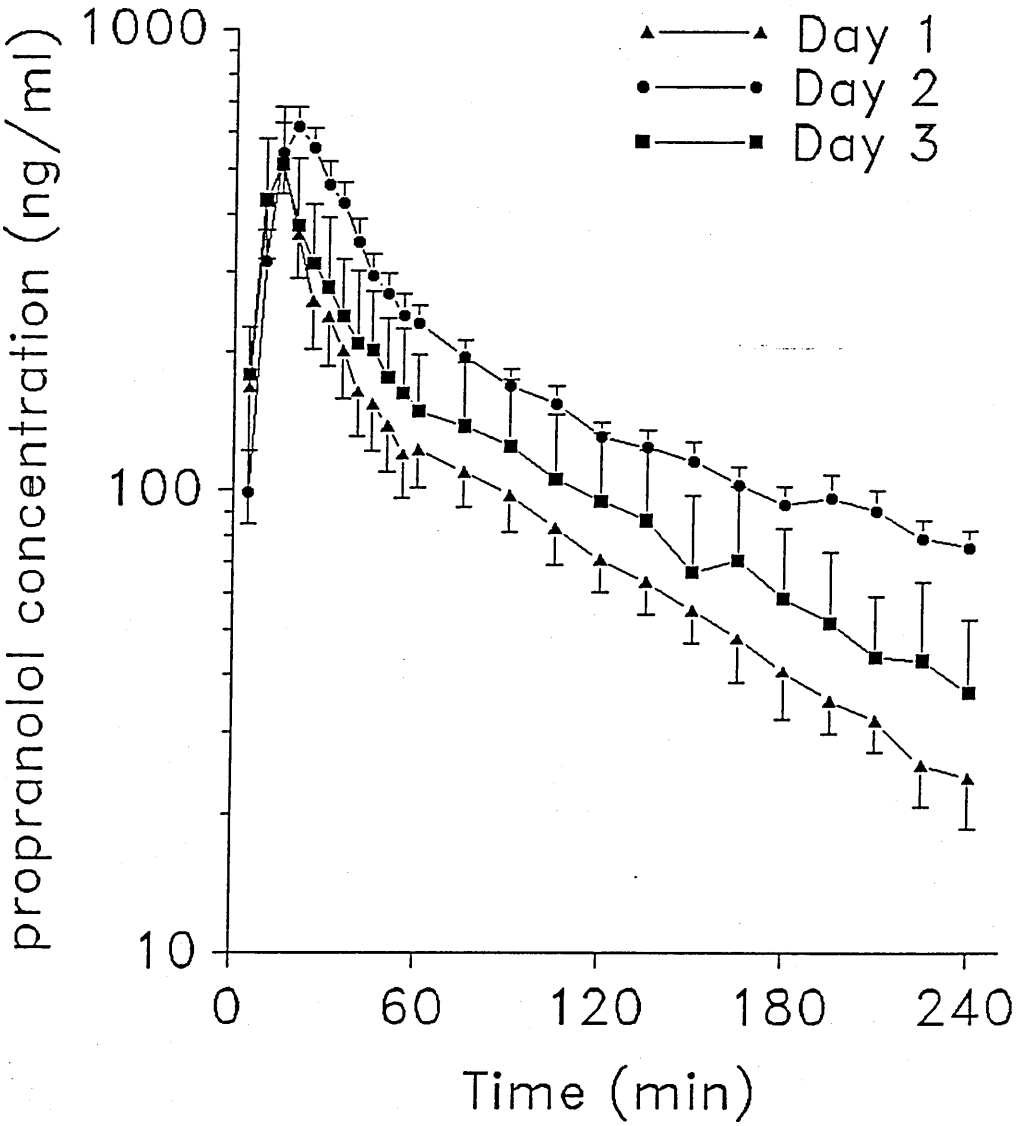


Figure 9 ISOFLURANE

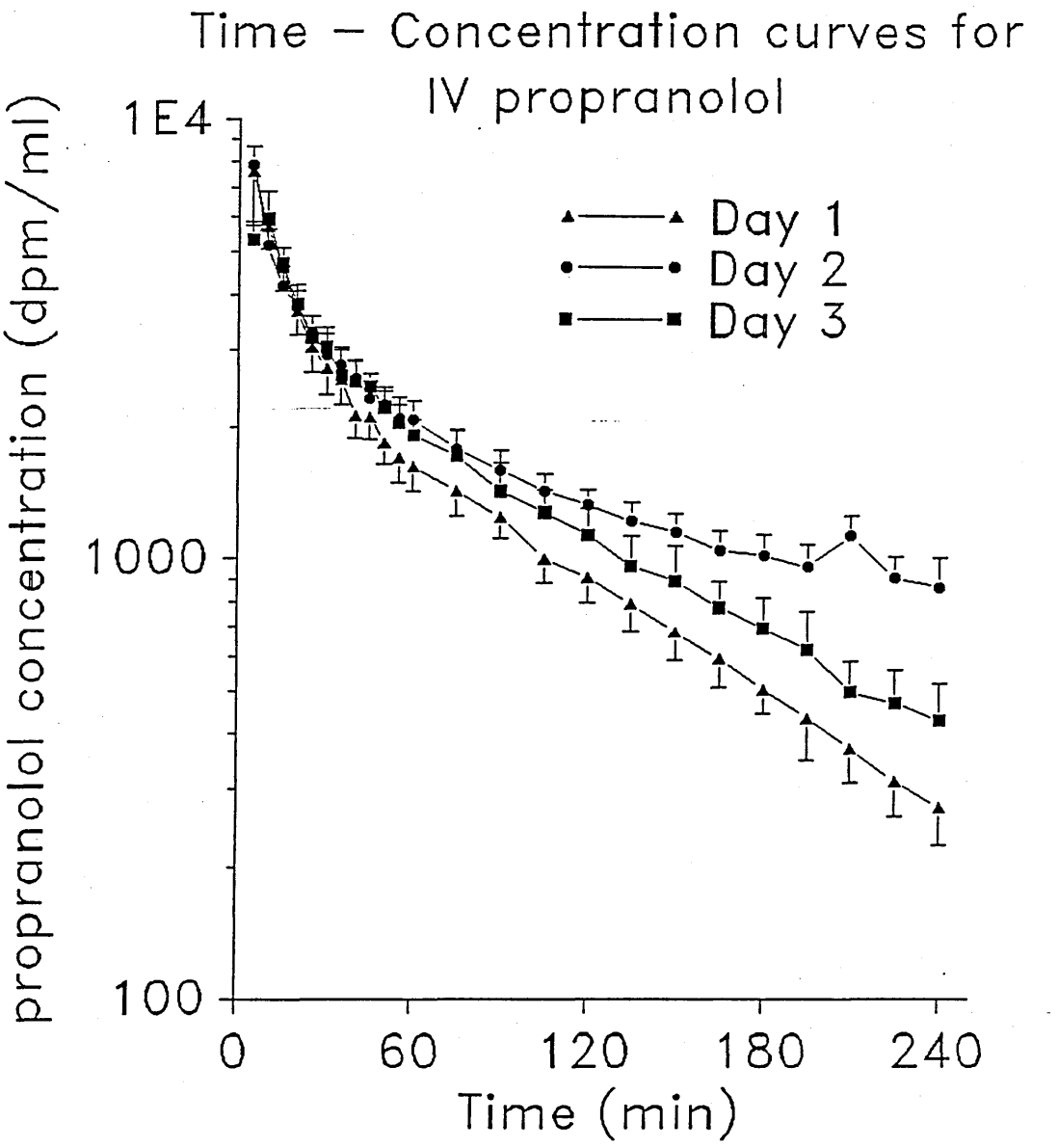


Table IX

Isoflurane Study Day 1

DOG NO.	1	2	3	4	5	6
$Dose_p$ (ng 10^7)	4.02	3.94	3.92	4.04	3.88	4.00
AUC_p (ng.min.10^4)	1.86	3.70	3.28	1.87	2.38	3.66
$Dose_{iv}$ (dpm.10^8)	1.94	1.86	1.48	1.40	1.67	1.32
AUC_{iv} (dpm.min.10^5)	3.84	2.50	4.21	3.06	3.42	4.43
Cl_{int} ($ml.min^{-1}$)	2160	1060	1200	2160	1630	1090
Cl_s ($ml.min^{-1}$)	500	740	350	460	490	330
H.P.F. ($ml.min^{-1}$)	660	2500	500	580	700	470
$T1/2_{iv}$ (min)	90	80	53	47	72	70
V_b (litre)	66	85	27	31	51	33
E.R.	0.77	0.69	0.70	0.79	0.70	0.70
% free	13.0	13.2	8.5	5.7	9.3	11.0

Table X

Isoflurane Study Day 2

DOG NO.	1	2	3	4	5	6
$Dose_p$ ($ng\ 10^7$)	4.00	3.93	4.00	4.03	4.21	3.97
AUC_p ($ng.min.10^4$)	7.33	4.99	6.68	5.21	4.88	5.17
$Dose_{iv}$ ($dpm.10^8$)	1.36	1.87	1.93	1.54	1.40	1.48
AUC_{iv} ($dpm.min.10^5$)	10.9	4.97	7.07	4.04	6.31	4.17
Cl_{int} ($ml.min^{-1}$)	550	790	600	770	860	770
Cl_s ($ml.min^{-1}$)	130	380	270	380	220	330
H.P.F. ($ml.min^{-1}$)	160	720	500	750	300	590
$T1/2_{iv}$ (min)	370	163	111	80	170	132
V_b (litre)	67	89	44	44	55	64
E.R.	0.77	0.52	0.54	0.50	0.74	0.56
% free	13.9	11.8	9.5	9.0	14.1	12.7

Table XI

Isoflurane Study Day 3

DOG NO.	1	2	3	4	5	6
$Dose_p$ (ng 10^7)	3.92	3.91	3.99	4.21	4.26	
AUC_p (ng.min.10^4)	8.62	2.05	3.15	1.10	2.35	
$Dose_{iv}$ (dpm.10^8)	1.77	1.94	1.90	1.72	1.58	
AUC_{iv} (dpm.min.10^5)	5.99	3.23	3.82	3.60	3.99	
Cl_{int} ($ml.min^{-1}$)	450	1910	1270	3820	1810	
Cl_s ($ml.min^{-1}$)	290	600	500	480	400	
H.P.F. ($ml.min^{-1}$)	850	880	820	540	520	
$T1/2_{iv}$ (min)	100	97	57	70	100	
V_b (litre)	43	84	41	49	57	
E.R.	0.65	0.68	0.60	0.87	0.78	
% free	13.1	13.6	8.0	7.9	11.6	

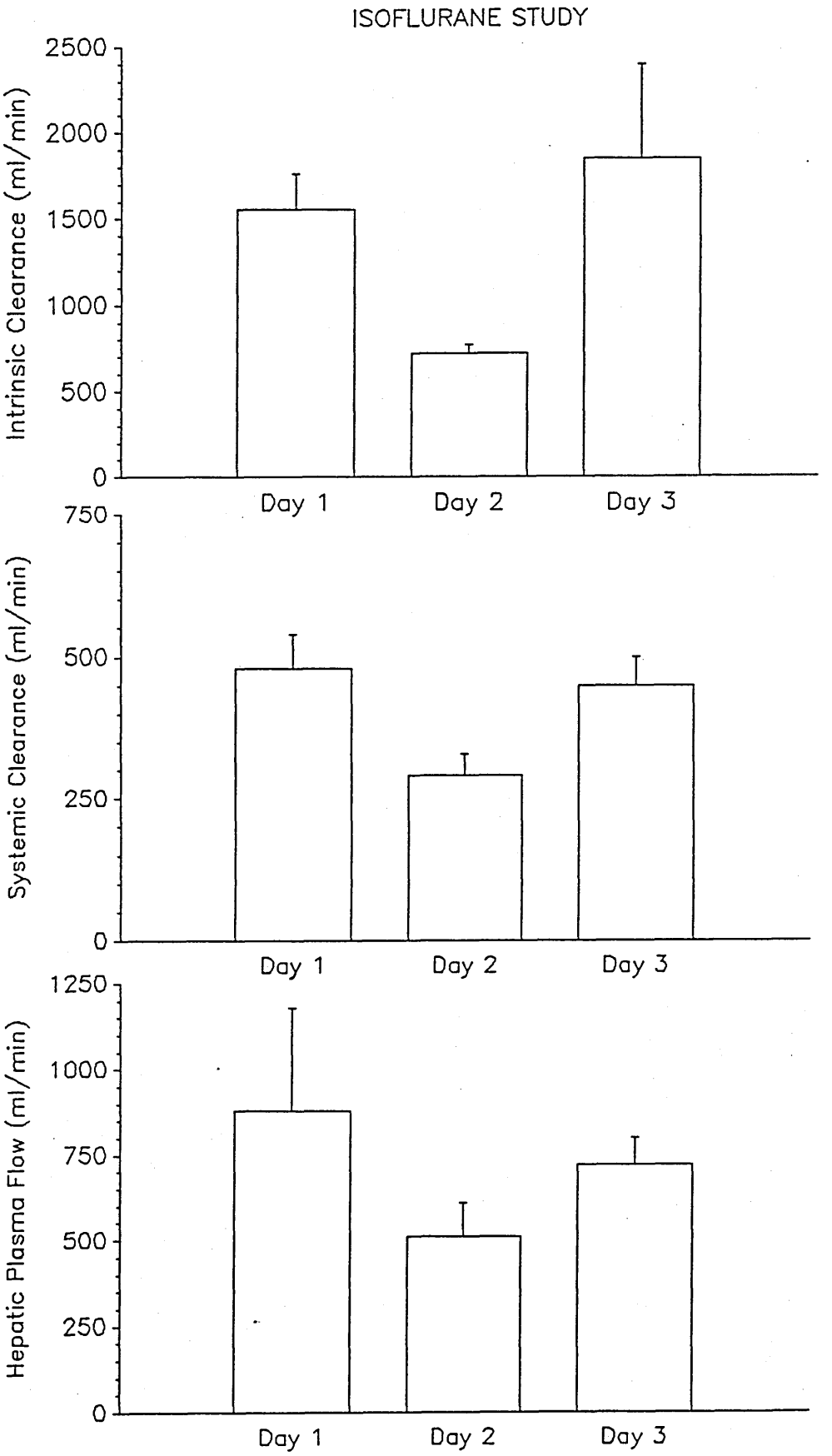
Table XII

Isoflurane study
Mean values (SEM)

DAY	1	2	3
Cl_{int} ($ml.min^{-1}$)	1550 (210)	720* (50)	1850 (550)
Cl_s ($ml.min^{-1}$)	480 (50)	290* (40)	450 (50)
H.P.F. ($ml.min^{-1}$)	880 (300)	510 (100)	720 (80)
$T1/2_{iv}$ (min)	69 (7)	172* (43)	85 (9)
V_b (litre)	49 (10)	60 (7)	55 (8)
E.R.	0.66 (.07)	0.61 (.05)	0.66 (.09)
% free	11.1 (.7)	11.9 (.8)	12.1 (1.5)

* $p<0.05$ compared with Day 1

Figure 10


ISOFLURANE STUDY
Intrinsic Clearance (ml/min)
2500
2000
1500
1000
500
0
Day 1
Day 2
Day 3
Systemic Clearance (ml/min)
750
500
250
0
Day 1
Day 2
Day 3
Hepatic Plasma Flow (ml/min)
1250
1000
750
500
250
0
Day 1
Day 2
Day 3

6.4 Enflurane anaesthesia

After initial studies with three dogs, which are not included in the results, it was found to be unsuitable to administer 2MAC of enflurane as this produced profound cardiac depression in the dogs. This was of a much greater magnitude than was found with the other agents. It was therefore decided to used 1.5 MAC in the enflurane study as this gave a comparable change in the cardiovascular parameters. The dose of propranolol was also reduced to 20mg given intraportally. This was still cardiodepressant and it was felt appropriate to continue the study for a third day in only two of the dogs.

Five male mongrel dogs of mean weight 20.8 +/-3.0 kg were studied.

The mean plasma concentration of the portally administered propranolol on the two study days is shown against time in Figure 11. This demonstrates an increase in plasma concentration and slowing of elimination of propranolol during enflurane anaesthesia. The individual concentration - time curves for the two dogs studied for a third day are not shown.

The mean plasma concentrations of the H3- labelled propranolol are shown against time in Figure 12. There

is again a slowing of elimination and increased concentration of propranolol.

The individual values for the five dogs studied for 2 days are shown in Tables XIII and XIV, and the values for the two dogs studied for a third day are in Table XV. The mean values for days 1 and 2 are shown in Table XVI. The changes in the mean values for intrinsic clearance, systemic clearance and hepatic plasma flow are shown in Figure 13.

The intrinsic clearance of the intraportally administered propranolol fell significantly from 3749 +/- 880 $ml.min^{-1}$ on Day 1 to 1197 +/- 206 $ml.min^{-1}$ during anaesthesia ($p<0.05$).

The systemic clearance of the intravenously administered propranolol also fell significantly from 672 +/- 85 $ml.min^{-1}$ on Day 1 to 314 +/- 40 $ml.min^{-1}$ on Day 2 ($p<0.05$).

The intravenous elimination half-life was significantly increased from 59 +/- 6 min on Day 1 to 167 +/- 28 min during anaesthesia ($p<0.05$).

There was no change in the apparent volume of distribution but the hepatic extraction fell from 0.80 +/- 0.02 to 0.72 +/- 0.02 during anaesthesia ($p<0.05$).

The calculated hepatic plasma flow fell significantly by approximately 50%
from 835 +/- 97 $ml.min^{-1}$ on Day 1 to 436 +/- 62 $ml.min^{-1}$ on Day 2 ($p<0.05$).

A mean dose of 9.25 +/- 0.58 MAC hours of enflurane was administered during anaesthesia.

Figure 11 ENFLURANE

Time – concentration curves for portal propranolol

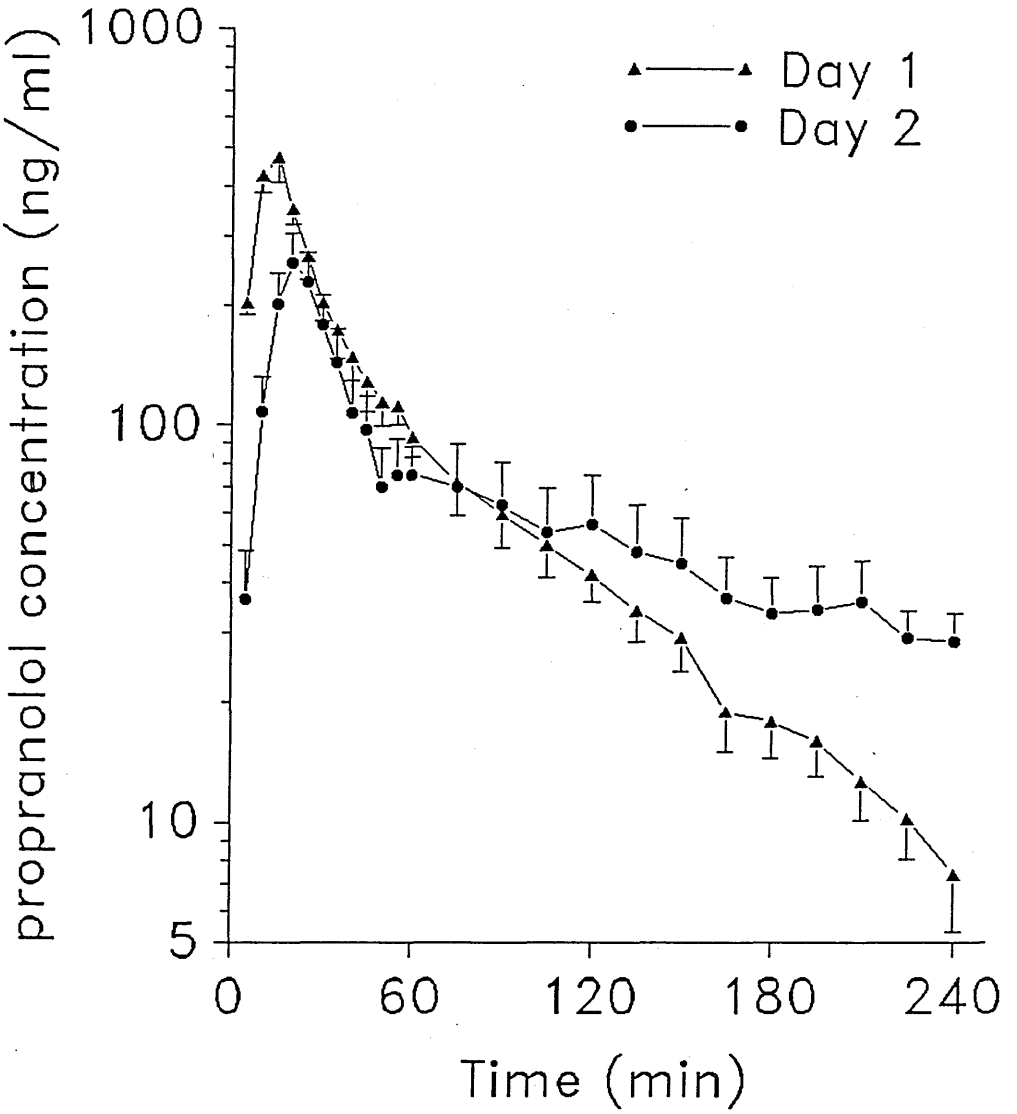


Figure 12 ENFLURANE

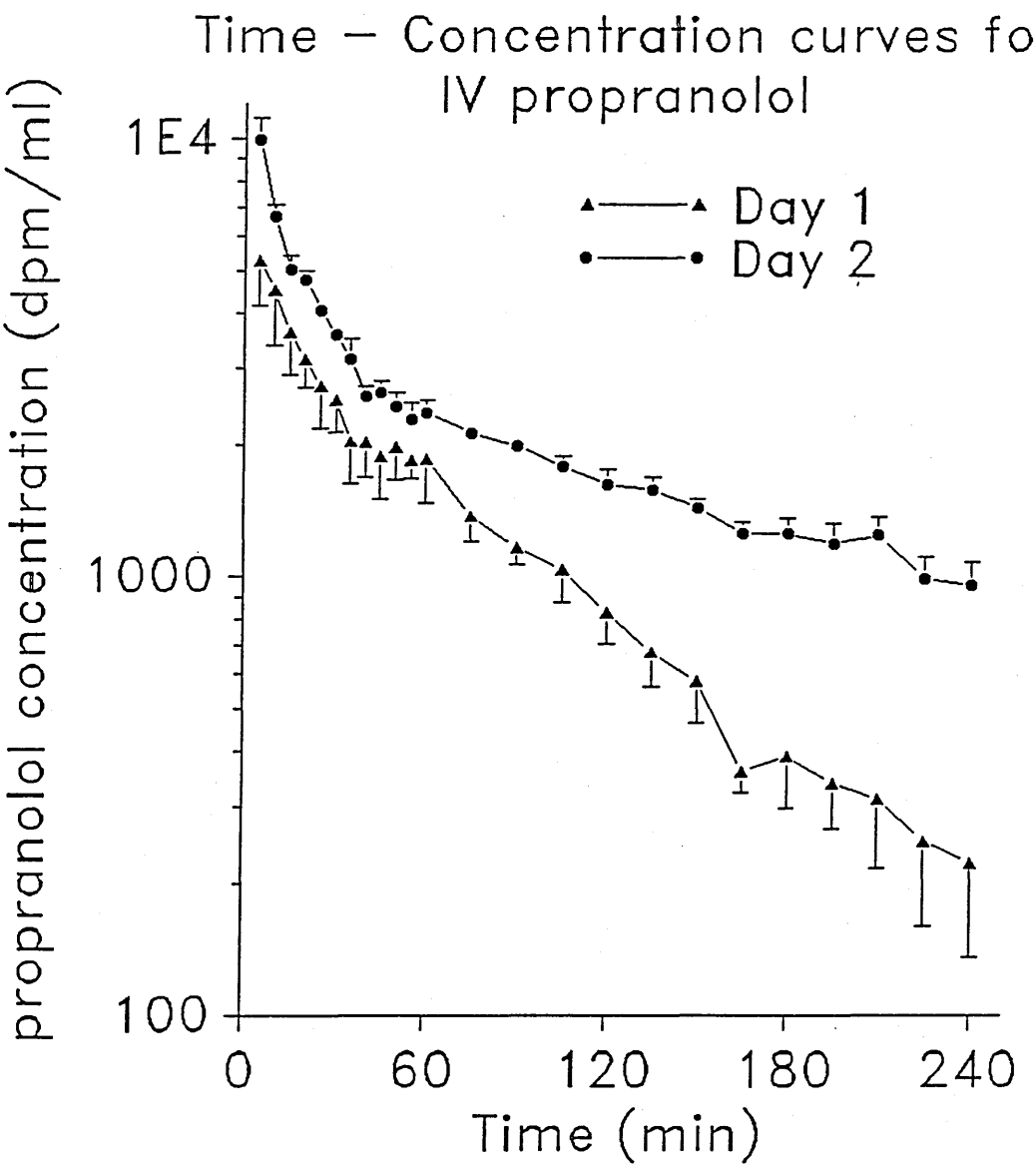


Table XIII

Enflurane Study Day 1

DOG NO.	1	2	3	4	5
$Dose_p$ ($ng\ 10^7$)	3.97	1.92	2.05	1.94	2.05
AUC_p ($ng.min.10^3$)	1.63	2.84	1.02	4.07	7.31
$Dose_{iv}$ ($dpm.10^8$)	1.43	1.86	1.98	1.74	2.10
AUC_{iv} ($dpm.min.10^5$)	2.63	2.22	4.77	2.04	2.95
Cl_{int} ($ml.min^{-1}$)	2440	6720	2010	4750	2800
Cl_s ($ml.min^{-1}$)	540	840	420	850	710
H.P.F. ($ml.min^{-1}$)	700	960	520	1040	950
$T1/2_{iv}$ (min)	53	49	83	61	49
V_b (litre)	41	59	50	76	50
E.R.	0.77	0.87	0.79	0.82	0.74
% free	8.2	5.7	6.1	7.6	6.0

Table XIV

Enflurane Study Day 2

DOG NO.	1	2	3	4	5
$Dose_p$ (ng 10^7)	3.98	1.98	1.94	1.94	1.91
AUC_p (ng.min.10^4)	3.50	1.27	3.35	1.12	1.95
$Dose_{iv}$ (dpm.10^8)	2.83	2.11	1.82	2.18	2.14
AUC_{iv} (dpm.min.10^5)	6.94	5.59	9.95	6.54	7.97
Cl_{int} ($ml.min^{-1}$)	1140	1560	580	1730	980
Cl_s ($ml.min^{-1}$)	410	380	180	330	270
H.P.F. ($ml.min^{-1}$)	630	500	270	410	370
$T1/2_{iv}$ (min)	141	101	270	159	162
V_b (litre)	83	55	71	76	63
E.R.	0.64	0.75	0.68	0.80	0.72
% free	10.8	11.0	10.0	10.5	8.2

Table XV

Enflurane Study Day3

DOG NO.	1	2	3	4	5
$Dose_p$ (ng 10^7)		2.02	1.93		
AUC_p (ng.min.10^4)		1.53	5.21		
$Dose_{iv}$ (dpm.10^8)		2.17	2.07		
AUC_{iv} (dpm.min.10^5)		3.20	9.20		
Cl_{int} (ml.min^{-1})		1320	370		
Cl_s (ml.min^{-1})		680	220		
H.P.F. (ml.min^{-1})		1400	570		
$T1/2_{iv}$ (min)		63	90		
V_b (litre)		61	29		
E.R.		0.48	0.39		
% free		7.3	5.8		

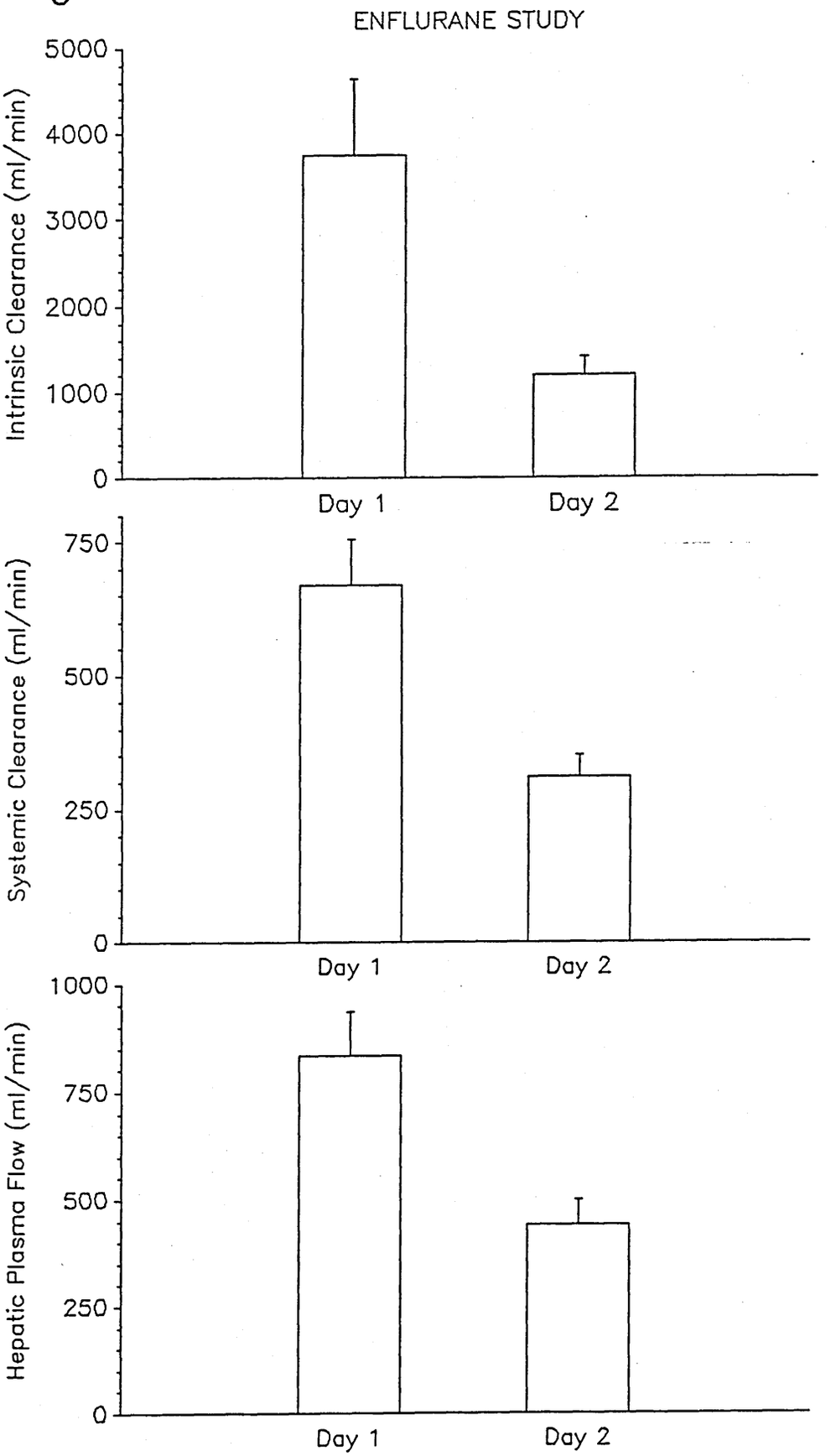
Table XVI Enflurane study

Mean values (SEM)

DAY	1	2	3
Cl_{int} ($ml.min^{-1}$)	3750 (880)	1200* (200)	
Cl_s ($ml.min^{-1}$)	670 (90)	310* (40)	
H.P.F. ($ml.min^{-1}$)	840 (100)	440* (60)	
$T1/2_{iv}$ (min)	59 (6)	167* (28)	
V_b (litre)	55 (6)	70 (5)	
E.R.	0.80 (.02)	0.72* (.02)	
% free	6.7 (.5)	10.1* (.5)	

* $p<0.05$ compared with Day 1

Figure 13


ENFLURANE STUDY
Intrinsic Clearance (ml/min)
5000
4000
3000
2000
1000
0
Day 1
Day 2
Systemic Clearance (ml/min)
750
500
250
0
Day 1
Day 2
Hepatic Plasma Flow (ml/min)
1000
750
500
250
0
Day 1
Day 2

6.5 Fentanyl - Atracurium - Nitrous Oxide Anaesthesia

Six male mongrel dogs of mean weight 21.3 +/-7.1 kg were studied.

The mean plasma concentration of the portally administered propranolol for each of the three study days is shown against time in Figure 14. This shows an increase in plasma concentration and slowing of propranolol elimination during anaesthesia which is almost fully reversed 24 hours later.

The mean plasma concentrations of the intravenously administered H-3 labelled propranolol are shown against time in Figure 15. This again shows an increase in concentration during anaesthesia which has returned to the pre-anaesthetic level by 24 hours.

The individual values for each study day are shown in Tables XVII-XIX , and the mean values for each day in Table XX. The changes in the mean values for intrinsic clearance, systemic clearance and hepatic plasma flow are shown in Figure 16.

The intrinsic clearance of the propranolol fell from a mean value of 1775 +/- 385 $ml.min^{-1}$ on Day 1 to 664 +/- 95 $ml.min^{-1}$ during anaesthesia, a fall of 63% ($p<0.05$). On Day 3 the mean value of 1649 +/- 445 $ml.min^{-1}$ was not

significantly different from that on Day 1.

The systemic clearance of propranolol fell by 35% from a mean value of 536 +/- 29 $ml.min^{-1}$ on Day 1 to 347 +/- 41 $ml.min^{-1}$ during anaesthesia ($p<0.05$). There was no significant difference between the Day 3 value of 615 +/- 84 $ml.min^{-1}$ and that of Day 1.

The mean elimination half-life of the intravenously administered propranolol increased from 65 +/- 6 min on Day 1 to 98 +/- 9 min on Day 2 ($p<0.05$). On day 3 the value was 65 +/- 4 min (NS).

The hepatic extraction of propranolol fell from 0.61 +/-0.04 on Day 1 to 0.46 +/-0.03 during anaesthesia and returning to 0.53 +/-0.08 on Day 3.

The volume of distribution did not change significantly throughout the study.

The free fraction of propranolol increased from a mean of 7.0 +/- 0.8% on Day 1 to 8.4 +/- 0.6% on Day 2 ($p<0.05$) and was 7.8 +/- 0.7% on Day 3 (NS).

The calculated hepatic plasma flow showed a non-significant decrease of 27% from 1044 +/- 233 $ml.min^{-1}$ on Day 1 to 762 +/- 99 $ml.min^{-1}$ during anaesthesia. The value for Day 3 was 1253 +/- 194 $ml.min^{-1}$ (NS).

The mean dose of fentanyl administered was 1.565 +/-0.15 mg and of atracurium 73.0 +/-6.5 mg. The mean duration of anaesthesia was 5.38 +/-0.08 hours.

Figure 14 FENTANYL

Time – concentration curves for portal propranolol

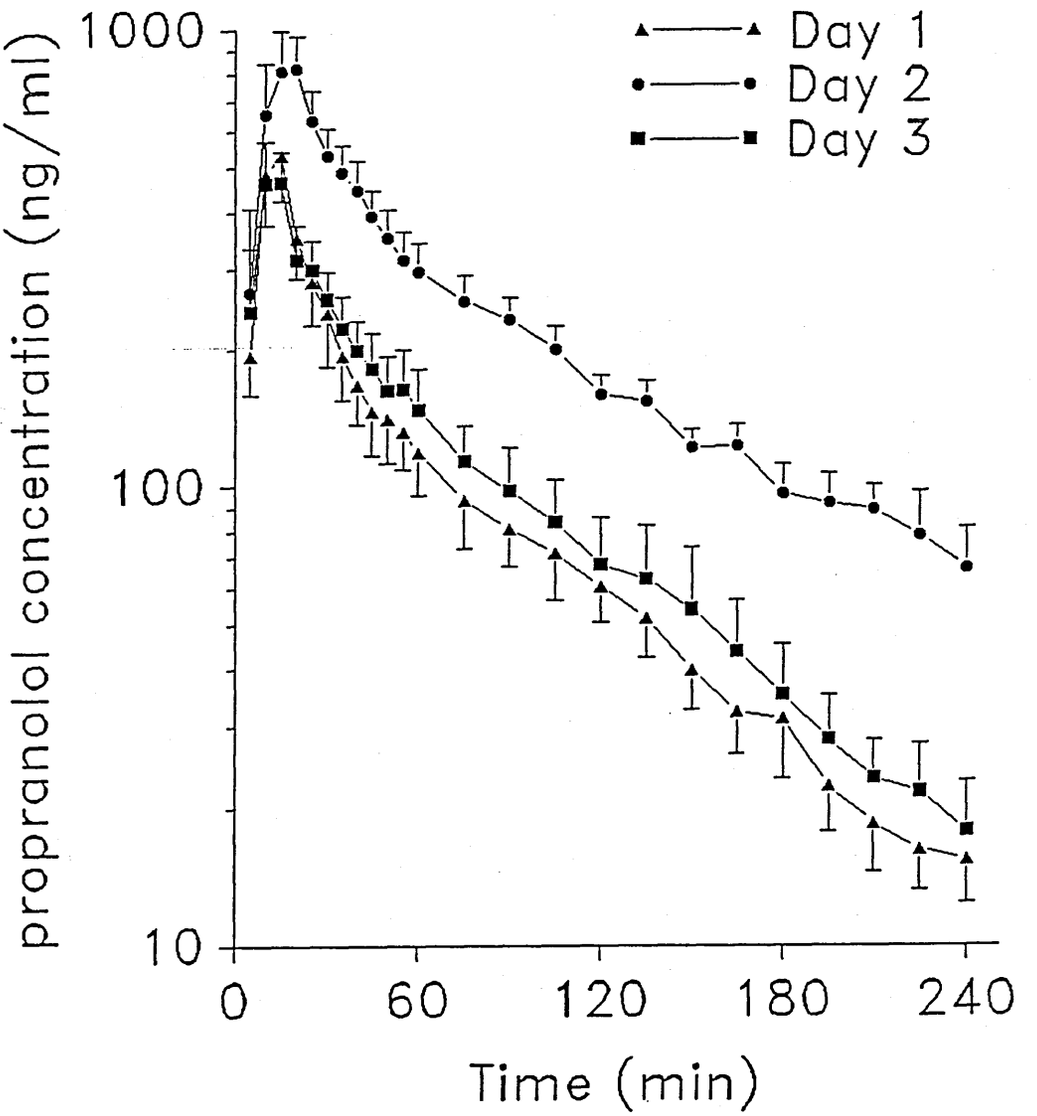


Figure 15 FENTANYL

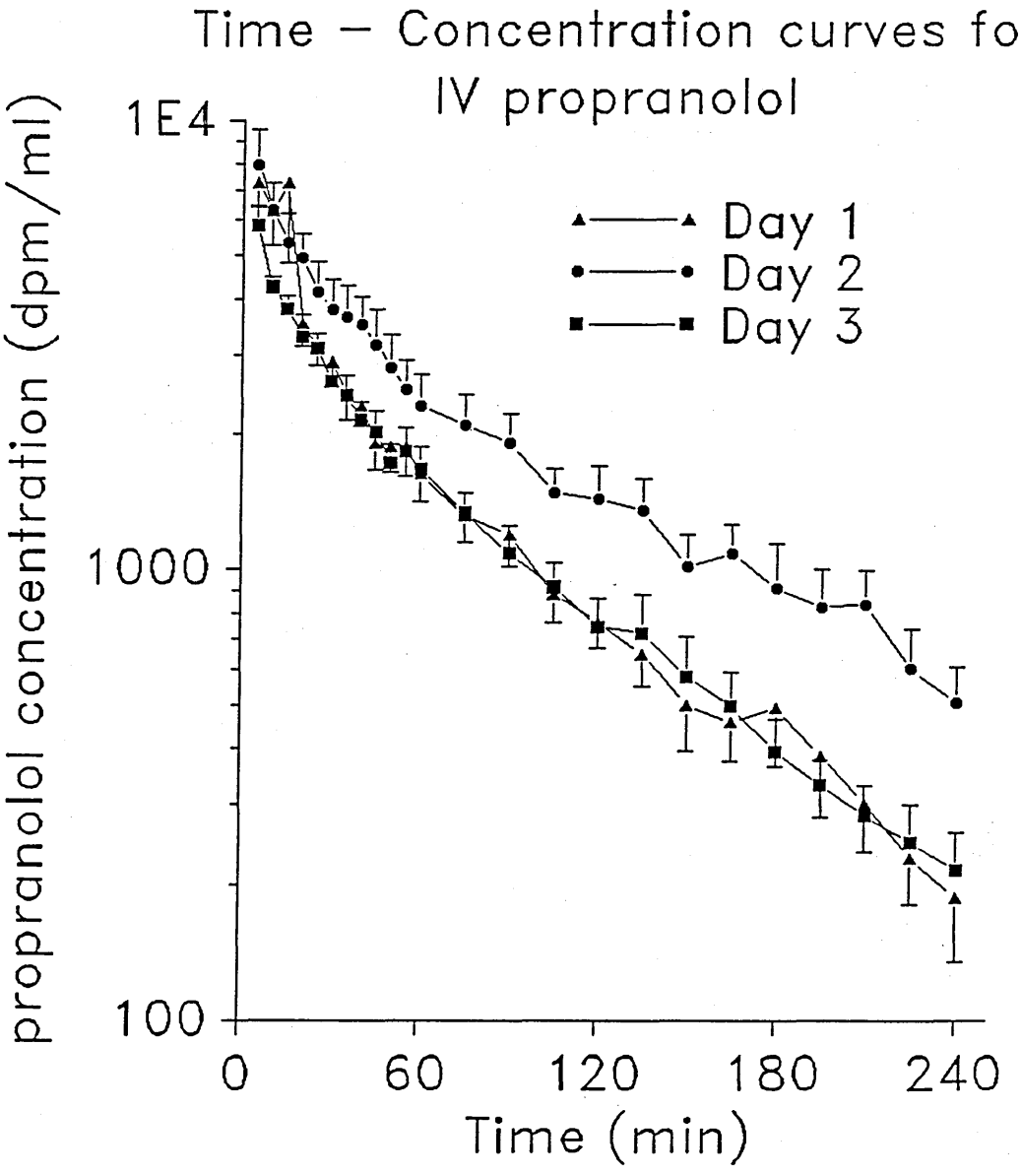


Table XVII

Fentanyl Study Day 1

DOG NO.	1	2	3	4	5	6
$Dose_p$ ($ng\ 10^7$)	4.06	3.89	4.01	3.96	3.91	4.01
AUC_p ($ng.min.10^4$)	1.41	1.94	6.49	3.15	3.63	1.42
$Dose_{iv}$ ($dpm.10^8$)	2.42	1.12	1.89	1.67	1.67	1.66
AUC_{iv} ($dpm.min.10^5$)	3.74	2.04	3.93	2.84	3.55	3.47
Cl_{int} ($ml.min^{-1}$)	2870	2010	620	1260	1080	2820
Cl_s ($ml.min^{-1}$)	650	550	480	580	480	480
H.P.F. ($ml.min^{-1}$)	840	760	2160	1100	840	580
$T1/2_{iv}$ (min)	81	66	79	40	68	52
V_b (litre)	76	53	55	34	46	36
E.R.	0.78	0.73	0.30	0.54	0.56	0.83
% free	10.3	7.6	5.8	5.1	5.4	5.4

Table XVIII

Fentanyl Study Day 2

DOG NO.	1	2	3	4	5	6
$Dose_p$ (ng 10^7)	3.98	3.93	3.90	3.92	3.97	3.92
AUC_p (ng.min.10^4)	5.36	6.49	6.35	9.15	7.86	3.62
$Dose_{iv}$ (dpm.10^8)	1.56	1.56	2.54	1.67	1.72	1.51
AUC_{iv} (dpm.min.10^5)	4.64	3.99	8.91	6.60	5.94	2.86
Cl_{int} (ml.min^{-1})	740	610	610	430	510	1080
Cl_s (ml.min^{-1})	340	390	280	250	290	530
H.P.F. (ml.min^{-1})	610	1110	530	620	680	1020
$T1/2_{iv}$ (min)	122	85	96	80	129	75
V_b (litre)	59	48	39	29	54	57
E.R.	0.55	0.35	0.53	0.41	0.43	0.51
% free	8.6	9.8	7.9		7.5	7.2

Table XIX

Fentanyl Study Day 3

DOG NO.	1	2	3	4	5	6
$Dose_p$ (ng 10^7)	3.92	3.89	3.93	3.94	4.01	3.93
AUC_p (ng.min.10^4)	4.62	6.48	1.43	3.21	3.33	1.21
$Dose_{iv}$ (dpm.10^8)	2.06	1.81	2.06	1.68	1.66	1.95
AUC_{iv} (dpm.min.10^5)	4.08	3.90	3.08	3.09	3.32	1.93
Cl_{int} ($ml.min^{-1}$)	850	600	2760	1230	1200	3260
Cl_s ($ml.min^{-1}$)	510	470	670	540	500	1010
H.P.F. ($ml.min^{-1}$)	1250	2090	880	980	850	1460
$T1/2_{iv}$ (min)	71	75	69	50	68	55
V_b (litre)	51	51	66	39	49	80
E.R.	0.40	0.22	0.75	0.55	0.58	0.69
% free	6.0	7.7	7.5	7.2	8.4	6.3

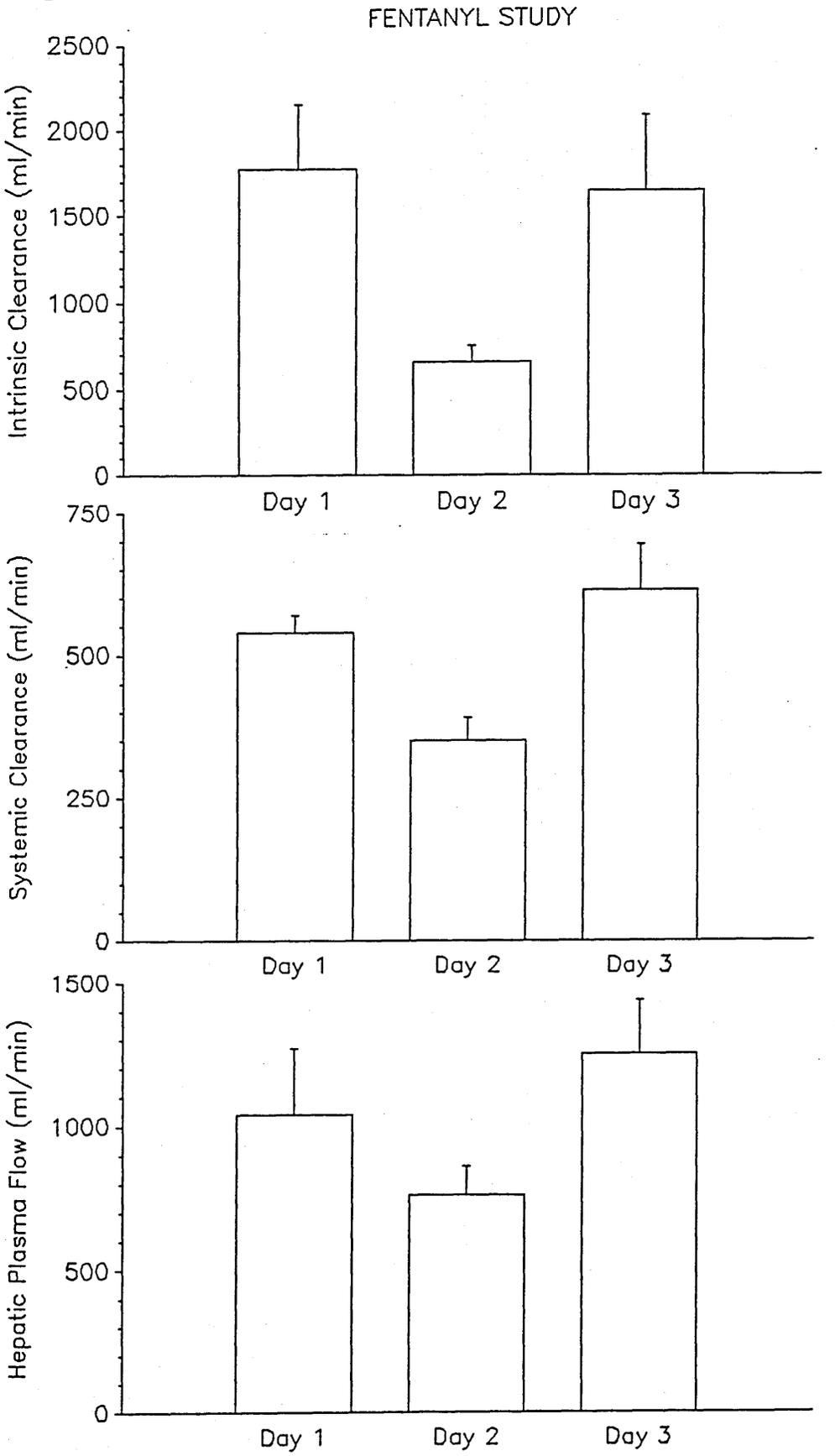
Table XX Fentanyl study

Mean values (SEM)

DAY	1	2	3
Cl_{int} ($ml.min^{-1}$)	1780 (380)	660* (100)	1650 (440)
Cl_s ($ml.min^{-1}$)	540 (30)	350* (40)	620 (80)
H.P.F. ($ml.min^{-1}$)	1040 (230)	760 100)	1250 190)
$T1/2_{iv}$ (min)	65 (12)	98* (23)	65 (12)
V_b (litre)	50 (6)	48 (5)	56 (6)
E.R.	0.61 (.04)	0.46* (.03)	0.53 (.08)
% free	7.0 (.8)	8.4* (.6)	7.8 (.7)

* $p<0.05$ compared with Day 1

Figure 16


FENTANYL STUDY
Intrinsic Clearance (ml/min)
2500
2000
1500
1000
500
0
Day 1
Day 2
Day 3
Systemic Clearance (ml/min)
750
500
250
0
Day 1
Day 2
Day 3
Hepatic Plasma Flow (ml/min)
1500
1000
500
0
Day 1
Day 2
Day 3

6.6 Comparison between the groups

The broad effect of all four anaesthetic regimens was similar. The effect of anaesthesia in the four groups on the disposition of propranolol as measured by the intrinsic clearance, systemic clearance, hepatic blood flow and elimination half-life, is shown in Table XI, where the results are expressed as percentages of the control value, ie. the Day 1 value in each group.

The values obtained for intrinsic clearance in all five studies are compared in Figure 17 and the change in intrinsic clearance from Day 1 to Days 2 and 3 in Figure 18. The values for systemic clearance are compared in Figure 19 and the change from Day 1 in Figure 20. The hepatic blood flow is shown in Figure 21 and the change from Day 1 in Figure 22. The calculated elimination half-life is shown in Figure 23 for all five groups.

Statistical comparison of the initial values (Day 1) for all five groups demonstrated no significant difference between the groups for systemic clearance, hepatic blood flow and intravenous half-life. ANOVA demonstrated a difference in intrinsic clearance between the groups. Subsequent inter-group analysis showed a significant difference for the values for intrinsic clearance between the enflurane and isoflurane groups ($p<0.05$).

The values for intrinsic and systemic clearance and intravenous half-life on Day 2 were not significantly different for all four anaesthetic agents, but all were significantly different from the control group. Hepatic blood flow in the fentanyl group was significantly different from the others ($p<0.05$). The extent of change from control to during anaesthesia ie Day 1 - Day 2 value, was not significantly different for all four anaesthetic regimens with respect to intrinsic and systemic clearance, intravenous half-life and hepatic blood flow.

A comparison of the values obtained in the halothane, isoflurane and fentanyl groups on Day 3 demonstrated no significant difference between the groups for intrinsic or systemic clearance. However the hepatic blood flow in the fentanyl group was significantly greater ($p<0.05$).

TABLE XXl

Comparison of effect of anaesthesia

Expressed as percentage of mean Day 1 value

	Cl_{int}	Cl_s	HPF	$T1/_{2iv}$
DAY 2				
Halothane	38	60	73	178
Isoflurane	47	60	58	249
Enflurane	32	47	52	283
Fentanyl	37	65	73	150
Control	88	109	126	81
DAY 3				
Halothane	52	70	90	107
Isoflurane	118	94	82	123
Enflurane				
Fentanyl	92	113	120	100
Control	66	97	135	91

Figure 17

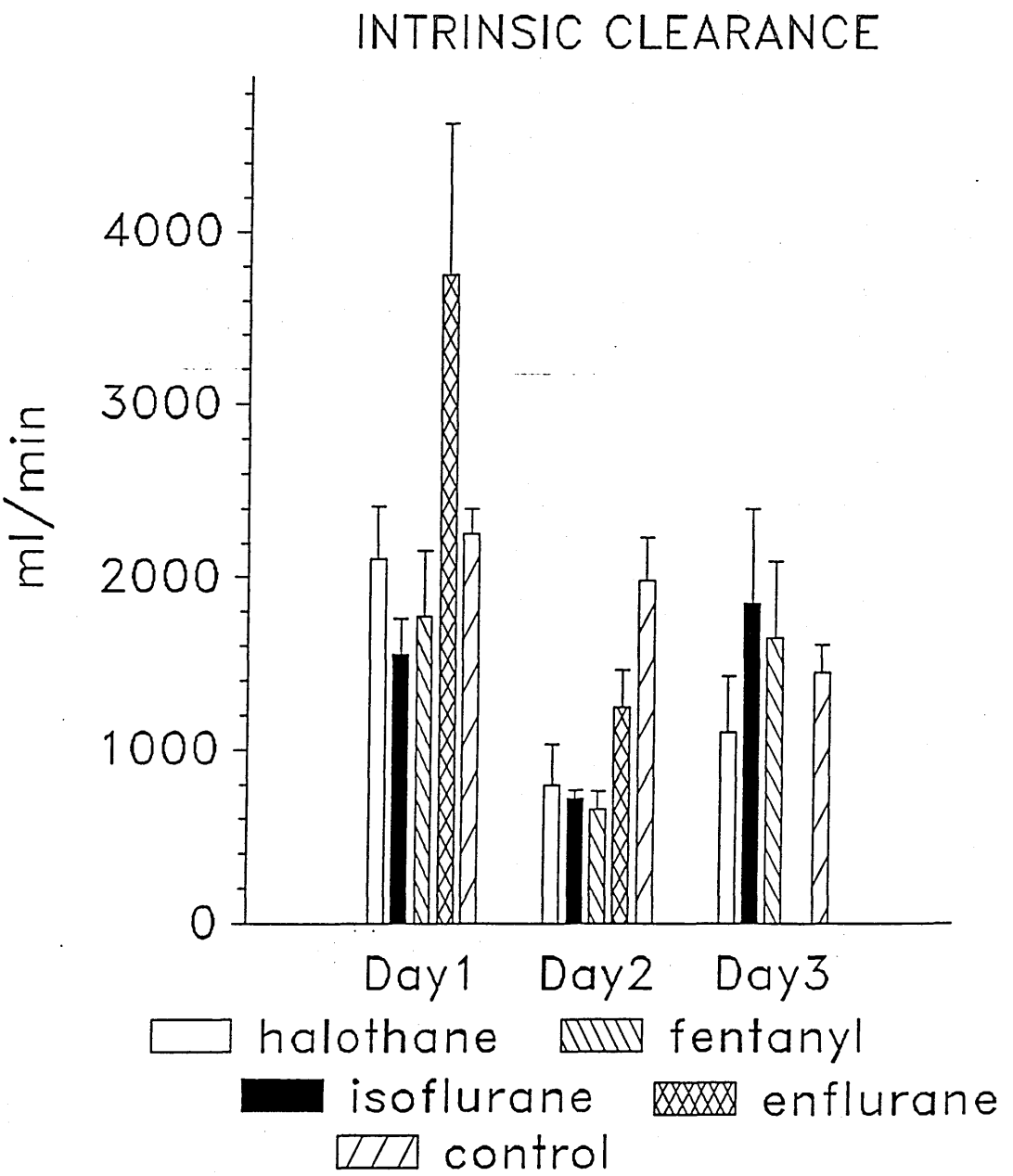

INTRINSIC CLEARANCE
ml/min
4000
3000
2000
1000
0
Day1
Day2
Day3
halothane
fentanyl
isoflurane
enflurane
control

Figure 18

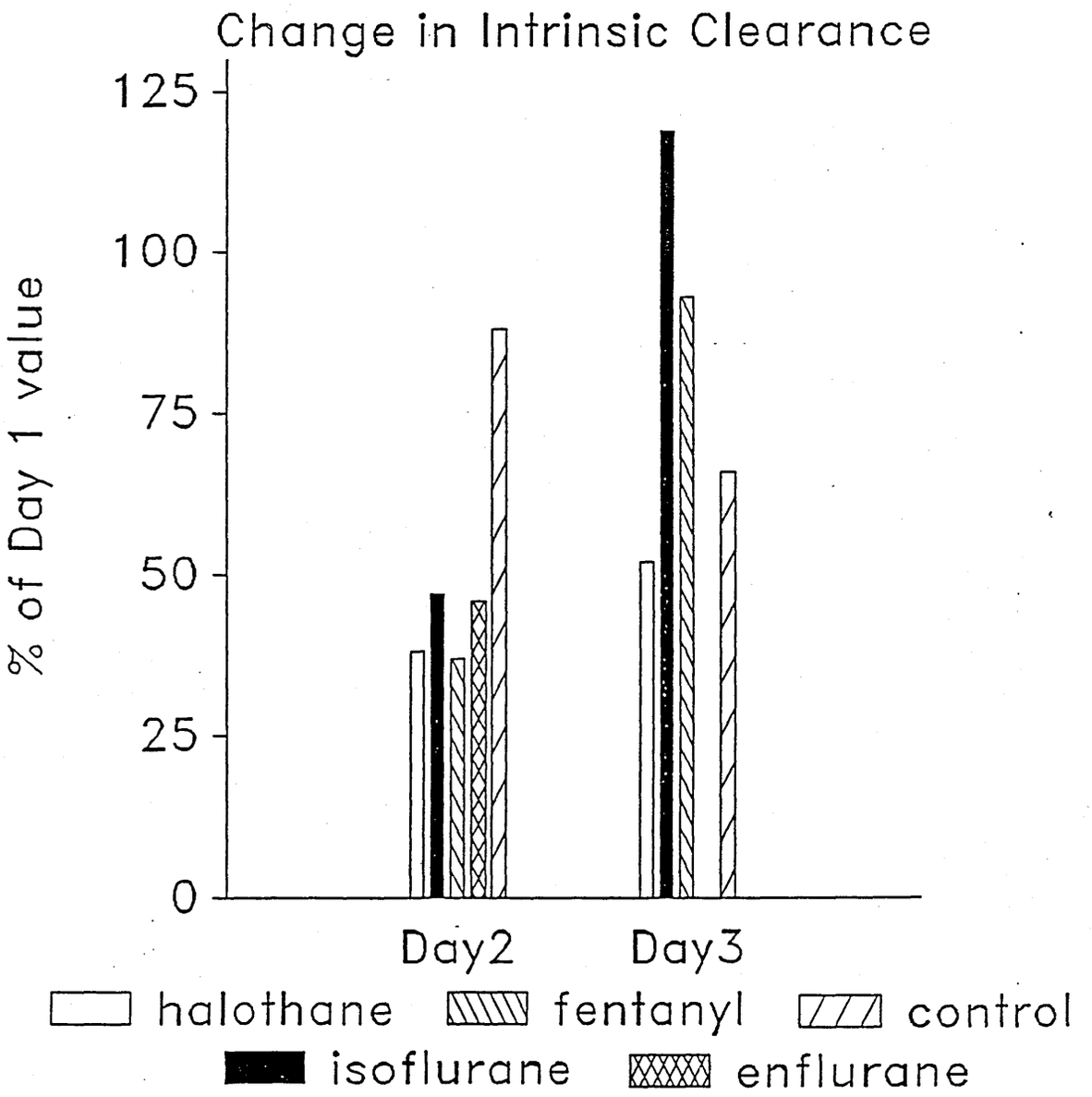

Change in Intrinsic Clearance
% of Day 1 value
125
100
75
50
25
0
Day2
Day3
halothane
fentanyl
control
isoflurane
enflurane

Figure 19

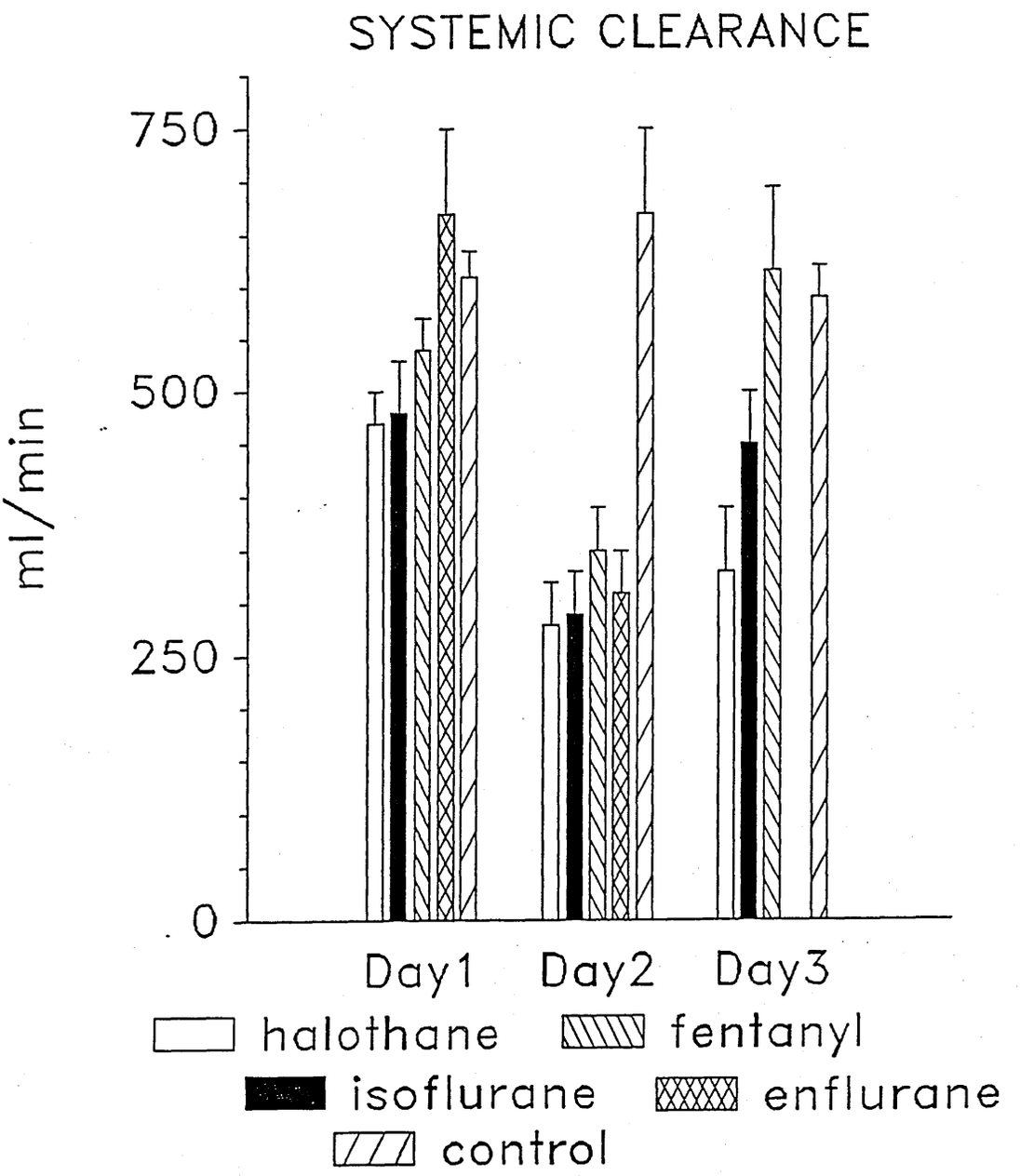

SYSTEMIC CLEARANCE
750
500
250
0
ml/min
Day1
Day2
Day3
halothane
fentanyl
isoflurane
enflurane
control

Figure 20

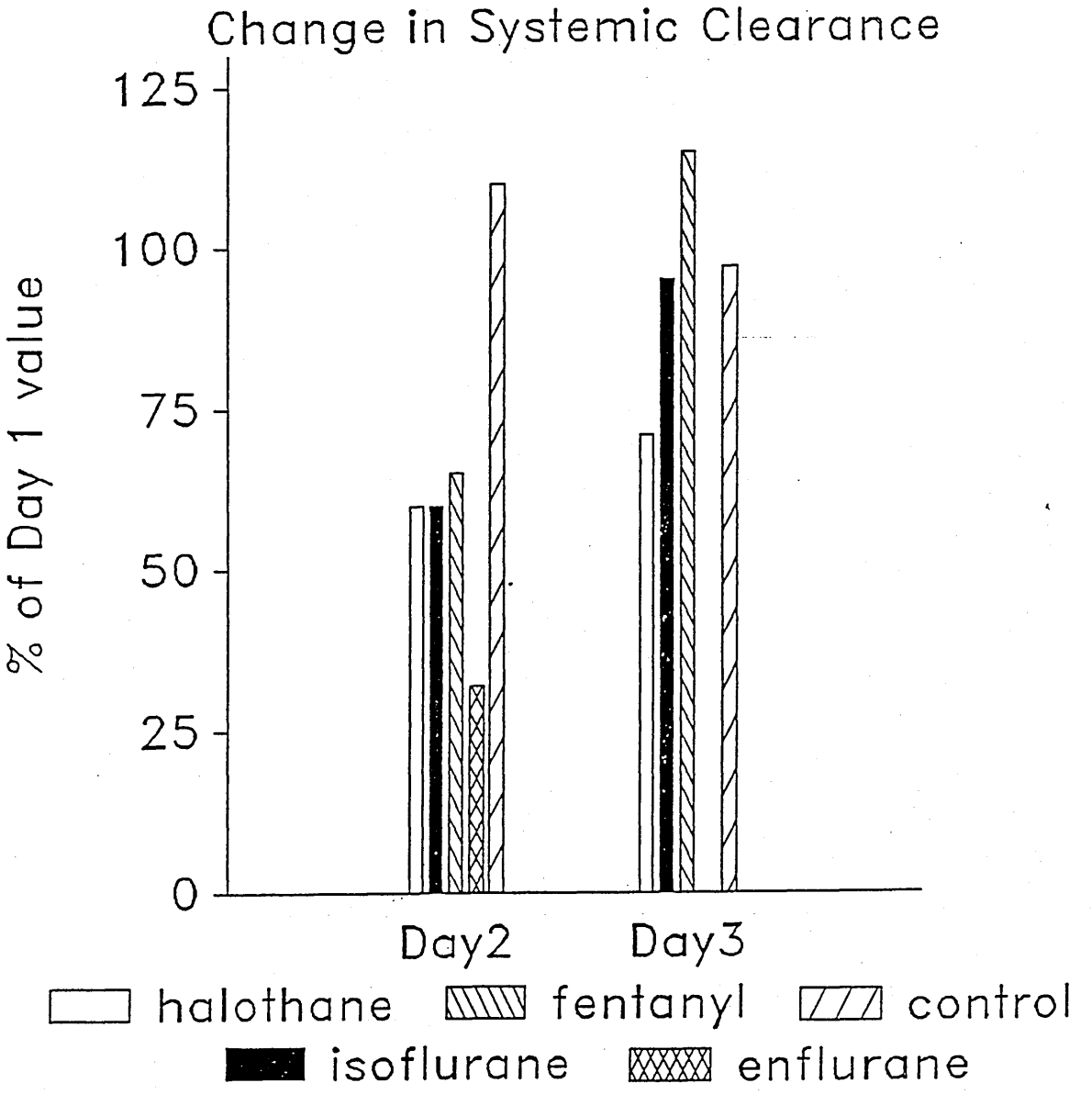

Change in Systemic Clearance
125
100
75
50
25
0
% of Day 1 value
Day2
Day3
halothane
fentanyl
control
isoflurane
enflurane

Figure 21

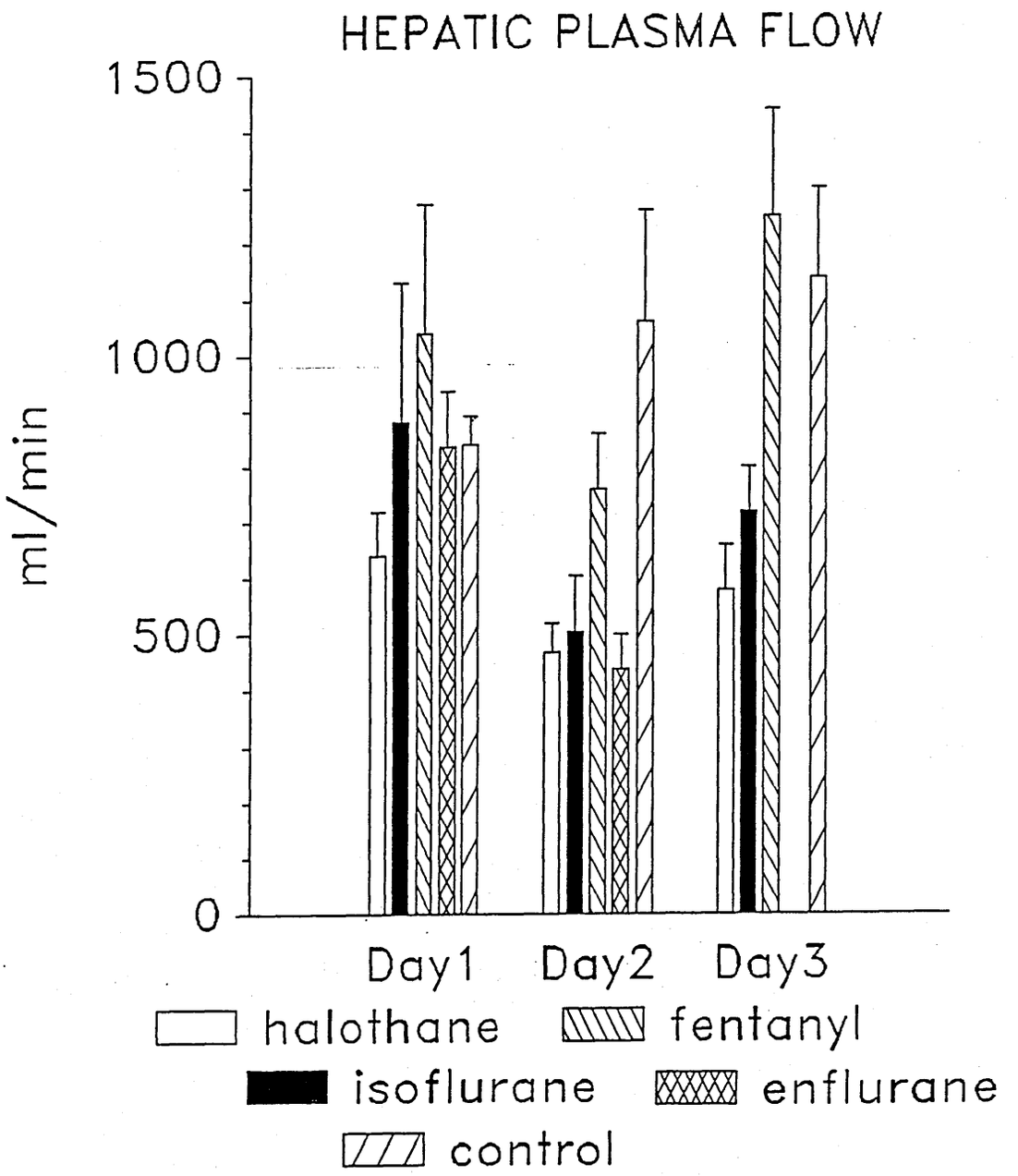

HEPATIC PLASMA FLOW
1500
1000
500
0
ml/min
Day1
Day2
Day3
halothane
fentanyl
isoflurane
enflurane
control

Figure 22

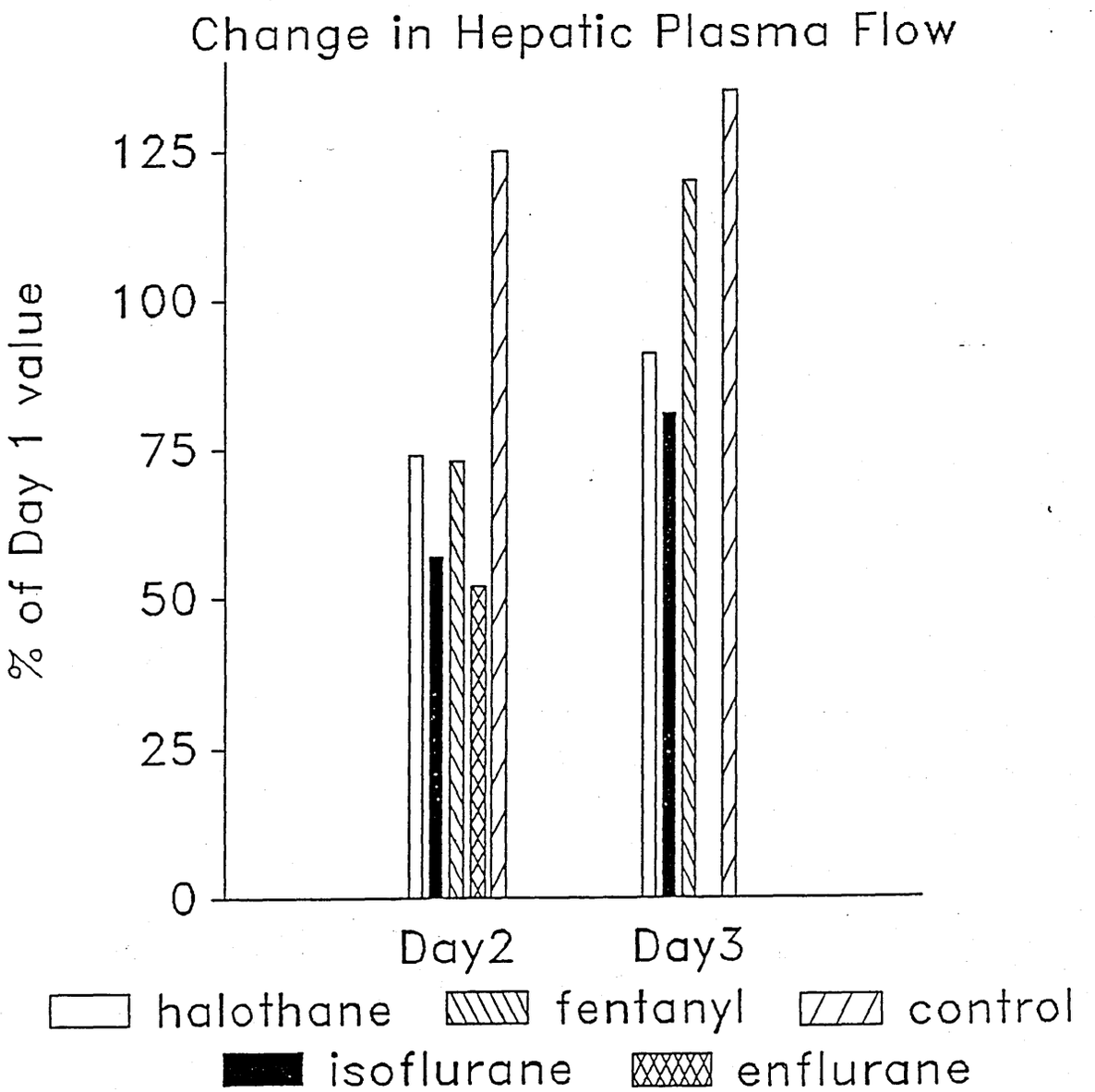
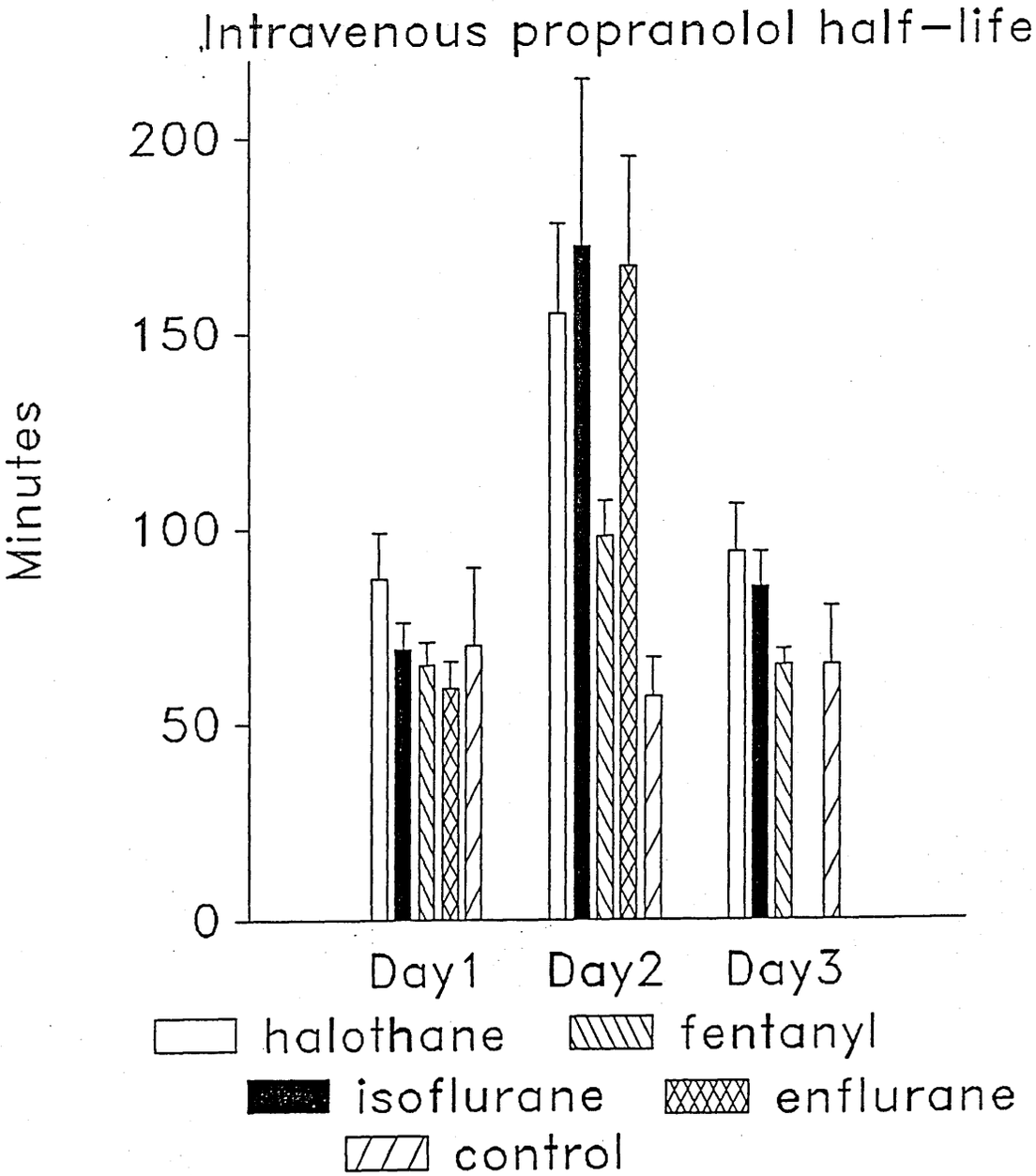

Change in Hepatic Plasma Flow
% of Day 1 value
125
100
75
50
25
0
Day2
Day3
halothane
fentanyl
control
isoflurane
enflurane

Figure 23


Intravenous propranolol half-life
Minutes
200
150
100
50
0
Day1
Day2
Day3
halothane
fentanyl
isoflurane
enflurane
control

6.7 Enprostil and Cimetidine Study

All nine subjects completed the three phases of the study.

The mean time concentration curve for the orally administered propranolol during the three phases, placebo, cimetidine and enprostil, is shown in Figure 24. The plasma concentration of propranolol was increased during the cimetidine phase. The oral clearance of propranolol was significantly decreased during the cimetidine phase to 2072 +/- 261 $ml.min^{-1}$ when compared to the placebo phase value of 4152 +/- 871 $ml.min^{-1}$ ($p<0.05$). The value during the enprostil phase, 4272 +/- 652 $ml.min^{-1}$ was not significantly different from the placebo value.

The time-concentration curve for the intravenous propranolol on the three study days is shown in Figure 25. The clearance of the intravenously administered H3-labelled propranolol was also significantly reduced duing the cimetidine phase to 727 +/- 64 $ml.min^{-1}$ from the placebo value of 897 +/- 83 $ml.min^{-1}$ ($p<0.05$). The enprostil value of 917 +/- 58 $ml.min^{-1}$ was not significantly different from the placebo value. The intravenous half-life of the labelled propranolol was 225 +/- 11 min during the placebo phase. This was

Figure 24

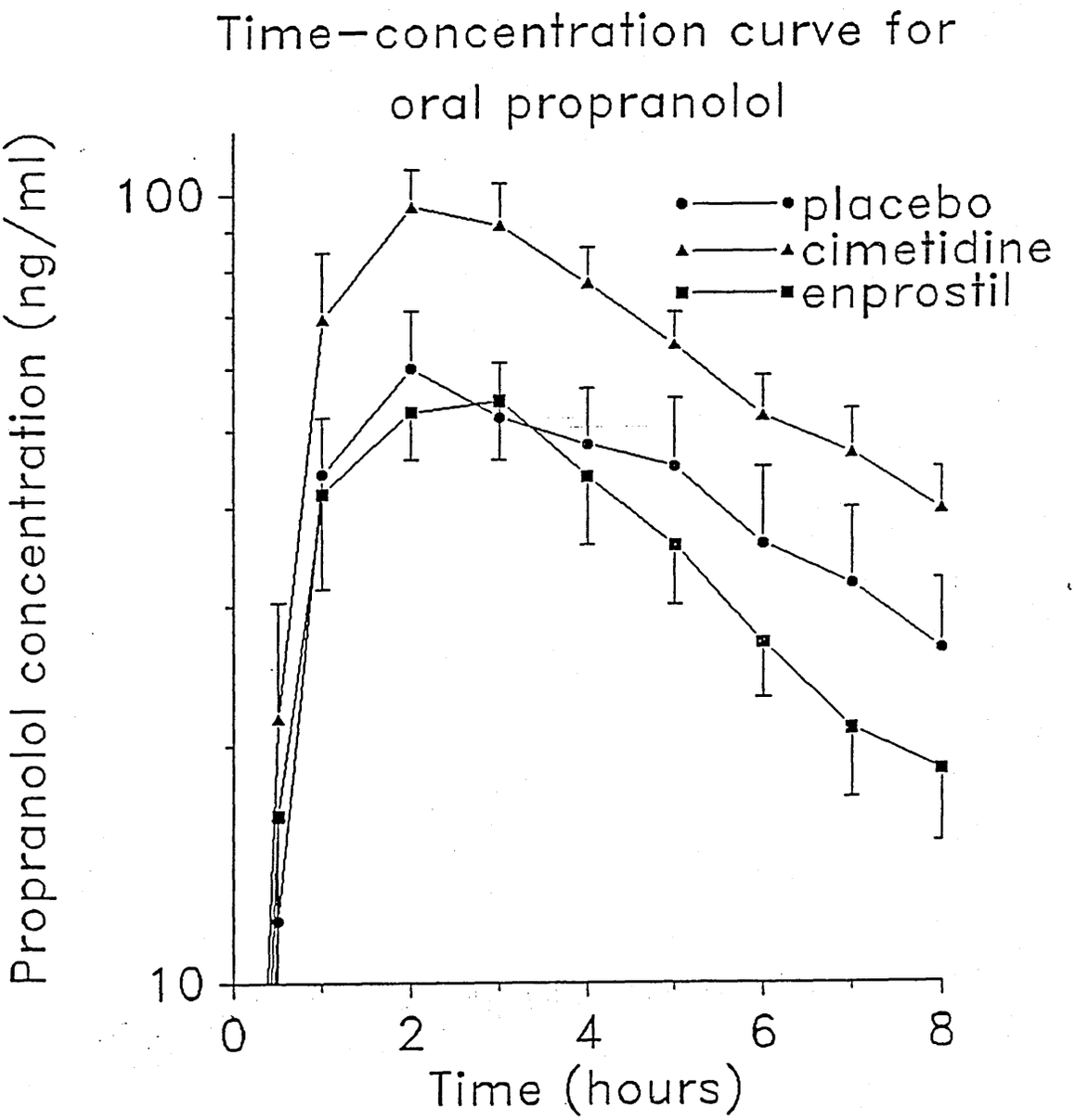
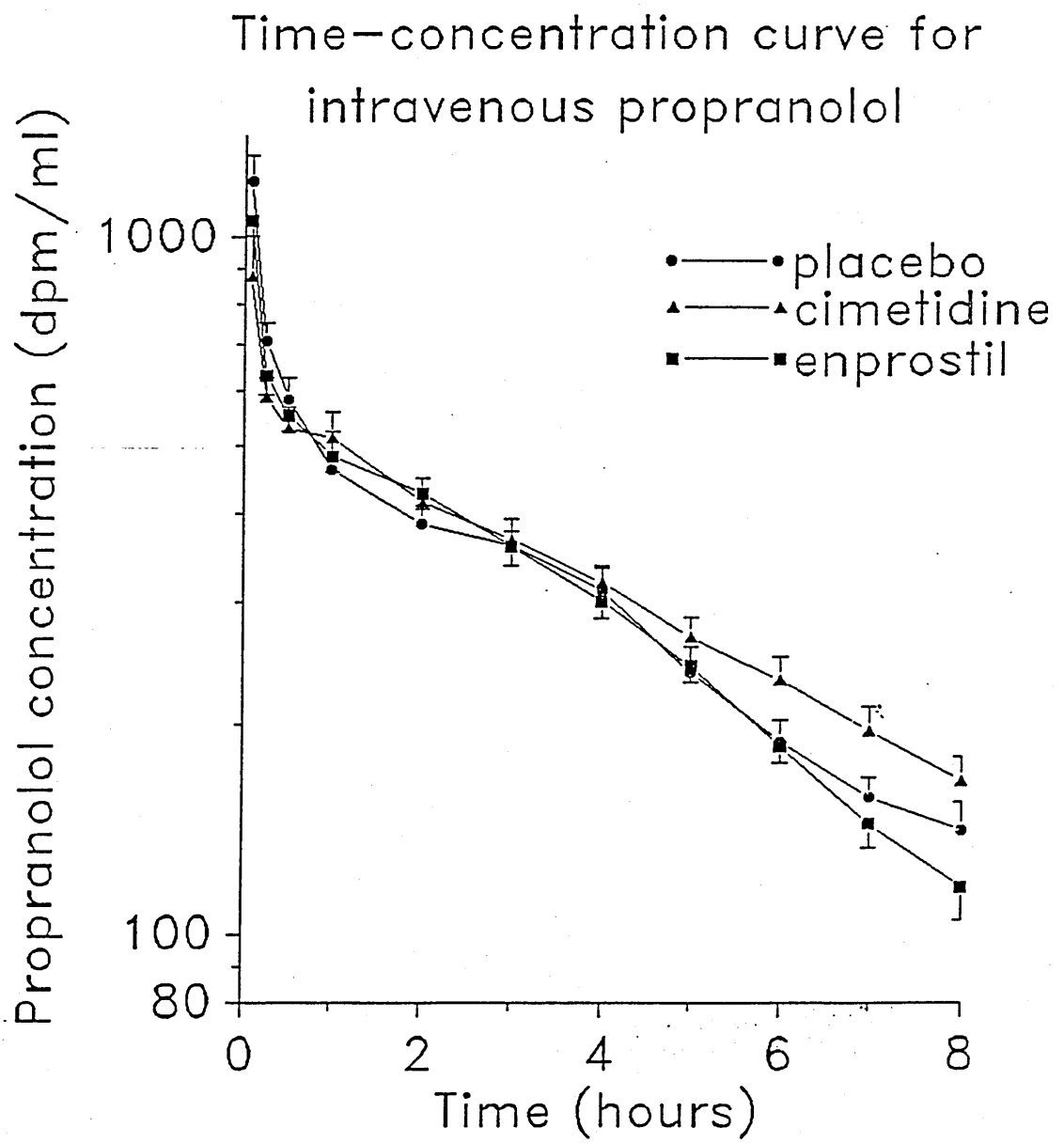

Time–concentration curve for
oral propranolol
Propranolol concentration (ng/ml)
100
10
placebo
cimetidine
enprostil
0
2
4
6
8
Time (hours)

Figure 25


Time–concentration curve for intravenous propranolol
Propranolol concentration (dpm/ml)
1000
100
80
0
2
4
6
8
Time (hours)
placebo
cimetidine
enprostil

significantly increased during the cimetidine phase to 270 +/- 12 min but not significantly different during the enprostil phase at 213 +/- 10 min.

The individual values for the three study phases are shown in Tables XXII-XXIV. The mean values for the three study phases are given in Table XXV. The mean values for intrinsic clearance, systemic clearance and hepatic plasma flow are shown in Figure 26.

The decrease in oral clearance during the cimetidine phase is reflected in the increase in bioavailability which increased from 26 +/- 3 % with placebo to 37 +/-3 % during the cimetidine phase ($p<0.05$). The enprostil treatment did not alter the bioavailability significantly (25 +/- 3%).

The plasma protein binding of propranolol was not significantly altered by any of the phases. The free fraction was 13.0 +/- 1.1% during placebo, 13.3 +/- 0.8% during cimetidine and 12.7 +/- 0.9% during enprostil phase.

The calculated hepatic plasma flow using the propranolol method was not significantly different during the three phases being 1211 +/- 98, 1167 +/- 93 and 1247 +/- 88 $ml.min^{-1}$ during the placebo, cimetidine and enprostil phases respectively. The hepatic plasma flow calculated from the indocyanine green clearance produced lower values but these were not significantly different between the three phases (Table XXVI). The values were

Table XXIIa Placebo Study Day

Subject	1	2	3	4	5
$Dose_o$ (ng 10^7)	8.0	8.0	8.0	8.0	8.0
AUC_o (ng.min.10^4)	2.35	4.78	1.04	1.68	0.90
$Dose_{iv}$ (dpm.10^8)	1.86	1.64	2.00	1.67	1.83
AUC_{iv} (dpm.min.10^5)	1.70	2.82	1.54	1.80	1.72
Cl_{int} ($ml.min^{-1}$)	3410	1680	7720	4760	8850
Cl_s ($ml.min^{-1}$)	1100	580	1290	930	1070
H.P.F. ($ml.min^{-1}$)	1620	900	1550	1150	1210
$T1/2_{iv}$ (min)	279	273	212	197	184
E.R.	0.68	0.65	0.83	0.81	0.88
% free	17.6	8.6	16.7	11.0	16.8

Table XXIIb Placebo Study Day

Subject	6	7	8	9
$Dose_o$ ($ng\ 10^7$)	8.0	8.0	8.0	8.0
AUC_o ($ng.min.10^4$)	4.08	4.16	3.22	1.74
$Dose_{iv}$ ($dpm.10^8$)	1.51	1.33	1.78	1.80
AUC_{iv} ($dpm.min.10^5$)	2.58	2.11	1.90	1.90
Cl_{int} ($ml.min^{-1}$)	1960	1930	2480	4600
Cl_s ($ml.min^{-1}$)	590	630	940	950
H.P.F. ($ml.min^{-1}$)	830	930	1510	1190
$T1/2_{iv}$ (min)	226	232	213	209
E.R.	0.70	0.67	0.62	0.79
% free	9.1	12.1	13.2	12.4

Table XXIIIa Cimetidine Study Day

Subject	1	2	3	4	5
$Dose_o$ (ng 10^7)	8.0	8.0	8.0	8.0	8.0
AUC_o (ng.min.10^4)	2.35	6.72	2.87	2.91	3.97
$Dose_{iv}$ (dpm.10^8)	1.88	1.52	1.85	1.93	1.41
AUC_{iv} (dpm.min.10^5)	1.70	3.29	1.95	2.41	2.37
Cl_{int} ($ml.min^{-1}$)	3000	1190	2790	2750	2020
Cl_s ($ml.min^{-1}$)	1040	460	950	800	600
H.P.F. ($ml.min^{-1}$)	1600	760	1440	1130	850
$T1/2_{iv}$ (min)	275	314	248	308	211
E.R.	0.65	0.61	0.70	0.71	0.70
% free	15.6	9.7	16.6	14.9	11.7

Table XXIIIb Cimetidine Study Day

Subject	6	7	8	9
$Dose_o$ ($ng\ 10^7$)	8.0	8.0	8.0	8.0
AUC_o ($ng.min.10^4$)	6.08	6.99	5.06	2.79
$Dose_{iv}$ ($dpm.10^8$)	1.51	1.62	1.21	1.39
AUC_{iv} ($dpm.min.10^5$)	2.70	2.56	1.80	1.69
Cl_{int} ($ml.min^{-1}$)	1320	1150	1580	2870
Cl_s ($ml.min^{-1}$)	570	630	670	820
H.P.F. ($ml.min^{-1}$)	990	1410	1170	1160
$T1/2_{iv}$ (min)	291	275	291	217
E.R.	0.57	0.45	0.58	0.71
% free	10.5	12.4	13.4	15.3

Table XXIVa Enprostil Study Day

Subject	1	2	3	4	5
$Dose_o$ (ng 10^7)	8.0	8.0	8.0	8.0	8.0
AUC_o (ng.min.10^4)	1.08	4.29	1.04	1.48	2.34
$Dose_{iv}$ (dpm.10^8)	1.81	1.65	2.00	1.56	1.64
AUC_{iv} (dpm.min.10^5)	1.52	2.82	1.54	2.00	2.13
Cl_{int} ($ml.min^{-1}$)	7390	1870	5310	5390	3420
Cl_s ($ml.min^{-1}$)	1190	750	1210	780	820
H.P.F. ($ml.min^{-1}$)	1420	1250	1570	910	1070
$T1/2_{iv}$ (min)	179	206	195	184	248
E.R.	0.84	0.60	0.77	0.86	0.76
% free	14.5	13.5	17.1	11.0	8.3

Table XXIVb Enprostil Study Day

Subject	6	7	8	9
$Dose_o$ (ng 10^7)	8.0	8.0	8.0	8.0
AUC_o (ng.min.10^4)	2.99	3.56	2.19	1.23
$Dose_{iv}$ (dpm.10^8)	1.81	1.93	1.82	1.42
AUC_{iv} (dpm.min.10^5)	2.25	2.03	1.94	1.76
Cl_{int} (ml.min^{-1})	2670	2250	3650	6500
Cl_s (ml.min^{-1})	810	950	940	810
H.P.F. (ml.min^{-1})	1160	1650	1270	920
$T1/2_{iv}$ (min)	267	228	210	196
E.R.	0.70	0.58	0.74	0.88
% free	12.9	10.3	12.6	14.2

Table XXV

Enprostil study
Mean values (SD)

Drug	Pla	Cim	Enp
Cl_{int} ($ml.min^{-1}$)	4150 (870)	2070* (260)	4270 (650)
Cl_s ($ml.min^{-1}$)	900 (80)	730* (60)	920 (60)
H.P.F. prop ($ml.min^{-1}$)	1210 (90)	1170 (90)	1250 (90)
H.P.F. icg ($ml.min^{-1}$)	620 (40)	610 (50)	560 (30)
$T1/2_{iv}$ (min)	225 (11)	270* (12)	213 (10)
E.R.	0.74 (.03)	0.63* (.03)	0.75 (.04)
% free	13.0 (1.1)	13.3 (0.8)	12.7 (0.9)

* $p<0.05$ compared with placebo

Pla Placebo phase
Cim Cimetidine phase
Enp Enprostil phase

H.P.F.prop Hepatic plasma flow propranolol method
H.P.F.icg Hepatic plams flow ICG method

Figure 26

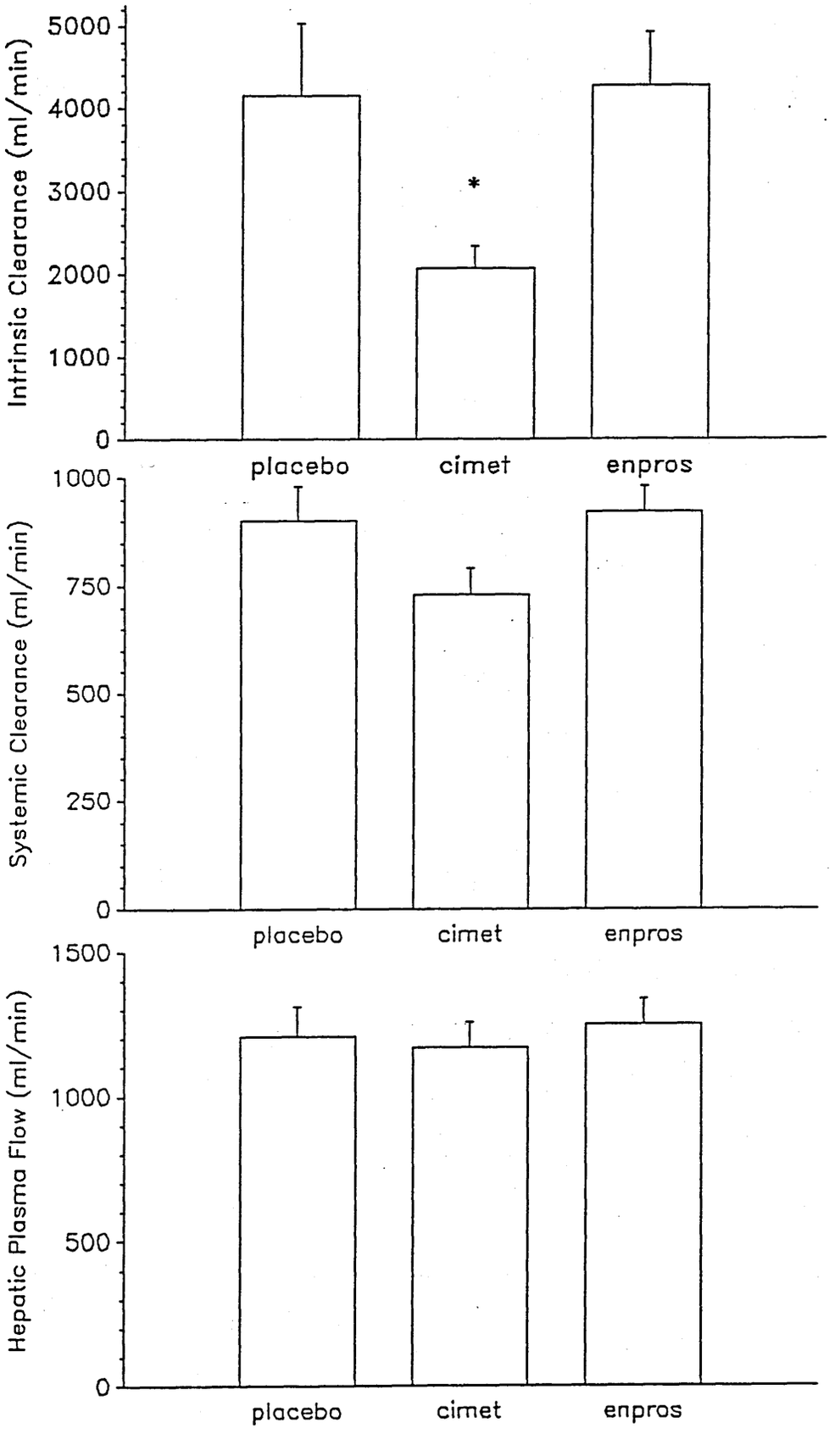

Intrinsic Clearance (ml/min)
5000
4000
3000
2000
1000
0
*
placebo
cimet
enpros
Systemic Clearance (ml/min)
1000
750
500
250
0
placebo
cimet
enpros
Hepatic Plasma Flow (ml/min)
1500
1000
500
0
placebo
cimet
enpros

Table XXVI

Propranolol and ICG

Hepatic plasma flow

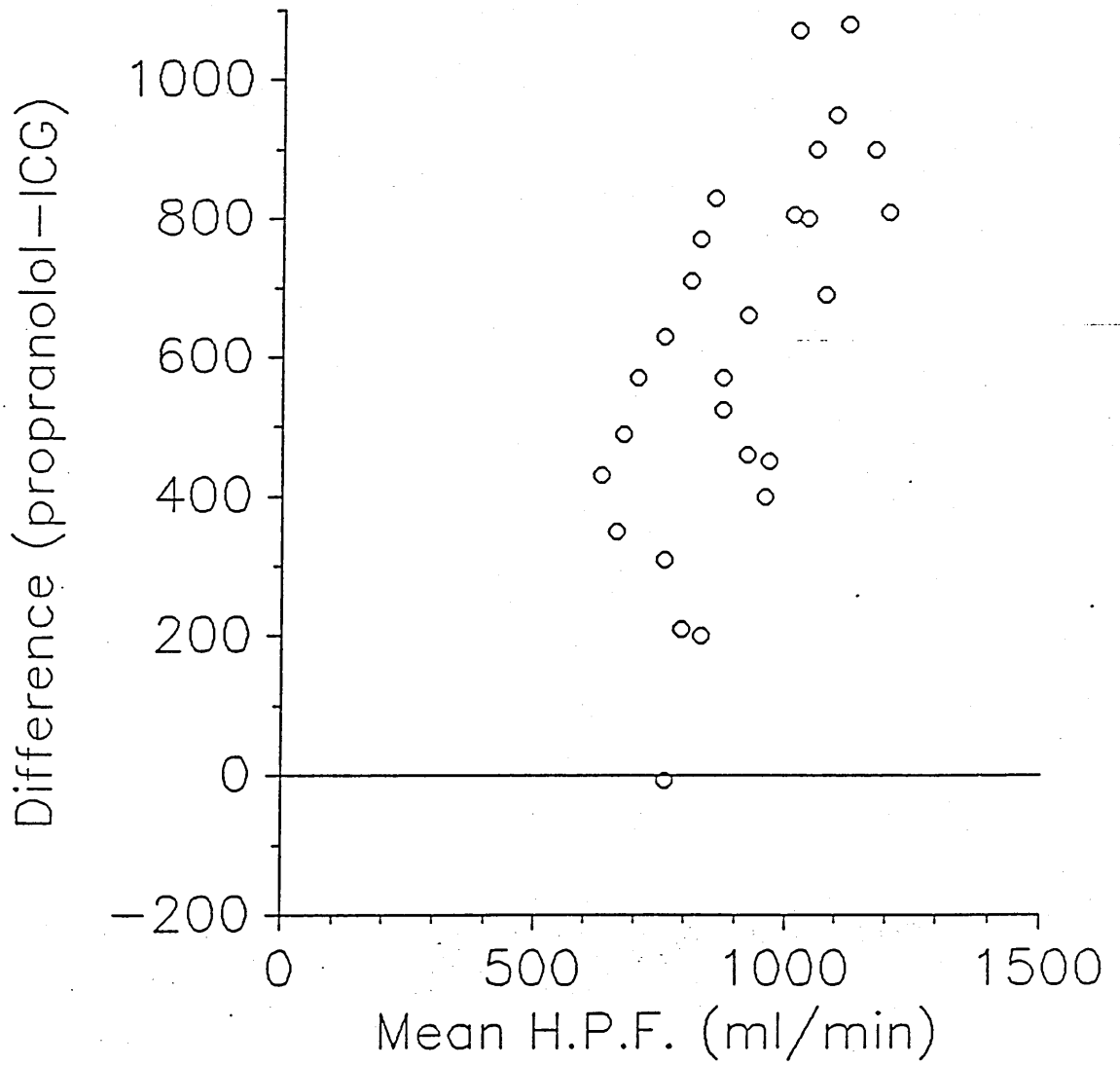
	Placebo		Cimetidine		Enprostil	
	PROP	ICG	PROP	ICG	PROP	ICG
1	1620	720	1600	800	1420	730
2	900	690	760	760	1250	590
3	1550	490	1440	640	1570	620
4	1150	690	1130	610	910	600
5	1210	440	850	410	1070	440
6	830	490	990	410	1160	590
7	930	730	1410	610	1650	580
8	1510	600	1170	450	1270	440
9	1190	740	1160	760	920	430
mean	1210	620	1170	610	1250	560
sem	90	40	90	50	90	30

Prop - Propranolol method
ICG - Indocyanine green method

620 +/- 40, 606 +/- 50 and 557 +/- 34 $ml.min^{-1}$ for placebo, cimetidine and enprostil phases respectively.

A comparison between the hepatic plasma flow values calculated by the two techniques was made using the method of Bland and Altman (316) (Figure 27). This method is applied to comparison of two ways of measuring the same variable. In order to avoid the possible false positive correlation inherent in measuring the same variable, the mean value for each pair of measurements is plotted against the difference between the same pair. Applied to this study it demonstrates that the difference between the value calculated by the ICG and the propranolol method increases as hepatic plasma flow increases.

Figure 27



The X-axis shows mean value for the two methods of calculating H.P.F. (ICG and propranolol).

The Y-axis shows the difference between the two values for each subject.

The graph shows that as H.P.F. increases the difference between the two methods increases.

Chapter 7

DISCUSSION

7.1 Dog studies

- 7.1.1 Action on enzymes
- 7.1.2 Action of anaesthesia
- 7.1.3 Hypoxia
- 7.1.4 Critique of methods
- 7.1.5 Recent studies
- 7.1.6 Summary

7.2 Human study

7.1 Dog studies

The disposition of propranolol was altered markedly during anaesthesia. The degree of change was similar during all four anaesthetic regimens. The changes were still present 24 hours after halothane anaesthesia but had returned to control values in the other study groups.

The principal finding of these studies was the marked inhibition of intrinsic clearance that occurred during anaesthesia. The fall was of similar magnitude (35-50% of control) with all four anaesthetic regimens. As propranolol undergoes only oxidative metabolism in the liver, this represents an inhibition of the activity of the hepatic cytochrome P450 enzymes by the anaesthetic agents. The mechanism of this inhibitory action is not immediately apparent. To explain this action several potential mechanisms must be considered including a direct effect of the anaesthetic agents on the hepatic enzymes, the effect of the anaesthetic state on the local environment of the hepatocytes and cellular hypoxia. These possibilities will be addressed.

7.1.1 Action on enzymes

The inhibition of the metabolic breakdown of a drug by the hepatic oxidative enzymes due to the action of another drug was described first nearly 30 years ago (317,318). Since these first studies many inhibitory interactions have been described. For example, a large study by Kato and colleagues (319), using five test substrates including pentobarbitone, hexobarbitone and meprobamate, demonstrated the inhibitory action of some 20 compounds and drugs on oxidative drug metabolism. More recently, the H2- receptor antagonist cimetidine has been shown to have an inhibitory action on the oxidative metabolism of many drugs. In anaesthesia an early study in this area of research demonstrated inhibition of pentobarbitone metabolism in rats both *in vitro* and *in vivo* by anaesthetic concentrations of diethyl ether (320). This inhibitory effect on oxidative reactions has been demonstrated using the halogenated agents both *in vitro* with a rat hepatocyte preparation exposed to halothane (10) and *in vivo* in rats during exposure to halothane and isoflurane using the aminopyrine breath test (20).

At a cellular level, the mechanism of this action has not been established. The potential modes of action for an inhibitor of microsomal enzyme function have been

outlined by Netter (321). These can be divided broadly into binding and chemical actions.

The possible actions due to binding include ;

a) binding of the inhibitor to the haem molecule and, therefore decreasing the oxygen binding capability. This effect is found with the inhibitor metyrapone (322).

b) competition between a substrate and the agent for the substrate binding site. This has been demonstrated for many inhibitors.

c) the reaction produces a metabolite which exhibits prolonged binding to the active site. This has been demonstrated with piperonyl butoxide (323).

The chemical actions on the microsomal enzyme system may include ;

a) inactivation of cytochrome P450 by transformation to cytochrome P420.

b) interference at a molecular level with the normal reactions involved in drug oxidation by inhibition of P-450 reduction

c) diversion of electrons from NADPH.

Competition for the substrate binding site is the most commonly described mechanism of drug interaction resulting in inhibition of metabolism and must be considered, therefore, as a possible explanation. It is interesting to note that, despite a very similar effect on propranolol metabolism, there is considerable difference in structure between the volatile anaesthetic

agents and fentanyl. However, the drugs and compounds known to cause inhibition of the hepatic oxidative enzymes show an even more diverse range of chemical structure (324). The halogenated ether anaesthetic agents enflurane and methoxyflurane have been shown to bind to cytochrome P450 (325). A property which is common to all four agents used in these studies is lipid solubility. This will allow these compounds to pass easily across the cell membrane into the cell and hence to the enzyme sites in the microsomes. It is possible that all of the agents may exert a similar effect on the microsomes which either inhibits the uptake of the substrate into the microsome or denies the substrate access to the enzyme site. This would be supported by the studies of Mather and colleagues (326) using a sheep model. They have noted that halothane anaesthesia causes a decrease in pethidine clearance but that bromsulphthalein clearance measured at the same time was unchanged despite a fall in hepatic blood flow. This may be explained by the different mechanisms involved in the elimination of pethidine and bromsulphthalein by the liver. Small basic drugs like pethidine enter the hepatocyte in their unbound form by diffusion down a concentration gradient and undergo oxidative metabolism. Large anions such as bromsulphthalein are transported into the cell while still bound to plasma proteins and usually undergo conjugative reactions. It would appear

that the affinity of the conjugating enzymes for bromsulphthalein is not altered by anaesthesia but that of the oxidative enzymes for pethidine is. As a side note this would suggest also that estimation of hepatic blood flow using indicator clearance methods must be interpreted with caution.

As conjugation takes place mainly in the rough endoplasmic reticulum and oxidation in the smooth endoplasmic reticulum the notion of a specific site of action would be supported. In his in vitro studies using rat hepatocytes, Brown described a much smaller degree of depression of conjugative metabolism than of oxidative metabolism (11). However, Brown rejected lipid solubility as the sole mechanism of action on the grounds that halothane produced the same magnitude of depression of metabolism on three barbiturates which had lipid solubility coefficients ranging from 39 - 250. Brown also noted the non-competitive nature of the inhibition, an all or nothing action (10). The same effect is found in these studies where the scale of depression of metabolism is similar for all four agents. This arguement would appear to be against competition for enzyme sites being the basis for the inhibitory action, as a graded response might be expected.

At a molecular level it has been shown that the fluoridated hydrocarbons interfere with the normal microsomal reactions. These compounds act as

pseudosubstrates which resist the action of the activated oxygen-cytochrome P450 complex but continue to stimulate NADPH oxidation (327-329). This uncoupling of the electron flow pattern may result in inhibition of the metabolism of other drugs.

It is interesting to note that, of the three inhalational agents, halothane produces a much longer acting effect on enzyme function. Halothane undergoes a much greater degree of metabolism in the body than the other volatile agents. Therefore, halothane and its metabolites will be present in the body for a longer time than the other drugs. The transient depression of metabolism by isoflurane has been described in other studies both *in vitro* (42,43) and *in vivo* (20). This supports the theory of a direct action of the agent or a metabolite on enzyme function, rather than an effect of anaesthesia per se.

7.1.2 Action of anaesthesia

The alterations in drug disposition may have resulted from changes produced by the state of anaesthesia, independent of the agent used. That is, the haemodynamic and metabolic changes which are a result of induction and maintenance of anaesthesia. This approach is supported by the finding that the change in the intrinsic clearance of propranolol produced during anaesthesia with fentanyl-atracurium-nitrous oxide was of the same magnitude as that produced with each of the inhalational anaesthetic agents. The haemodynamic and metabolic factors which may have influenced the disposition of propranolol will be considered.

The haemodynamic changes which may have occurred during the anaesthesia phase of these studies would result from the effect of controlled ventilation and from the effects of the individual agents on regional blood flow (330). During normal (spontaneous) respiration in the dog hepatic venous blood flow is the principal contribution to venous return during expiration (331). However, the pattern of venous return is altered by controlled (positive pressure) ventilation (332-334). This produces an increase in hepatic venous pressure and splanchnic vascular resistance which results in a decrease in hepatic blood flow

(52,53,67,334,335). This effect is increased in the presence of hypocarbia (140,336). The effects of the various agents were summarised in Chapter 1. At an inspired concentration of 2MAC, halothane and enflurane, in the absence of surgery, appear to depress total hepatic blood flow to a similar degree, some 20-50% (55,140). Isoflurane at an inspired concentration of 2MAC appears to have a smaller effect on total hepatic blood flow (59). The changes in total hepatic blood flow are due mainly to the decrease in portal flow which results from the decrease in cardiac output found during anaesthesia with these agents. The measured change in hepatic blood flow of 25-45% found in these studies was consistant with that described in other studies (52,53,55,140,334-338). This change was insufficient to account entirely for the change demonstrated in the clearance of propranolol. However, the decreased hepatic blood flow did contribute to the overall decrease in the measured systemic (intravenous) clearance. In addition, in the halothane study a similar degree of change to that found during anaesthesia was still present 24 hours after anaesthesia, at which time the dogs were awake and breathing normally. Further evidence that blood flow changes are only a minor mechanism for the change in disposition comes from studies using isolated perfused bovine liver (339). A fall in clearance occurred in the presence of anaesthesia despite the perfusing flow being

held constant.

7.1.3 Hypoxia

An alteration in the normal hepatic metabolism resulting from anaesthesia or anaesthetic agents is a further possible mechanism. This could be due to an hypoxia at cellular level. In an isolated perfused rat liver preparation, the metabolic clearance of antipyrine has been shown to be related linearly to oxygen delivery and consumption (340). Systemic hypoxia was not present in any of the study animals as arterial blood gases were measured at regular intervals during each study. The inhalational agents were administered in oxygen alone, and 33% oxygen was used in the fentanyl-atracurium-nitrous oxide study. Hyperoxia has been shown to have no effect on hepatic enzyme function (140).

However, it is possible that anaesthesia results in a relative intra-hepatic hypoxia and subsequent reduction in enzyme activity (341). Inhibition of propranolol and antipyrine elimination consistent with reduced hepatic enzyme function has been demonstrated during hypoxia in isolated perfused rat liver and hepatocyte preparations (340-344). This may be due to a redistribution of the intrahepatic blood flow or due to interference in the utilisation of oxygen by the hepatocytes. The effect of anaesthesia on the hepatic microcirculation is difficult

to determine. It has been suggested that halothane can cause a selective hepatic arterial constriction (337,338). In contrast, isoflurane appears to preserve hepatic arterial flow but a similar effect on metabolism is found (59). As the effect of hypoxia on drug metabolism can be detected in isolated, therefore denervated, perfused liver, it would appear more likely that the defect is in the utilisation of the oxygen by the hepatocyte.

Studies which have measured the hepatic oxygen supply, uptake and consumption during anaesthesia may support this theory. A fall in the oxygen supply to the liver, due to decreased hepatic blood flow, which is compensated for by an increase in the hepatic extraction of oxygen (an increased arterio-venous difference) resulting in no significant change in hepatic oxygen consumption has been demonstrated during anaesthesia with halothane (57,345,346), enflurane (57,62) and isoflurane (60). This would support the site of the defect being at the level of oxygen utilisation within the hepatocyte. This could be produced by the mechanisms suggested above such as binding to receptors or alteration of the cell membrane. Hepatic oxygen consumption and utilisation measurements were not made during this series of studies. However, a similar anaesthetic technique and dose of anaesthetic agent to that used in the studies described above was used in

this series.

7.1.4 Critique of methods

The mean hepatic plasma flow was calculated in these studies from plasma propranolol concentrations. In the dog the blood:plasma ratio for propranolol is 0.9 ; plasma concentrations are thus slightly greater than blood concentrations. Hence, the mean hepatic plasma flow measurements in these studies may be regarded as approximating to mean hepatic blood flow. The measured values are consistent with those previously described for the dog (57,59). The changes demonstrated during anaesthesia are similar to those described in humans during anaesthesia and in the absence of surgery (52,53,55). Beta-adrenoceptor blockade has been shown to produce a fall in hepatic blood flow of up to 20% (348,349). In these studies the plasma propranolol concentrations were sufficient on all three study days to cause near maximal beta-adrenoceptor blockade. It is unlikely that the increase in propranolol concentration during anaesthesia would produce the measured decrease in hepatic plasma flow. Some previous studies in humans have described larger falls in hepatic blood flow during anaesthesia. These studies may have overestimated the change in hepatic blood flow if flow was calculated from the systemic clearance of an intravenouly administered indicator substance. This would occur if the hepatic

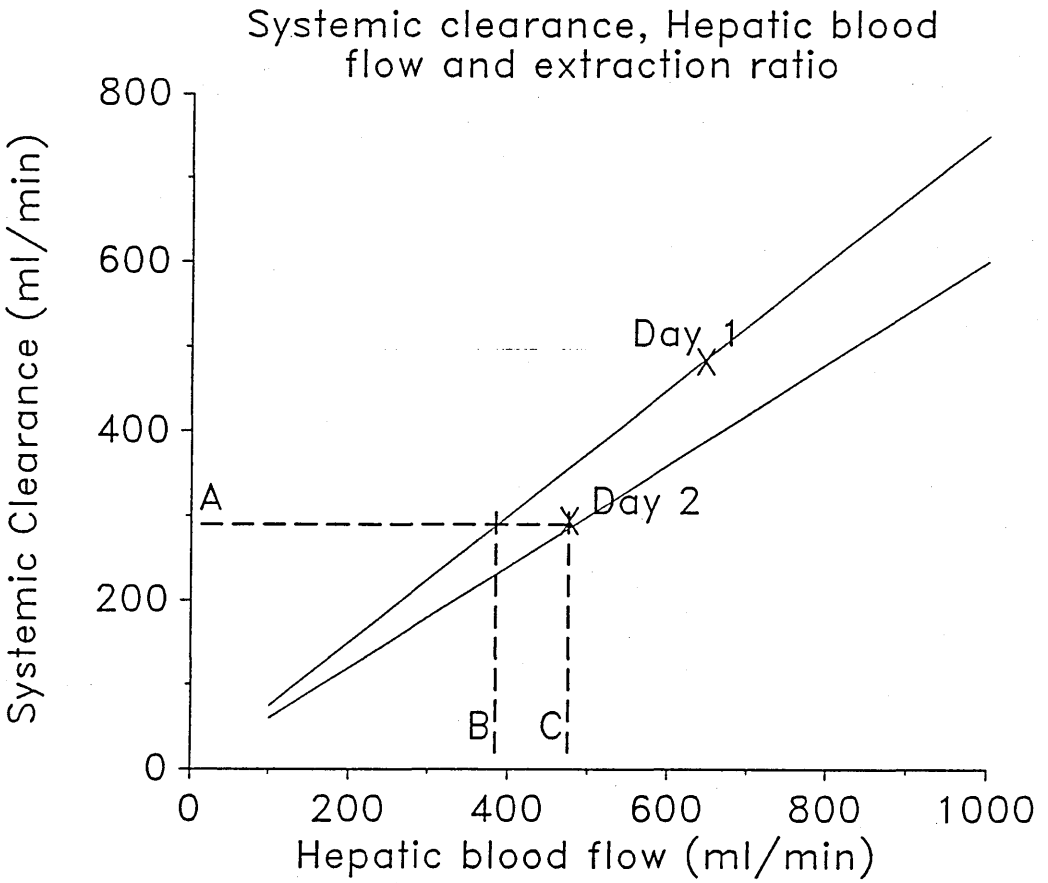
extraction of the indicator also was decreased during anaesthesia. The hepatic extraction ratio at a fixed intrinsic clearance will increase as hepatic blood flow decreases (176). A similar series of lines describing the resultant curvilinear relationship between systemic clearance and hepatic blood flow demonstrates a shift to the right as extraction ratio decreases. If, in the presence of a decrease in extraction, hepatic blood flow was calculated using the new systemic clearance value and the original extraction ratio a falsely low value for hepatic blood flow would be obtained.

The change in the systemic clearance during anaesthesia therefore reflects the changes in both hepatic blood flow and intrinsic clearance. For drugs of very high hepatic extraction, systemic clearance and hepatic blood flow have a near linear relationship (Cls = QE) and intrinsic clearance is independent of hepatic blood flow. However, as extraction ratio decreases, the influence of hepatic enzyme function in determining systemic clearance gradually increases. As Cls = (1-E)Clint, it can be seen that if E is very small Cls = Clint. This is shown in the results of these studies where the change in systemic clearance is too large to be explained by change in hepatic blood flow alone. The inter-relationship of these three variables can be illustrated using the results of the halothane study. The change from Day 1 to Day 2 in the values for

systemic clearance and hepatic blood flow are influenced by the change in hepatic extraction and provide a unique solution for the relationship on each Day (Figure 28). From the calculated systemic clearance on Day 2 (Point A), hepatic blood flow calculated assuming the extraction ratio had not changed would derive a much lower value (Point B) than that which takes account of the decrease in hepatic extraction (Point C).

Anaesthesia with halothane, enflurane and fentanyl-atracurium- nitrous oxide caused an increase in the free fraction of propranolol of 2-4%. This had returned towards the pre-anaesthetic value within 24 hours. The effect was not seen during isoflurane anaesthesia. The change demonstrated represents an increase of 20-30% in the unbound propranolol. A change of this magnitude has important implications regarding the potential for toxicity of drugs present during anaesthesia. The free fraction of propranolol during the study period is lower than has been described previously where levels of 15-25% have been found. The lower values found in these studies are consistent with the increase in acute phase reactant proteins, such as alpha 1-acid glycoprotein, which would be expected following the surgery to place the cannulae five days earlier (110,350,351). This is supported by the finding that samples taken from some dogs before the surgery demonstrated a free fraction of propranolol of around 15%. Any variability in the

Figure 28



The lines describe the relationship between systemic clearance and hepatic blood flow with a fixed extraction ratio (Day 1 0.75, Day 2 0.60). Using the calculated Cl_s for Day 2 (Point A) the derived hepatic blood flow would be falsely low (Point B) if it was assumed E.R. had not changed. Point C takes account of the change in E.R.

response to surgery was minimised by starting the study with all dogs on the fifth day after surgery. Plasma concentrations of alpha 1-acid glycoprotein were not measured in these studies.

The change in clearance of propranolol, as there is no change in volume of distribution, is reflected in a prolongation of the elimination half-life of propranolol.

The methods used in these studies were designed to allow comparison within each study group and between groups. The design was successful in the control, halothane, isoflurane and fentanyl groups but problems were encountered with the enflurane study. The dogs were unable to tolerate enflurane at 2 MAC in the presence of beta-adrenergic blockade. Even when the dose of propranolol was reduced and the enflurane reduced to 1.5 MAC, three of the five dogs were not fit enough for a third study day. The cardiac depressive effects of higher doses of enflurane in dogs has been noted by other workers (57). These changes in protocol make it difficult to compare directly the results of the enflurane study with the others. However, the scale of change in the pharmacokinetic variables is similar to that in the other studies in this series.

The time between surgery for cannulation of the vessels and the pharmacokinetic study was five days in all animals. The post-surgical changes, discussed in the

introduction, of increased protein binding and a small increase in clearance which has been ascribed to enzyme induction by some workers (7-9), would be present at this time. However, these changes would be fairly stable throughout the study period (110) and are relatively small in comparison to the changes produced in the pharmacokinetic variables by anaesthesia. For example, the free fraction of propranolol which decreased between surgery and the study days demonstrated an increase during anaesthesia in all the studies with a return to control value 24 hours later.

The metabolic state at the time of study has been shown to influence the pharmacokinetic of some drugs (289,290). All the dogs were fed with the same brand of dog food and biscuits at set times in relation to the study to minimise any influence of this variable.

7.1.5 Recent studies

Several other studies of the effect of anaesthesia on drug disposition have appeared since this project was started. Runcieman and Mather have developed a chronically cannulated sheep model with which the have looked at hepatic drug clearance of chlormethiazole, pethidine and mepivacaine (28,352-356). With this model they have evaluated the changes produced by anaesthesia using volatile agents and intravenous agents such as propofol. A similar pattern of altered disposition has been found with all these techniques and the extent of change is comparable to that found in this series of studies. Using halothane anaesthesia at 1.5 MAC in the sheep, the mean hepatic clearance of pethidine fell to 60% of control value. A decrease of similar magnitude was described for enflurane and isoflurane anaesthesia. Both propofol and thiopentone, by infusion, produced a reduction in the hepatic clearance of pethidine of around 20% (37). These workers also described changes in renal and pulmonary drug clearance during anaesthesia which was ascribed to altered excretion and blood flow.

Merin and co-workers have shown that the disposition of verapamil in the dog is altered during anaesthesia to an extent similar to that described here for propranolol (25,358). In the presence of halothane,isoflurane or

enflurane anaesthesia, the clearance of verapamil is reduced by about 40% and the apparent volume of distribution decreased by 35%. Hepatic blood flow was not measured in these studies, but the cardiovascular effects of verapamil were potentiated by higher concentrations of halothane anaesthesia (358). Intravenous lignocaine produced an increase in the apparent volume of distribution and in the clearance of verapamil (359). In a separate study this group demonstrated no change in hepatic arterial blood flow and a 35% fall in portal blood flow during enflurane anaesthesia in dogs (360). Isoflurane anaesthesia has been shown to produce a significant decrease in total hepatic blood flow with no change in oxygen consumption resulting in lower hepatic venous and hepatic surface oxygen tensions (361).

Further studies have been undertaken in Vanderbilt University using the same canine model. It has been shown that anaesthesia using a continuous infusion of propofol produces an effect on the disposition of propranolol (362). A 40% reduction in the intrinsic clearance of propranolol was produced but there was no change in hepatic blood flow. There was a significant increase from 8.5 to 14.0% of the free fraction of propranolol during propofol anaesthesia. In addition, the study with halothane anaesthesia has been repeated with a refinment of the HPLC analysis which allows

detection of both isomers of propranolol (363). The overall effect was the same as in the original study but it was found that the metabolism of the pharmacologically active l-isomer was much more markedly inhibited (75%) than that of the d-isomer (63%). In contrast, spinal anaesthesia with tetracaine produced no significant effect on propranolol disposition in the dog (364). The acute effect of halothane anesthesia on the disposition of morphine has been studied with a similar canine model (365,366). Halothane anaesthesia produced a decreased hepatic clearance of morphine which was ascribed to the decrease in hepatic blood flow and not to a decrease in metabolic clearance as the hepatic extraction of morphine increased during anaesthesia. The authors concluded that halothane has less effect on conjugative than on oxidative metabolism.

A series of studies in the rat has shown a comparible effect on lignocaine and propranolol metabolism (367). Halothane, enflurane and isoflurane all produced a similar degree of depression of propranolol clearance. However, isoflurane had a smaller effect on lignocaine clearance than halothane or enflurane.

The effect of infusion of intravenous anaesthetic agents on canine hepatic blood flow has been studied by Thomson and colleagues (368). A dose related decrease in hepatic blood flow was found with infusion of thiopentone, althesin or etomidate. With Althesin and

etomidate infusions at low rates hepatic arterial flow appeared to decrease before systemic effects were found. Etomidate has been shown to have a reversible inhibitory effect on in vitro rabbit hepatocyte cytochrome P450 activity resulting from binding of etomidate to the cytochromes (369).

These studies suggest that anaesthesia produced by either the inhalational or by the intravenous agents appears to produce a reduction in hepatic drug metabolising enzyme activity. The effect is dose related but appears to be of a consistent magnitude across a number of study groups and species. The effect is greater than can be explained by changes in hepatic blood flow. An increased pharmacological effect has been demonstrated with some test drugs.

7.1.6 Summary

The studies described here have demonstrated that general anaesthesia using a volatile agent or a balanced anaesthesia technique, alters significantly the disposition of propranolol. This effect would appear to be of a similar magnitude for all the agents but is present for a longer period post-operatively following halothane anaesthesia. Propranolol is a drug of high hepatic extraction which undergoes oxidative metabolism in the liver, which, when given simultaneously intravenously and intraportally, can be used to define the metabolic and flow dependent elements of hepatic drug clearance. The principal effect of anaesthesia was a marked inhibition of the rate of metabolism of propranolol. The action of the anaesthetic agents appears to be an inhibition of the cytochrome P450 system, which is responsible for oxidative drug metabolism. At a cellular level, the mechanism of this change is not fully understood but appears to involve impairment of the utilisation of oxygen by the microsomes. The clinical significance of this effect is an increase in the bioavailability of, a rise in the plasma concentrations and a prolongation of the elimination of propranolol. It has become more common to continue concurrent drug therapy throughout the period

of operation. If, as is likely, anaesthesia has a similar effect on other drugs which undergo hepatic oxidative metabolism care must be exercised to alter the dosage schedule of drugs which are continued through the peri-operative period.

7.2 Human study

This study demonstrated a reduction in the clearance of propranolol administered orally which was consistent with a reduction in hepatic drug metabolising capability. No effect on hepatic blood flow was found. The prostaglandin analogue, enprostil appeared to have no effect on enzyme activity or hepatic blood flow.

Cimetidine has been shown to impair the elimination of a large number of drugs (221) including propranolol (275,276). This study confirms that the co-administration of cimetidine reduces the oral clearance of propranolol due to inhibition of hepatic oxidative enzyme activity. This results in increased bioavailability and elevation of plasma concentrations of propranolol.

Previous studies have demonstrated conflicting conclusions on the effect of cimetidine on hepatic blood flow. Some studies have suggested that cimetidine causes a decrease in hepatic blood flow (275,296) and some have found a similar effect with ranitidine (370,371). Other investigators have, however, failed to demonstrate any consistent effect of the H2 antagonists on hepatic blood flow (301,303). The results of these previous studies are difficult to interpret as many used small numbers of patients, did not include a placebo control, were not

double-blind and did not randomise the order of drug administration. Considerable interindividual variation in the baseline hepatic blood flow was found. This study involved nine subjects who received the drugs and placebo in a double-blind, randomised fashion with a balanced order of drug administration. Using this design we demonstrated no effect of cimetidine on hepatic blood flow.

The role of prostaglandins in the control of drug metabolising activity is poorly defined. In vitro studies have suggested that prostaglandins can alter the hepatic metabolism of a number of drugs including benzpyrine, aminopyrine and hexobarbitone (309,310). The haemodynamic effects of prostaglandins are defined more clearly. Gastric blood flow appears to be partially under the control of prostaglandins as it is altered by the administration of the cyclo-oxygenase inhibitors aspirin and indomethacin (306,307). A reduction in the clearance of indocyanine green, which was attributed to a reduction in hepatic blood flow has been demonstrated in man following aspirin or indomethacin administration (372). The prostaglandin analogue, enprostil, may have been expected to cause a decrease in either hepatic enzyme activity or in hepatic blood flow. However, no effect was demonstrated on either variable.

Two different methods of estimation of hepatic blood flow were used in this study. The value for hepatic

blood flow measured by dual-route propranolol was consistently higher than that measured using indocyanine green clearance. This has not been remarked on before but review of other published data appears to confirm this finding(275,296,373). The explanation of this difference is not immediately clear. It must be noted that the two methods measure hepatic blood flow over quite different time periods. The indocyanine clearance was measured over a 20 minute period after the subjects had been resting for 1 hour following an overnight fast. The propranolol method measures the hepatic blood flow over a prolonged period with sampling continued for 8 hours and the time-concentration curve extrapolated from the last data point. It is likely that there was an increase in blood flow after the subjects were fed at 4-5 hours into the study period. The estimation of hepatic blood flow using indocyanine clearance assumes a hepatic extraction of 100%. Considerable interindividual variation has been shown in the hepatic extraction of ICG with many subjects having extraction ratios of less than 100% (168,169). The ICG method will therefore tend to underestimate hepatic blood flow. The difference in estimated blood flow between the two methods appears to increase as hepatic blood flow increases.

This study is included to demonstrate the feasability of using the dual-route administration method for studies in man in the peri-operative period. A known

enzyme inhibitor was administered to the subjects to test the ability of the method to detect the resultant change in oral clearance. Hepatic blood flow was unchanged by the drugs used but consistent values were found for the subjects and these appear to be more reproducable than those estimated from indocyananine green clearance. It is clear that propranolol is not an appropriate drug for administration to patients undergoing surgery and anaesthesia but other drugs of similarly high hepatic extraction but with much less systemic effect would be suitable model drugs with which to apply this methodology to studies in man during the peri-operative period.

APPENDICES

Pharmacological Abbreviations

Xdiv	- Dose of drug given intravenously
AUCiv	- Area under the time-concentration curve for intravenous drug to infinity
Xdp	- Dose of drug given portally
AUCp	- Area under time-concentration curve for portal drug to infinity
Xdo	- Dose of drug given orally
AUCo	- Area under time concentration curve for oral drug to infinity
Cls	- Systemic or intravenous clearance
Clp	- Apparent portal clearance
Clint	- Intrinsic clearance
Cliv	- Intravenous clearance
E	- Hepatic extraction ratio
F	- Bioavailability
T1/2iv	- Elimination half-life
k	- Elimination rate constant
Vb	- Apparent volume of distribution after equilibrium is achieved
HPF	- Hepatic plasma flow
fb	- Fraction of bound drug

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CONSENT

ENPROSTIL (RS-84135-00-00-3) AND CIMETIDINE: A COMPARISON OF EFFECTS ON HEPATIC BLOOD FLOW

Explanation of Study

I understand that I have been asked to participate in a five week research study that will involve a maximum of nine healthy male volunteers. The purpose of this study is to evaluate and compare the effects of a marketed drug (cimetidine) and an investigational drug (enprostil) on the rate at which blood flows through my liver. These drugs will be compared to a placebo, which is inactive.

I understand that no other drugs, alcohol, aspirin products, coffee or caffeine products should be consumed 72 hours before admission to the study or during the study.

Procedures

I understand that this study is divided into three (3) six day phases. I will receive each one of the study medications once. Prior to phase one I will have complete physical and laboratory examinations. The physical exam will be repeated at the end of the study while the laboratory exam will be repeated at the conclusion of each phase.

I understand that in order to measure my liver blood flow I will be injected with an indicator dye called ICG on day 5 and a cardiovascular drug called propranolol on day 6. ICG is commonly used to study liver function. Propranolol is widely prescribed for high blood pressure and is removed from the blood primarily by the liver. I understand that blood samples will be drawn at the following intervals: Day 5 at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15 and 20 minutes; Day 6 at 0, 5, 15, 20 minutes and 1, 2, 3, 4, 5, 6, 7 and 8 hours.

Risks and Precautions

I understand that enprostil should not be handled by asthmatics or pregnant women. It should be kept out of reach of children. I understand that side effects that might occur during this study could include wheezing, nausea, vomiting, diarrhea, headache, warm feeling in the stomach, stomach cramps and slightly increased risk of blood clotting. Other, previously unreported or unknown, side effects, including drug allergy, may also occur.

During the collection of blood samples, I may experience pain and/or bruising at the site on my arm where blood is drawn. Local infections may rarely occur.

Benefits

I cannot expect any benefits from my participation in this study.

Withdrawal from Study

I acknowledge that my participation in this research project is voluntary and that I have the right to withdraw at any time. It has been explained to me that if the study physician feels that it is in my best interest to be withdrawn from the study, he will do so immediately. I further understand that my participation in the study may be ended at any time with or without my consent.

Medical Care/Problems Related to this Study

I understand that in the event I suffer any medical problem which, in the opinion of the investigator, is related to this clinical study or to the preliminary physical examination or laboratory work, emergency medical care will be provided at no charge to me. However, no other compensation is available. I may obtain medical treatment from the study physician or he will assist me in obtaining treatment. I have been assured that every effort will be made to prevent any injury that could result from this study by the study physician.

I understand that if any questions arise related to this study or to research subjects' rights I am to contact the study physician (Dr. _____) at the following phone numbers _____.

Release of Medical Records

I understand that it may be necessary for the United States Food and Drug Administration to inspect my medical records as they relate to this study. I therefore consent to the release of my medical records to Syntex or other regulatory agencies with the understanding that these records will be used only in connection with carrying out obligations relating to this clinical study and that they shall not be used for any other purpose.

Confidentiality

Syntex will not disclose the contents of my medical records to any other party other than the Food and Drug Administration. I understand that a report of the results of this study will be made to the United States Food and Drug Administration and possibly to other regulatory agencies with confidentiality of subject identification preserved. The results of these studies may also be used for medical and scientific publication but my identity will not be disclosed.

Consent

Before giving my consent by signing this form, I have read it and I have been sufficiently informed of the drugs used, the inconveniences, the hazards, dangers and adverse effects that might occur from the use of the drugs described in the consent form. I have spoken directly to the study physician who has answered to my satisfaction all of my questions concerning this study. Based on this information I voluntarily agree to participate in this study. I have received a signed copy of the consent form.

_____ _____
Patient's Signature Date

_____ _____
Study Physician's Signature Date

_____ _____
Witness Signature Date

4

SCHEDULE OF EVENTS

	72 Hours Prior and During Study	Baseline	Phase I Day 1	Phase I Day 6	Phase II Day 15	Phase II Day 20	Phase III Day 29	Phase III Day 34	Follow-Up**
s/Alcohol	X								
Laboratory Tests*		X		X		X		X	
l Examination**		X						X	
on		X							
d Consent		X							
x Week			X		X		X		
Phase				X		X		X	

ine laboratory tests: RBC, Hgb, Hct, WBC, differential, platelet estimation, serum creatinine, uric , BUN, alkaline phosphatase, calcium, phosphorus, cholesterol, total bilirubin, total protein, min, globulin, serum testosterone, SGOT, SGPT, LDH, serum electrolytes and urinalysis.

based on symptoms or laboratory abnormalities throughout the study

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