LIGNOCAINE EXTRACTION RATIO AND CLEARANCE AS AN INDICATOR OF HYPOXIC HEPATIC INJURY

A study using the in situ and the isolated perfused pig liver

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WORK HARDER

HOPE FOR LUCK

NEVER GIVE UP

Vietnamese Proverb

ABSTRACT

The metabolism of lignocaine to monoethylglycinexylidide has been found useful as an indicator of hepatic function in association with liver transplantation. It has been postulated that this might be due to the common effect of hypoxic damage on liver function and lignocaine metabolism.

The aim of this work was to establish whether hepatic lignocaine elimination was impaired by hypoxia and whether lignocaine extraction ratio and clearance could be used as an indicator of hepatic function. This was studied using the isolated pig liver perfused via the hepatic artery and portal vein. To establish whether the pig liver could be used as a possible human model for this investigation and whether lignocaine had any detrimental effects on liver function and blood flow in vivo, hepatic lignocaine elimination and the effects of lignocaine administration on hepatic function and blood flow were studied in the anaesthetized pig, surgically prepared to allow sampling across the liver and direct hepatic blood flow measurement.

Hepatic lignocaine elimination was then studied in the isolated perfused liver to determine whether this was similar to that found in vivo.

The definitive studies required preliminary investigations not available from the literature to determine the feasibility of comparing in vivo and ex vivo hepatic function using the same liver. In addition, by studying the decay of lignocaine after bolus dose administration the necessary pharmacokinetic parameters to achieve similar constant hepatic affluent lignocaine concentrations in vivo and in the isolated preparation could be determined.

The preliminary investigations showed that a sequential experiment using the same liver to compare in vivo and ex vivo function was inappropriate as the energy state of isolated perfused livers previously studied in vivo was significantly different from that in livers perfused immediately.
The decay of lignocaine after a bolus dose in vivo and ex vivo could be described by a two compartment open model and in both preparations the derived pharmacokinetic parameters from this analysis were used to achieve similar constant hepatic affluent concentrations over the study period used to determine hepatic lignocaine elimination.

Lignocaine extraction ratio by the in situ pig liver was similar to that reported in man and together with hepatic clearance and intrinsic clearance was similar to that determined in the isolated state when different livers were used for this comparison.

There was no detrimental effect of lignocaine administration on hepatic function and blood flow in vivo.

Lignocaine extraction ratio and clearance and monoethylglycinexylidide formation were significantly impaired in livers subjected to hypoxia. Lignocaine elimination correlated strongly with hepatic cellular ATP, energy charge and ATP/ADP ratio as well as with hepatic potassium release but less strongly with aspartate aminotransferase release when this relationship was tested using the combined data from hypoxic and normoxic livers ex vivo.
These correlations were positive for hepatic adenine nucleotide status and negative for hepatic potassium and aspartate aminotransferase release.

Neither hepatic alanine aminotransferase release nor lactate utilization were significantly affected by hypoxia.

Lignocaine extraction ratio, hepatic oxygen consumption, ATP content, bile flow and potassium release were shown to be equivalent, more highly sensitive, and earlier indicators of hypoxic hepatic injury than hepatic aspartate aminotransferase release in the isolated perfused pig liver.

DECLARATION

I declare that this thesis is my own, unaided work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other University.

Berend Mets

December 1991

To

Ulane Neveling

and

Rosemary Hickman

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All the experiments in Chapters 4,5,6,7 and 8 described in the thesis proper as well as the calibration and validation experiments included in the Appendices A and E were performed by the candidate personally. The analysis of lignocaine and its metabolites (section 3), was performed as stated above under my direct supervision. The pharmacokinetic analysis of lignocaine decay in vivo and ex vivo was my own unaided work.
The entire processing of this document was my own work.

GLOSSARY OF TERMS

ADP	Adenosine-5-diphosphate
ALT	Alanine Aminotransferase
AMP	Adenosine-5-monophosphate
AST	Aspartate Aminotransferase
ATP	Adenosine-5-triphosphate
BSP	Bromosulphthalein
CL	Hepatic Clearance
CLintrinsic	Intrinsic Hepatic Clearance
CV	Coefficient of Variation
EC	Energy Charge
EHBF	Effective Hepatic Blood Flow
EMGX	Ethylmethylglycinexylidide
ER	Extraction Ratio
FF	Free Fraction
FGFR	Fresh Gas Flow Rate
GX	Glycinexylidide
HA	Hepatic Artery
HAQ	Hepatic Artery Flow
Hb	Haemoglobin
HBF	Hepatic Blood Flow
Hct	Haematocrit
HPLC	High Performance Liquid Chromatography
HV	Hepatic Vein
ICG	Indocyanine Green
IPPL	Isolated Perfused Pig Liver
K+	Potassium
LU	Lactate Uptake
MEGX	Monoethylglycinexylidide
$P0_2$	Partial Pressure of Oxygen
$PC0_2$	Partial Pressure of Carbon Dioxide
PV	Portal Vein
PVQ	Portal Vein Flow

SD..... Standard Deviation

SEM..... Standard Error of the Mean

TAN..... Total Adenine Nucleotides

THBF..... Total Hepatic Blood Flow

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CHAPTER 1: INTRODUCTION AND SCOPE OF THESIS

A. INTRODUCTION

1.1. THE EVALUATION OF LIVER FUNCTION

"Conventional liver function tests rarely assess liver function, rather they detect the presence or absence of liver disease" (Branch, 1982). Because of this, the hepatologist is at a disadvantage when compared to the nephrologist who can readily estimate renal function (Crom et al, 1986). In an attempt to better quantify hepatic function, investigators have administered exogenous compounds as well as drugs to determine their fate (Tygstrup 1966)(Vesell, 1984)(Van Waeg et al, 1988). However, the "ideal drug probe which is both innocuous and specific in its liver activity assessment" has yet to be found (Barstow and Small, 1990). The hope that a single compound can be used to assess the multitude of functions of the liver and be used as a single index of liver function is unlikely to be realised as liver function is not unitary (Milowszewski et al, 1970). Because of the varying effects of different disease processes on hepatic function (Roberts and Schenker, 1983)(Hepner et al, 1977), Galambos and Wills, (1978) have stated that "there is a need for accurate clinical tests which can estimate the specific liver function most likely affected by the suspected disease process."

The metabolism of lignocaine to monoethylglycinexylidide (MEGX) has been found useful as an indicator of hepatic function in association with liver transplantation. It has been postulated that this might be due to the common effect of hypoxic damage on liver function and lignocaine metabolism (Oellerich et al, 1991).

1.1.1 THE ASSESSMENT OF HEPATIC FUNCTION

The discussion of the clinical assessment of hepatic function using conventional tests will be limited largely to the actual tests used for this purpose in the experiments to be described. In addition, in the relevant sections emphasis will be placed on the tests used experimentally to assess hepatic function.

For the purpose of this discussion clinical liver function assessment is divided into

(A) Conventional tests

(B) Specialised tests

1.1.2 CONVENTIONAL TESTS

The term "conventional tests" is used here to include only those tests that a clinician might order as part of a routine "liver function" screen, such as those incorporated in a SMAC (Simultaneous Multiple Analyser and Computer) battery of tests. This group can be subdivided roughly into:

(A) Tests of hepatocellular damage

(B) Tests of cellular synthesis

(C) Tests of excretory function and hepatobiliary obstruction.

1.1.2.A. Tests of Hepatocellular Damage

The tests used to detect and assess cellular injury are based on the measurement of cellular contents which have leaked into the circulation. Clinically this estimation is based on the measurement of enzyme activity in plasma (Price and Alberti, 1985). The enzymes that are most commonly employed as indicators of hepatocellular damage are **aspartate aminotransferase (AST)**[1] and **alanine aminotransferase (ALT)**[2]. Although a large number of other hepatic enzymes such as isocitrate dehydrogenase, alcohol dehydrogenase, guanase, sorbitol dehydrogenase, glutathione S-transferase as well as the liver specific isoenzyme of lactate dehydrogenase have been suggested, changes in these enzymes generally parallel those of AST and ALT and do not offer greater sensitivity but may be more complex to measure and thus tend not to be used routinely (Price and Alberti, 1985).

AST is found as a cytoplasmic and mitochondrial isoenzyme but is usually measured in serum as total AST. In human liver cells the distribution of AST is 81% as the mitochondrial isoenzyme and 19% as the soluble (cytoplasmic) isoenzyme whilst that measured in plasma in health is predominantly the cytoplasmic isoenzyme (Rej, 1978). The relative importance of ALT and AST as indicators is still debated as varying hepatic insults result in differential increases of these enzymes (Coodley, 1971)(Baron, 1970). However, when massive hepatocellular necrosis occurrs, AST concentrations exceed those of ALT, probably as a result of the disruption of mitochondria. Also, ALT is regarded as more liver specific than AST which is more prevalent in other tissues (Price and Alberti, 1985) including red bloodcells (Silva and Pannal, 1975) from which it may be released during haemolysis. Although it has been suggested that the ratios of the transaminases may be useful in establishing hepatic status (Brown, 1988) this is denied by others (Baron, 1970)(Price and Alberti, 1985).

---

1 Formerly designated serum glutamic oxaloacetic transaminase (SGOT) which referred to the products of this transaminase reaction.

2 Formerly designated serum glutamic pyruvic transaminase (SGPT). Both transaminases (transferases) are enzymes that catalyze transfer of an amino group from an alpha-amino acid to an alpha-keto acid.

1.1.2.B. Tests of Cellular Synthesis

This implies the measurement of parenchymal cell function. The first example is that of **albumin** which is formed exclusively in the liver and so the measurement of plasma albumin is regarded as an indicator of liver cell function. This may be complemented by measurement of plasma **urea** as indicator of hepatic nitrogen metabolism (Baron, 1970). These parameters may be of of use in assessing longstanding hepatic disease (in the absence of renal disease) as in chronic cirrhosis both plasma albumin and urea are decreased. Although a low urea may indicate acute hepatic necrosis (Baron, 1970) in general, measurement of urea is of little use in patients with liver disease (Price and Alberti, 1985). Plasma albumin is not a useful indicator of acute hepatic insults because its half-life is 14-20 days, and its level may be affected by nutrition, renal disease and changes in extracellular fluid volume. Prealbumin has a half-life of 1.9 days and a higher sensitivity for indicating acute hepatic dysfunction (Goldberg and Brown, 1987).

The **prothrombin time** can be used as an indicator of hepatic synthetic function as the vitamin K dependent factors II, VII, IX and X are all exclusively synthesised by the liver (Chisholm, 1985). However, this too can be affected by other factors than liver function such as malabsorption, bile obstruction and antibiotic therapy. The prothrombin time is of value in both acute and chronic liver disease due to the short half life (6 hours) of prothrombin (Brown, 1988).

Fibrinogen is synthesised exclusively by the liver and is present in measureable amounts in plasma. Nevertheless plasma levels are of little use in the diagnosis of hepatic dysfunction although low values do indicate extensive liver damage (Price and Alberti,1985). The half life of fibrinogen is approximately 5 days (Chisholm, 1985).

Although one of the major functions of the liver is **glucose homeostasis,** none of the commonly used liver function tests involves carbohydrate metabolism, partly because of the large functional reserve of the liver. Fasting blood glucose has proved a poor discriminant of hepatic integrity; only showing a significant inconstant fall in severe liver disease (Price and Alberti, 1985).

Gluconeogenic precursors such as **lactate** may be abnormal in patients with liver disease (Royle and Kettlewell, 1978)(Stewart et al, 1983). However, the occurrence of hyperlactatemia in association with hepatic disease is contentious as it may be attributed to perfusion failure (Kruise et al, 1987). As a routine test there appears to be little benefit in using this assay in the clinical situation (Price and Albertti, 1985).

Plasma **cholesterol** levels have been suggested as indicators of hepatic-synthetic function (Barstow and Small, 1990). The reduced plasma cholesterol (measured as cholesteryl esters) in hepatic parenchymal disease is due to decreased lecithin-cholesterol acyltransferase activity (Harry et al, 1985), however its role in the assessment of hepatocellular damage has been questioned (Price and Alberti, 1985).

Gamma-glutamyl transferase (GGT) is a sensitive indicator of liver disease and so may be used as a screening test. However, the plasma level of this enzyme may be raised in patients taking certain drugs as well as alcohol and thus may give an indication of microsomal enzyme induction rather than hepatic

function (Price and Alberti, 1985). As the plasma GGT is dependent on both induction of hepatic GGT and disturbance of plasma membranes and these two functions are not necessarily associated, plasma GGT may be an unreliable index of either (Park, 1982).

1.1.2.C. Tests of Excretory Function and Hepatobiliary Obstruction

One of the functions of the liver is to clear bilirubin, formed largely from senescent red cells, and make this compound more water soluble through conjugation for elimination via the kidneys. Reduced excretion by the liver is observed in many forms of parenchymal liver disease (Brown, 1988) resulting in a rise in plasma conjugated bilirubin which can be used as an indicator of impaired hepatic function. However, conjugated hyperbilirubinaemia may also be caused by intra hepatic or extrahepatic biliary obstruction under which circumstances this rise in bilirubin does not reflect impaired parenchymal function [See Schafner and Popper, (1985) for a review].

The enzymic assessment of hepatobiliary obstructive disease using alkaline phosphatase, 5'-nucleotidase and gamma-glutamyl transferase will not be discussed as this is not pertinent to this thesis. [See Price and Alberti (1985) for a review].

1.1.3 SPECIALISED TESTS

There are many aspects of liver function that are not measured by conventional tests such as its role in the metabolism of hormones, trace elements and vitamins, its storage function for iron, copper, lipids and glycogen and its immunological function.

The clinical utility of serum bile acid measurement which held out the hope of improved sensitivity over conventional tests in assessing hepatic disease (Korman et al, 1974) is still debated as measurement is complex and appears not to be better than conventional tests for diagnosing hepatic diseases (Goldberg and Brown, 1987).

In summary it is clear from the above short review that the conventional "liver function tests" are more useful for documenting liver dysfunction either with respect to hepatocellular damage or with respect to the inability to excrete bilirubin than they are in documenting and quantifying liver function and making a specific diagnosis. With regard to the assessment of liver function, the prothrombin time may be a noteable exception although this test may lack specificity.

To enhance the diagnostic specificity of liver disease using conventional liver function tests a number of investigators have turned to discriminant function analysis (Hamilton, 1977)(Winkel et al, 1975) whilst others have turned to the study of the hepatic elimination of administered substrates.

1.1.4 DRUGS ADMINISTERED AS INDICATORS OF HEPATIC FUNCTION

1.1.4.A. General

There are a number of requirements which should be satisfied for a drug to be used as a test of hepatic function (Branch, 1982)(Barstow and Small, 1990).

(1) The drug should be nontoxic in both healthy people and in patients with liver disease and lack (detrimental) pharmacological effects.

(2) It must be feasible to administer the drug intravenously or if administered orally it should be completely and rapidly absorbed.

(3) The rate-limiting steps of elimination of the drug should be affected by hepatic disease but not by other drugs or conditions.

(4) It should be eliminated only, or primarily, by the hepatic route.

(5) It should be possible to measure the parent compound and/or its metabolites in plasma, saliva, urine or exhaled breath.

(6) The drug should not be protein bound unless it has a high extraction ratio.

(7) The drug should be inexpensive.

(8) It should be possible to administer the drug by a noninvasive method suitable for routine clinical use.

(9) The analytical equipment for assaying the drug should be commonly available.

(10) The drug should be readily available without requiring permission for investigational use.

To date (1990) according to Barstow and Small, the ideal drug for this purpose has not been reported on.

A number of drugs have been investigated in this regard. In order to classify these, they can probably best be divided according to the site of measurement namely, breath, saliva, urine and plasma.

1.1.4.B. Indicators of Hepatic Function Measured in Breath

Radioisotope-labelled aminopyrine (dimethylaminoantipyrine) was the first drug used in a breath test to assess liver function (Hepner and Vesell, 1974). The aminopyrine breath test has been extensively evaluated since and found to be a sensitive indicator of hepatic function (See Vesell, 1984 for review). Diazepam (Hepner et al, 1977) as well as caffeine, galactose and phenacetin have also been assessed in this regard (See Baker et al, 1983 for review). However, breath tests as indicators of hepatic function are performed in only a few medical centers. With respect to the aminopyrine breath test this may be due to the continuing concern about the safety of aminopyrine (Vesell, 1984) as well as the long half life of ^{14}C when used as a label for breath test substrates. In spite of not being invasive the tests as now performed require timed collection and analysis of a number of samples and are more expensive than standard tests of liver function (Baker et al, 1983).

1.1.4.C. Indicators of Hepatic Function Measured in Saliva and Urine

As drugs can be readily measured in urine this avenue for the analysis of drug concentration to establish hepatic function has been used for antipyrine and aminopyrine (Vesell et al, 1975) as well as more recently for methacetin in children (Krumbiegel et al, 1988). Antipyrine (Vesell,1984) and caffeine concentration (Jost et al,1987) have been measured in saliva to successfully evaluate hepatic function.

1.1.4.D. Indicators of Hepatic Function Measured in Plasma

Although the disposition and metabolism of quite a number of exogenous substances have been studied for detecting changes in drug metabolising capacity in man (Park, 1982) only relatively few drugs have been evaluated as indicators of hepatic function either by assessing these in various disease states or through correlation with other indicators of liver function.

In this context liver function has been assessed using sulfobromophthalein (See Kaplan, 1987 for review), the galactose elimination capacity (Jost et al, 1987), galactose clearance (Kawasaki et al, 1988), the galactose tolerance test (Van Waeg et al, 1988) indocyanine green (ICG) clearance (Jost et al, 1987)(Oellerich et al, 1990), ICG extraction ratio (Caesar et al, 1961)(Burns et al, 1991), antipyrine and aminopyrine (Vesell, 1984) and diazepam decay (Hepner et al, 1977) as well as, dimethadione/trimethadone ratio (Tanaka et al, 1987) caffeine decay (McDonagh et al, 1991), allopurinol decay (Van Waeg et al, 1988) and by the formation of monoethylglycinexylidide (MEGX) after the administration of lignocaine (Oellerich et al, 1987)(Schroeder et al, 1989).

1.1.5 LIGNOCAINE METABOLISM TO MONOETHYLGLYCINEXYLIDIDE AS AN INDICATOR OF HEPATIC FUNCTION

Oellerich et al, (1987) established that there was a significantly lower 15 minute MEGX concentration in patients with cirrhosis, and a significantly higher concentration in hepatic transplant donors[3] after the administration of an intravenous bolus dose of of lignocaine hydrochloride ($1 mgkg^{-1}$) than in control patients. These investigators went on to show that this test was a better predictor of primary non-function of the subsequently transplanted liver than ICG clearance and galactose elimination capacity (Burdelski et al, 1987) as well as a better predictor than the latter tests and conventional liver function tests of 120 day graft survival (Oellerich et al, 1989). In addition, Schroeder et al (1989) showed that recipients with histologic diagnoses of rejection or ischaemia had lower MEGX concentrations after lignocaine administration. This test has also been used to assess hepatic function in adults (Oellerich et al, 1990) and children (Gremse et al, 1988) with chronic liver disease.

---

3 Ascribed to the administration of life supporting drugs to the donor patients which may have enhanced hepatic blood flow.

As the integrity of the hepatic cytochrome P450 system is of particular importance in lignocaine metabolism (vide infra), Oellerich et al, (1991) speculate that the impaired functioning of the transplanted liver may be due to a lowered cytochrome P450 IIIA activity. This could be explained on the basis of hypoxic injury which may affect the liver in the peri-transplant period (Tygstrup et al, 1975) as destruction of cytochrome P450 structures can be caused by acute severe hypoxia (Srivastava et al, 1980).
An investigation of the effects of hypoxic hepatic injury on the metabolism of lignocaine would help to answer this pertinent question. Therefore, this aspect was studied further and thus hepatic lignocaine elimination will be reviewed in detail.

1.2 LIGNOCAINE

1.2.1 HISTORY

Lignocaine was first synthesised by Lofgren and Lundqvist in 1943 in Sweden and was introduced into clinical practice as a local anaesthetic in 1948 (Gordh, 1949). This agent has become the most commonly used local anaesthetic in the United Kingdom (Atkinson et al, 1987).
The first reported use of lignocaine as an antiarrhythmic agent was by Southworth et al, (1950). To date it remains the parenteral drug of choice for the management of ventricular arrhythmias (Pieper and Rodman, 1987).
Impaired lignocaine clearance as a result of hepatic disease was first documented by Thomson et al, (1971) setting the stage for the first report (Oellerich et al, 1987)[4], documenting the formation of MEGX (vide supra) after a bolus dose of lignocaine as a potential indicator of hepatic function.

1.2.2 CHEMISTRY AND METABOLISM[5]

The chemical structure of lignocaine is an aromatic group, 2,6-xylidine, which is coupled to diethyl glycine via an amide bond (Figure 1.1). It has a molecular weight of 234.3. Lignocaine does not possess a chiral center and so does not exhibit optical isomerism (Tucker et al, 1990).
In man the high clearance of lignocaine is attributed almost exclusively to the liver (Rowland et al, 1971)(Stenson et al, 1971). Here, lignocaine is metabolised by oxidative-N-deethylation to monoethylglycinexylidide (MEGX) followed by hydrolysis to 2,6-xylidine (Hollunger, 1960) or further

4 In the English press. An earlier report appeared in German: (Oellerich, 1987). In:Funktion und Funktionsdiagnostik der Leber (Seidel, D & Lang, H eds.) Heidelberg, Germany, Springer, pp 53-55.
5 The pharmacokinetics and relevant haemodynamic and toxic effects of lignocaine are discussed in the relevant sections. See Collinsworth et al, (1974) and Pieper and Rodman (1987) for reviews.

deethylation to glycinexylidide (GX). Aromatic ring hydroxylation accounts for the formation of 3-hydroxy-lignocaine, 3-hydroxy-MEGX as well as 4-hydroxy-2.6-xylidine in man (Keenaghan and Boyes, 1972) whilst N-hydroxy derivatives are also formed (Mather and Thomas, 1972). In man the major metabolic pathway of lignocaine appears to be via deethylation and the formation of MEGX (Beckett et al, 1966)(Hermansson et al, 1980). The probable site for this reaction in man is the cytochrome P450 IIIA4 enzyme (Bargetzi et al, 1989).

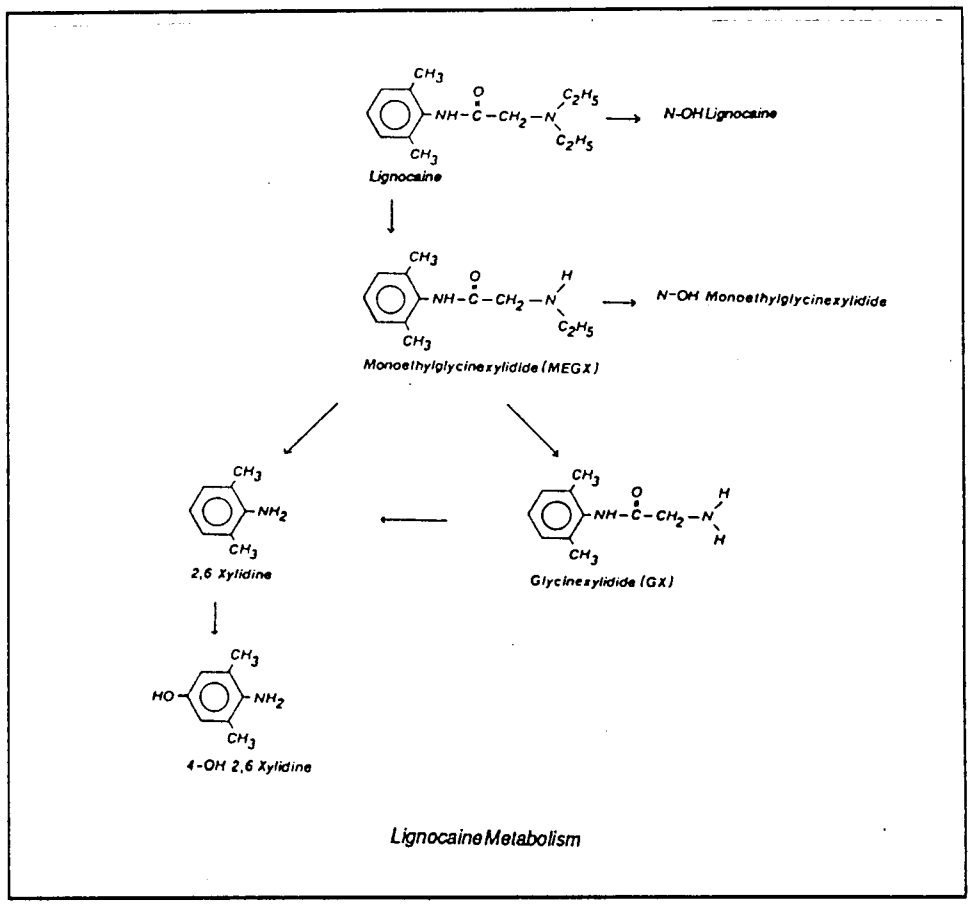

Figure 1.1 The metabolism of lignocaine.

There are however important species and sex differences as to the major metabolic path of lignocaine (Von Bahr et al, 1977) (Keenaghan and Boyes, 1972).

There is no evidence of enterohepatic recirculation of lignocaine in man (Collinsworth et al, 1974). Whilst the metabolism of lignocaine in humans appears to be independent of dose after a single administration (Bennet et al, 1982) and has been shown to be independent of concentration over the human

antiarrhythmic therapeutic range (2-6ugml^{-1})(Pieper and Rodman, 1987) in the rat (Pang and Rowland, 1977b), there is increasing evidence that hepatic lignocaine metabolism may be time dependent (Bauer et al, 1982)(Tam et al, 1987). This may be due to lignocaine (Saville et al, 1989) or MEGX (Lennard et al, 1983) induced enzyme inhibition.

Hepatic lignocaine metabolism has been studied in a number of animal species in vivo namely the monkey (Benowitz et al, 1974) cat (Lautt and Skelton, 1977), dog (Lelorier et al, 1977), the sheep (Mather et al, 1986) and in the isolated perfused rat liver ex vivo (Tam et al, 1987) but *appears not to have been studied in the pig.*

The effect of hypoxia on hepatic metabolism of lignocaine is unknown.

1.2.3 CONCEPT OF LIGNOCAINE EXTRACTION RATIO AND CLEARANCE

The hepatic clearance (CL) of a drug can be determined at steady state by a knowledge of the liver blood flow (Q) and its hepatic extraction ratio (E) which in turn can be calculated from a knowledge of the affluent (Cin) and effluent (Cout) blood concentrations using the following formula (Wilkinson and Shand, 1975)

$$CL = Q \times \frac{(Cin - Cout)}{Cin} = QE$$

The hepatic elimination of a drug depends on hepatic blood flow, intrinsic clearance (CLintrinsic) and the fraction of drug unbound in blood (FF) as well as the permeability of the hepatocytes to the drug (Pang and Rowland, 1977a). The intrinsic clearance reflects the inherent ability of the organ to remove drug in the absence of flow limitations (Wilkinson and Shand, 1975). In perfused liver preparations or in vivo systems, the calculation of intrinsic clearance depends on the model of hepatic clearance chosen (Smallwood et al, 1990). This is because, to define the precise mathematical relationship amongst the parameters of hepatic extraction, which remain controversial, (Roberts and Rowland, 1986), it is necessary to know the concentration of drug in the liver water. As this cannot be measured directly various mathematical models to approximate this have been proposed (Smallwood et al, 1990). It appears that certain of these models are more appropriate to different drugs (Smallwood et al, 1990). The hepatic elimination of lignocaine can probably best be described using the "well stirred" model (Pang and Rowland, 1977b)(Ahmad et al, 1983) also known as the venous equilibrium model (Rowland et al, 1973). In terms of this model, hepatic elimination is related to the concentration of drug in the hepatic venous effluent (which is in equilibrium with that throughout the liver) and hepatic intrinsic clearance can be calculated using the following formula: (Roberts and Schenker, 1983)(Mather et al, 1986)

$$CLintrinsic = \frac{QE}{1-E}$$

Returning to the factors that may influence the hepatic elimination of lignocaine it is clear that as its extraction ratio in man is 0.64 (Stenson et al, 1971) lignocaine is regarded as an intermediate to high extracted drug and thus its elimination will be both flow and intrinsic clearance dependent (Huet and Villeneuve, 1983). However, due to its high extraction ratio relative to the fraction of unbound drug [30% under normal circumstances [Tucker and Mather, (1975), Holley et al, 1984)] the elimination of lignocaine is non-restrictive (ie not restricted by protein binding)(Wood, 1986) and will be unaffected by changes in plasma protein binding (Wood, 1986)(Williams and Mamelok, 1980). Further, diffusion into the hepatocyte is unlikely to impede lignocaine elimination under normal circumstances as Chen et al, (1980) has shown that this is not saturable over a wide range of concentrations.

1.2.4 THE EFFECTS OF DISEASE ON HEPATIC LIGNOCAINE EXTRACTION RATIO AND CLEARANCE

Only those drugs whose elimination is affected by liver disease offer the potential for being used as quantitative tools to evaluate the extent of hepatic dysfunction (Branch, 1982). *It is one of the hypotheses of this thesis that lignocaine extraction ratio and clearance may serve as a useful indicator.* Therefore it is pertinent to review briefly the clinical studies relating to lignocaine in this regard.

The significant impairment of systemic lignocaine clearance in patients hospitalised for treatment of decompensated alcoholic cirrhosis (Thomson et al, 1971)(Thomson et al, 1973) referred to in the introduction was confirmed in a patient with chronic aggressive hepatitis in whom the rate of lignocaine (and antipyrine) elimination was only 8% of normal (Adjepon-Yamoah et al, 1974). In the study of Thomson et al, (1973) impaired elimination of lignocaine could however not be correlated with severity of liver disease as assessed by conventional liver function tests.

Huet and Villeneuve (1983) studied lignocaine and ICG hepatic clearance and found that in chronic liver disease these were not impaired whilst in cirrhosis there was a significant decrease in both lignocaine and ICG extraction ratio and intrinsic clearance.

Williams et al, (1976) found no significant difference in lignocaine systemic clearance during and after acute hepatitis. There was no correlation between the pharmacokinetic parameters determined for lignocaine and conventional indicators of hepatic function.

In a study of patients with chronic hepatitis B infections (with raised transaminases as the only abnormal laboratory test) both lignocaine and ICG systemic clearances were significantly higher when compared with those of volunteers and correlated well with each other (Huet and Lelorier, 1980).

Colli et al (1988) compared theophylline and lignocaine clearance in patients with cirrhosis and found that the clearance of both drugs was significantly impaired but that altered lignocaine disposition seemed to reflect better the severity of liver disease and its overall functional impairment.

Similarly when comparing the half lives of antipyrine, paracetamol and lignocaine elimination in patients with chronic liver disease in an effort to relate them to biochemical indices of hepatic function, Forrest et al, (1977) found that "the half-life of lignocaine was always the most prolonged and was a highly sensitive indicator of hepatic dysfunction." Furthermore, the half lives of all three drugs were significantly correlated with the serum albumin level and the corrected prothrombin time ratio but not with plasma alanine aminotransferase, alkaline phosphatase or bilirubin concentrations.

It is clear that liver disease may impair elimination of lignocaine. The mechanism for impaired drug metabolism is still debated (Wilkinson and Shenker, 1976)(Colli et al, 1988). In chronic liver disease this may be due to a decreased intrinsic metabolic capacity as well as impairment of liver blood flow with portal systemic shunting (Pessayre et al, 1978)(Huet and Villeneuve, 1983). The "intact hepatocyte" hypothesis (Wood et al, 1979)(Branch, 1982) has been proposed which suggests that the impaired drug elimination may be due to the combination of functional intra hepatic shunts and a reduced number of functioning hepatocytes. This hypothesis gains support from the in vitro finding that in a wide variety of liver diseases there was a loss of hepatocytes but that in those remaining, the individual hepatocytes' capacity to metabolise lignocaine was well preserved (Meyer et al, 1991). In contrast the "sick hepatocyte hypothesis" postulates that in diseased liver, there are complex biochemical changes within the hepatocyte (Bircher, 1983). Despite the substantial volume of literature devoted to the effect of liver disease on drug metabolism *the role of hypoxic liver injury on hepatic drug metabolism in intact livers has received very little attention* (Jones et al, 1984). This inspite of the fact that ischaemic and hypoxic stress to the liver is of major clinical importance (Marotto et al, 1988) and may play an important role in alcoholic liver injury (Bradford et al, 1986), one of the most important aetiological factors in cirrhosis (Lieber and Salaspuro, 1985).

1.2.5 THE EFFECT OF LIGNOCAINE ON HEPATIC FUNCTION AND BLOOD FLOW

Lignocaine administered as a constant infusion to a whole blood concentration of 2.0ug ml^{-1} in man has been shown to increase hepatic blood flow (Wiklund, 1977). Although Tarba and Cracium (1990) have recently reported an increase in hepatic mitochondrial basal respiratory rate as well as ultramicroscopic hepatocelluar changes in association with the in vitro administration of lignocaine, *there appears not to have been an investigation into the effects of lignocaine on normal hepatic function and ultrastructure in vivo.*

1.3 THE PIG AND THE ISOLATED PERFUSED PIG LIVER PREPARATION AS EXPERIMENTAL MODELS

1.3.1 THE PIG

The pig has remarkable anatomical and physiological similarities to man (Bustad, 1967) with respect to the cardiovascular system (Rendas et al, 1978) and digestive systems as well as with respect to nutritional requirements and mineral metabolism (Dodds, 1982)(Lundeen et al, 1983). The pig and man have similar values for haematological and biochemical parameters (Hickman et al, 1970) and share some blood groups (Liem et al, 1964). Bustad, (1967), has said "in almost every way the pig offers a closer analogy to man than do those laboratory favourites, the rat and dog". Despite these similarities the porcine liver has been little used for drug metabolic studies which are usually performed using the (isolated) rat liver (Gores et al, 1986). This may be unfortunate as one of the major problems in medical research is the difficulty experienced in extrapolating observations in the experimental laboratory to man because the animals used so often differ in many respects from humans (Terblanche et al, 1970).

1.3.2 STUDIES PERFORMED IN THE PIG

The pig has been used extensively to study hepatic transplantation, (Terblanche et al, 1970)(Blankensteijn et al, 1990), hepatic physiology and function (Hardison et al, 1967)(Ham et al, 1969) and for research on human disease (Dodds, 1982). The pig liver has been used in the past for temporary extracorporeal assistance in patients with hepatic coma (Eiseman, 1965)(Ham et al, 1969), and more recently to study the effect of anaesthetic drugs on hepatic blood flow (Lundeen et al, 1983). In addition, the hepatic clearance of alcohol, (Elmslie et al, 1971)(Keiding et al, 1979) galactose (Keiding et al, 1982)(Welch and Parbhoo, 1973) bromosulpthalein (Abouna et al, 1969)(Drapanas et al, 1966) and indocyanine green (Winkler et al, 1970)(Tygstyrup et al, 1971) have been studied using the isolated perfused pig liver.
The limited use of this animal for the study of the hepatic metabolism of drugs is surprising. Perhaps its usefulness in this regard has yet to be realised.

1.3.3 ADVANTAGES AND DISADVANTAGES OF THE PIG AS AN EXPERIMENTAL MODEL

A number of theoretical advantages of the use of the pig in medical research have been highlighted above. The larger size (22-25 Kg) of the pig allows the use of both invasive monitoring and conventional "adult" anaesthetic systems but makes it somewhat more unwieldy than the rat.
The larger size of the liver (±700g) permits direct hepatic arterial and portal venous flow measurement, and makes the cannulation of these vessels for isolated liver perfusion more feasible. In the rat, the liver is

usually perfused solely through the portal vein (Tam et al, 1987)(Pang et al, 1986). Ahmad et al, (1984) showed that this may have significant effects on lignocaine availability. Further, in a pig liver of this size the taking of multiple biopsies to assess hepatic function does not appear to significantly affect functioning liver mass.

When compared with the rat, the pig is of course more expensive to obtain and maintain. A further problem is that of anaesthetic induced Malignant Hyperpyrexia (MH) which has an incidence of 3.4% in the Landrace x Large White pig (Harrison et al, 1969) although it appears that the primary defect of MH does not affect the liver adversely (Britt et al, 1978).

1.3.4 THE USE OF THE ISOLATED PERFUSED PIG LIVER AS A MODEL

The isolated perfused liver is likely to deteriorate with the passage of time (Gores et al, 1986) and does not necessarily reflect in vivo liver function (Tygstrup et al, 1971). *This needs to be assessed for the particular liver function in question.* For example, Winkler et al. (1970), have shown no difference in vivo and ex vivo for ICG elimination whilst Tygstrup et al, (1971) found differences in galactose elimination when similar models were used. As there is significant inter animal variation in liver function (Van Dyke et al, 1983) and drug metabolism (Williams et al, 1976)(Vesell, 1984) *this comparison may well be best performed using the same liver in vivo and ex vivo.*

The use of the isolated perfused liver has been suggested for the study of the effects of hypoxia (Jones, 1981) on hepatic metabolic function. This not only avoids metabolic interactions with the body (Tygstrup, 1975), but also circumvents the potential instability of an in vivo preparation subjected to hypoxia (Taskin et al, 1972)(Larsen et al, 1976). A further advantage of this system is that it allows precise control (and measurement) of hepatic blood flow and perfusate oxygenation, carbonation and temperature. The latter may significantly affect hepatic metabolism (Larsen, 1971).

B. AIMS AND SCOPE OF THESIS

1.1 AIMS AND SCOPE OF THIS THESIS

The metabolism of lignocaine to MEGX has been shown to be a useful indicator and predictor of hepatic function in association with liver transplantation. During this procedure the liver may be damaged by hypoxia which is known to damage the cytochrome P450 system, the principal enzyme system for lignocaine metabolism. It is possible that hypoxic damage to the liver is the common factor affecting both lignocaine metabolism and liver function after liver transplantation. This thesis investigates the effect of hypoxia on lignocaine metabolism and MEGX formation as well as the relationship of lignocaine elimination with other indices of hepatic function using the isolated perfused pig liver preparation.

The pig, although little used to date for the study of the metabolism of drugs, may, due to its anatomic and physiologic similarities with man, prove to be a useful model. The isolated preparation has the advantage that it allows specific study of the effects of hypoxia ex vivo with precise control of variables that could be deranged by hypoxia. However, as this preparation may not reflect various aspects of in vivo function, lignocaine elimination ex vivo may not be representative of the in vivo state. This could be assessed using the same liver to avoid interindividual variation in the capacity for drug metabolism.

An indicator used to assess hepatic function should be devoid of detrimental effects on hepatic function and blood flow. Lignocaine has been shown to enhance hepatic blood flow in man when infused to concentrations in the low antiarrhythmic therapeutic range, but the effects on hepatic blood flow and function at higher lignocaine concentrations have not been assessed.

1.1.2 OBJECTIVES OF THIS THESIS

The objectives of this thesis are to determine:

(1) a. What aspects of in vivo and ex vivo hepatic function measurements differ when the same liver is used for this comparison.

b. Whether the methodology employed to compare in vivo and ex vivo hepatic function can be used for the comparison of lignocaine metabolism.

(2) Whether lignocaine extraction ratio and clearance in the pig are similar to that reported for man.

(3) The effects of lignocaine at constant concentrations of ± 5ugml^{-1} on hepatic blood flow and function in vivo.

(4) Whether lignocaine extraction ratio and clearance are similar in vivo and ex vivo

(5) The effect of hypoxia on lignocaine extraction ratio and clearance and MEGX formation in the isolated perfused pig liver.

(6) Whether lignocaine extraction ratio and clearance correlate with other indices of hepatic function when the data from the studies in the normoxic and hypoxic livers are combined.

1.2 EXPERIMENTAL STUDIES PERFORMED.

Before studying in vivo and ex vivo hepatic lignocaine extraction ratio and clearance for the objectives stated, it appeared necessary to establish the stability of the two preparations and as it had been postulated that the in vivo and ex vivo comparison could best be made using the same liver, whether this was feasible.

1.2.1 Comparative Study of In Vivo and Ex Vivo Hepatic Physiology and Function

In vivo hepatic function and blood flow were studied in anaesthetized pigs by means of transhepatic sampling and direct blood flow measurement. The liver was then resected and studied according to the same protocol immediately afterwards as an isolated perfused preparation. A further group of livers were studied in the isolated state only for comparison with the last mentioned group.

1.2.2 Determination of the Pharmacokinetic Parameters for the Achievement of Constant Drug Concentrations In Vivo and Ex Vivo To compare in vivo and ex vivo lignocaine extraction ratio and clearance at the same target constant concentration in the upper therapeutic range, the decay of lignocaine after a bolus dose in both in vivo and ex vivo preparations was studied to determine the pharmacokinetic parameters to achieve this goal. The decay data was analysed using an iterative non-linear regression computor programme (PCNONLIN).

1.2.3 The Hepatic Extraction and Clearance of Lignocaine In Vivo and the Effect of Lignocaine on In Vivo Hepatic Blood Flow and Function

Two groups of anaesthetized pigs were studied, (a) for the determination of hepatic extraction ratio and clearance after lignocaine administration and (b) a group receiving saline in place of lignocaine for comparison with (a) to determine the effects of lignocaine on hepatic blood flow and function.

1.2.4 Hepatic Extraction and Clearance of Lignocaine in the Normoxic and Hypoxic Isolated Perfused Pig Liver

Using the previously determined blood flows and temperature, two groups of livers were perfused either under normoxic or hypoxic conditions and lignocaine hepatic extraction ratio and clearance studied at similar constant hepatic affluent lignocaine concentrations.

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CHAPTER 2: EXPERIMENTAL MATERIALS AND METHODS

SUMMARY

This section gives a broad outline of the materials and methods used in both the in vivo and ex vivo experiments. A detailed description is given only where this is common to all the experiments performed. In the interest of brevity and clarity detailed methodology for particular experiments are only described in the appropriate sections.

2.1 ANIMALS

Castrated Landrace x Large White pigs were obtained at the age of ten to twelve weeks from a single source farm. To facilitate transportation they were sedated with an intramuscular injection, 1ml/ 20kg, of a butyrophenone (Stresnil, 40mg in 1ml, Janssen Pharmaceutica). No other medications were administered. The animals were held at the University of Cape Town Animal Unit for a minimum of seven days prior to experimentation. Here they were kept indoors in an enclosure with underfloor heating. They were fed a standard commercial ration (Pig Growers Pellets, HiGro 16 Meadow Feeds; see appendix F.1.2 for constituents). In an attempt to standardize the size of the livers studied, only animals of between 21-25kg in weight were used. The animals were weighed just prior to the study using a Weaner pig weighing scale (Feedquip Engineering, Klapmuts.) The pigs were starved for 24 hours before surgery but were allowed free access to water. This minimised stomach contents allowing easier surgical access to the porta hepatis and reduced the chances of aspiration during induction of anaesthesia.

2.2 ANAESTHESIA

2.2.1 INDUCTION

The animals were manually restrained and anaesthesia was induced by the administration of 500mg-750mg of thiopentone (Intraval Sodium, May Baker) through a cannulated vein in the ear until there was loss of the eyelash reflex. The pigs were then intubated using a size 7mm (internal diameter) uncut Portex Blue Line (Kent, England) endotracheal tube and respiration was assisted with an ambubag for transportation from the animal unit to the JS Marais Surgical Laboratory of the University. Here the animals' lungs were mechanically ventilated, in initial experiments using a Blease Pulmoflator, Deansway model (England) and

subsequently an Ohio Anaesthesia Ventilator (Wisconsin, USA). A wide bore Ryles' tube was passed to drain any residual stomach contents.

2.2.2 MECHANICAL VENTILATION

In an attempt to standardize mechanical ventilation of the lungs within and between experiments all animals were ventilated at standard settings of inspiratory flow rate, tidal volume and frequency. In the experiments where the Blease Pulmoflator was used, (Chapter 4 and 5) the tidal volume was set at 260ml monitored with a Wrights Respirometer (British Oxygen Company), and the respiratory frequency at 18 breaths per minute (bpm), timed by a stopwatch. All other ventilator settings were kept constant. The $PaCO_2$ was maintained between 38-42mmHg by adjusting respirator deadspace by means of interposing tubing of varying lengths between the Y-piece of the ventilator hose and the endotracheal tube.
The Ohio Anaesthesia ventilator was used in all subsequent experiments (Chapters 6-8). In these experiments the $PaCO_2$ was kept between 35-40mmHg again by adjusting dead space after the tidal volume had been set at 10ml kg^{-1} and the respiratory rate at 25 bpm. All other ventilator settings were standardized.

2.2.3 MAINTENANCE ANAESTHETIC

Isoflurane was chosen as a suitable maintenance anaesthetic (See section 2.6.2) in combination with nitrous oxide in oxygen. Isoflurane (Abbott Laboratories) was administered by means of a new, factory calibrated, Isotec 3 Vaporiser (Ohmeda) kindly donated by Abbotts (South Africa). The end tidal anaesthetic concentration was monitored using an Engstrom EMMA agent monitor (Chapters 6 and 7) which had been calibrated using a gravimetric method (Appendix A.2.1).

2.2.4 TEMPERATURE MONITORING AND MAINTENANCE

The rectal temperature was monitored using a calibrated (Appendix A.3.4) thermometer (Yellow Springs-telethermometer, USA) and was maintained by humidification and heating of inspired gases using a MR 600 Dual Servo Heated Respiratory Humidifier (Fisher and Paykel Ltd, Auckland). In addition the operating table was warmed using a conventional fan heater and if required a radiant heater was used. In all animals, feeding catheters (Fr8) were introduced into the internal jugular vein for intravenous access and the carotid artery for continuous monitoring of the mean arterial blood pressure using initially a bourdon gauge (calibrated against a standard sphygmomanometer) and later via a transducer using a Hellige Servomed SMK154-3 Monitor (Freiburg, Germany). The accuracy of this system was confirmed against a standard sphygmomanometer (Appendix A.3.3).

Continuous electrocardiography was used to monitor heart rate and rhythm (Electrodyne PMS-5D, Massachusetts, USA) in initial experiments and subsequently the above mentioned Hellige Servomed SMK154-3 Monitor was used for this purpose.

Regular arterial samples were drawn from the carotid cannula to monitor blood PO_2 and PCO_2 as well as acid base status. Blood pH was maintained above 7.43 by administration of 4.2% sodium bicarbonate when necessary.

2.3 SURGICAL PREPARATION

2.3.1 IN VIVO STUDY

2.3.1.A. Surgery

In addition to the cannulation of the carotid artery and internal jugular vein described above, a catheter was passed down the R internal jugular vein through the heart and into the inferior vena cava to cannulate the left hepatic vein for the study described in Chapter 5 and in subsequent experiments (Chapter 6 and 7) via the left internal jugular vein into the median hepatic vein. A long mid-line laparotomy incision was performed to allow the placement of the appropriate catheters to permit transhepatic sampling of blood for analysis and to allow placement of perivascular flow cuffs around the portal vein and hepatic artery.

Through a midline incision the bowel was retracted and placed in a plastic bag to limit evaporation and temperature loss. Cannulation of the appropriate hepatic vein was confirmed by direct palpation and a branch of the splenic vein adjacent to the pancreas was identified and back cannulated to place a catheter in the portal vein. The hepatic artery and portal veins were partially mobilised to allow placement of perivascular flow cuffs, taking care to maintain as far as possible the nervous plexus on the hepatic artery.

2.3.1.B. Blood Flow Measurement

In all experiments hepatic arterial blood flow was measured using an ultrasonic flowmeter (T201D Transonic Systems Inc) and a 2mm 2RS series cuff. This system was found to be accurate, (Appendix A.1.1.D) displayed arterial flow digitally, and had an auto zero facility allowing the setting to zero of the equipment without vessel occlusion. (Operators Manual Transonic^R T101D/T201D: Blood Flow Meters for Investigative Use). This fact was confirmed in experiments by manual compression of the relevant vessel.

Portal venous blood flow was measured initially using an SEM 275 electromagnetic flow meter (SE Laboratories, Feltham,England) This flow meter had an analogue scale. When calibrated (Appendix A.1.1.B.) the flow meter performed linearly over the range measured ($r = 0.998$). [Portal venous blood flow

was determined during the actual experiments by noting the percentage deflection on the analogue scale and converting this to absolute flow using the calibration line y= 9.58x + 11.1]. Zero deflection of the analogue scale was confirmed in vivo by manual compression of the portal vein at the start of each experiment. This equipment was used for the measurement of blood flow in the physiological study (chapter 5). However, for all subsequent studies (Chapter 6 and 7) a second larger (6mm) ultrasonic flow probe was acquired and used in the second channel of the T210D ultrasonic flow meter (Appendix A.1.1.C.). This system was found to have a variation of ± 5% over the measured range and performed linearly (r = 0.998).

2.3.2 EX VIVO STUDY

The surgical technique used to remove the liver for isolated perfusion is described. In consecutive experiments where the same liver was studied in vivo and then immediately afterwards in the isolated perfused state (ex vivo) (Chapter 5) the same surgical technique and preparation was employed at the end of the in vivo study period.

2.3.2.A. Liver Mobilisation

Through a midline incision the bowel was retracted and placed in a plastic bag. The falciform ligament was cut and dissection was continued around the left diaphragmatic border of the liver with incision into the left transverse ligament. Blunt and sharp dissection allowed identification of the hepatic artery and portal vein. In the pig the hepatic artery lies consistently to the left of the portal vein. Variable gastric branches of the hepatic artery and the pancreatico-duodenal branch of the coeliac trunk were identified and ligated. Periportal lymphatics were cut and left to drain as ligation or cautery of these resulted in oedema of the porta hepatis with the possibility of a rise in portal venous pressure during perfusion. The periportal tissues were then freed from the surrounding structures and the common bile duct was identified and cannulated for bile collection. The cystic duct was ligated to avoid further drainage of bile to the common bile duct from the gallbladder. The pancreatico-duodenal tributaries of the portal vein were ligated and at this time the hepatic artery could be identified anterolateral to the portal vein. The tissue adherent to these two vessels was resected to obtain a sufficient length for later cannulation. The infra-hepatic vena cava was identified and cleared of the surrounding loose membrane for subsequent cannulation. The liver was further mobilised by dissection of the posterior diaphragmatic attachments and ligation of the phrenic veins with ring resection of the pericaval diaphragmatic attachments. The liver was thus freed save for its vascular attachments namely the supra-hepatic and infra-hepatic inferior venae cava, the hepatic artery and the portal vein.

2.3.2.B. Liver Cannulation:

Before cannulation was begun a 'rest period' of at least 15 minutes was given to allow the liver to recover from the manipulations of surgery. The liver's return to a state of adequate perfusion was assessed on the criterion of homogenous colour of the liver surface. The pig was then heparinised with 2.5mgkg^{-1} porcine heparin sodium (Labethica). Cannulation was performed as quickly as possible in view of the potential detrimental effect of ischaemia and care was taken to exclude all air from the perfusion cannulae as this could have profound effects on hepatic artery and portal vein perfusion pressures. The hepatic artery was cannulated using an Fr9 feeding catheter. If the hepatic artery was too small to allow this an Fr8 catheter was used instead. All catheters were filled with saline to exclude air but saline flushing of the liver prior to perfusion was not carried out. The portal vein was cannulated using a length of silastic tubing with an internal diameter of 6.25mm (Propan Pharmaceuticals). The supra and infra-hepatic venae cavae were clamped and the liver removed with incision of these vessels allowing drainage of intra hepatic blood and injected saline. The liver was then placed in a basin and the supra and infrahepatic inferior venae cava were catheterised with a 'Y-tube' for drainage of hepatic venous effluent. (see Figure 2.1). The liver was immediately transferred to the perfusion chamber where perfusion was established within 5-10 minutes.

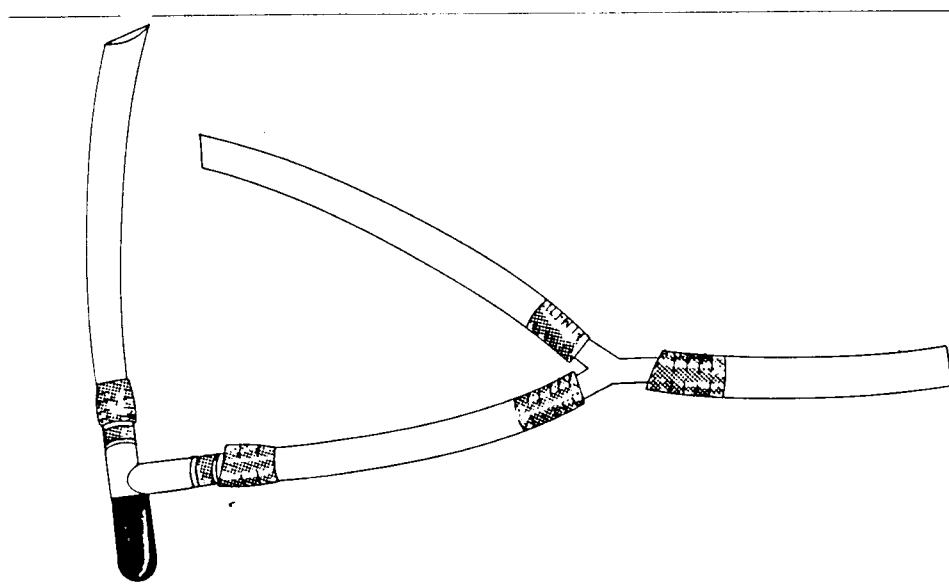

Figure 2.1 'Y'tube used for cannulating the supra hepatic (vertical tube) and infrahepatic (diagonal tube) vena cava.

2.4 THE PERFUSION CIRCUIT:

2.4.1 DESIGN:

The circuit components can best be described with reference to the accompanying diagrams (Figure 2.2 (a)&(b)).

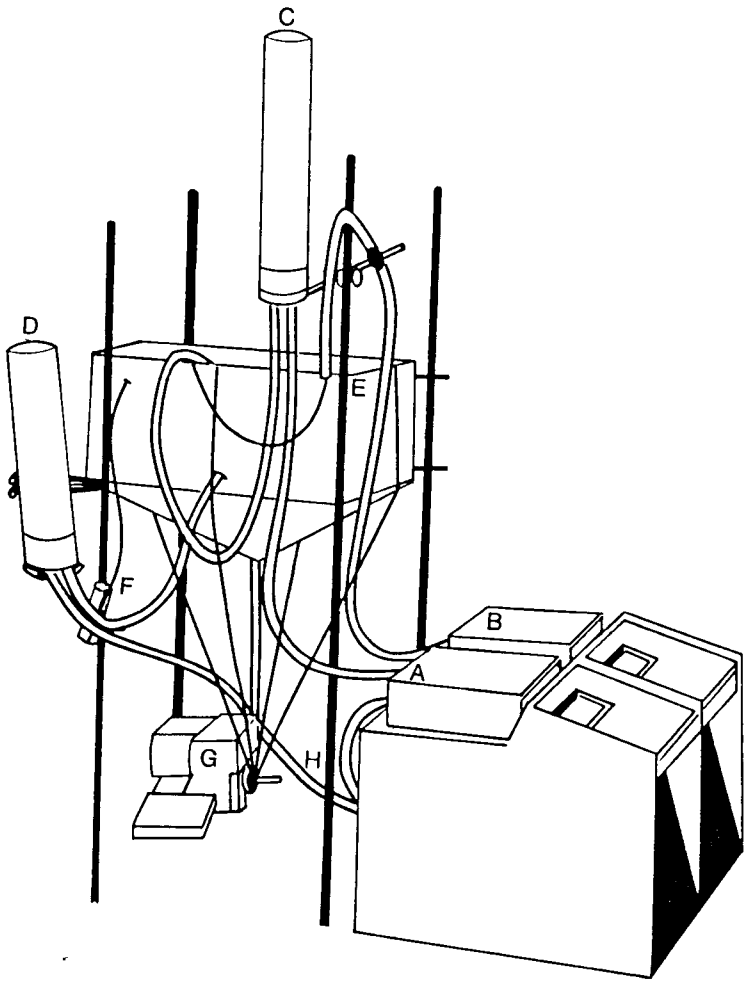

Figure 2.2(a) Isolated liver perfusion system.

A = Roller pump supplying portal vein reservoir
B = Roller pump supplying HA cannula
C = Portal venous reservoir allowing variable adjustment of height above the liver
D = Hepatic venous reservoir
E = Perfusion chamber
F = Collecting syringe for bile
G = Electric motor with eccentric fly wheel to pull gauze diaphragm in chamber by means of 4 wires
H = Tubing returning hepatic venous effluent from (D) and ascitic fluid from (E) back to the oxygenator

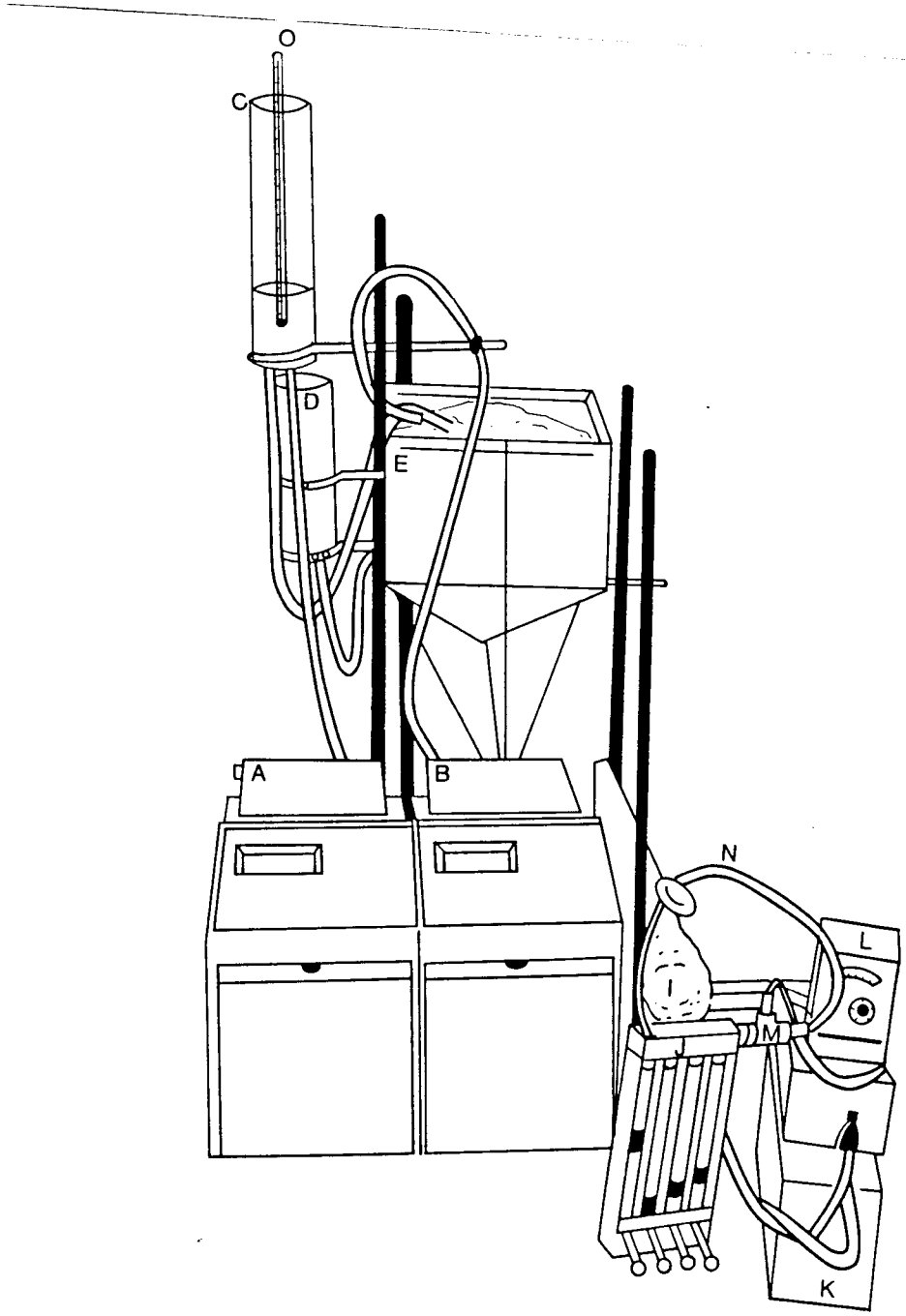

Figure 2.2(b) Isolated liver perfusion system
A-H See Figure 2.2(a)
I = Oxygenator (Rygg Kyvsgaard)
J = Rotameter block supplying nitrogen, oxygen and carbon dioxide to the oxygenator
K = Thermostat controlled water pump to warm perfusate via heating coils of oxygenator
L = Polarographic oxygen meter.
M = Polarographic oxygen probe between rotameter probe and oxygenator inflow.
N = Oxygenator inflow O = Mercury in glass thermometer.

The circuit was designed to allow normothermic perfusion of an isolated liver with warmed, oxygenated and carbonated perfusate comprising fresh porcine blood and a physiological electrolyte solution. Sampling points in the circuit allowed measurement of blood constituents and drug concentrations.

2.4.2 CIRCUIT COMPONENTS:

2.4.2.A. The Oxygenator:

For these experiments Rygg Kyvsgaard 'bubble type' oxygenators (Venotherm Adult/Paediatric Oxygenator: Polystan-Copenhagen) were used (See accompanying diagram Figure 2.3). This allowed a low priming volume (2 liters) and ensured adequate oxygenation.

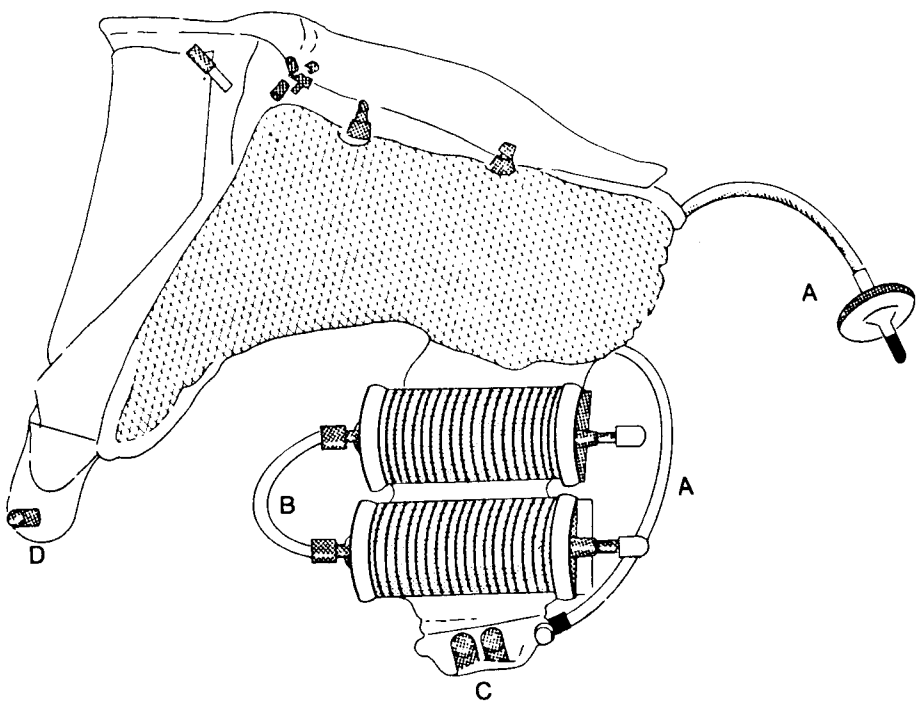

Figure 2.3 Rygg Kyvsgaard oxygenator.

A = Gas inflow tubing
B = Two heating coils
C = Oxygenator 'venous' inlet
D = Oxygenator 'arterial' outlet

Control of perfusate temperature was possible as the oxygenator incorporated a heating coil [Figure 2.3-(B)] which was supplied with warm water using a recirculating thermostat-controlled water pump [Figure 2.2 (b)-(K)] (Heto, Denmark). Deoxygenated blood entered the oxygenator [Figure 2.3-(C)] and was then

oxygenated and carbonated. These gases were supplied through tubing (B). Oxygenated and carbonated warm blood left the oxygenator through (D).

2.4.2.B. The Rotameter Block:

The rotameter block Figure 2.2(b)-(J) allowed control of the inflow of air or nitrogen, oxygen and carbon dioxide which were 'bubbled' through the oxygenator by passing it via tubing (N) and so in turn controlled the perfusate partial pressure of oxygen and carbon dioxide. In initial experiments (Chapter 4 and 5) gas flow rates were set at 600mlmin^{-1} for air and oxygen and 60mlmin^{-1} for carbon dioxide. This was adjusted according to subsequent bloodgas analysis of the perfusate sampled from the HA cannulae to maintain a PaO_2 of 150-200mmHg, and a $PaCO_2$ of 38-42mmHg whilst the pH was maintained above 7.43 by the administration of appropriate quantities of sodium bicarbonate. In later experiments (Chapter 8 and 9) different flow rates were used (Section 8.2.2.B.) and the administered percentage of oxygen was monitored at the rotameter outlet by passing this through a polarographic oxygen probe (M) connected to an Ohio Oxygen Monitor 201 (USA) designated (L) in the diagram.

2.4.2.C. Two Roller Pumps:

The roller pumps (Sarns, USA) Figure 2.2(a&b)-(A&B) were used to emulate the in vivo physiology as closely as possible, administering flow (pulse pressure ± 8mmHg^{-1}) to the hepatic artery through silastic tubing (2.5mm internal diameter) whilst a second pump (A) supplied a reservoir (C) allowing gravity drainage of perfusate to the portal vein by means of 6.25 mm internal diameter silastic tubing. In later experiments (Chapters 8 and 9) the hepatic arterial flow to the livers was increased and so 6,25 mm tubing was used in both pumps as it was impossible to supply the flow required through the smaller diameter tubing. The rollers of each pump were set at the recommended occlusion such that a column of fluid 90cm high would fall at less than 2.5cm in 1 minute. Individual stroke volumes were calculated for each pump (Appendix A.1.2). The desired hepatic artery and portal vein perfusate flow per minute could thus be set by calculating the appropriate number of revolutions required for the particular pump and setting the pump speed accordingly. During the course of a perfusion the pump speed was checked every 15 minutes and adjusted to maintain a constant speed (Appendix A.1.2.D.). At the end of each perfusion, the initial set pump speed was again confirmed and the actual flow delivered to the liver determined in triplicate at this setting by collecting the flow during one minute at the final setting for each pump. The mean values so determined were used in all further calculations relevant to this experiment.

2.4.2.D. The Liver Chamber:

The liver chamber (Figure 2.2 (a&b)-(E) which was 25cm by 37.5cm and 25cm deep was fixed to the perfusion trolley. It had a sloping base with a single exit tube which drained back into the circuit by a

1 This pulse pressure was as a result of the action of the roller pumps on the silastic tubing and could be monitored by the deflection on a bourdon gauge connected in line.

length of silastic tubing. This allowed return to the perfusate of spilt blood or ascitic drainage from the liver. The chamber was closed by a transparent perspex cover (not shown in the diagram) allowing easy inspection of the liver and the maintenance of moisture and temperature. A number of holes through the chamber permitted the passage of perfusion tubing. The liver was supported within the chamber on a diaphragm of fibre-glass gauze which was attached within the chamber by elastic hooks from each corner. In order to simulate the movement of the pig diaphragm in vivo, the chamber diaphragm was moved rhythmically by connecting its four sides to a motor with an eccentric fly wheel (G) allowing an excursion of 5cm at 20 cycles per minute. This may have a beneficial effect on the microcirculation of the diaphragmatic surface and peripheral perfusion of the livers' edge.

2.4.2.E. The Tubing:

Standard silastic tubing 0.625cm (i.d.) was used to make up the circuit except for a section of 50 cm in length feeding through the arterial pump which was 0.25 cm (i.d.) (but see 2.4.2.C. above). This smaller size was used in the hepatic arterial pump to ensure that similar pump speeds could be used despite the lower flow volume necessary for hepatic arterial perfusion. Tubing was joined by means of portex connectors while 3 way taps were connected to in-line T connectors to allow sampling from the HA, PV and HV catheters.

2.4.2.F. The Full Circuit:

This is best understood by reference to the schematic diagram Figure. 2.4. In summary: warmed oxygenated and carbonated perfusate passed from the oxygenator through a single piece of silastic tubing which divided in two, one division carried perfusate through the hepatic artery pump directly to the hepatic artery, the second division conveyed perfusate to the portal venous pump and so to the portal venous reservoir, from where the perfusate drained by gravity to the portal vein. The height of the portal venous reservoir could be adjusted [Figure 2.2a-(C)] so increasing or decreasing the portal venous pressure whilst the volume in the reservoir was kept constant at all times (arbitrarily chosen at 5cm of perfusate)[2]. This reservoir contained a calibrated mercury in glass thermometer [Figure 2.2b-(O)] (Appendix A.3.4) and the perfusate temperature was adjusted to 37-38 degrees Celsius as appropriate by adjustment of the temperature of the circulating water in the water pump. Blood drained from the liver through the 'Y-tube' (Figure 2.1) into the hepatic venous reservoir which was fixed at a standard level below the liver to avoid hepatic congestion. Perfusate drained from this reservoir by means of silastic (i.d. = 6.25 mm) tubing to the oxygenator to be rewarmed and reoxygenated. Any ascitic fluid or blood that had spilt into the liver chamber ran into this line (Figure 2.2a-(H)) and was also returned to the oxygenator, thus completing the circuit.

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2 Variations in this level resulted in variable discrepancies in portal vein and hepatic artery lignocaine concentrations in preliminary experiments.

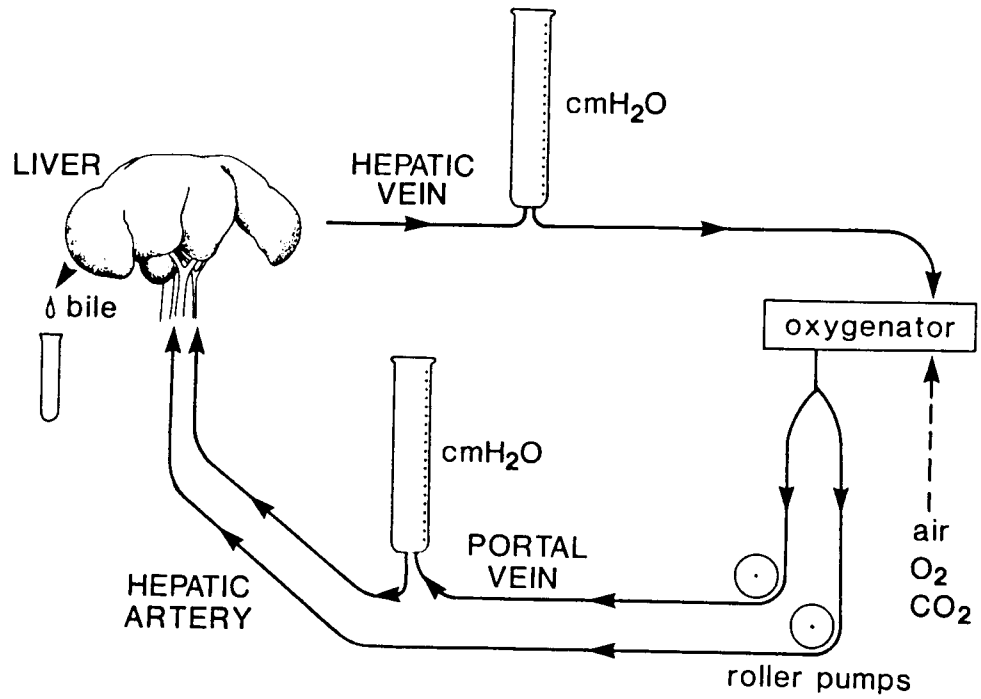

Figure 2.4 Schematic diagram of isolated pig liver perfusion.

2.4.3 PREPARATION OF THE CIRCUIT:

2.4.3.A. The circuit:

Prior to every perfusion the circuit was reassembled in a standard fashion using the same tubing, which had been thoroughly cleaned and deproteinated using Superdecontamine (Interscliences S.A. Brussels) before being left to dry in a drying chamber. New three-way taps for sampling of perfusate constituents were incorporated using T connectors placed in-line in the tubing supplying the hepatic artery cannula and portal vein cannula and in the tubing draining the liver to the hepatic venous reservoir. A new oxygenator was placed in the circuit and the rotameter flow rates were appropriately set.

2.4.3.B. The Perfusate:

The quantity of perfusate was calculated to allow for the withdrawal of approximately 125ml of perfusate for biochemical and lignocaine analysis whilst not overfilling the circuit ab initio. The perfusate was composed of 1600ml of fresh porcine heparinised ($5000uL^{-1}$) abatoir blood plus 600ml of Plasmalyte B (a physiological solution; see appendix F.1.1 for composition) as well as 10ml of 4.2% sodium bicarbonate. The blood was collected from the local abattoir on the morning of perfusion from at least five donor pigs.

No attempt was made to attain sterility as this was impossible. The blood was strained through gauze and the volume measured in an Erlenmeyer flask after which it was decanted via the hepatic venous reservoir into the perfusion circuit. In anticipation of insertion of the liver in the circuit the perfusate was slowly circulated, oxygenated and carbonated and warmed to a temperature of 37-38 degrees Celsius. The acid base status was assessed and pH corrected to > 7.43 with sodium bicarbonate 4.2% (0.5 $mEqml^{-1}$) prior to insertion of the liver.

2.5 CONDUCT OF A STANDARD PERFUSION EXPERIMENT

2.5.1 STABILIZATION PERIOD

A period of approximately half an hour was required to ensure a return of the perfusate temperature to 37 or 38 degrees and to establish optimal flow at the arbitrarily defined pressure levels. Perfusate flows were slowly increased while maintaining arbitrary pressure levels of < 25 cm of H_20 in the portal venous reservoir measured as the height in cm of blood above the liver surface[3] and 100-170mmHg in the hepatic arterial line. The flows were increased to a setting of 41 revolutions per minute (rpm) of the HA pump and 34 rpm in the PV pump in initial experiments but in later experiments were adjusted to deliver a standard flow per measured gram of liver. The former initial flow settings were established in preliminary studies as being adequate to assure a homogenously perfused liver and were the average flows achieved when not exceeding the arbitrary chosen pressure limits with the perfusate composition described. Once the perfusate temperature had returned to the designated temperature, as determined by continuous measurement in the portal vein reservoir, it was maintained at this level by adjustment of the water pump rheostat as necessary. Once this temperature was reached the acid base status of the perfusate was checked and adjusted as described above (Section 2.4.2.B.).

2.5.2 EXPERIMENTAL PERIOD

The experimental period for all studies was two hours.

2.5.2.A. Physical Parameters Measured

(1) Pressure:

Hepatic artery pressure was measured continuously using a bourdon gauge (previously calibrated against a standard sphygmomanometer) connected into the hepatic artery line via a second 3 way tap in addition to the one used for sampling.

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3 The specific gravity of the standard perfusate was determined to be 1.063 gm/ml (appendix B.10)

Portal vein pressure was measured continuously as the height in centimeters of the portal venous perfusion level above the liver using a standard ruler attached to the portal venous reservoir. As the liver was moving up and down on the cycling diaphragm the highest point in the cycle was taken as reference zero point for measurement.

(2) Flow:

Hepatic arterial and portal venous flow were determined as described in section 2.4.2.C. above. Hepatic venous blood flow was computed as the sum of portal venous and hepatic arterial flow.

(3) Temperature:

Temperature was measured continuously using a calibrated mercury in glass thermometer placed in the portal venous reservoir.

(4) Bile Volume:

Bile flow was collected in a graduated container. To improve the accuracy of volume measurement the bile was aspirated half hourly into a 2ml or 1ml (tuberculin) syringe.

(5) Liver Weight:

At the end of a standard perfusion the gall bladder was dissected and removed to assess more accurately liver mass. The cannulae were disconnected from the liver which was then left to drain on the gauze diaphragm of the perfusion chamber for a standard time of 15 minutes before weighing on a scale (Berkel Africa Pty, Ltd.) (See appendix A.3.5 for calibration).

2.5.2.B. Biochemical Analysis:

The biochemical analyses described here were those that were routinely performed in each perfusion to determine perfusate composition and to assess liver function. (See appendix B for the methods used to perform the specific tests mentioned here.)

All samples were taken from the in line three-way taps described above after the initial tap deadspace had been drawn up in a second syringe to ensure that a mainstream sample was obtained.

Samples were drawn at 0, 1 and 2 hours.

Perfusate samples were drawn from the hepatic artery port to determine:

(1) Blood gases (PaO_2 and $PaCO_2$) as well as acid base status (pH), total bicarbonate and base excess.

(2) Sodium and Potassium, **(3) Total Protein,** **(4) Albumin,**

(5) Osmolality, **(6) Lactate,** **(7) Glucose.**

(8) Aspartate aminotransaminase, **(9) Haemoglobin and Haematocrit.**

At the same time intervals transhepatic samples were drawn from the HA, PV and HV taps for determination of lactate concentration (in duplicate) and oxygen content in order to calculate hepatic lactate utilisation and oxygen consumption.

2.5.2.C. Biopsies

At the same time intervals that perfusate samples were taken as described above, biopsies (for adenine nucleotide status) were taken from the free edge of the liver at three standard sites of the left lobe (1)(2)(3) in all ex vivo studies (Figure 2.5). These were taken using Wollenberg type clamps precooled in liquid nitrogen. The biopsies were immediately stored in liquid nitrogen for subsequent batch analysis.

When biopsies for adenine nucleotide status were taken in vivo (Chapter 5) these were taken from standard sites on the free edge of the median lobe (A)(B)(C) (Figure 2.5) as in that study the left hepatic vein had been catheterised. In the studies described in Chapter 8 the single control in vivo biopsy was taken from site (x) of the left lobe.

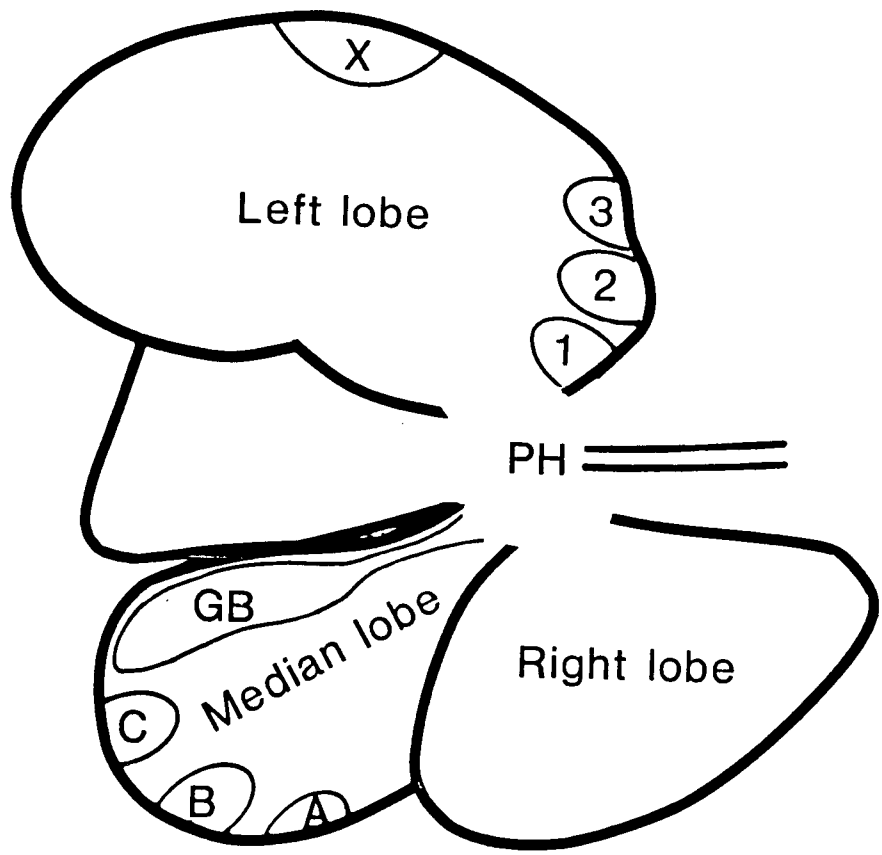

Figure 2.5 Schematic diagram of standard sites used for liver biopsies.

2.5.3 EXCLUSION CRITERIA

Experiments were discontinued and data not considered for analysis if there were technical problems such as air embolism or inability to achieve the required flows without a portal pressure greater than 25 cm H20, or if there was macroscopic evidence (blotching) of inadequate perfusion of greater than 5% of the liver surface at the end of the experimental period.

2.6 DISCUSSION OF METHODOLOGY

2.6.1 ANIMALS

As the success of liver perfusion is held to depend in large measure on the standard of preparation of the donor animal (Hickman, 1972), meticulous attention was given to this. In order to standardize the liver preparations as far as possible the same strain of pigs was used from a single source farm, whilst males only were used to avoid possible sex differences on drug metabolism (Gillette, 1971).

2.6.2 ANAESTHESIA

2.6.2.A. Maintenance Anaesthetic

As in vivo drug metabolism and pharmacokinetics were to be studied over periods from 2 and 5 hours respectively an anaesthetic technique was necessary which would meet the following criteria:

(1) A stable state of anaesthesia of adequate depth to minimise fluctuations in haemodynamic status and so hepatic blood flow in order to achieve a constant lignocaine concentration (Wilkinson, 1975)(Zierler, 1961) for the determination of lignocaine extraction and clearance.

(2) Minimal (or no) inhibition of hepatic drug metabolism and substrate synthesis. This inhibition has been found in rats with the use of halothane (White et al, 1976)(Franks et al, 1988).

(3) The anaesthetic agents used should be minimally metabolised to limit interference with the chromatographic analysis of plasma for lignocaine and its metabolites.

(4) The agents should not adversely affect hepatic blood flow and oxygen supply.

(5) Minimal protein binding of the administered anaesthetic would be a further theoretical advantage as displacement of a study drug from binding sites could enhance hepatic extraction.

In the case of lignocaine however, where the extraction ratio is greater than the unbound drug in the circulation (Tucker and Mather, 1975) its hepatic extraction would be insensitive to this variable (Wilkinson and Shand, 1975).

2.6.2.B. Anaesthetic Options

The options for general anaesthesia are to use an inhalational anaesthetic technique employing one of the currently available volatile agents (halothane, enflurane or isoflurane) with or without nitrous oxide or an intravenous technique.

An intravenous induction agent (thiopentone, etomidate, ketamine) or an opioid (morphine, pethidine, fentanyl) could be considered as an adjunct for maintenance of anaesthesia. However, most of these agents produce a significant number of metabolites, and are variably protein bound [Stanley, 1981 (a&b)] incurring problems as described above. Further, there is evidence that fentanyl increases hepatic oxygen consumption (Gelman et al, 1987a).

Thus it appeared that the use of an inhalational agent should be preferred.

2.6.2.C. Isoflurane

Isoflurane appears to have advantages over the other two available inhalational agents (halothane and enflurane) in that it is the least metabolised, less than 0,2% appearing as urinary metabolites, which is one tenth that for enflurane and one hundredth that for halothane (Eger, 1981). Furthermore, there is no evidence to date that isoflurane has the inhibitory effects on drug metabolism and substrate synthesis found with halothane. Indeed, whilst inhibition of ICG clearance was noted with halothane this was not the case with the use of isoflurane (Gelman et al, 1984).

Effects of inhalational agents on hepatic blood flow should also be considered. This however constitutes only one of the many factors which may influence hepatic blood flow during laparotomy and mechanical ventilation (Gelman, 1987b)(Gelman, 1989). Interpretation of the relative advantage of the one inhalational agent over the other in this regard is difficult. Gelman et al (1984) suggest that halothane has a more adverse effect on liver blood flow than isoflurane. Hughes et al, (1980) found that there was no significant difference in total hepatic blood flow with the use of equipotent concentrations of halothane and enflurane but that hepatic arterial blood flow was better maintained with enflurane. A review of two studies by Lundeen et al (1983) and Tranquilli et al (1982) did not indicate a clear difference between isoflurane and enflurane in this respect.

Isoflurane appears to maintain hepatic arterial flow at preanaesthetic (awake) levels in pigs at the concentrations administered in the present study (Lundeen et al, 1983). Further, this agent maintains hepatic oxygen supply better than halothane and enflurane (Nagano et al, 1990a) and has a better protective effect against hepatic ischaemia (Nagano et al, 1990b).

Thus, isoflurane appeared to be the preferred agent. It was decided to administer 1.5% isoflurane with 30% oxygen in nitrous oxide as this would provide adequate anaesthesia for laparotomy in pigs (Lundeen et al,1983).

2.6.3 SURGICAL PREPARATION

Since catheterisation of the hepatic artery could impair hepatic blood flow and because the composition of arterial blood in major vessels is similar (Rowland, 1972a), the carotid artery was used as an hepatic artery equivalent for sampling purposes[4].

The composition of blood sampled from the hepatic vein by means of hepatic venous catheterisation was regarded as representative of hepatic effluent. This assumption and technique has been adopted by numerous authors, including Stenson et al, (1971), Difazio and Brown, (1972), Wiklund et al, (1975), Hoorn-Hickman et al, (1979), and Mather et al, (1986). However Bradley et al, (1945), Sapirstein and Reiniger (1956) and Goldstein et al (1971) have warned that hepatic venous sampling may not adequately reflect global hepatic venous effluent. Goldstein et al, (1971) have described a technique of supra hepatic vena caval sampling one minute after infra hepatic occlusion, suggesting that in this way a "mixed hepatic venous blood sample" can be obtained. This method was not adopted as according to the authors it induced haemodynamic changes, which could affect lignocaine steady state concentrations. Further, the authors suggested that hepatic metabolism might temporarily have been altered as a result of this methodology.

2.6.4 MEASUREMENT OF HEPATIC BLOOD FLOW

There are a number of methods by which hepatic blood flow can be determined, which can roughly be divided into indirect and direct methods.

2.6.4.A. Indirect Methods

Hepatic blood flow in the intact animal can be measured indirectly using the method described by Bradley et al, (1945) and an indicator such as bromosulphthalein (BSP) or ICG (Winkler and Tygstrup, 1960). This method, involving hepatic venous catheterisation and determination of hepatic extraction of the indicator, has been criticised as being inaccurate for ICG (Skak and Keiding, 1987), whilst a method utilising only clearance without a measure of extraction is likely to be even less accurate (Groszman, 1983). These methods do not allow the differentiation of hepatic arterial (HAQ) and portal venous flow (PVQ). This problem has been addressed by a number of investigators using multiple catheters placed at laparotomy under anaesthesia. Huet et al, (1973) used Cr^{51} labelled red bloodcells as indicator whilst Katz and Bergman (1969) used para-aminohippuric acid as indicator. Runciman et al, (1984) used radioactive ^{125}I-iodohippurate as indicator but found that this technique resulted in 'streaming' and thus PVQ had to be

4 With respect to in vivo data sampling from this site will be designated HA denoting it as hepatic arterial equivalent.

estimated from the relative concentrations of dye at steady state in the hepatic arterial, portal vein and hepatic venous vessels.

Another indirect method of hepatic blood flow determination is by means of injected radioactive microspheres (Lundeen et al, 1983). This is complex and the number of measurements are limited to the ability of the gamma counter to separate out nuclides whilst hepatic blood flow cannot be determined instantaneously. It requires sacrificing of the animal, and is expensive (Runciman et al, 1984). In addition, using this method portal venous flow is not determined but assumed to equal splanchnic flow.

2.6.4.B. Direct Methods

Drapanas et al (1960) compared directly measured hepatic blood flow determined using an electromagnetic flow meter and perivascular flow probes, with the values of blood flow determined using BSP and the method of Bradley et al, (1945) in anaesthetized dogs. These investigators recognised the following advantages of using flow probes:

(1) Instantaneous hepatic blood flow determinations were possible and

(2) differential PVQ and HAQ measurements were available.

(3) Single measurements were reproducible and did not require serial determinations or the plotting of extraction curves.

To this could be added that the constancy or stability of blood flow could be ascertained (in real time) as this has been noted to vary widely (Zierler et al, 1961).

Perivascular flow probes can be used for hepatic blood flow determination at the time of laparotomy (Fujita et al, 1989) or after implantation at a later time in the awake animal (Fettman et al, 1984). The latter method may be less accurate as:

(1) undetected malpositioning of the probe may lead to erroneous hepatic blood flow readings.

(2) It may not be possible to confirm the zero reading of the system, as occlusion of the relevant vessels is not possible in the intact animal.

(3) The implantation of perivascular probes may alter regional blood flow by rendering pulsatile vessels rigid and by interfering with their autonomic nervous supply (Runciman et al, 1984).

2.6.4.C. Method Adopted

It would appear that the direct measurement of hepatic blood flow has a number of distinct advantages over indirect methods and thus this methodology was adopted in this thesis.

Although it may be considered preferable to study hepatic drug metabolism and function in the conscious animal in the absence of the potential derangement due to anaesthesia and surgery, this study was performed under anaesthesia as liver biopsies could then be taken, bile flow measured and the above considerations suggested that hepatic blood flow measurement was likely to be more accurate.

2.6.5 ISOLATED LIVER PERFUSION

2.6.5.A. Non-pulsatile Hepatic Arterial Flow

The pulse pressure in the HA of 8 mmHg (section 2.4.2.C.) may be regarded as non-pulsatile flow. This method of perfusion has been used in this (Hickman et al, 1971) and other laboratories (Jablonski et al, 1971)(Abouna et al, 1969). The question arises whether the absence of pulsatile hepatic arterial flow in the isolated liver may contribute to impaired hepatic perfusion, and so impaired hepatic function, and whether this contributes to the steady rise in hepatic arterial pressure that has been noted with time (Hickman et al, 1971). This rise in pressure (which may reflect an increase in vascular resistance) in both the HA and PV has been postulated to be due to; (a) the development of microscopic thrombi within the vasculature, (b) hypoxic damage resulting in hepatocyte swelling and blocking of sinusoids or (c) due to the compression of sinusoids as a consequence of the liver lying on a diaphragm (Winkler et al, 1971). This may have different functional effects dependent upon whether all vascular channels increase their resistance to the same extent, or alternatively, vessels in some areas only, close completely, resulting in an effective decrease of functioning tissue mass (Winkler et al, 1971). Thus it is not clear whether the increase in HA pressure that may occur during isolated liver perfusion can be attributed to the use of non-pulsatile flow. That pulsatile hepatic arterial flow may have beneficial effects was shown by Drapanas et al, (1966) who noted a marked improvement in liver appearance when pulsatile flow (40-60mmHg) was used.

2.6.5.B. Hepatic Arterial and Portal Vein Pressure Limits for Perfusion

In the isolated perfused liver preparation HA and PV pressure is dependent on the rate of perfusate flow, perfusate composition (haematocrit), the vascular resistance of the organ as well as the size of the cannulated vessel and the cannula size. The latter two points are particularly relevant to the hepatic artery. The hepatic artery was cannulated using an Fr9 feeding catheter. If the hepatic artery was too small to allow this an Fr8 feeding catheter was used instead. It had been established that when perfusate was pumped through the respective catheters at standard flow rates in the absence of a liver the flow pressure recorded was in the order of 50 mmHg higher when the Fr8 catheter was used compared with the Fr9 catheter. Thus the pressure measured was to a large extent dependant on the HA catheter size used. The arbitrary chosen pressure limit of 170 mmHg for the Fr8 (smaller) catheter was chosen as successful perfusions in preliminary experiments using standard flow rates had hepatic arterial pressures below this level. Welch and Parbhoo (1973) maintained hepatic arterial pressures below 200 mmHg. The arbitrary pressure limit for the PV pressure of 25 cm H_2O was arrived at in a similar fashion. Jablonski et al, (1971) used a pressure head of 22 cm H_2O. The portal vein pressure in the intact animals was found to be 15 $\pm$ 1.7 cm H_20 (n=5) in the in vivo experiments reported in Chapter 7.

Jablonski et al, (1971) and Tygstrup et al (1971) used the portal vein pressure as an acceptance criterion of adequate perfusion while other workers have assessed the gross appearance of the isolated liver (Vang et al, 1986)(Jablonski et al, 1971)(Drapanas et al, 1966). Both of these methods of assessing isolated liver perfusion were used in the present experiments (section 2.5.3).

2.6.5.C. Sites Chosen for taking of Liver Biopsies

The standard biopsy sites (Figure 2.5, Page 2.15) used for the determination of hepatocellular adenine nucleotide status and sited on the liver edge were chosen as these sites were shown macroscopically to be consistently well perfused in preliminary experiments. In addition, sampling from these peripheral sites allowed immediate closure of the wound using catgut to avoid bleeding from the liver. We did not establish whether there was a correlation between these peripheral sites and central sites. There is as yet no literature that discusses this issue but the use of biopsies from the right or left lobe has been the subject of a recent study (Hickman) which indicates that it is immaterial which lobe is used for this biopsy. In the studies reported by Kamiike et al, (1988) and Lanir et al (1988), liver biopsies for adenine nucleotide status were also taken from the periphery.

2.6.5.D. Absence of Isoflurane and Nitrous Oxide Ex Vivo.

The absence of these agents from the ex vivo liver perfusion may well have an influence on the comparison of in vivo and ex vivo liver function which is studied in Chapter 5 and Chapter 8. Hepatic blood flow and possibly substrate metabolism may be affected by these agents (section 2.6.2.C). Their affect on hepatic blood flow would be negated ex vivo, where this is controlled, unless these agents also have an influence on intrahepatic blood flow. It is not clear from the literature at present whether these agents have an effect on drug and substrate metabolism and this is certainly an area for further investigation. It should be added that, although it would have been possible to administer these agents via the oxygenator to the isolated liver it was elected not to do this as this would have complicated the subsequent administration of a hypoxic gas mixture in later experiments.

2.6.6 EXPERIMENTAL PERIOD

The experimental period of two hours was chosen both in vivo and ex vivo (total period of three hours including stabilisation and rest period) as the isolated perfused pig liver is regarded as remaining viable for this period by a number of workers as measured by oxygen consumption (Abouna et al, 1969)(Hickman et al, 1971) bile flow (Abouna et al, 1969) and hepatic ATP and energy charge as well as galactose elimination

capacity (Winkler et al, 1986) although ongoing damage is evident in terms of a rise in AST (Hickman et al, 1971).

CHAPTER 3: LIGNOCAINE AND METABOLITE ANALYSIS

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CHAPTER 3: LIGNOCAINE AND METABOLITE ANALYSIS

SUMMARY

The method of extraction of lignocaine and its major metabolites from plasma as well as their quantitation using a high performance liquid chromatography (HPLC) system is described. Two HPLC methods were developed both using EMGX as internal standard; Method 1: for quantitation of lignocaine only (coefficient of variation [cv] = 7.6%) which was used to assess the decay of lignocaine after a bolus dose in vivo and ex vivo, and Method 2: for quantitation of lignocaine (cv = 6.1-7.0%), MEGX (cv = 13.1-13.9%) and GX (cv=15.3-15.9%) used to study lignocaine metabolism in all subsequent steady state experiments. In addition, identification of five further metabolites of lignocaine namely, 3-hydroxy-lignocaine, 3-hydroxy-MEGX, lignocaine oxide, 2,6 xylidine, and 4-hydroxy-2,6-xylidine in the hepatic venous effluent from the pig liver was possible using this methodology. In order to determine whole blood lignocaine concentrations the plasma to red blood cell partition coefficient for lignocaine was determined for pigs blood and found to be 1.63 and independent of concentration over the range studied.

3.1 MATERIALS AND APPARATUS

3.1.1 REAGENTS

Lignocaine (2-diethylamino-2',6'-acetoxylidide) and the metabolites monoethylglycinexylidide (MEGX), 3-hydroxy-lignocaine, 3-hydroxy-MEGX, glycinexylidide (GX), lignocaine oxide, 2, 6 xylidine, and 4-hydroxy-2,6-xylidine as well as the internal standard ethylmethylglycine xylidide (EMGX) were kindly donated by Astra Pharmaceuticals, Soldertalje, Sweden.

Acetonitrile (Mallinkrodt, Kentucky, USA) was of HPLC grade whilst all other reagents and chemicals used were of reagent grade or better.

3.1.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Lignocaine and the metabolites MEGX and GX were determined by HPLC with ultraviolet detection. The method was a modification of that described by Luzzi et al (1984) and Wisnicki et al (1979). Separation was achieved on a 25cm x 4.6mm VYDAK reverse phase C18 column (particle size 5uM) with a C18 precolumn (particle size 30-40uM) (Uptight,Washington,USA). Detection was with a Spectra-Physics SP8450 uv/vis detector set to read at 205nm at a range of 0.02 AUFS (Absorbance Units Full Scale). Peak heights were measured using a Spectra Physics SP4290 integrator. The mobile phase was either 0.04M Na_2HPO_4 (pH 5.5) Acetonitrile 93:7 or 0.04M Na_2HPO_4 (pH 4.0) Acetonitrile 89:11. The flow rate was maintained at 2.0ml min^{-1} using a Spectra Physics SP8800 ternary pump attached to a Spectra-Physics SP8780 autosampler with a 100ul sample loop.

3.2 EXPERIMENTAL

3.2.1 STANDARD SOLUTIONS

Stock standard solutions Lignocaine (4 $mg.ml^{-1}$), MEGX (0.5$mg.ml^{-1}$), GX (0.5$mg.ml^{-1}$) and EMGX (0.16$mg.ml^{-1}$) were made up in 0.04M Na_2HPO_4 (pH 5.5) Acetonitrile 93:7. Aliquots (200uL) were stored at -20^{o}C for no longer than 3 months. Plasma standards were made up daily by using the stock standards to spike fresh pooled multidonor (n = 5) pig plasma in appropriate quantities to achieve Lignocaine, MEGX, GX and EMGX concentrations of 5ug ml^{-1}, 2.5ug ml^{-1}, and 0.5ug ml^{-1}, and 0.2ug ml^{-1}, respectively.

3.2.2 PREPARATION OF EXPERIMENTAL SAMPLES

Two milliliters of heparinized porcine blood or perfusate samples were placed in cuvettes (Rund Kuvetten, Germany) and occluded using corks[1] for transport to the analytical laboratory within an hour of the termination of the experiment. The samples were centrifuged at 3000 rpm for 6 minutes and then the plasma was decanted into 1.5 ml Eppendorf tubes (Hamburg, Germany) and refrigerated at -20^{o}C till the time of analysis, within 4 weeks of sampling.

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1 It has been reported that falsely lowered lignocaine plasma concentrations may occur when the blood sample was allowed to make contact with the stopper of the VacutainerR collection tube (Stargel et al 1979) hence corks were used to avoid this.

3.2.3 SAMPLE EXTRACTION

The sample for injection on to the chromatographic column was extracted using C18 Bondelut cartridges (Analytichem Harbor City, CA, U.S.A.). The sample was drawn through the cartridge by means of a vacuum system (Vacelut Harbor City, CA, U.S.A.).

1) The Bondelut cartridges were rinsed with 2 x 1ml MeOH followed by 2 x 1ml of H_2O and preconditioned using 1ml of 0.04 M Na_2HPO_4 buffer (pH 9.5) passed through the cartridge.

2) 500ul of the above mentioned buffer and 40ul of EMGX internal standard (in an appropriate concentration to achieve 0.2 ug ml^{-1} in the final solution) were added to 500ul plasma sample[2], vortexed for 15 seconds, transferred to the cartridge and left to stand for 5 minutes.

3) The sample was then drawn onto the column under vacuum, rinsed with 1 x 1ml buffer (pH 9.5) and eluted with 2 x 200ul Acetonitrile into a sample vial.

4) The Acetonitrile was evaporated off under nitrogen after which the sample was reconstituted in 500ul mobile phase.

5) 100ul in volume was injected on to the column for analysis.

3.2.4 LIGNOCAINE AND METABOLITE ANALYSIS

Two methods of analysis were developed.

Method (1) for the analysis of lignocaine only which was used to analyze samples for the study of lignocaine decay after bolus dose injection in vivo and in the isolated perfused pig liver.

Method (2) was used to determine the concentrations of lignocaine as well as MEGX and GX and in addition to identify the presence of the other metabolites of lignocaine named above (Section 3.1.1).

3.2.4.A. Chromatographic Conditions

Detection Wavelength	205nm
Chart Speed	0.5cm min^{-1}
Flow Rate	2.0ml min^{-1}
Temperature	ambient[3]

The mobile phase used for method (1) was 0.04M Na_2HPO_4 (pH 5.5): Acetonitrile 93:7. and in method (2) was 0.04M Na_2HPO_4 (pH 4.0): Acetonitrile 89:11.

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2 Note: In samples that were found or predicted to be outside the calibration range this volume was appropriately adjusted to achieve a concentration within this range. The concentration so determined was corrected by the appropriate dilutional factor.

3 The room where the analyses were performed was air-conditioned; approximate temperature 23-25 degrees Celsius.

3.2.4.B. Quantitation

Peak heights of the substance in question and the internal standard EMGX were electronically measured at the corresponding retention times allowing determination of the peak height ratio (PHR). The value of the concentration of the substance was then determined from the calibration curve of PHR determined for known standard concentrations of the substance against a standard concentration of EMGX. The limit of sensitivity was defined as the lowest concentration of a substance at which the peak height to noise ratio was > 3.

3.2.4.C. Extraction Efficiency

Extraction efficiency (percentage absolute recovery) of drug from pig plasma was determined for lignocaine, EMGX, MEGX and GX by spiking plasma and mobile phase with identical samples of each. The ratio of the peak heights upon HPLC analysis of the extracted plasma sample to the peak height of the identical sample in mobile phase was determined and computed as a percentage. A study at a given concentration (Table 3.1) was completed on the same day, thus assessing intra-assay variability.

3.2.4.D. Calibration Curves

Initial calibration curves were performed for both Methods (1) and (2) to determine:

1) Linearity of the system over the chosen concentration range.

2) The interassay precision (reproducibility).

3) The range of concentrations within which control samples injected before and after an analysis "run" needed to be for acceptance of the results.

3.3 RESULTS AND DISCUSSION

3.3.1 CHROMATOGRAMS

3.3.1.A. Method (1)

The chromatogram (Figure 3.1) shows clear separation with retention times of:

Lignocaine: 12.07 min EMGX: 8.71 min

3.3.1.B. Method (2)

There is again clear separation (Figure 3.2) of lignocaine and its metabolites with retention times of:

Lignocaine: 22.82 min MEGX: 8.45 min

GX 4.39 min EMGX 15.11 min

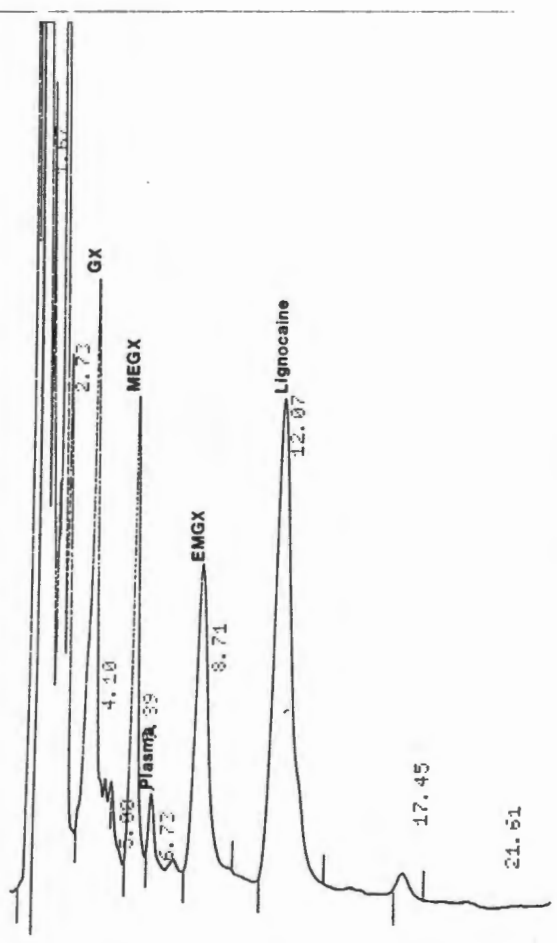

Figure 3.1 Chromatogram of lignocaine & metabolites (method 1)

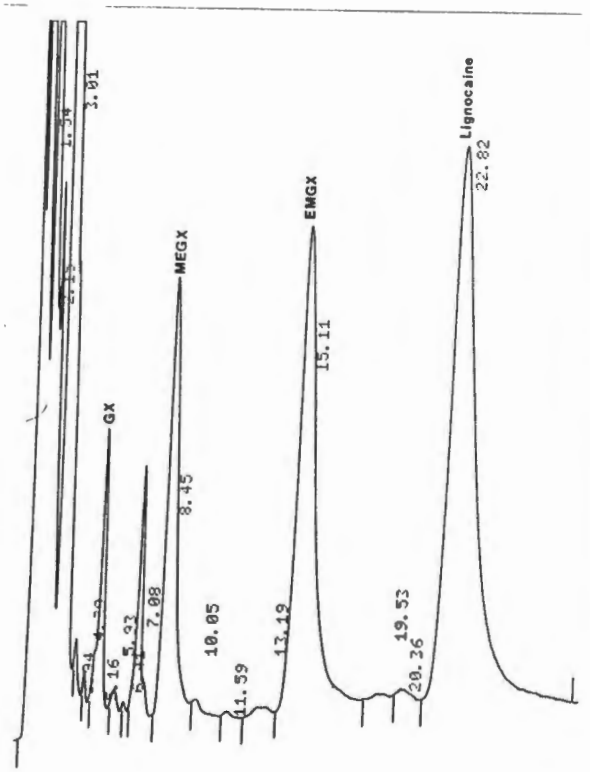

Figure 3.2 Chromatogram of lignocaine & metabolites (method 2)

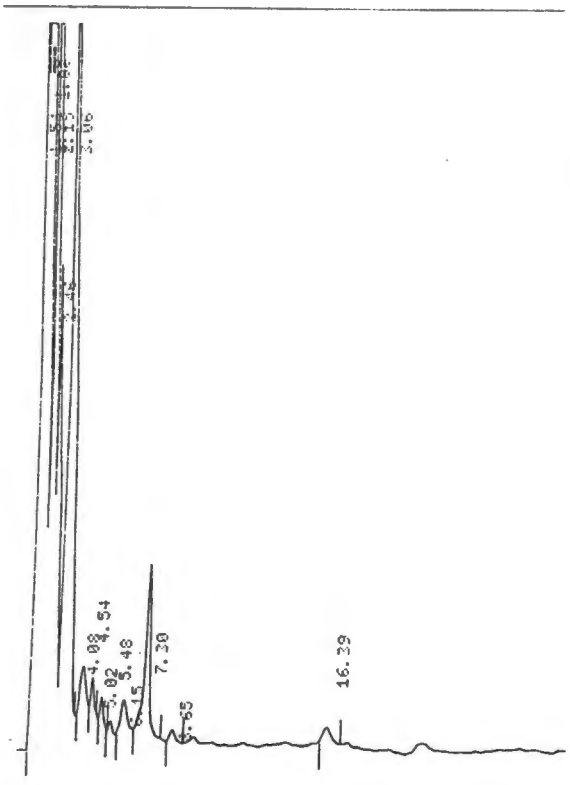

Figure 3.3 Chromatogram of multi donor pig plasma

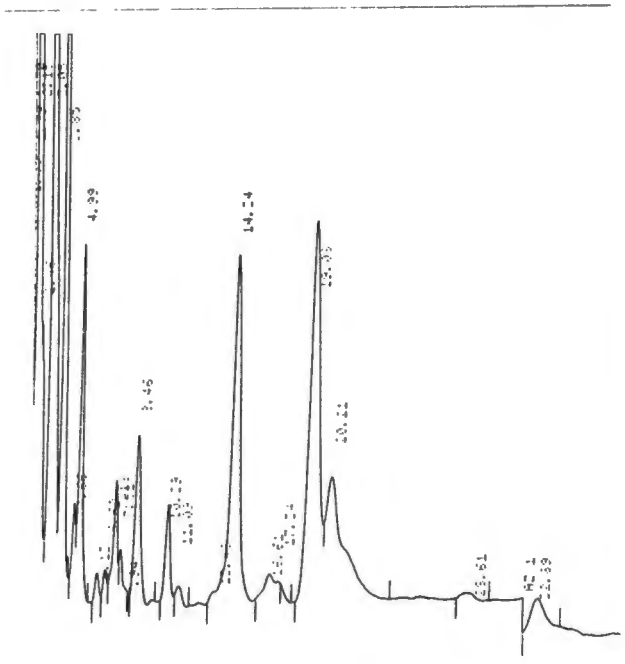

Figure 3.4 Chromatogram of multidonor pig plasma after hypoxia of the liver for a period of one-half hour

The chromatograms of multidonor pig plasma in the absence of lignocaine and its metabolites (Figure 3.3) shows that interference with the determination of lignocaine, EMGX, MEGX and GX is unlikely. However, in practise this was not always the case. In the ex vivo experiments variable new peaks arose upon analysis of perfusate that had been sampled from livers subjected to hypoxia (Fig 3.4). Further, the use of multidonor pigs blood did not allow standardization of the perfusate and so at times variable peaks on chromatographic analysis occurred due to this factor as well. Fortunately these apparently did not interfere with either lignocaine or EMGX analysis

3.3.1.B. Metabolites

Figure 3.5 shows a chromatogram of pigs plasma spiked with lignocaine, the internal standard EMGX and the metabolites (MEGX), 3-hydroxy-lignocaine, 3-hydroxy-MEGX, (GX), 2, 6 xylidine, and 4-hydroxy-2,6-xylidine showing that good separation was achieved. The intention with respect to this thesis was only to establish (but not to quantitate and so no standard curves were performed) whether the mentioned metabolites which have been described in man, the rat and the guinea pig (Keenaghan and Boyes, 1972) were also present in the hepatic effluent of the pig. The chromatogram (Figure 3.6) of a sample taken from

the hepatic venous effluent of an isolated perfused liver 10 minutes after a bolus dose of 40mg of lignocaine hydrochloride suggests that these metabolites were indeed present. Whilst 3-hydroxy-lignocaine appeared to be present, as a small peak was evident at the appropriate retention time (7.64 minutes), this was more clearly demonstrated on further analysis. The methodology used to achieve this separation was essentially as described in Method 2 above, however a different mobile phase was used namely: Acetonitrile 90:10 ph 4.65. A more elegant way of separating potential contaminant peaks from the metabolites to confirm their presence would have been to use a gas chromatograph with a mass spectrometer as detector. This equipment was however not available for the present analysis.

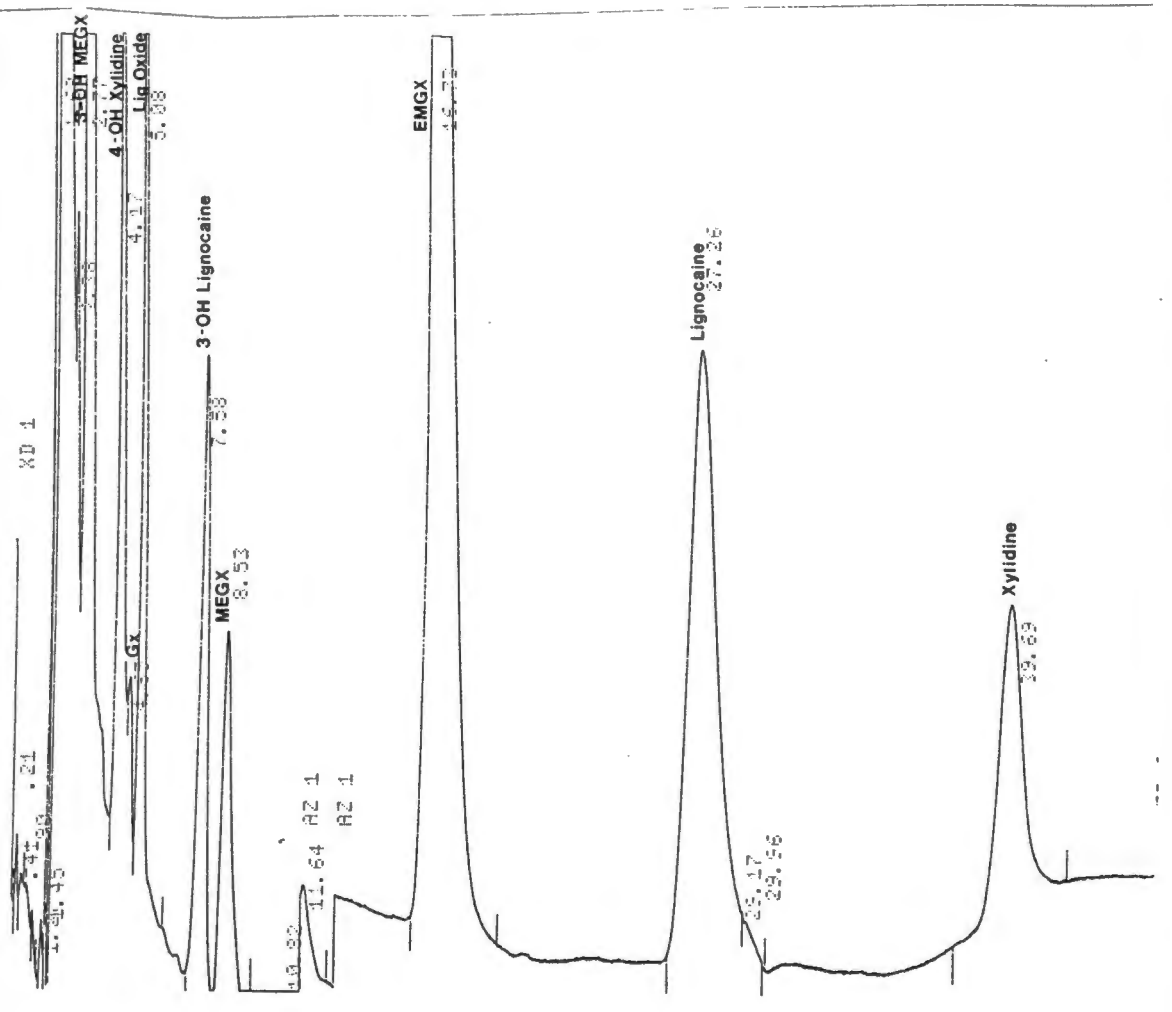

Figure 3.5 Chromatogram of pig plasma spiked with lignocaine and its' metabolites.

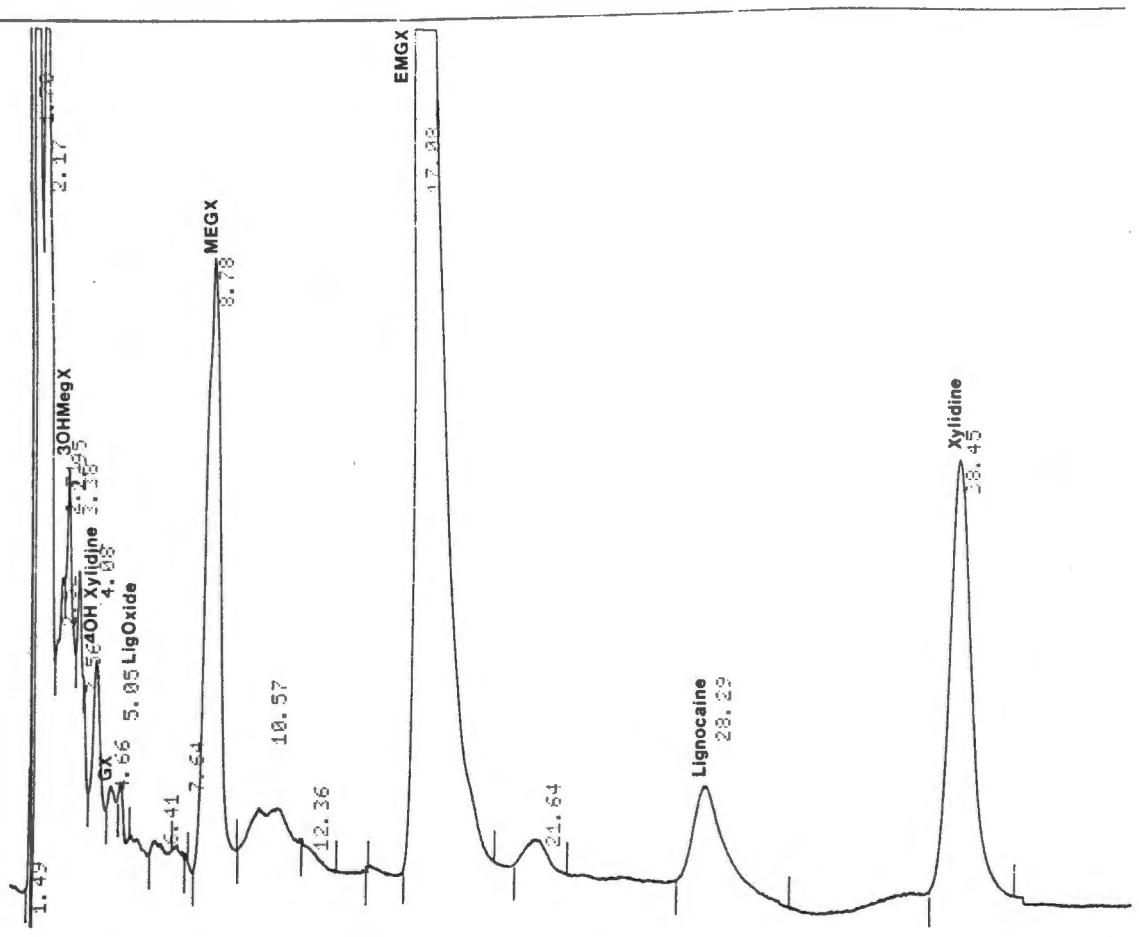

Figure 3.6 Chromatogram of hepatic venous effluent after lignocaine administration to the isolated perfused pig liver.

3.3.2 EXTRACTION EFFICIENCY (PERCENT ABSOLUTE RECOVERY)

Table 3.1

Percent absolute recovery of lignocaine MEGX, GX and EMGX

Substance	Concentration	Recovery	SD	Intra-assay Coef. Var
Method (1) (n=6)				
Lignocaine	10ug ml^{-1}	109%	4.3%	7%
Lignocaine	2.5ug ml^{-1}	104%	7.1%	6.9%
Lignocaine	0.16ug ml^{-1}	115%	3.3%	2.8%
EMGX	0.2ug ml^{-1}	93%	2.7%	3%
Method (2) (n=6)				
Lignocaine	10ug ml^{-1}	104%	3.2%	3.1%
EMGX	5ug ml^{-1}	86%	2.5%	2.9%
MEGX	2.5ug ml^{-1}	51%	2.1%	4.2%
GX	2.5ug ml^{-1}	47%	1.5%	3.2%
Lignocaine	5ug ml^{-1}	92%	1.7%	1.8%
EMGX	2.5ug ml^{-1}	77%	6.4%	8.3%
MEGX	1.25ug ml^{-1}	45.%	3.8%	8.4%
GX	1.25ug ml^{-1}	83%	5.3%	6.4%
Lignocaine	2.5ug ml^{-1}	98%	2.6%	2.6%
EMGX	0.2ug ml^{-1}	100%	3.1%	3.1%
MEGX	0.5ug ml^{-1}	45%	2.8%	6.3%
GX	0.5ug ml^{-1}	58%	4.9%	8.6%

The percent absolute recovery and intra-assay coefficient of variation (Coef. Var.) at the concentrations indicated are given above. All studies at one concentration were performed on the same day. The overall intra-assay coefficient of variation computed from the data in Table 3.1 was for Lignocaine, Method [1] (5.6%): Method [2] (2.5%). EMGX Method [1] (3%): Method [2] (4.8%). MEGX (6.3%) and GX (6.1%) over the range shown.

3.3.3 CALIBRATION CURVES

3.3.3.A. METHOD: 1

Calibration curves were performed on four successive days (run 1-4) in one week using a lignocaine concentration range of 40ng.ml^{-1} to 5.125 ug ml^{-1} see table C.1 (Appendix C) This data was submitted to

the MRC Biostatistics unit (Professor JS Maritz) for analysis to determine linearity, the variation during one week, and the range within which control samples should remain.

(i) Statistical Analysis

The first model considered for a straight line relationship between the concentration of lignocaine (C) and the peak height ratio (R) (measured lignocaine concentration/ measured internal standard) was: $R = \beta C +$ random error. (β = the slope parameter)

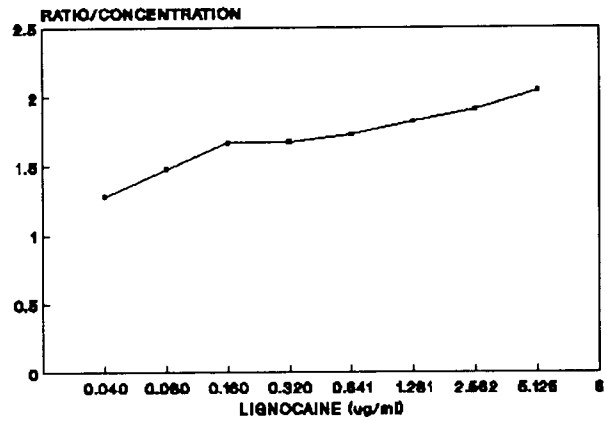

Figure 3.7 Lignocaine standard curve, method 1.

An accurate plot of the data for run 1 described above (see table C.1 Appendix C) indicated that this relationship was not truly a straight line. According to this model a plot of R/C against C should exhibit no trend. Figure 3.7 shows such a plot for Run 1 indicating clearly that there is an upward trend in the plotted points. In order that a calibration line may be used for the calculation of lignocaine concentrations, from a knowledge of the peak height ratio, a straight line over the calibration range is necessary. To achieve a straight line relationship for the present system a natural logarithm transformation of the data may be required. Thus an alternative way of writing this model would be: $\ln R = \ln\beta + \ln C +$ random error

Therefore a plot of $Y = \ln R$ vs $X = \ln C$ should give points close to a straight line with a slope of 1. Figure 3.8 shows Y vs X plots for the 4 runs and indicates that these lines fit the data very well.

Table 3.2 summarizes the estimated parameters of the fitted lines written in the form: $Y = \alpha + \beta(X - \text{meanX}) +$ error (where α and β are the intercept and slope parameters and (a) and (b) their estimates).

The estimated slopes (b) do not differ significantly ($F_{3,24} = 2.12$) but the intercepts (a) are significantly different ($F_{3,24} = 6.15$, $p < 0.01$). This suggests that there is random variation between the calibration lines from day to day apart from the random error variation associated with individual ratio determinations.

Table 3.2

Estimated parameters of lines fitted to lignocaine standard curves*

Intercept=a	s.e.(a)	slope=b	s.e.(b)	error variance
-0.2710	0.0160	1.0830	0.0101	0.002080
-0.4098	0.0370	1.1069	0.0231	0.010732
-0.3200	0.0210	1.0618	0.0135	0.003678
-0.3719	0.0210	1.0586	0.0114	0.002604

* Written in the form $Y = \alpha + \beta$ (X-meanX) + error. See text for details

(ii) Routine Calibration

As mentioned above (section 3.2.4.D.) it was necessary to ascertain, (1) the precision of the estimation of the unknown lignocaine concentration in a sample taken for analysis, (2) the frequency of calibration required, as well as to determine (3) a method to ensure that the calibration line had not changed dramatically.

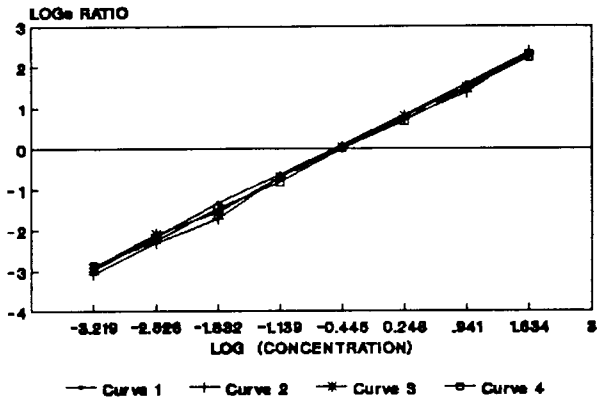

Figure 3.8 Lignocaine standard curves, method 1.

The interassay **coefficient of variation** (cv) of the determination of lignocaine concentration from a standard calibration line would be **7.6%**. This is based on the following statistical considerations: The concentration of lignocaine (X_{hat}) is established from: $\mathbf{X_{hat} = [(Y - \alpha)/\beta] + mean\ X}$, where α and β are estimated from a calibration line and Y is the observed ratio of an unknown lignocaine concentration. Then $var(X_{hat}) \approx \sigma^2/\beta^2 + [var(a/\alpha)]/\beta^2 + [(X\text{-}meanX)^2/\beta^2]var(b/\beta)$ where σ^2 is the error variance. Now $var(a/\alpha)$ is estimated by $s^2/8$ and $var(b/\beta)$ is estimated by $s^2 / \Sigma (Xi - meanX)^2$ where s^2 is the pooled residual variance obtained from fitting of the straight lines for runs 1-4 and equals 0.004773. Substituting the appropriate variance estimates and putting β and α equal to the respective mean values of the estimates (a) and (b) we get: $var(X_{hat}) \approx 0.004110 + 0.000514 + (X + 0.79235)^2(0.000204)$.

For the range of concentrations used in the calibration runs the largest value of the third term is 0.001201, giving $var(X_{hat}) = 0.005825 = (0.07632)^2$ ie a final estimate of coefficient of variation of 7.6%.

Calibration plots were run with each experiment analyzed. However, as analyses could not be performed by the technician daily, calibration plots were repeated at least weekly and daily standards were included with each run to establish whether earlier recalibration was necessary. From the statistical analysis performed these test standards taken at the center of the calibration range for observed Y = ln(ratio) should fall within the interval: $a + bX \pm 2(0.004773 + 0.003076)^{\frac{1}{2}}$ i.e. $a + bX \pm 0.18$.

(iii) Discussion and Conclusion

(1) The statistical analysis above indicates that a natural logarithm transformation of the lignocaine concentration and peak height ratio is required to achieve linearity over the standard concentration range of $40ng.ml^{-1}$-5.125 ug ml^{-1} of lignocaine when measured using the HPLC method described.

(2) The interassay coefficient of variation is 7.6%.

(3) A daily check of the calibration could be done by determining a peak height ratio for a concentration near the center of the calibration range and ensuring that the observed Y = ln (ratio) fell in the interval a + bX ± 0.18.

This method was adopted for both method 1, (and method 2) a calibration curve, using single samples, was performed weekly and only those analyses accepted where the provisions described in (3) above were satisfied. Moreover in the 15 standard curves performed using method 1 the linear (least squares) correlation coefficient was never less than 0.995 and more usually 0.998 or better.

The daily variation in the HPLC assay shown by the above mentioned statistical analysis is a normal phenomenon and may be multifactorial in origin. This is probably due largely to changes in the column or possibly due to ambient temperature changes (Meyer VR, 1988).

Table 3.3

Method parameters for the measurement of lignocaine concentration in plasma (method 1)	
Precision (interassay) (n=4)	5.7% at 5.12ug ml^{-1} 7.7% at 2.56ug ml^{-1} 6.0% at 1.28ug ml^{-1} 3.3% at 1.72ug ml^{-1} 7.9% at 0.32ug ml^{-1}
Limit of Sensitivity	40ng.ml^{-1}
Recovery of Lignocaine	109% at 10ug ml^{-1} 104% at 2.5 ug ml^{-1} 115% at 0.16ug ml^{-1}
Recovery of Internal Standard 93% at 0.2ug ml^{-1}	

3.3.3 B. METHOD 2

Method (2) was used to analyze the concentration of lignocaine and its metabolites at steady state both in vivo and ex vivo.

Three standard curves each were performed for lignocaine (156ng.ml^{-1} - 5.0 ug ml^{-1}) and the metabolites, MEGX (82ng.ml^{-1} - 2.64ug ml^{-1}) and GX (16ng.ml^{-1} - 578 ng.ml^{-1}) again using EMGX as the internal standard over the ranges as indicated in brackets. (Tables C.2, C.3, and C.4; Appendix C)

This data was subjected to the same statistical analysis as for method (1) above with similar findings. Only a brief summary will be given here. For further details please see Appendix C.2 (Detailed statistical analysis of standard curves for lignocaine MEGX and GX for HPLC method :2).

LIGNOCAINE

1) Coefficient of variation 6.1-7.0%.

2) Daily check calibration Y = ln(ratio) should fall in the interval a + bX ± 0.16.

Table 3.4

Method parameters for the measurement of lignocaine concentration in plasma (method 2)	
Precision (interassay) (n=3)	9.3% at 5.0ug ml^{-1} 9.0% at 2.5ug ml^{-1} 4.8% at 1.25ug ml^{-1} 11.1% at 0.625ug ml^{-1} 7.0% at 0.312ug ml^{-1}
Limit of Sensitivity	78ng.ml^{-1}
Mean Recovery of lignocaine	98%

MEGX

1) Coefficient of variation 13.1-13.9%.

2) Daily check calibration Y = ln(ratio) should fall in the interval a + bX ± 0.26.

Table 3.5

Method parameters for the measurement of MEGX concentration in plasma	
Precision (interassay) (n=3)	6.5% at 2.64ug ml^{-1} 13.0% at 1.32ug ml^{-1} 11.2% at 0.661ug ml^{-1} 11.0% at 0.165ug ml^{-1} 19.0% at 0.082ug ml^{-1}
Limit of Sensitivity	19ng.ml^{-1}
Mean Recovery of MEGX	47%

GX

1) Coefficient of variation 15.3-15.9%.

2) Daily check calibration Y = ln(ratio) should fall in the interval a + bX ± 0.25.

Table 3.6

Method parameters for the measurement of GX concentration in plasma	
Precision (interassay) (n=3)	7.2% at 578ng.ml^{-1} 10.1% at 289ng.ml^{-1} 4.0% at 145ng.ml^{-1} 2.4% at 72ng.ml^{-1} 16.5% at 31ng.ml^{-1}
Limit of Sensitivity	19ng.ml^{-1}
Mean Recovery of GX	62%

(i) Discussion and Conclusion

(1) As was the case with the analysis of lignocaine in Method 1 a natural logarithm transformation was again required to achieve linearity over the indicated range and in all 22 standard curves used for data analysis the correlation coefficients for MEGX and Lignocaine exceeded 0.97 and 0.99 respectively and for MEGX was usually greater than 0.99 and for lignocaine usually greater than 0.999.

(2) Standard curves were performed for each experiment and at least weekly if there was a delay in completion of a run. Daily standards were included in each analysis run as a check to establish whether earlier recalibration was necessary and data accepted according to the criteria set for method 1.

(3) The correlation coefficients of the standard curves for GX were of the same order as for MEGX and lignocaine above but as the analysis of experimental samples progressed it became apparent that the concentrations of GX could not be accurately determined as not only were the concentrations of this metabolite in the samples low but the shorter retention time of this substance resulted in significant interference with plasma constituents. It was thus decided to abandon further evaluation of this metabolite for this thesis.

Calibration samples and experimental samples in vivo were assayed singly and all ex vivo samples, where MEGX was determined, in duplicate.

3.4 PLASMA TO BLOOD PARTITIONING COEFFICIENT OF LIGNOCAINE WITH RESPECT TO PORCINE BLOOD

3.4.1 OBJECTIVE

The determination of hepatic clearance of a substance is best done using whole blood data (Tucker and Mather, 1975)(Rowland, 1972b). Thus in order to determine the whole blood concentration from the plasma concentration the partition coefficient between blood and plasma in pig blood must be determined. Furthermore it must be established whether this partition coefficient is concentration dependent. Tucker and Mather (1975) found the lignocaine blood to plasma concentration ratio to be independent of drug concentration in human blood.

3.4.2 MATERIALS AND METHOD

The method of Billig et al, (1989) was used.

Thirteen samples of 10ml of fresh porcine blood were injected into test tubes, to which were added different quantities of lignocaine to achieve anticipated plasma concentrations within the experimental range. An equivalent volume of the whole blood to the necessary volume to spike the sample (range 367.5 - 7.0 ul) was aspirated to ensure that the test volume remained 10ml. The test tubes were immediately covered with plastifilm and left for half an hour and regularly agitated to ensure adequate mixing. The specimens were then centrifuged in standard fashion, the plasma separated and extraction performed for immediate analysis of lignocaine concentration by HPLC analysis performed in triplicate for each sample. We confirmed that binding of lignocaine (in plasma or H_2O) to the glass test tubes used in these experiments was negligible.

3.4.3 RESULTS

3.4.3.A. Lignocaine

Table 3.7

Plasma to blood partitioning coefficient for lignocaine in pig blood

Calculated conc:(1) ($ug\ ml^{-1}$)	Actual mean conc: ($ug\ ml^{-1}$)	Red Blood Cell uptake:(2) ($ug\ ml^{-1}$)	Red Blood Cell Conc:(3) ($ug\ ml^{-1}$)	Plasma/RBC Coef:(4)
1) 40.80	31.57	9.23	23.73	1.33
2) 33.99	27.42	6.57	16.89	1.62
3) 26.22	22.67	3.55	9.12	2.48
4) 19.43	15.59	3.84	9.87	1.57
5) 14.54	11.8	2.74	7.04	1.68
6) 11.65	9.50	2.15	5.52	1.72
7) 9.71	7.71	2.0	5.14	1.50
8) 7.48	5.50	1.89	4.41	1.25
9) 5.99	4.49	1.5	3.50	1.28
10) 4.50	3.57	0.93	2.17	1.64
11) 2.99	2.45	0.54	1.26	1.94
12) 1.79	1.45	0.35	0.81	1.79
13) 0.79	0.60	0.19	0.44	1.37

(1) in plasma in the absence of red cell uptake.
(2) Difference between calculated and actual measured plasma concentration.*
(3) [(Red blood cell uptake)x(1 - Hct)]/Hct = the amount of drug lost from plasma/ red blood cell volume.
(4) Plasma concentration/ red blood cell concentration.
Samples 1-7 Haematocrit = 28% (n=5). Samples 8-13 Haematocrit = 30% (n=5).
* This plasma concentration difference is attributed to the uptake by or binding to red blood cell constituents.

This table presents the data used to determine the plasma\rbc coefficient for lignocaine in porcine blood. The values given under the heading 'Calculated concentration' are those anticipated from the different quantities of lignocaine added to the sample. The 'Actual mean concentration' is the mean lignocaine concentration of 3 samples determined by HPLC after lignocaine administration. The 'Red Blood Cell' uptake is the difference between these two values from which the amount of lignocaine bound to or taken up by blood cells can be calculated by multiplying this value by the plasma volume [(1-Hct) x 10ml].'

Least squares regression of the plasma concentration of lignocaine on the plasma to red blood cell partition coefficient yielded a line with a slope no different from zero ($y = 0.005x + 1.57$) ($r = 0.167$, $P = 0.584$). This confirmed in pigs the finding in humans by Tucker and Mather (1975) that the red blood cell uptake of lignocaine by porcine red cells was independent of concentration over the range of lignocaine concentrations studied.

The mean ($\pm$ SEM) plasma to red blood cell partition coefficient was 1.63 ± 0.09.

3.4.4 DETERMINATION OF BLOOD CONCENTRATION

The plasma concentration can be converted to blood concentration using a conversion factor (lambda) [drug concentration in whole blood/drug concentration in plasma].

Lambda will vary with the haematocrit and is dependent on the plasma to red blood cell partition coefficient.

$$\textbf{Hence: lambda} = \frac{H}{K} + (1\text{-}H) \quad \textbf{(Wiklund, 1977) ... (1)}$$

Where K is the plasma to red blood cell partition coefficient.

Thus as whole blood lignocaine concentration varies with the haematocrit, this was determined for each sample taken for lignocaine analysis using the haematocrit (determined in triplicate over the sampling period) and the individual plasma lignocaine concentrations and taking K to be 1.63 as determined here.

Wiklund determined K to be 1.6 for lignocaine in human blood from the work of Tucker and Mather (1975) cited above.

The value of K = 1.63 as determined for pig blood over a wide concentration is thus almost identical to that in humans and assuming this to be the same would have been acceptable.

CHAPTER 4: DETERMINATION OF PHARMACOKINETIC PARAMETERS FOR THE ACHIEVEMENT OF A CONSTANT DRUG CONCENTRATION IN THE IN VIVO AND EX VIVO PREPARATION

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CHAPTER 4: DETERMINATION OF PHARMACOKINETIC PARAMETERS FOR THE ACHIEVEMENT OF A CONSTANT DRUG CONCENTRATION IN THE IN VIVO AND EX VIVO PREPARATION

SUMMARY

The aim of this investigation was to study the decay of lignocaine after a bolus dose in both the in vivo and ex vivo preparations to determine the pharmacokinetic parameters required to achieve comparable constant drug concentrations in the two models using a method described by Mitenko and Ogilvie, (1972) for man. The decay of lignocaine in the anaesthetized pig was well described by a two compartment open model. The pharmacokinetic analysis of lignocaine in the isolated perfused pig liver was complicated by a wash in curve as well as recirculation phenomena resulting in a poorer fit of the data to this model. The parameters determined were used to successfully achieve constant plasma lignocaine concentrations near the target concentration of 5ug ml^{-1} within one hour of continuous lignocaine administration in both preparations.

4.1 INTRODUCTION

4.1.1 AIM OF STUDY

The aim of this study was to determine the pharmacokinetic parameters for the in vivo and ex vivo preparations necessary to calculate a lignocaine administration regimen which would reliably achieve a constant hepatic affluent plasma lignocaine concentration of 5ug ml^{-1} at the earliest possible time of an experiment. A constant or steady state plasma concentration was required because the extraction and clearance of a drug across the liver is best studied when the rate of drug binding is zero (Pang, 1980). An equivalent concentration in both preparations studied over a similar time period would permit a comparison of hepatic elimination independent of concentration and time dependent effects (Bauer et al, 1982). It was necessary to achieve this as soon as possible because of the limited period of viability of the isolated perfused preparation (section 2.6.4).

The target concentration of 5ug ml^{-1} was chosen as this is at the upper end of the therapeutic range.

4.1.2 METHOD OF ACHIEVING A CONSTANT DRUG CONCENTRATION

In adult humans the usual method for attaining a steady state lignocaine plasma concentration consists of giving an intravenous lignocaine bolus dose of 50 - 100 mg and then infusing this drug at a rate of 1 - 2 $mgmin^{-1}$. Vaughan and Tucker (1976) in an excellent review have derived the ideal intravenous drug input required to achieve and maintain a constant plasma drug concentration and applied this theoretically to lignocaine therapy for patients with myocardial infarction. However it appears that others have found in practice that a bolus dose and intravenous infusion regimen may result in an initial decline in plasma concentration, achieving a plateau only after 2 hours (Salzer et al, 1981). To attempt to avoid this a number of methods employing exponentially declining infusions (Sebaldt et al, 1984)(Riddell et al, 1984)[1] as well as the use of a three step method of lignocaine administration (Salzer at al, 1981) have been described for use in man. Using these methods a steady state was not achieved before 60 minutes and usually later.

Mitenko and Ogilvie (1972) have shown that the quickest method of achieving a constant plateau concentration was by following an initial bolus of a drug with an infusion rate equal to Beta (β) times the initial infusion rate, where β is the slower disposition constant in a two compartment model. They demonstrated in humans that when theophylline was administered in this fashion a steady state was achieved in less than 30 minutes.

This appeared to be an attractive method to establish a constant drug concentration in the pig and the isolated perfused pig liver (IPPL) at the earliest possible time.

The method described requires that the pharmacokinetics of the drug can be described by a two compartment open system model (Gibaldi and Perrier, 1975). This is the case for many exogenous compounds and has also been assumed by others for lignocaine (Salzer et al, 1981)(Rowland et al, 1971)(Thomson et al, 1973) in humans and in sheep (Morishima et al, 1979) in the monkey (Benowitz et al, 1974) and in the horse (Engelking et al, 1987).

4.1.3 DESIRED PHARMACOKINETIC PARAMETERS

In order to use the described method it was necessary to determine:

a) Whether lignocaine decay in both preparations could be described by a two compartment model and if so:

b) The necessary pharmacokinetic parameters in both the pig and the IPPL.

According to Mitenko and Ogilvie (1972)

$$\textbf{The loading dose (R)} = \frac{V1 \times K10 \times C}{Beta} \quad (1)$$

1 A description of this method using the data derived here is given in appendix D. (Page D.20)

where C is the plasma concentration of drug required at steady state and V1 the volume of distribution of the central compartment (vide infra), whilst K10 is the elimination rate constant and Beta the slow disposition constant.

The infusion rate (Q) to maintain steady state is:

$$Q = V1 * K10 * C \quad (2)$$

These parameters for pigs and for the isolated perfused pig liver preparation are not available from the literature and had therefore to be determined.

4.1.4 THE TWO COMPARTMENT PHARMACOKINETIC MODEL

For review see Greenblatt and Koch-Weser (1975).

The two compartment pharmacokinetic model assumes that with respect to drug disposition the body can be divided into a central compartment of small apparent volume (V1) and a larger peripheral compartment of volume V2. (See figure 4.1).

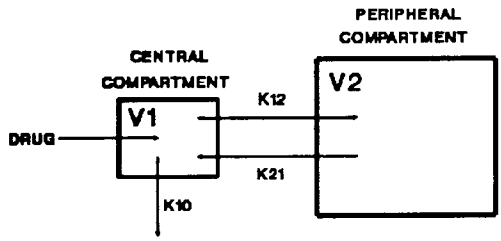

Figure 4.1 Schematic diagram of the two compartment open model.

Drugs enter and are eliminated from the system only via the central compartment but reversible transfer occurs between the central and peripheral compartment.

The transfer and exit of the drug in this system occurs under "first order kinetics" i.e. the rate of transfer is proportional to the concentration of the drug. In this model K12 and K21 are the first order constants associated with drug transfer between the two compartments and K10 the elimination rate constant.

4.2 MATERIALS AND METHODS

4.2.1 IN VIVO STUDY

Five male Landrace x Large White pigs of 22-25kg in weight were anaesthetized using thiopentone and isoflurane as described in section 2.2. An indwelling venous cannula was placed in the right internal jugular

vein and a carotid artery cannula was placed for sampling for lignocaine analysis. A laparotomy for transhepatic cannulation and measurement of hepatic blood flow was not done in these studies. An empirical bolus dose of 120 mg of lignocaine hydrochloride was used. This was aspirated from a freshly opened ampoule of lignocaine hydrochloride 10% (Remicaine, Transvaal) using two pre-calibrated Gilson pipettes (1ml and 200ul) and introduced into a 20ml syringe to be diluted with 20ml of saline immediately prior to administration to the animal. This was then administered into a fast running intravenous line over a period of two minutes timed with a stop watch. Thereafter samples were taken from the carotid artery line via a fresh three-way tap following aspiration of deadspace blood. Sampling for lignocaine analysis was performed for 5 hours after the bolus injection at the following time intervals:
At 2 minute intervals for the first 20 minutes, then at 5 minute intervals for 40 minutes, followed by 15 minute intervals for 2 hours and 30 minute intervals for 2 hours; a total of 30 samples.

4.2.2 EX VIVO STUDY

4.2.2.A. Introduction

As there is no indication in the literature as to the likely bolus dose that would be appropriate for use in the isolated perfused pig liver (IPPL) or the appropriate frequency of sampling, a number of preliminary experiments were performed, a short account of which is given here.

4.2.2.B. Determination of Bolus Dose and Sampling Frequency for the IPPL

Three standard isolated perfused liver studies with arbitrarily chosen increasing bolus doses of lignocaine hydrochloride administered into the hepatic venous reservoir Figure 2.2a-(D) were performed. Doses administered were 13 mg, 26mg and 32mg. Samples for analysis were taken from the HA cannula at 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105, 120 minutes. The decay of lignocaine was found to be rapid and the intervals of sampling chosen too long to yield sufficient data for pharmacokinetic analysis. Thus in the study described below forty milligrams of lignocaine hydrochloride was given as a bolus dose and sampling was performed at 1/2 minute intervals for the first fifteen minutes, then at 5 minute intervals for 45 minutes and then at 10 minute intervals for the next hour.

4.4.2.C. Conduct of Experiment

Three standard liver perfusions were performed. At time zero a 40mg bolus dose of lignocaine as the hydrochloride salt was administered into the hepatic vein reservoir. This was done by aspirating 400ul from a freshly opened ampoule of 10% lignocaine hydrochloride with a previously calibrated Gilson pipette. At this time a stop watch was started and all subsequent sampling was performed at the times stated above.

4.3 RESULTS AND DISCUSSION

4.3.1 IN VIVO STUDY

The decay of lignocaine with time after a bolus injection of 120mg in five male pigs anaesthetized with isoflurane is illustrated on an arithmetic (Figure 4.2) and semilogarithmic (Figure 4.3) plot respectively. These decay curves show neither a wash in phenomenon nor evidence of recirculation as seen in the isolated perfused pig liver preparation (vide infra).

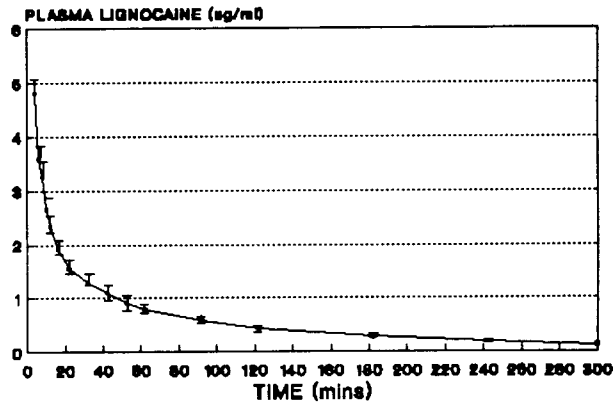

Figure 4.2 Mean (± SEM) plasma lignocaine concentration decay after a bolus dose in 5 pigs plotted on an arithmetic scale.

4.3.2 EX VIVO STUDY

4.3.2.A. Liver Viability

The three livers studied ex vivo were similar in weight; 772g, 762g and 741g respectively. The parameters used to assess liver function and perfusate composition over the two hour study period (Table 4.1) in this group of three livers [Lignocaine Decay Group: (1)] were no different from the control group (2) of perfused livers studied in Chapter (5) Experiment (B) (Section 5.5). However, there was one difference of interest, namely that in the lignocaine group there was no decrease of potassium concentration and no increase in sodium concentration with time as occurred in the control group (2) and had also been noted in another group of isolated perfused livers which had not received lignocaine (Section 5.3.3.D.). This may be as a result of the small numbers in this study but it is possible that lignocaine exerts an effect in this regard.

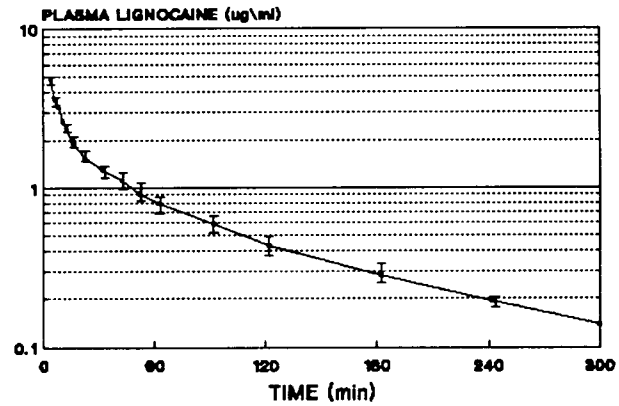

Figure 4.3 Mean (± SEM) plasma lignocaine concentration decay after a bolus dose in 5 pigs plotted on a logarithmic scale.

Table 4.1

Liver Function Indices and Perfusate Composition for Isolated Livers Used to Study Lignocaine Decay (Group 1) Compared to Control Group (2)

Liver Function Indices:

Parameter	Experiment	Time (hrs)0	1	2
Oxygen	(1)	2.7 ± 0.8	2.6 ± 0.3	2.5 ± 0.4
Cons.($ml O_2 100g^{-1}$)	(2)	2.8 ± 0.2	2.5 ± 0.2	2.5 ± 0.2
Bile Flow	(1)	-	3.9 ± 0.1	2.4 ± 0.5
($ml\ hr^{-1}$)	(2)	-	5.6 ± 0.7	3.0 ± 0.6
K± ($mmol\ L^{-1}$)	(1)	6.3 ± 0.7	* 5.3 ± 0.5	4.8 ± 0.3
	(2)	5.1 ± 0.4	* 4.0 ± 0.3[a]	3.4 ± 0.3[a]
AST	(1)	95 ± 22	154 ± 62	199 ± 94
(UL^{-1})	(2)	111 ± 31	155 ± 49	173 ± 49
ATP	(1)	6.80 ± 0.89	6.5 ± 1.39	6.39 ± 8.6
($uM\ gm\text{-}liver^{-1}$)	(2)	5.04 ± 0.44	5.16 ± 0.26	6.76 ± 0.44
ADP	(1)	2.16 ± 0.15	2.23 ± 0.21	2.26 ± 0.11
($uM\ gm\text{-}liver^{-1}$)	(2)	2.62 ± 0.37	2.45 ± 0.30	2.56 ± 0.29
AMP	(1)	1.04 ± 0.13	1.20 ± 0.08	0.98 ± 0.01
($uM\ gm\text{-}liver^{-1}$)	(2)	1.02 ± 0.24	0.99 ± 0.20	0.94 ± 0.14
Energy	(1)	0.789 ± 0.002	0.755 ± 0.041	0.773 ± 0.020
Charge	(2)	0.737 ± 0.033	0.745 ± 0.025	0.783 ± 0.026
TAN	(1)	10.00 ± 1.17	9.96 ± 1.48	9.61 ± 0.92
($uM\ gm\text{-}liver^{-1}$)	(2)	8.73 ± 0.81	8.61 ± 0.40	10.27 ± 0.31

Perfusate Composition:

Sodium	(1)	136 ± 1	138 ± 1	141 ± 1
($meq\ L^{-1}$)	(2)	136 ± 2	142 ± 1[a]	144 ± 1[a]
Tot. Prot.	(1)	59 ± 1	56 ± 1	56 ± 1
($gm\ 100ml^{-1}$)	(2)	53 ± 5	53 ± 2	54 ± 2
Albumin	(1)	36 ± 1	36 ± 1	36 ± 1
($gm\ 100ml^{-1}$)	(2)	34 ± 2	34 ± 1	34 ± 1
Osmolality	(1)	270 ± 18	271 ± 26	276 ± 14
($mosmls\ L^{-1}$)	(2)	284 ± 5	284 ± 9	283 ± 6
Haemoglobin	(1)	10.8 ± 0.2	11.3 ± 0.4	10.5 ± 0.5
(g%)	(2)	11.3 ± 0.9	11.7 ± 0.4	11.8 ± 0.5
Urea	(1)	29 ± 3.4	39 ± 7.3	47 ± 4.7
($mg\ 100ml^{-1}$)	(2)	26 ± 4.9	31 ± 5.7	36 ± 6.5
pH	(1)	7.43 ± 0.03	7.44 ± 0.03	7.40 ± 0.02
	(2)	7.41 ± 0.03	7.40 ± 0.02	7.40 ± 0.02
Hep.Venous	(1)	131 ± 5.8	131 ± 7.0	123 ± 8.6
PO_2 (mmHg)	(2)	136 ± 3.2	137 ± 7.9	141 ± 6.4

Mean ± (SEM) Lignocaine decay group (1) (A=3) vs Control group (2) n=6. Data analysed by repeated measures analysis of variance used to determine within and between group differences. * = significant difference between groups. (a) = significant difference between 0 hours and 1 or 2 hrs. (b) = significant difference between time 1 and 2 hours. P = < 0.05.

Oxygen Cons. = Hepatic Oxygen Consumption, K+ = Potassium, AST = Aspartate Aminotransferase. Hep. Venous PO_2 = Hepatic Venous PO_2

4.3.2.B. Lignocaine Decay

The composite plot of the time course of lignocaine (n=3) after a bolus dose of 40mg in the isolated perfused pig liver preparation (Figure 4.4) shows an initial wash in curve followed by a subsequent decay curve. The wash in curve indicates marked variablility between the experiments whilst the subsequent decay curve shows more uniformity. This initial variability, despite the similarity in all respects of the isolated perfused liver preparations may be the effect of depositing the bolus of lignocaine into the hepatic venous reservoir and a variable rate of release of lignocaine from this reservoir. (This reservoir is a reservoir in name only as liver effluent draining into it drains immediately via a second outlet to the oxygenator without a collection of fluid building up in the container and so this possiblity had not been anticipated). Inspection of the composite decay curve (Figure 4.4) reveals that the rapid decay of lignocaine is slowed after about 4 minutes and a secondary "hump" occurs. This is probably a recirculation effect. This recirculation effect can be explained thus: The initial very high plasma concentration measured prior to entry into the liver results in a high concentration of lignocaine in the hepatic venous effluent (as it is not totally extracted by the liver) returning to the oxygenator. This return of a significant quantity of lignocaine to the oxygenator tends to raise again the concentration of lignocaine in the perfusate leaving the oxygenator which is so measured in the hepatic artery cannula and results in the secondary hump seen. This recirculation effect was seen in each of the individual decay curves (Displayed in Appendix D.6, Figure D.4) and may explain the poor fit of conventional pharmacokinetic modelling using this data (vide infra).

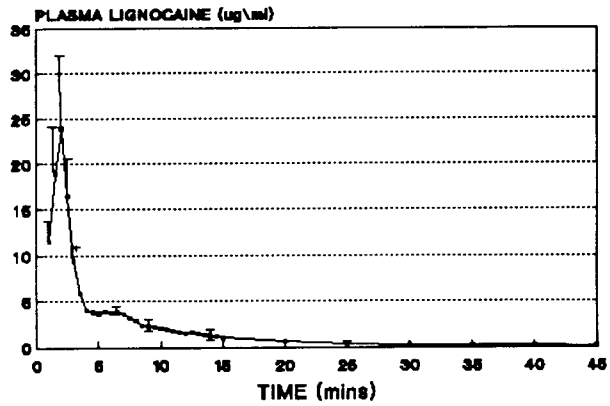

Figure 4.4 Mean (± SEM) plasma lignocaine concentration decay after a bolus dose in 3 isolated perfused pig livers.

4.4 INTERPRETATION OF RESULTS

4.4.1 INTRODUCTION

The prime objective of this pharmacokinetic analysis was to establish the parameters necessary to achieve a predicted constant plasma drug concentration in both the in vivo and ex vivo preparations. No

exhaustive attempt was made to elucidate fully the pharmacokinetics of lignocaine in either preparation beyond what was necessary to this goal. Further, as lignocaine does not exhibit optical isomerism (Tucker et al, 1990) there was no need for enantiomer specific pharmacokinetic analysis (Tucker and Lennard, 1989).

4.4.2 PRINCIPLES OF PHARMACOKINETIC ANALYSIS

4.4.2.A. Graphic Analysis

According to Boxenbaum et al, (1974) the first step in the pharmacokinetic analysis of a decay curve is to start with a graphical analysis of the data in order to attempt to formulate a preliminary model; be this a one or two compartment or possibly a more complex model. This is done by plotting the decay data on a semilogarithmic plot of concentration versus time. If this plot indicates that the decay could be described by two exponentials then the data is likely to fit a two compartment model. However the most "parsimonius" model should be aimed for (Daniel and Wood, 1971). From such a plot, a rough estimate of certain parameters can be readily determined by the process of curve stripping (Wagner, 1975)(Gibaldi and Perrier, 1975)(See appendix D.1). These parameter values can then be used as the 'initial values' required by various iterative computer programs to start a pharmacokinetic analysis. The assessment of the initial values is critical (Boxenbaum et al, 1974) as they are determinant for the final parameter values as well as for the precision of the parameter estimates. Convergence to the true parameter value will only be achieved if the parameter estimates were sufficiently close to this value.

4.4.2.B. Weighting

Weighting of the data points is a further consideration about which there is considerable debate and uncertainty as to which is the best available method (Gibaldi and Perrier, 1975).

According to Daniel and Wood (1971), weighting is not required when all observations have the same (though possibly unknown) variance. Wagner (1975) indicates that when the observations do not have the same variance each point should be weighted inversely as the square of its variance. This is however only useful when fitting averaged data where the variances may be calculated for each point. In actual practice Wagner performs the fitting of the same data to the same equations using weights equal to 1, 1/observation and 1/ (observation)^2 and then makes the final choice on the basis of several criteria such as systematic deviation of weighted residual plots versus time (a feature of PCNONLIN, vide infra) as well as the consistency of the estimated parameter values from subject to subject. This approach was followed in the analysis to be described.

4.4.2.C. Selection of a Pharmacokinetic Model

According to Boxenbaum et al (1974) there exist no absolute criteria for the selection of a pharmacokinetic model, but two aims are generally sought:

(i) The scatter of the observed data points about the theoretical curves should be randomly distributed and,

(ii) The sums of the weighted squared deviations from the fitted equations should be "reasonably minimal".

Berman, (1966) maintains that the use of the sum of squares as a measure of comparison of fit between two models maybe helpful but is not always very sensitive. Both authorities agree however that plotting to assess systematic deviations, although not quantifiable is a most useful measure of fit.

In this regard Boxenbaum et al (1974) suggest that an appropriate measure to assess scatter is to plot the weighted residual against the calculated value in order to establish systematic deviations. This plot, a feature of PCNONLIN (vide infra), has been used in this analysis. When systematic deviations exist the model is regarded as inconsistent and should be modified (Berman, 1966). However, if this is not the case the fit is regarded as good but the calculated parameters may still show large uncertainties. The model is then regarded as nonunique or ill conditioned (Berman, 1966).

With respect to the second criterion of Boxenbaum, [(b) above] he suggests that to test whether the weighted sum of squares have been sufficiently reduced to justify fitting of additional parameters i.e. a more complex model, the F ratio test may be used: where

$$F = \frac{(WSSj - WSSk)}{WSSk} \times \frac{Dfk}{Dfj - Dfk}$$

and WSSj is the weighted sum of squared deviations obtained with the jth set of parameters, WSSk is the weighted sum of squares obtained with the kth set of parameters and Df is the number of degrees of freedom which is equal to the number of data points used to fit the curves minus the number of parameters fitted (Dfj > Dfk).

If the calculated F is less than the critical value derived from standard tables [numerator has (Dfj-Dfk) degrees of freedom and the denominator has Dfk degrees of freedom] it can be concluded that the weighted sum of squared deviations is not significantly different and that there is thus no justification to fit a more complex model. A 5% level of significance is adopted.

4.4.2.D. Pharmacokinetic Computer Program, PCNONLIN

The pharmacokinetic analysis of the decay of lignocaine was performed using PCNONLIN Version 3 (SCI Software, Kentucky USA). This is an iterative program for nonlinear regression analysis which can be run on an IBM compatible personal computer. It was used in the default mode, namely the Gauss Newton method with Levenberg modification (PCNONLIN Manual). It features a number of standard compartmental models for pharmacokinetic analysis of data, which will be referred to in the following text by number and which are described more fully in the appendix (Section D.2). To aid analysis of data a number of standard plots are produced by this program, namely;

(1) Observed Y versus weighted calculated Y

(2) Weighted calculated Y versus weighted residual

(3) Time (x) versus weighted residual Y

4.4.2.E. Criteria for Model Choice

In the analysis performed here competing models were assessed by

(i) Criteria of Fit

(1) AIC criteria (Akaike, 1974)
(2) Corrected sum of squares
(3) Sum of squared residuals
(4) Correlation coefficient

"AIC" stands for Akaike Information Criteria and is an estimate of a measure of fit of the model. It gives a mathematical formulation for the principal of parsimony in model building. When there are a number of competing models the minimum information theoretical criteria estimate is defined by the model and the maximum likelihood estimates of the parameters which give the minimum of AIC as defined by:

AIC = [(-2) log-(maximum likelihood) +2 (number of independently adjusted parameters within the model)] (Akaike, 1974).

An F test for (2) and (3) was performed as suggested by Boxenbaum (1974) and Berman (1966).

(ii) Visual Assessment of Systematic Deviation of Plots 1-3

Described above (Section 4.4.2.D.).

4.4.2.F. Data Definition Of Parameters

The extent to which the determined pharmacokinetic parameters define the data can be determined by:

(i) The confidence interval and standard error. These estimates, generated by PCNONLIN, are only approximate as they are based on the linearisation of a non-linear model (PCNONLIN instruction manual).

(ii) Correlation Matrix.

If there is a high correlation amongst one or more pairs of estimates this indicates that the univariate confidence limits are underestimations of the uncertainty in the parameter estimates. Thus the estimates may not be reliable.

4.4.3 PHARMACOKINETIC ANALYSIS OF IN VIVO DATA

4.4.3.A. Introduction

Five animals were studied in this group. For each animal a total of 30 samples were drawn however in the interests of economy in only one study were all the samples analysed for lignocaine concentration by

HPLC. This study served as a template to assess which samples should be analysed in the other 4 studies in which 16 samples were analysed in each.

4.4.3.B. Analysis of Lignocaine Decay

In the interests of brevity only the essential points of this analysis are described here. (The appendix, section D.3, D.4, D.5, contains all further information concerning this analysis.)

A logarithmic plot of the mean lignocaine plasma concentration versus time (Figure 4.3) revealed that a two or three compartment model was likely to be appropriate for all five in vivo studies.

This hypothesis was tested using the methodology described above. Initial values were determined by curve stripping of this plot (Appendix D.1). These values were then used to assess the fit of this data to a one, two, and three compartment model using PCNONLIN models 1, 8, and 18 respectively. (For details of models see appendix D.2.) Weighting with $1/y$ and $1/y^2$ did not improve the fit.

Fit of the data to a one, two or three compartment model is compared in Table 4.2. according to the criteria for choosing a model discussed above.

Table: 4.2

Goodness of Fit of Mean Lignocaine Decay Data In Vivo

(Model 1)	ONE Compartment (Model 8)	TWO Compartment (Model 18)	THREE Compartment
AIC	18.1	-28.5	-33.6
Corrected Sum Of Squares	28.3	28.3	28.3
Sum Of Squared Residuals	2.41	0.1018*	0.0590*
Correlation Coefficient	.968	.998	.999
Degrees Of Freedom	14	12	10
		Visual Assessment	
Plots			
1	NL+++	L++	L++
2	SD++	R	R
3	SD++	R	R

* = F Test different from one compartment model. $P < 0.05$
L=linear. NL=nonlinear. R=random scatter. SD=systematic deviation

Plot 1 = Observed Y versus weighted calculated Y.
Plot 2 = Weighted calculated Y versus weighted residual.
Plot 3 = Time (x) versus weighted residual Y.

See appendix D.3 for graphs of these plots

The decay of lignocaine in this study in the anaesthetized pig appears to be better described by a multi-compartment model as the AIC criteria and the sum of squared residuals is less in the two and three compartment models than in the one compartment model. The F test is not significantly different for the corrected sum of squares but is for the sum of squared residuals when the two and three compartmental models are compared with the single compartmental model. Most importantly the plots (Appendix D.3) indicate a better fit for the two and three compartment models. The question arises whether the data is fitted better by a three compartment model than a two compartment model. Visual assessment of the plots 1-3 reveals that there is little to choose between them. Further, although the AIC value is less for the three compartment model the F test dose not reach statistical significance and thus according to Boxenbaum (1974) there is no justification for using the more complex model. In conclusion it was accepted that a two compartment model adequately describes the decay of lignocaine in the pig. The lignocaine decay in each pig was separately modeled to determine the derived pharmacokinetic parameters. (See appendix D.4 & D.5).

4.4.3.C. Derived Pharmacokinetic Parameters

Table: 4.3

Derived Pharmacokinetic Parameters: Lignocaine Decay in The Pig

	MEAN	SEM
V1(L kg^{-1})	**0.638**	**0.076**
V2(L kg^{-1})	1.515	0.180
Vdarea(L kg^{-1})	2.152	0.140
K10 (min^{-1})	**0.0419**	**0.008**
K12(min^{-1})	0.0915	0.016
K21(min^{-1})	0.0529	0.005
AUC(ug ml^{-1} min)	171.5	21.4
K10 $t_{\frac{1}{2}}$ (min)	19.9	4.8
α(min^{-1})	0.1786	0.029
β(min^{-1})	**0.0124**	**0.0016**
$t_{\frac{1}{2}}\alpha$ (min)	4.42	0.8
$t_{\frac{1}{2}}\beta$ (min)	59.6	7.3
Cmax (ug ml^{-1})	6.06	0.47
A (ug ml^{-1})	5.16	0.74
B (ug ml^{-1})	1.76	0.11
A'(ug ml^{-1})	6.13	0.96
B'(ug ml^{-1})	1.78	0.11

V1 = volume of central compartment, V2 = volume of peripheral compartment, Vdarea = dose / β(AUC)*, K10, K12 and K21 = first order constants see section 4.1.4 AUC = Area under the concentration time curve. $t_{\frac{1}{2}}$ = the half-life. α and β are the distribution and elimination phase hybrid disposition rate constant respectively, while A and B are their coefficients. A' and B' are the coefficients recalculated to compensate for the drug infusion time (see section 4.4.6.A.). C max is the maximum mean concentration achieved.

* **Greenblatt and Koch-Weser, (1975).**

Table 4.3 (above) summarizes the derived pharmacokinetic parameters in vivo.

4.4.3.D. Calculation of Loading Dose and Infusion Rate

For the determination of the loading dose and subsequent infusion rate according to Mitenko and Ogilvie (1972) the parameter values of importance are; V1 = 0.638 L kg^{-1}, β = 0.0124, and K10 = 0.0419.
Theoretically the loading dose (R) of lignocaine hydrochloride would be calculated as

$$= \frac{V1 \times K10 \times C}{Beta} \quad X\ 1.232^{1} = 13.27 mgkg^{-1}$$

and the infusion rate (Q) = V1 x K10 x C x 1.232 would be 0.164 $mgkg^{-1}min^{-1}$ to achieve a plasma concentration of 5 ug ml^{-1}.

4.4.4 PHARMACOKINETIC ANALYSIS OF EX VIVO DATA

4.4.4.A. Analysis of lignocaine decay

The pharmacokinetic analysis of lignocaine decay in the IPPL using PCNONLIN was more complex than that for the in vivo experiment. Therefore, in the interests of brevity only essentials of analysis are discussed here (Appendix D.6 contains a full description of the pharmacokinetic analysis performed).
The greater complexity may be explained by the initial wash in period as well as the effect of recirculation on the lignocaine decay curve (Figure 4.4) which resulted in a poor fit in comparison with the in vivo data. However the analysis suggested that within the constraints of the models used the time course of lignocaine after a bolus injection was better described by a two compartment model with constant iv input and first order output (Model 9) than by a one compartment model with constant iv input and first order output (Model 2). The high AIC, and corrected sum of squares as well as the sum of squared residuals values found in this analysis suggest that the data might be better described by a more complicated model.

4.4.4.B. Derived Pharmacokinetic Parameters

Table 4.4 gives a summary of the mean derived pharmacokinetic parameters determined using PCNONLIN. The parameters of interest are again the volume of the central compartment (V1), the elimination rate constant (K10) and Beta.

4.4.4.C. Calculation of Loading Dose and Infusion Rate

Applying the above formulae (Section 4.4.3.D.) the administered bolus dose of lignocaine hydrochloride to achieve a concentration of 5 ug ml^{-1} in the hepatic artery would be 39.42 mg.

---

1 To achieve a constant concentration of 5ug/ml of lignocaine base in the perfusate these formulae must be multiplied by a factor of 1.232 being the ratio of the molecular weights of lignocaine hydrochloride (the administered form), and lignocaine base (the form analysed).

Whilst the infusion rate would be 2.80 mg per minute started simultaneously.

Table: 4.4

Derived Pharmacokinetic Parameters: Lignocaine Decay In The Isolated Perfused Pig Liver

	MEAN	SEM
V1 (L)	**1.128**	**0.792**
K10 (min^{-1})	0.404	0.216
K12(min^{-1})	0.321	0.113
K21(min^{-1})	0.206	0.090
AUC(ug ml^{-1} min)	88.6	8.01
K10 $t_{\frac{1}{2}}$ (min)	**2.1**	**0.68**
α(min^{-1})	0.859	0.50
β(min^{-1})	**0.071**	**0.01**
$t_{\frac{1}{2}}\alpha$ (min)	0.817	0.07
$t_{\frac{1}{2}}\beta$ (min)	9.866	0.86
Cmax (ug ml^{-1})	20.41	5.65
A (ug ml^{-1})	34.28	12.59
B (ug ml^{-1})	3.56	1.29

Symbols as for table 4.3.

4.4.5 ACHIEVEMENT OF A CONSTANT DRUG CONCENTRATION IN VIVO AND IN THE ISOLATED PERFUSED PIG LIVER

4.4.5.A. Introduction

As stated earlier Mitenko and Ogilvie (1972) have shown that the fastest way to achieve a plasma concentration plateau is to administer a bolus infusion and simultaneously start a maintenance infusion at a rate beta times the initial bolus dose, where beta is the slower disposition constant. In the live animal this large bolus (± 320 mg) is likely to result in significant toxic effects if administered too rapidly due to a high peak plasma concentration. To overcome this problem Wagner (1974) has suggested a method by which initially a constant rate infusion at a rate Q1 is given over time (t) after which the rate of infusion is abruptly changed to a lower rate, Q2, which is maintained as long as a steady state is required. Wagner in agreement with Mitenko and Ogilvie states that the loading dose (R) should be equal to the infusion rate (Q2) divided by beta.

$$\text{ie } R = Q2 / \beta$$

Wagner has mathematically determined a critical ratio for (Q1/Q2) which provides the most rapid attainment of this plateau concentration. This is:

$$\frac{Q1}{Q2} = \frac{1}{1 - e^{-\beta t}}$$

Thus once the infusion rate (Q2) has been determined the infusion rate necessary to administer the loading dose over time (t) can be calculated.

In this study the empirical time chosen for t was 10 minutes whilst beta was the mean value determined in the study above, namely, 0.0124 (Table 4.3).

In the actual experiments the initial (Q1) and subsequent infusion rates (Q2) were calculated using the formulae and parameters mentioned above and using the weight of the animal measured just prior to induction of anaesthesia.

4.4.5.B. Achievement of a Constant Drug Concentration in Vivo.

In later experiments (Chapters 6, 7 and 8) the methods of Mitenko and Ogilvie (1972) and Wagner (1974) described here, were used to rapidly achieve a constant plateau concentration in both the in vivo and IPPL preparations in order to determine hepatic elimination of lignocaine at similar mean hepatic affluent concentrations from 5 consecutive measurements. For the purpose of this work therefore, a constant (or steady state) lignocaine concentration was defined as a coefficient of variation of less than 10% (Tam et al, 1987) of the 5 consecutive lignocaine measurements in both vessels. This is illustrated graphically in Figure 4.5 which was generated using data from 3 in vivo experiments (described in chapter 6). In these three initial experiments all samples taken were analysed in order to establish when a constant concentration was consistently present. This was found to be the case over the second hour of lignocaine administration.

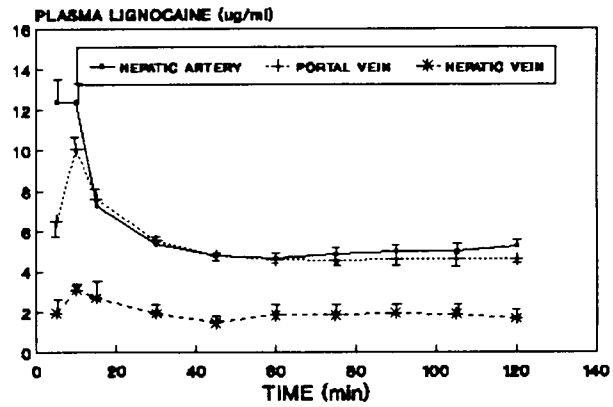

Figure 4.5 Mean (± SEM) plasma lignocaine concentrations in 3 anaesthetized pigs in the hepatic artery, portal vein and hepatic vein during a two stage lignocaine infusion.

4.4.5.C. Achievement Of A Constant Drug Concentration In The Isolated Perfused Pig Liver Preparation

(i) Lignocaine Administration

In contrast to the in vivo study the loading dose and infusion rate to achieve a plasma (perfusate) concentration of 5ug ml^{-1} in the IPPL were determined independent of the weights of the donor pigs.

As mentioned earlier it was thought that the disparities in the profiles of lignocaine decay seen in the isolated perfused liver preparation may have been contributed to by administering the lignocaine into the

hepatic venous reservoir with a resultant delay in passage to the oxygenator due to a possible 'reservoir effect '. To exclude this possibility two three way taps were placed in the tubing leading from the hepatic venous reservoir to the oxygenator Figure 2.2a-(H). The first tap was used to administer the bolus whilst the second was used for the continuous infusion.

The calulated loading dose of lignocaine hydrochloride was 39.32 mg so 40 mg was administered. This was diluted to facilitate administration as follows; 400 uL was aspirated from a freshly opened ampoule of Lignocaine Hydrochloride 10% using a calibrated Gilson pipette. This was then pipetted into an empty capped syringe 10ml syringe (Promex, South Africa) from which the plunger had been removed. The plunger was replaced by removing the cap and gently expelling air through the syringe nozzle whilst the lignocaine remained in the dependent part of the syringe. Ten millilitres of saline was then aspirated into the syringe. At time zero this was injected into the tubing directly supplying the oxygenator over a 10 second period.

The calculated infusion rate was determined to be 2.8 mg of lignocaine hydrochloride per minute. This was administered as 10% lignocaine hydrochloride using a precalibrated (Appendix A.3.1) infusion pump (Vial Medical SE 200, Paris) loaded with a 50ml Terumo syringe. The infusion pump was started at least one hour prior to switching the three way tap to go online to ensure that possible "deadspace" was eradicated. At the time indicated the second aperture of the three way tap through which the lignocaine had been escaping was capped and the tap turned to infuse the lignocaine.

(ii) Experiment

A single experiment was performed using the standard liver perfusion technique described in chapter 2 to assess whether and when a constant lignocaine concentration could be achieved using the methodology and parameters determined in this study.

(iii) Results.

All criteria for successful liver perfusion were met in this single liver perfusion and the biochemical values were similar to those of the control studies. Constant plasma lignocaine concentrations according to the above criteria (4.4.5.B) were present during the second hour of the lignocaine infusion. This is illustrated in Figure 4.6.

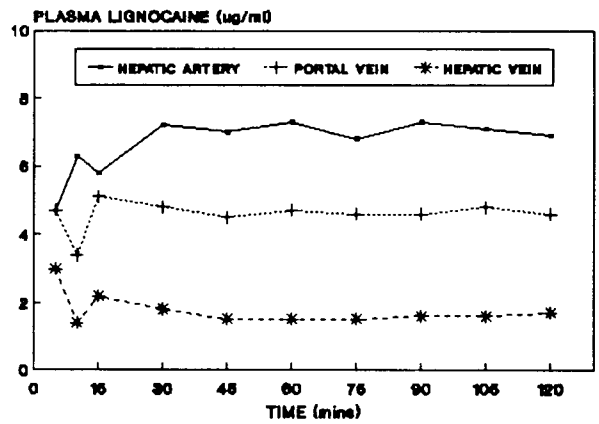

Figure 4.6 Plasma lignocaine concentration in the hepatic vessels of a single isolated perfused pig liver after a bolus dose and during a continuous infusion of lignocaine.

The reason for the difference in the hepatic artery and portal vein lignocaine concentrations is not clear. In later isolated liver experiments where the hepatic arterial to total blood flow ratio was higher this was not a consistent finding and was found not to be statistically significant (Table 8.4).

4.4.5.D. Conclusion

As a constant plasma concentration was achieved in both preparations for the second hour of lignocaine administration this period was chosen as the study period for the determination of hepatic lignocaine elimination in all subsequent experiments in vivo and ex vivo.

Data from studies performed later in this thesis showed that equivalent constant systemic plasma concentrations of lignocaine were achieved both in vivo [5.9 ± 0.5 ug ml^{-1} (n=7)] and ex vivo [5.6 ± 0.4 ug ml^{-1} (n=7)]. These concentrations were a little higher than the target plasma concentration of 5ug ml^{-1}. Nevertheless, this work indicates that the methodology described by Mitenko and Ogilvie (1972) and Wagner (1974) can be used successfully to achieve comparable constant plateau concentrations within one hour of continuous lignocaine administration in the anaesthetized pig and isolated perfused pig liver preparation.

In the ex vivo study as the elimination half life was short (± 10 min) another method of achieving a constant plasma concentration (Css) within one hour would have been by infusing a calculated maintenance infusion at a rate determined by (Css x V1 x K10). A steady state concentration would then have been obtained in 50 minutes (5 half lives).

4.4.6. COMMENTS ON PHARMACOKINETIC MODELLING

4.4.6.A. In Vivo Administration of Bolus Dose versus Infusion.

The kinetic analysis of the decay of lignocaine was performed in the present study after the administration of lignocaine hydrochloride over a period of 2 minutes to avoid potential toxicity of a dose of 120mg in a 25 Kg pig. Sampling for plasma lignocaine analysis was first performed 2 minutes after the completion of lignocaine administration. Pharmacokinetic theory assumes rapid administration of a bolus dose (Greenblatt and Koch-Weser, 1975) but the administration of lignocaine has been given over 3 minutes (Tucker and Mather,1975) and over one minute (Engelking et al, 1987)(Morishima et al, 1979) by others. Loo and Riegelman, (1970) have described a method for compensating for this infusion time as this may have an influence on the calculated intercept terms A and B which are the coefficients of α and β (the slopes of the distribution and elimination curves respectively). The calculation described was applied to the present data and the compensated values for these terms are given as A' and B' respectively (Table 4.3, Page 4.13 and Table D.2 Appendix D, Page D.11).

4.4.6.B. Compartmental versus Noncompartmental Pharmacokinetics

A more flexible approach for defining lignocaine kinetics both in vivo and ex vivo might have been to use "moment analysis" instead of the compartmental analysis which was used in this thesis. Statistical moment analysis of drug concentration time data following a single dose of a drug was first described by Yamoaka et al, in 1978. In contrast one of the first kinetic analyses of what is now known as the two compartment model was described by Teorel in 1937. Thus "moment analysis" is a relatively new way of describing the time course of drugs through the body. Hence a large body of kinetic data in the literature is presented in the 'conventional' form. As the intention was to assess lignocaine kinetics in the pig as well as the isolated perfused pig liver in order to use the method described by Mitenko and Ogilvie (1972) and Wagner (1974) to achieve a steady state lignocaine concentration at a predicted level, and as this required the determination of various conventional pharmacokinetic parameters, the method of analysis described in the thesis was adopted. (Beta-the slow phase hybrid disposition constant cannot be directly determined using moment analysis [Gibaldi, 1991]). In addition, the analysis performed in the thesis allowed the comparison of lignocaine kinetics in the pig with that of other animals and man (Section 4.5, below) using 'conventional' pharmacokinetic data from the literature. It must be born in mind however, that the conventional pharmacokinetic data could have been transformed into statistical moments, with the advantage of allowing the comparison of data fitted to differing compartmental models (Yamaoka et al, 1976).

4.5 COMPARISON OF LIGNOCAINE KINETICS IN THE PIG, OTHER ANIMALS AND MAN

One of the hypotheses of this thesis is that the pig is a good model for human lignocaine metabolism. As the disposition kinetics of lignocaine in the pig has not been reported it would be useful to compare this where possible with data from human studies and to assess this in the light of the limited number of studies done in other animals. Tables 4.5 and 4.6 draw such comparisons.

4.5.1 PROBLEMS OF DIRECT COMPARISON BETWEEN DIFFERENT STUDIES

Direct comparison of studies in different animals by different investigators must be done with circumspection. In order to minimise the potential differences the only data presented here is that where lignocaine decay was studied after an intravenous bolus dose (over a maximum of 3 minutes). However, other factors that may influence drug kinetics include; duration of sampling, assay methodology, sample size and sensitivity, drug dosage (and hence duration of detectable drug concentrations) sampling regimens, and kinetic analytical methods, to name but a few. In addition, the four animal studies and the human studies reported here were all performed in the awake state in contrast to the anaesthetized state of

the present study in the pig. Anaesthesia may have an effect on drug clearance and so drug pharmacokinetics principally in two ways.

4.5.1.A. Effects of Anaesthetic Agents

Anaesthetic agents may directly inhibit drug metabolism, affect disposition kinetics by decreasing hepatic blood flow, or alter protein binding. The latter is only of importance in poorly extracted drugs (Wilkinson and Shand, 1975) as in highly extracted drugs such as lignocaine, elimination is not restricted to unbound drug in the circulation (Tucker and Mather, 1975). With respect to an inhibitory effect on drug metabolism, halothane decreases the intrinsic clearance of propranolol (Reilly et al, 1985), and increases the half-life of ketamine, (White et al, 1976) fentanyl (Borel et al, 1982) and intravenous lignocaine (Bentley et al, 1983). This agent was thus avoided in favour of isoflurane which when compared with halothane in dogs had a less deleterious effect on hepatic blood flow and whilst halothane significantly prolonged ICG half-life this appeared not to be the case when isoflurane was used (Gelman et al, 1984).

4.5.1.B. Effects of Mechanical Ventilation

Mechanical ventilation on its own has been shown in man to prolong the half life and decrease the clearance of lignocaine (Richard et al, 1986) whilst not affecting the volume of distribution. This is probably due to a decrease in cardiac output and so hepatic blood flow. Thus although the anaesthetic technique used was chosen to limit potential interfering effects on lignocaine disposition this possibility must be borne in mind when comparing other studies.

4.5.2 COMPARISON OF LIGNOCAINE DISPOSITION

The distribution half lives ($t_{\frac{1}{2}}\alpha$) are remarkably similar in the animal studies (Table 4.4) and about half that in humans (Table 4.5) whilst the elimination half lives are more disparate in animals compared to the apparently consistent value in humans of around 100 minutes. The monkey has the shortest $t_{\frac{1}{2}}\beta$ (15 minutes) and the pig the longest (60 minutes). The volume of the central compartments (V1) in the pig (0.64 L kg^{-1}) and monkey (0.44 L kg^{-1}) is similar to that in man (0.44-0.53 L kg^{-1}) whilst the total volume of distribution (Vdarea) in the animals (excluding the newborn lamb) is remarkably similar. The larger volume of distribution of lignocaine in the new born lamb has also been reported in neonates (Mihaly et al, 1978). It is noteworthy especially as young pigs (± 12 weeks) were used in this work that both Morishima et al (1979) and (Mihaly et al, 1978) conclude that the prolonged half life of lignocaine in the newborn is not due to the impaired capacity for drug metabolism (clearance) but rather due to the large volume of distribution which results in a smaller fraction of drug being available to the clearing organ at any one time resulting in a slower rate of elimination.

Table: 4.5

Disposition Kinetics of Lignocaine in Animals after Intravenous Bolus Administration

AUTHOR		Morishima 1979		Benowitz 1974	Engelking 1987
ANIMAL	**PIG**	**SHEEP**	**LAMB (newborn)**	**MONKEY**	**HORSE**
NUMBER	5	7	7	3	3
BOLUS ($mg\ kg^{-1}$)	5.1	5	10	2-3	0.43
$T_{\frac{1}{2}}\alpha$	4	5	5	2	4
$T_{\frac{1}{2}}\beta$	60	31	51	15	37
V1 ($L\ kg^{-1}$)	0.64	0.90	1.42	0.44	-
V2($L\ kg^{-1}$)	1.52	-	-	-	-
Vdarea($L\ kg^{-1}$)	2.15	1.84	3.94	-	2.33
TOTAL CLEARANCE ($L\ min^{-1}$)	0.60	-	-	0.27*	-
UNIT CLEARANCE ($ml\ min^{-1}\ kg^{-1}$)	26	39	50	66*	44

$T_{\frac{1}{2}}$ = half-life in minutes, V1 & V2 = volume of distribution of the central and peripheral compartment respectively, Vdarea = dose (AUC)β. Total clearance = dose/AUC where AUC=area under plasma lignocaine concentration vs time curve. All volume and clearance figures refer to plasma data except * = blood clearance. For conversion of pig volume and clearance data to whole blood data divide by τ = 0.87 ± 0.02 where τ = whole blood to plasma drug concentration ratio determined for pig blood (Section 3.4.4) (Mean haematocrit = 33.5 ± 1 in pigs studied)

Table: 4.6

Disposition Kinetics of Lignocaine in Man after Intravenous Bolus Administration

AUTHOR	Rowland 1971	Tucker 1975	Thomson 1973	Pieper† 1987
NUMBER	10	5	10	75
BOLUS (mg)	50	200	50	25-200
$T_{\frac{1}{2}}\alpha$	7	10	8	-
$T_{\frac{1}{2}}\beta$	108	10	108	96
$T_{\frac{1}{2}}\tau$	-	96	-	-
V1($L\ kg^{-1}$)	0.44	-	0.53	0.48
TOTAL CLEARANCE ($L\ min^{-1}$)	0.72	0.79*	-	-
UNIT CLEARANCE ($ml\ min^{-1}\ kg^{-1}$)	9	12*	10	16

$T_{\frac{1}{2}}$ = half-life in minutes, V1 = volume of distribution of the central compartment, Total clearance = dose/area under plasma lignocaine concentration vs time curve. All volume and clearance figures refer to plasma data. * Corresponding values for plasma converted from whole blood data.† = pooled data.

Unit hepatic clearance after a bolus dose is remarkably consistent in the human studies performed (± 12mL $kg^{-1}min^{-1}$) but is substantially less than that determined for animals. Here the pig model is closer to humans, whilst the monkey has the highest value for clearance.

Thus in summary lignocaine kinetics after a bolus was best described by a two compartment model in the pig which confirmed the findings of the above mentioned authors (Table 4.5) however De Jonge et al (1972) found in cats and Tucker and Mather (1975), in humans that the decay of lignocaine could best be described using a three compartmental model. Comparing studies in humans and comparable studies of lignocaine decay in animals with this pig model reveals that the distribution half life and unit clearance of lignocaine in the pig is closer to that in humans than the other animals while the central volume of distribution together with that determined for monkeys is similar to that in man.

4.5.3 COMPARISON OF LIGNOCAINE CLEARANCE IN VIVO AND EX VIVO

This experiment had not been designed expressly to compare in vivo and ex vivo lignocaine clearance, but it is interesting to note that the total plasma clearance (dose ÷ AUC) of lignocaine (base) in the two preparations was 605.4 ± 77 ml min^{-1} in vivo and 371.5 ± 31 ml min^{-1} ex vivo. Although this was not statistically different the trend may suggest that hepatic clearance of lignocaine is impaired ex vivo or that extrahepatic lignocaine clearance is occurring in vivo. Data determined in chapter 6 suggest that the latter is true as extra hepatic clearance of lignocaine was found to occur whilst in vivo and ex vivo lignocaine clearance, determined using a direct method, was no different. This supports the need for studying hepatic extraction by the liver directly in a suitably designed experiment.

CHAPTER 5: COMPARATIVE STUDY OF IN VIVO AND EX VIVO HEPATIC PHYSIOLOGY AND FUNCTION

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CHAPTER 5: COMPARATIVE STUDY OF IN VIVO AND EX VIVO HEPATIC PHYSIOLOGY AND FUNCTION

SUMMARY

In this pilot experiment, preliminary to the study of lignocaine extraction and clearance, hepatic blood flow and function was compared in vivo and ex vivo by making use of the same liver in a sequential study. This study was performed to determine (a) whether in vivo and ex vivo hepatic function was different when the same liver was used, (b) whether the preparations were stable and if not to devise appropriate modifications to achieve this, and (c) the appropriateness of using the same liver for this comparison by comparing the ex vivo liver group with a group of isolated livers not previously studied in vivo. The in vivo and ex vivo livers were different in ATP content, energy charge, oxygen consumption and bile flow. Ex vivo the livers remained apparently viable over the two hour study period maintaining TAN content whilst decreasing perfusate potassium concentration. The in vivo preparation was less stable as hepatic blood flow and plasma composition varied with time, probably as a result of inappropriate fluid therapy. Use of the same liver in a sequential study resulted in a detrimental effect on the adenine nucleotide status of these livers ex vivo when compared with livers studied only in the isolated state, suggesting that this study design is inappropriate for comparing in vivo and ex vivo hepatic lignocaine extraction and clearance.

5.1 INTRODUCTION

5.1.1 MOTIVATION

This study was designed as a preliminary to the comparison of hepatic lignocaine metabolism in vivo and ex vivo (Chapter 8). Certain studies have suggested that the function of the isolated perfused liver cannot be regarded as identical to function in vivo (Tygstrup et al, 1971)(Welch and Parbhoo, 1973)(Elmslie et al, 1971) and is likely to deteriorate with the passage of time (Gores et al, 1986). As significant interanimal variability in liver function (Van Dyke et al, 1983) and interindividual variation in hepatic drug metabolism

(Vesell, 1984) has been observed it was decided to determine whether hepatic function was also different when in vivo and ex vivo function was compared using the same liver. Further, it was pertinent to document potential deterioration of either preparation over the study period. Thus the aim of this pilot study was to:

(a) Determine changes in and differences between the in vivo and ex vivo preparations over the envisioned two hour study period with respect to:

(1) Hepatic blood flow,

(2) Plasma or perfusate composition and

(3) Indices of liver function

and thereafter to attempt to improve the models for the definitive studies involving lignocaine.

(b) Determine the feasibility of using the same liver for the in vivo and ex vivo comparison.

(c) Determine the correlates of hepatic function in the absence of lignocaine both in vivo and ex vivo.

5.1.2 STUDY DESIGN

As the same liver was to be used both in vivo and ex vivo a sequential study of liver function was thought appropriate. Thus hepatic function was first studied in vivo in the anaesthetized animal and then immediately after this as an isolated perfused preparation (Experiment A). Implicit in using the same liver to compare in vivo and ex vivo hepatic function was that the prior in vivo study did not damage the liver. In order to investigate this as yet unanswered question the group of pig livers perfused after an initial in vivo study was compared with a second group of perfused livers studied only ex vivo (Experiment B).

5.1.2.A. Sequential Study

There are inherent advantages and disadvantages in the proposed study design.

(i) Advantages of immediate comparison of liver function

(a) Anaesthetic and surgical impairment of hepatic function is minimised

Anaesthesia and surgery, especially upper abdominal surgery, decrease hepatic blood flow (Gelman, 1976) (Brown,1988) and may impair hepatic function (Brown, 1988) possibly due to hepatic oxygen deprivation (Gelman, 1987b). Hepatic ischaemia has been shown to result in significant biochemical and long term morphological changes (Swenson et al, 1967). By using a sequential study and so avoiding the detrimental effect of a second anaesthetic (Misra et al, 1972) and the need for repeat surgery on a subsequent occasion (for the purpose of liver harvesting) it was hoped to minimise further potential liver impairment and so a further possible discrepancy between the in vivo and ex vivo liver.

(b) The potential detrimental influence of infection and the need for antibiotic prophylaxis is avoided

With immediate study of the liver the potential for any detrimental effects of infection on liver function is excluded. This obviates the need for antibiotic prophylaxis which might affect ex vivo liver function as different antibiotics have varying effects on liver enzyme induction and inhibition (Calvey and Williams, 1985). Further, the presence of antibiotics in plasma samples taken for lignocaine analysis might complicate chromatographic analysis.

(c) Liver enzyme induction by administered anaesthetic agents is likely to be minimal

Enzyme induction by a drug such as thiopentone, used to anaesthetize the animal, is unlikely to effect the study ex vivo as this peaks from 24-48 hours after exposure (Gillette, 1971)(Cooksley and Powell, 1971) and will thus not result in a discrepant effect on in vivo and ex vivo hepatic function.

(ii) Disadvantages of immediate comparison of liver function

(a) The build up of an administered substance may affect its subsequent hepatic extraction

A potential disadvantage of studying the hepatic extraction and clearance of an administered substance in this fashion is that build up of the substance may occur after the first administration in vivo which could affect the subsequent extraction of the substance on repeat administration ex vivo.

(iii) Advantages and disadvantages of delayed comparison of liver function using the same liver

These follow largely from the foregoing.

In the event of a delayed comparison of in vivo and ex vivo function being performed, the question arises how long the ex vivo study should be delayed after a prior in vivo study. This decision would rest on the time taken for:

(a) the effects of anaesthesia and surgery on hepatic blood flow and function to return to normal as well as

(b) the total elimination of all administered drugs and their metabolites.

With regard to the former (a) as far as known this has not been determined and thus investigators appear to assign arbitrary times to this of between 2 (Mather et al, 1986) and 6 weeks (Lundeen et al, 1983). With regard to the latter consideration, (b) to avoid the potential inhibitory effect of metabolites on drug elimination as has been suggested for MEGX with respect to lignocaine (Thomson et al, 1987) the time required for the elimination of the respective metabolites should be known.

It is clear that a delayed study would be more complex and expensive, requiring the prolonged storage of animals, possibly the use of prophylactic antibiotics and that repeat surgery performed for liver resection is likely to be more difficult as a result of adhesions developing after the prior surgical operation.

5.1.3 ASSESSMENT OF HEPATIC FUNCTION IN THE EXPERIMENTAL LIVER

The liver has many functions (Abouna et al,1969) each of which may deteriorate separately. Thus most investigators use a variety of tests. Gores et al, (1986) suggest that for the assessment of isolated perfused rat liver viability, serial measurements of bile flow, oxygen extraction, pH, and perfusate concentrations of potassium as well as the determination of the activities of cytosolic enzymes provide an adequate global assessment of viability. In addition to these tests other workers have assessed the gross appearance of the isolated liver (Vang et al, 1966)(Jablonski et al, 1971)(Drapanas et al, 1966). The clearance of various administered compounds has been used to assess viability including galactose (Winkler et al, 1971)(Welch and Parbhoo, 1973)(Tygstrup et al, 1971)(Abouna et al, 1969); ammonia (Jablonski et al, 1971)(Abouna et al, 1969)(Eiseman et al, 1961); indocyanine green (Winkler et al, 1971)(Tygstrup et al, 1971) and bromosulpthalein (Ham et al, 1969)(Abouna et al, 1969)(Jablonski et al, 1971). Adenine nucleotide status has been used by a number of investigators, (Rabol et al, 1974)(Kamiike et al, 1988)(Tygstrup et al, 1971). Successful transplantation would appear to be the best test (Hickman et al, 1971) but this is in general not practicable.

In the present experiments all the tests recommended by Gores et al, (1986), (see above) were used. Aspartate aminotransferase was measured as the cytosolic enzyme.

Hepatic adenine nucleotides (ATP, ADP, AMP) were measured allowing calculation of the adenylate energy charge proposed by Atkinson, (1968) which appears to be a good indicator of liver viability especially when assessed in conjunction with the total adenine nucleotide (TAN) status (Kamiike et al, 1988). The rise in urea concentration was measured and taken as urea production. Except for the brain, the liver is the only site of urea formation (Ganong, 1983). Jablonski et al, (1971) monitored a rise of urea on serial sampling in 9 out of 10 IPPL perfusions, but the increase could not be related to the success of perfusion as judged by hepatic blood flow, hepatic arterial pressure and bile flow. Hems et al, (1966) assessed the performance of the liver by the rate of urea synthesis after administration of ammonia arguing that together with gluconeogenesis this is the most exacting synthetic function of the liver in terms of ATP requirements. Although the measurement of the clearance of exogenous substances may be useful in the assessment of hepatic function (vide supra) this might interfere with the subsequent evaluation of lignocaine metabolism as loading with certain substrates (ammonia and amino acids) may increase oxygen consumption by the isolated liver (Tygstrup et al, 1975). These tests were therefore not employed.

However, as the clearance of lactate is regarded as a useful indicator of hepatic function (Drapanas et al, 1966)(Abouna et al, 1969)(Vang and Drapanas, 1966) it was decided to establish whether clearance of endogenous lactate could be used as a comparative indicator of in vivo and ex vivo function.

5.2 MATERIALS AND METHODS

5.2.1 PROTOCOL

Six male pigs between 21-24kg were studied in Experiment (A) (in vivo vs ex vivo comparison) and a further 6 in Experiment (B) (ex vivo vs ex vivo comparison). Hepatic function, blood flow and plasma or perfusate composition were studied under anaesthesia (group 1) and then immediately afterwards as an isolated perfused preparation (group 2) in Experiment (A) whilst in Experiment (B) these were studied only ex vivo.

5.2.2 IN VIVO PREPARATION

The animals were anaesthetized and ventilated as described in sections (2.2.1, 2.2.2 & 2.2.3). The pigs were surgically prepared for liver resection, transhepatic sampling and continuous hepatic blood flow measurement (sections 2.3.1 A & B). This was followed by a half-hour stabilisation period followed by a two hour hepatic function study period.
During the course of the in vivo study intravenous fluid replacement was given via the internal jugular vein catheter [Plamalyte B with dextrose (2.5%), Appendix F.1.1] This was administered at a fixed rate of one litre per hour.

5.2.3 EX VIVO PREPARATION

After the two hour in vivo study, (Experiment A) or immediately (Experiment B), the liver was resected as described earlier (section 2.3.2) and isolated perfusion established (section 2.5). A further two hour hepatic function study was then performed ex vivo. In Experiment A the time interval between the in vivo and ex vivo study periods was approximately two hours.

5.2.4 HEPATIC FUNCTION ASSESSMENT

Transhepatic and hepatic artery samples for biochemical analysis (section 2.5.2.B.) were taken both in vivo and ex vivo during the course of each two hour study period. The first, at time zero and then at one hour and two hours respectively. In both studies samples were drawn in a standard order: hepatic artery, portal vein and then hepatic vein. The hepatic vein samples taken in vivo were aspirated over 30 seconds to minimise the possibility of entraining non hepatic venous blood from the inferior vena cava. Hourly liver biopsies were taken to assess adenine nucleotide status (section 2.5.2.B.).

5.2.5 CALCULATIONS

Liver uptake of lactate was calculated as the:
(HA concentration - HV concentration) x HA flow min^{-1} + (PV concentration - HV concentration) x PV flow min^{-1} and expressed as the uptake (+) or release (-) per 100g liver mass.
Oxygen content and Energy Charge were calculated as described (Appendix B.8 and B.3.2) and oxygen consumption determined using the formulae above with oxygen content substituted in place of concentration.

5.2.6 STATISTICS

Statistical analysis was performed by Dr DO Chalton of the Medical Research Council Biostatistics Unit using the SAS statistical package (SAS Institute Inc.,USA). Repeated measures analysis of variance was used to determine within and between group differences (Crowder and Hand, 1990). Pearsons correlation coefficient was used to generate correlation matrices between all data separately in vivo and ex vivo. $P < 0.05$ was regarded as significant. Results are expressed as mean ± 1 standard error of the mean.

5.3 RESULTS (EXPERIMENT A)

Six animals made up this study, fulfilling the criteria for successful isolated liver perfusion defined in section 2.5.3.

5.3.1 HEPATIC BLOOD FLOW

The portal vein flow (PVQ) and hence the total hepatic blood flow (THBF) measured in vivo (Figure 5.1) showed a downward trend after the first 60 minutes of the study period which became statistically significant at 120 minutes. In contrast, the hepatic artery flow (HAQ) did not deteriorate with time. The mean arterial percentage of THBF taken over the two hour study was 25.9 ± 1.3%.

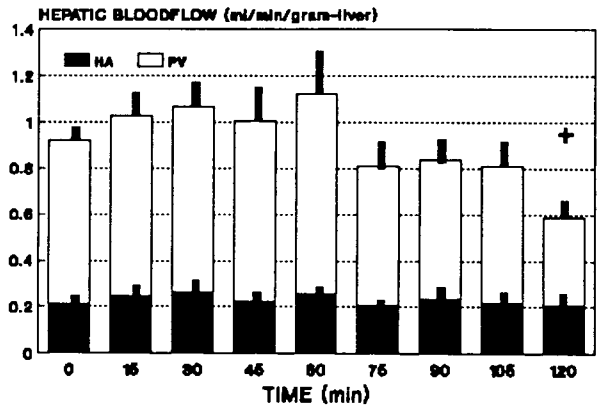


Figure 5.1 In vivo hepatic arterial and portal venous blood flow (n=6) over the two hour study period. + = PV and total hepatic blood flow (HA + PV) significantly different from blood flow at times 0,15,30,45 and 60 minutes.

In the isolated liver where the flows were set and unaltered the arterial percentage of total flow was 17.8 ± 0.1$ml g^{-1} min^{-1}$ which was significantly less than in vivo, whilst the total flow was 0.84 ± 0.03$ml g^{-1} min^{-1}$ which was 92% of the mean flow of 0.91 ± 0.16$ml g^{-1} min^{-1}$ in vivo and was not significantly different.

5.3.2 PLASMA AND PERFUSATE COMPOSITION.

Comparison of the blood pH, temperature, haemoglobin, and haematocrit as well plasma perfusate glucose (Table 5.1) showed no significant difference or variation with time between the in vivo and ex vivo study. This was true for the HA, PV and HV concentrations of lactate as well.

Table 5.1

In Vivo Plasma and Ex Vivo Perfusate Composition

Parameter	Study Time (Hrs) 0		1	2
Sodium (mmol L^{-1})	in	134 ± 3	* 134 ± 3	* 134 ± 3
	out	136 ± 1	* 139 ± 1^{a}	* 143 ± 3^{ab}
Total Protein	in	* 44 ± 4	* 42 ± 3	* 39 ± 2^{a}
(g $100ml^{-1}$)	out	* 54 ± 3	* 58 ± 3	* 57 ± 2
Albumin	in	36 ± 2	* 33 ± 1^{a}	* 32 ± 1^{a}
(gm $100ml^{-1}$)	out	36 ± 1	* 36 ± 1	* 36 ± 1
Osmolality	in	255 ± 6	* 243 ± 5^{a}	* 245 ± 6^{a}
(mosmols L^{-1})	out	260 ± 6	* 256 ± 7	* 264 ± 6
Haematocrit	in	35 ± 1	34 ± 3	36 ± 2
	out	35 ± 1	34 ± 1	36 ± 1
Haemoglobin (g%)	in	11.6 ± 0.3	11.5 ± 0.5	12.1 ± 0.6
	out	11.5 ± 0.5	11.9 ± 0.4	12.1 ± 0.3
Glucose	in	278 ± 43	216 ± 5.2	247 ± 52
(mg $100ml^{-1}$)	out	331 ± 125	235 ± 9.6	212 ± 86
Lactate (HA)	in	21.8 ± 6.1	22.2 ± 6.3	23.5 ± 6.8
(mgm $100ml^{-1}$)	out	19.3 ± 6.5	18.0 ± 4.6	19.6 ± 3.1
Lactate (PV)	in	23.9 ± 8.0	21.8 ± 7.9	25.8 ± 8.6
(mgm $100ml^{-1}$)	out	25.8 ± 6.9	18.3 ± 5.5	21.0 ± 3.8
Lactate (HV)	in	19.4 ± 6.5	13.0 ± 3.9	16.03 ± 2.2
(mgm $100ml^{-1}$)	out	24.7 ± 4.5	16.30 ± 7.7	18.0 ± 7.8
Hepatic Venous	in	* 48.6 ± 11.4	* 63.8 ± 9.5	* 42.6 ± 12.92
PO_2(mmHg)	out	* 103.4 ± 3.7	* 104.9 ± 6.1	* 103.4 ± 11.4
Urea (mg $100ml^{-1}$)	in	15.8 ± 2.4	15.1 ± 2.1	* 14.8 ± 2.3
	out	16.5 ± 2.4	19.0 ± 2.3	* 21.3 ± 4.4
Temperature	in	38.1 ± 0.2	38.2 ± 0.4	38.3 ± 0.4
(degrees celcius)	out	38.5 ± 0.2	38.3 ± 0.1	38.1 ± 0.1
pH	in	7.41 ± 0.2	7.40 ± 0.2	7.41 ± 0.2
	out	7.41 ± 0.2	7.44 ± 0.8	7.42 ± 0.2

Mean ± (SEM) n = 6. in = in vivo, out = exvivo. * = significant difference between in vivo and ex vivo study. (a) = significant difference with time = 0. (b) = significant difference between time 1 and 2 hours. p = < 0.05

However the sodium concentration, total protein, albumin and osmolality did differ and vary with time in certain instances; despite similar plasma and perfusate sodium, albumin and osmolality values at time (0), significant differences occurred at the 1 and 2 hour sampling times because of a rise in sodium ex vivo and a fall in albumin concentration and osmolality in vivo. The total protein concentration was significantly higher ex vivo at time (0), remaining stable with time but the concentration decreased in vivo. Although the urea concentration rose with time ex vivo and fell in vivo these trends did not achieve statistical significance but did result in a signifcant difference between the groups after 2 hours. Hepatic venous PaO_2 was significantly less in vivo. (This was not significantly correlated with oxygen consumption.)

5.3.3 PARAMETERS OF LIVER FUNCTION AND INJURY

5.3.3.A. Oxygen Consumption

The unit oxygen consumption (Figure 5.2) ex vivo, (2.6 ± 5.7ml $min^{-1}100gliver^{-1}$ at time 0), remained stable over the two hour study period but was significantly less than in vivo where there was a significant decrease with time (6.5 ± 0.9ml $min^{-1}100gliver^{-1}$ at time=0 to 4.9 ± 0.5ml $min^{-1}100gliver^{-1}$ two hours later). In vivo there was a positive correlation of hepatic oxygen consumption with the aspartate aminotransferase (AST) and urea concentration and an inverse correlation with hepatic ATP, energy charge and sodium concentration (Table 5.2.A). Ex vivo there was also a negative correlation with hepatic ATP as as well as total adenine nucleotides (TAN), pH, hourly bile flow and potassium concentration (Table 5.2.B).

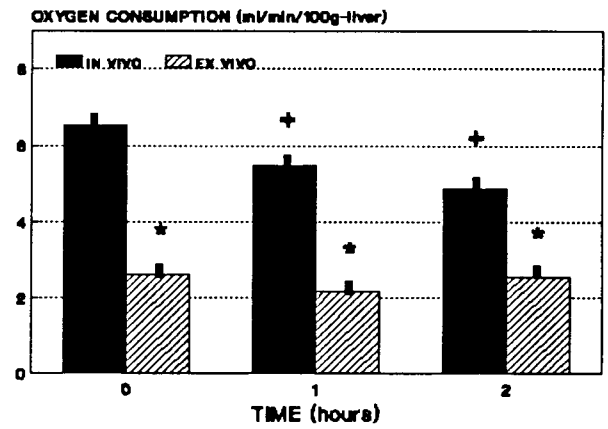


*Figure 5.2 Hepatic oxygen consumption by the same livers in vivo and ex vivo. * = difference in vivo and ex vivo and + = difference with time from 0 hours.*

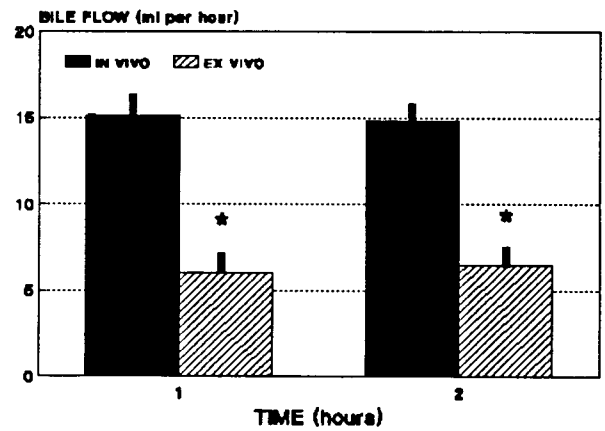


*Figure 5.3 Hourly bile flow measured in the same livers in vivo and ex vivo. * = difference in vivo and ex vivo.*

5.3.3.B. BILE FLOW

There was a significant decrease in hourly bile volume from 6.0 ± 1 ml in vivo to 1.5 ± 0.1ml ex vivo at time=0, but no variation with time in either preparation (Figure 5.3). There was a positive correlation of hourly bile flow with sodium concentration and an inverse correlation with oxygen consumption ex vivo (Table 5.2.B). The weak correlation both in vivo and ex vivo with pH did not achieve significance (p=0.08 in both).

Table 5.2.A.

Correlations between functions of the pig liver....in vivo.

	AST	pH	BF	K+	O_2con	Na+	Lac U.	TAN	ATP	EC	Urea	$HVPO_2$
AST		-.23	-.12	-.12	+.49	-.29	-.00	+.05	-.18	-.22	+.38	-.10
pH			+.51	-.33	+.08	-.14	+.16	-.14	-.11	+.01	-.15	
BF				-.09	+.21	+.33	+.22	-.23	+.15	+.32	-.34	-.20
K+					-.35	+.20-	-.17	+.28	+.19	+.12	-.42	-.15
O_2 Con						-.52	+.38	-.45	-.46	-.47	+.65	+.39
Na+							-.11	-.05	+.69	+.67	-.51	
Lac U.								-.22	+.21	+.19	+.13	-.35
TAN									-.01	-.02	-.32	+.08
ATP										+.95*	-.25	-.15
EC										+.80*	-.21	-.17

AST = Aspartate Aminotransferase, BF = bile flow, K+ = potassium concentration and Na+ = sodium concentration, O_2 con = oxygen consumption, Lac U = Lactate uptake, TAN = Total Adenine Nucleotides, EC = energy charge, $HVPO_2$ = hepatic venous PO_2
Correlation coefficients are marked___,___* for significance at the P<0.05 and P < 0.01 level (n=18).

Table 5.2.B

Correlations between functions of the pig liver....ex vivo.

	AST	pH	BF	K+	O_2con	Na+	Lac U.	TAN	ATP	EC	Urea	$HVPO_2$
AST		-.23	-.35	-.44	+.41	+.28	+.29	-.13	-.34	-.34	+.60*	-.29
pH			+.52	+.33	-.55	+.22	-.36	+.11	+.57	+.57	-.30	
BF				+.48	-.63	+.76*	-.14	+.10	+.44	+.35	+.02	-.20
K+					-.65*	-.54	+.02	+.61	+.63	+.24	-.15	
O_2 Con						-.16	-.04	-.67*	-.58	-.17	+.39	-.36
Na+							-.05	-.09	+.12	+.14	+.36	
Lac U.								-.01	-.50	-.67*	+.08	-.52
TAN									+.62	+.13	-.28	-.35
ATP										+.80*	+.70*	-.76*
EC											-.03	+.79

AST = Aspartate Aminotransferase, BF = bile flow, K+ = potassium concentration, Na+ = sodium concentration, O_2 cons = oxygen consumption, Lac U = Lactate uptake TAN = Total Adenine Nucleotides, EC = energy charge, $HVPO_2$ = hepatic venous PO_2
Correlation coefficients are marked___,___* for significance at the P<0.05 and P < 0.01 level (n=18).

5.3.3.C. Adenine Nucleotide Status

The hepatic adenosine triphosphate (ATP) level (Figure 5.4) ex vivo, (4.1 ± 0.7uM-gm-liver^{-1}) was significantly less than in vivo (6.3 ± 0.5uM-gm-liver^{-1}) at time=0 where an increase occurred with time over the first hour of the study. Despite this the total adenine nucleotides (TAN) (ATP + ADP + AMP) did not differ or vary with time; (12.5 ± 0.6 in vivo and 11.7 ± 0.6 uM-gm-liver^{-1} ex vivo at time=0)(Figure 5.5). Adenosine diphosphate (ADP) and adenine monophosphate (AMP) were stable over the two hour period and no different between the two groups; although hepatic AMP tended to be higher ex vivo (Figure 5.4).

Energy charge (EC) was less ex vivo at 0 and 2hours (Figure 5.5). The values were 0.620 ± 0.033 in vivo vs 0.551 ± 0.061 ex vivo at time=0 and 0.658 ± 0.016 and 0.549 ± 0.070 respectively at 2 hours.

ATP was negatively correlated with hepatic oxygen consumption both in vivo and ex vivo and positively correlated with potassium and pH ex vivo (Table 5.2.A & B). Hepatic energy charge was negatively correlated with oxygen consumption in vivo and positively correlated with pH ex vivo.

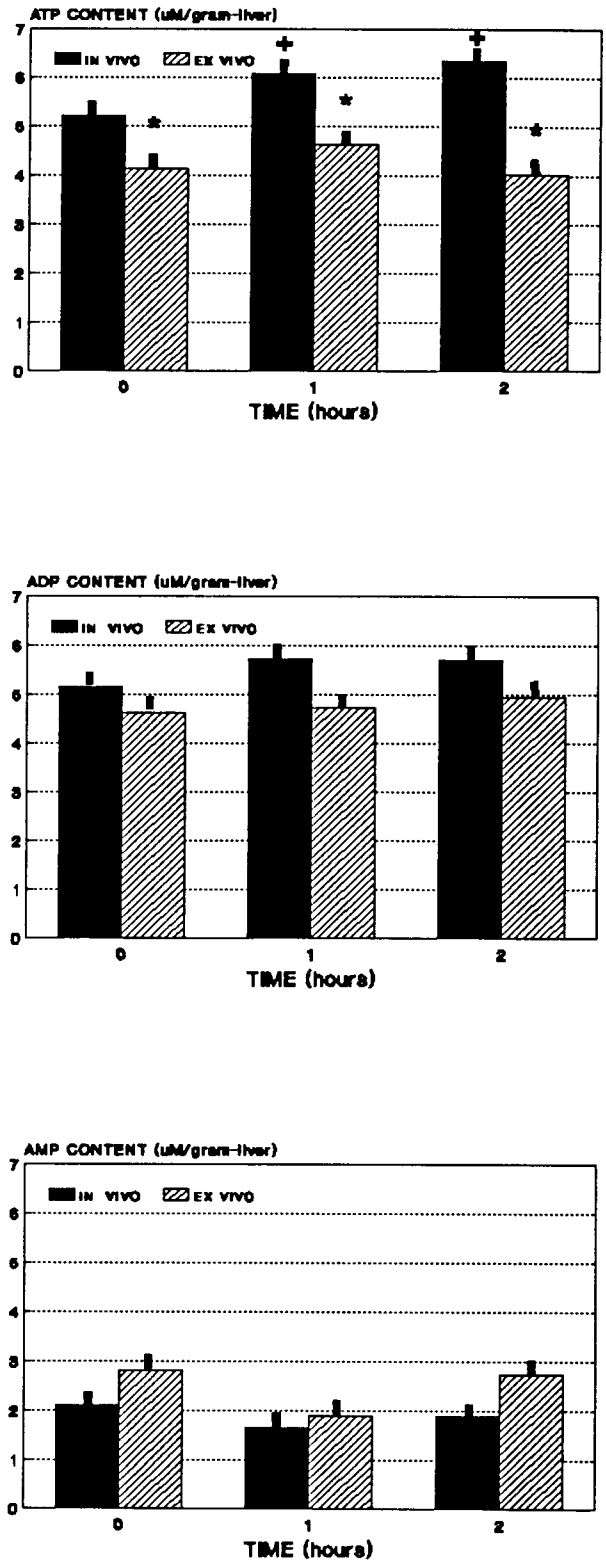


*Figure 5.4 Hepatic adenine nucleotide content in the same liver in vivo and ex vivo. * = difference in vivo and ex vivo. + variation with time from time = 0.*

5.3.3.D. Potassium

In vivo there was a significant hourly rise in potassium concentration while ex vivo there was an hourly decrease. Hence, despite an initial difference in concentration at time zero, (3.7 ± 0.1mmol L^{-1} in vivo vs 6.4 ± 0.3mmol L^{-1} ex vivo) after two hours the potassium concentrations were similar in the two studies, (4.6 ± 0.3 vs 4.8 ± 0.1 mmol L^{-1}) respectively (Figure 5.6). Ex vivo potassium correlated inversely with oxygen consumption and with sodium concentration and positively with TAN and ATP (Table 5.2.B).

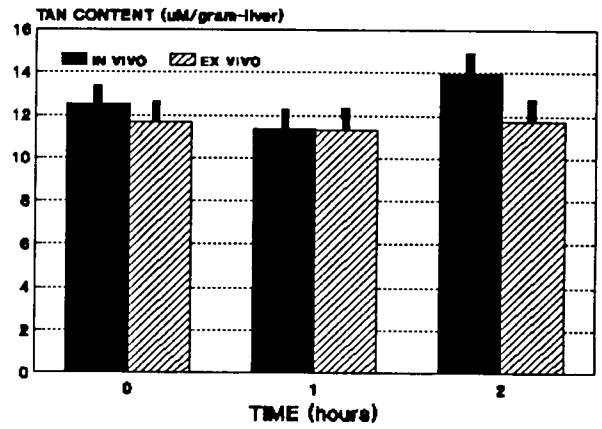


Figure 5.5 Hepatic TAN content and energy charge in the same livers in vivo and ex vivo.

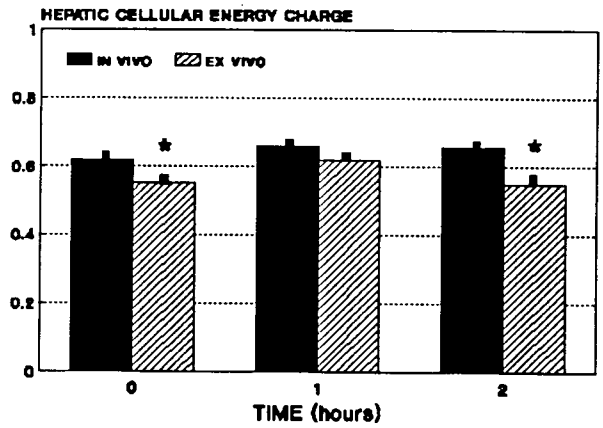
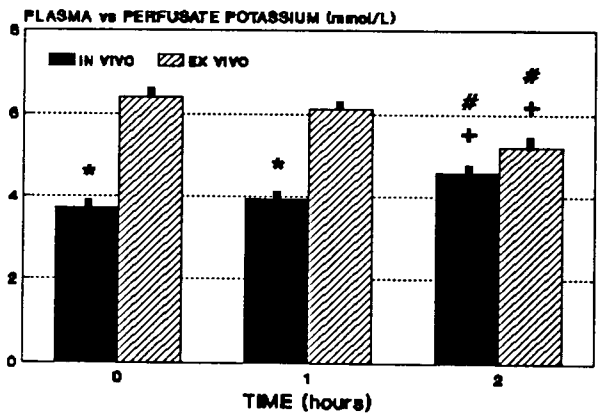


*Figure 5.6 Plasma and perfusate potassium concentration in vivo and ex vivo. * = difference in vivo and ex vivo. + variation with time from time = 0. # = difference with time from 1 hour.*



5.3.3.E. Aspartate Aminotransferase

The AST concentration was significantly higher ex vivo at all time points studied, (76 ± 5 vs 50 ± 6 UL^{-1} at time=0). Ex vivo the perfusate AST concentration increased with time reaching significance after two hours at a level of 99 ± 10 UL^{-1}) (Figure 5.7). AST correlated positively with oxygen consumption in vivo (Table: 5.2.A).

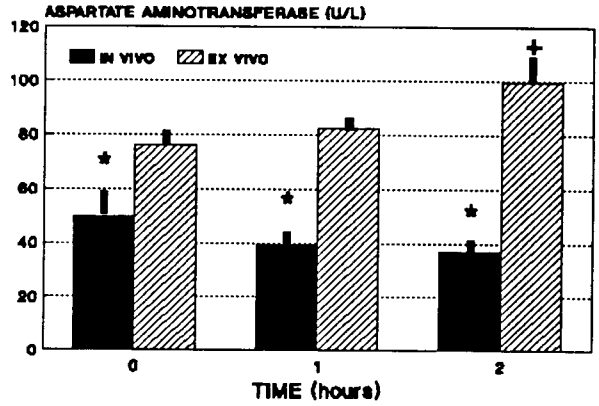


*Figure 5.7 Plasma and perfusate aspartate aminotransferase concentration in vivo and ex vivo. * = difference in vivo and ex vivo. + variation with time from time = 0.*

5.3.3.F. Hepatic Lactate Uptake:

The HA, PV and HV lactate concentrations (Table 5.1) and lactate utilisation in vivo and ex vivo (Figure 5.8) were similar and did not vary significantly with time. [Lactate utilisation in vivo at time: 0,1 and 2 hours was (5.6 ± 3.6), (3.3 ± 2.4) and (5.4 ± 2.0), and (1.0 ± 1.0), (1.7 ± 1.7) and (3.6 ± 1.9)mg $min^{-1}100gliver^{-1}$ ex vivo respectively]. However in both in vivo and ex vivo preparations there was no consistent uptake of lactate by the liver as within studies both lactate release and uptake occurred. The mean values of lactate uptake may not clearly reflect this and thus figure 5.8 has been constructed to illustrate this point. Lactate uptake correlated inversely

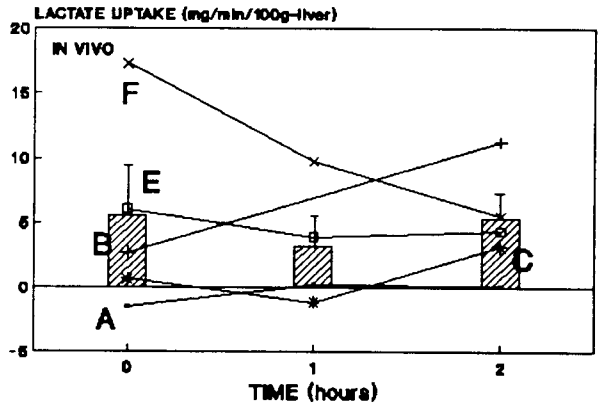
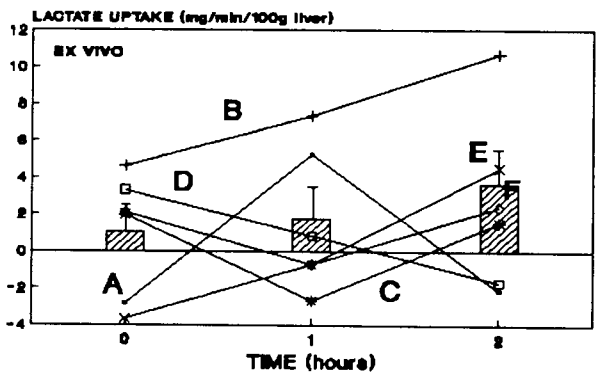


Figure 5.8 Lactate uptake (+) and release (-) by the same livers (A-F) in vivo and ex vivo. Individual liver utilisation as well as group means are represented.



with hepatic venous O_2 and energy charge ex vivo (Table 5.2.A). There was no correlation with hepatic blood flow in vivo.

5.3.4 SUMMARY OF RESULTS

Although the in vivo and ex vivo mean total hepatic blood flow were similar over the two hour study period total hepatic blood flow decreased with time in vivo but remained stable ex vivo whilst the hepatic artery to total hepatic blood flow ratio was less ex vivo. The plasma and perfusate composition was essentially similar at the start of the respective studies except for a higher perfusate protein concentration which persisted. Plasma osmolality, total protein and albumin then decreased while perfusate sodium increased with time resulting in significant differences with regard to these values as well as to urea concentration at the end of the two hour study period.
Hepatic oxygen consumption, ATP content, energy charge and hourly bile flow were significantly less ex vivo while hepatic TAN content, and lactate uptake were no different throughout the study. Initial perfusate potassium and AST concentrations were higher ex vivo but the former decreased resulting in similar concentrations at the end of the study while AST increased still further with time.

5.4 DISCUSSION (EXPERIMENT A)

5.4.1 INTRODUCTION

Although several other workers have compared function of the liver in vivo with that ex vivo (Tygstrup et al, 1971)(Welch and Parbhoo, 1973) using different livers, the only other reported study performed using the same liver (Elmslie et al, 1971) assessed ethanol and bromosulphalein elimination but did not compare the parameters studied here. Further, these investigators perfused only the portal vein ex vivo which may have significant implications for hepatic function (Drapanas et al, 1966).

5.4.2 GOALS OF PILOT STUDY

The purpose of this pilot study was:
(a) To determine whether the in vivo and ex vivo preparations remained stable in terms of blood flow, plasma or perfusate composition and liver function indices over the study period and
(b) where in vivo liver function remained stable, use these parameters as a basis for assessing the viability and stability of the isolated perfused liver as a model for in vivo hepatic function.

(c) To determine what factors undermined stability of the in vivo and ex vivo preparations (if any) and attempt to correct these in future experiments.
(d) To determine whether the same liver could be used in a sequential experiment to compare the two models.

5.4.3 HEPATIC BLOOD FLOW

Since hepatic blood flow in vivo may be affected by the extent of surgery, the method of ventilation, the $PaCO_2$ and the anaesthetic technique used (Gelman, 1987b)(Gelman, 1989) these factors were standardized as far as possible as described in sections (2.2.2) and (2.2.3). Hepatic blood flow in vivo varied significantly with time, declining after the first 60 minutes of the study (Figure 5.1). There was considerable variation in hepatic blood flow between experimental animals. This has been reported for anaesthetized dogs (Katz and Bergman, 1969) and conscious sheep (Runciman et al, 1984). In this respect the in vivo preparation could not be regarded as stable in comparison with the ex vivo preparation where hepatic blood flow could be controlled. Stability of hepatic blood flow is an advantage for the study of the hepatic extraction of highly extracted drugs as it allows more readily the achievement of a constant concentration (Wilkinson, 1975).
The reason for the unstable hepatic blood flow in vivo is not immediately apparent but may have been due to inappropriately standardised fluid therapy with inadequate knowledge of cardiac filling pressures. Although this could be a time dependent effect, Stenson et al, (1971) measured cardiac output on two occasions in awake humans 100 minutes apart and could not detect a time dependent change in cardiac output.
The mean total hepatic blood flow in vivo of 0.91 ml gm^{-1} was less than that reported by Tygstrup et al (1971) of 1.03 ml gm^{-1} and 1.125 ml gm^{-1} measured with microspheres in dogs (Gelman et al 1984). The latter difference could be due to a species difference (Richardson and Withrington, 1981), or on the basis of a different mode of ventilation and hence mean intrathoracic pressure ($PaC0_2$ was held in the same range as in the present experiments)(Gelman et al, 1989) or because of a different technique of hepatic blood flow determination; Gelman et al (1984) determined hepatic blood flow using radiolabelled microspheres where portal venous flow is not determined but assumed to equal splanchnic flow (Runciman et al, 1984).
In the isolated preparation, total hepatic blood flow of 0.84ml gm^{-1} was similar to that in vivo and was comparable to that reported by others (Abouna et al 1969)(Winkler et al 1971) whilst the portal venous component was similar to that described by Elmslie et al (1971). Although a total flow of 1.0ml gm^{-1} is thought to be optimal (Hardison et al, 1967) the value in the present study was considerably greater than the 0.5ml gm^{-1} which is regarded as inadequate (Drapanas et al 1966).

The hepatic arterial contribution to in vivo flow was 25.9% and 17.8% ex vivo which was significantly different. In man and the dog it is approximately 30% (Greenway and Stark, 1971) while in the pig, Tygstrup et al (1971) determined a ratio of 40%. These discrepant values may be the effect of different inhalational agents upon this ratio (Gelman 1987b), the effect of the perivascular flow probes (Runciman et al, 1984) as used in the present experiments or the inaccuracy of the ICG infusion extraction clearance method (Winkler and Tygstrup, 1960)(Skak and Keiding, 1987) used by Tygstrup et al (1971) in determining this ratio.

To improve the in vivo preparation an attempt must thus be made to maintain a more stable hepatic blood flow whilst to effect a better comparison of in vivo and ex vivo preparations for the purpose of assessing lignocaine elimination, not only the total unit hepatic blood flow (Lautt and Greenway, 1987) but also the HA to THBF ratio should be similar (Ahmad et al, 1984).

The instability of the hepatic blood flow in vivo may have been due to:

(a) Inadequate replacement of fluid losses due to a standardized method of fluid administration. This could be addressed by replacing fluid according to sampling losses and in addition replacing fluid as dictated by measuring pulmonary capillary wedge presssure and cardiac output using a pulmonary artery catheter.

(b) Movement or respiratory efforts in conflict with mechanical ventilation of the non-paralysed animal.

(c) The use of a relatively unsophisticated ventilator in these experiments.

The methods adopted as a result of these findings in order to achieve a more stable hepatic blood flow will be discussed further in chapters 6 and 7.

5.4.4 PLASMA AND PERFUSATE COMPOSITION

(i) Literature Review of Perfusate Composition for Isolated Liver Perfusion

Various perfusates have been used by investigators in isolated liver perfusion. In order to achieve a standardized perfusate various constituents can be mixed. This method has been commonly employed in small animal (rat) liver perfusions; e.g. Hems et al (1966) used Krebs Henseleit physiological saline, bovine serum albumin (3.9gm/100ml) and washed human red cells to achieve a haemoglobin concentration of 2.5% in a total perfusate of 150ml. This medium with minor modifications has been used by others (Keiding and Chiaranti et al, 1978) (Lennard et al, 1983). This technique has the advantage of standardization but would be expensive for large organ perfusion (pig liver) where from 1500 ml (Elmslie et al, (1971) to 4000 ml is used (Eiseman et al 1961). For pig liver perfusion blood is usually the major component. Human blood (Drapanas et al, 1966)(Tygstrup et al, 1971) and pig blood from donor animals (Eiseman et al, 1961)(Jablonski et al, 1971) or from the animal prior to liver removal (Ham et al, 1969) as well as blood from individual pigs at abbatoirs (Jablonski et al, 1971) has been used. Although some investigators have cross matched the blood prior to administration (Eiseman et al, 1961)(Jablonski et al, 1971) others have not

(Ruwart et al, 1979) as this does not appear to be necessary (Hickman, 1972). The blood is heparinised and administered without further additives (Eiseman et al, 1961) or it may be mixed with Dextran (Drapanas et al, 1966)(Jablonski et al, 1971)(Abouna et al, 1969), or physiological saline (Jablonski et al,1971)(Tygstrup et al, 1971).

In order to maintain normal bile flow some authors have infused choleretic agents (Jablonski et al, 1971)(Welch and Parbhoo, 1973). Insulin is added by some investigators (Welch and Parbhoo, 1973) (Hickman and Terblanche, 1985)(Thomsen and Larsen, 1983).

Most of the investigators mentioned maintain the perfusate at physiological pH by the addition of sodium bicarbonate and maintain the perfusate temperature at 37-39 degrees celsius. Jablonski et al (1971): 37 degrees; (Tygstrup et al, (1971), Ham et al, (1969) and Abouna et al, (1969): 38 degrees and Drapanas et al, (1966) 39 degrees used perfusate temperatures as indicated.

In pig liver perfusions the perfusate is usually oxygenated using either a disc oxygenator (Drapanas and Vang, 1966) (Jablonski et al, 1971)(Eiseman et al, 1961) or a Rygg Kyvsgaard type "bubble" oxygenator (Tygstrup et al, 1971)(Elmslie et al, 1971) by administering a mixture of 95% O_2 and 5% CO_2. No mention of adjusting these mixtures to achieve a standard range for either PaO_2 or PCO_2 is made in the cited reports.

(ii) Motivation for Perfusate Chosen

In order to compare in vivo and ex vivo liver function and eventually lignocaine extraction and clearance, the perfusate composition should ideally be as close to natural blood as possible. A mixture of fresh porcine blood and a physiological saline solution mixed in proportion so as to achieve a haematocrit of around 33% was decided on as Hickman et al, (1970) had found a hematocrit of 31.3 $\pm$ 4.4% in 46 pigs. The ratio of 1600 blood to 600 physiological saline (Plasmalyte B,) was determined as suitable in a number of preliminary experiments while the total volume was sufficient to maintain an adequate prime for the system while allowing for the loss of perfusate due to sampling. Ten ml of sodium bicarbonate 4.2% was added ab initio as experience had shown that this was needed to combat the acidosis present at the start of a perfusion.

As oxygen transport to cells is inversely proportional to the affinity of haemoglobin for oxygen and this affinity is decreased by 2,3 diphosphoglycerate (2,3 DPG) which is depleted in stored blood (Benumof, 1990) an attempt was made to minimise this variable by using only fresh abbatoir blood harvested on the day of experimentation. This was anticoagulated with porcine sodium heparin (Labethica) 5,000 iu per liter. For technical reasons, single, same sex donors could not be used and at least 5 donors of variable sex were used at every collection. This may have had an advantage in minimising any effect of individual pigs on perfusate composition.

The perfusate temperature was maintained at 38 degrees Celsius.

(iii) Stability of Plasma and Perfusate Composition

With respect to pH, temperature, glucose, lactate and haemoglobin concentration as well as the haematocrit value (Table 5.1) the plasma and perfusate were comparable and remained stable. The similarity in haematocrit may be of importance as hepatic oxygen utilisation (Hardison et al, 1967)(Riedel et al, 1983), and bile salt independent bile flow (Thomsen and Larsen, 1983) are affected by changes in haematocrit. It was found however, that in this study haematocrit correlated significantly only with bile flow ex vivo ($r=0.64$).

The foregoing and the similar initial (time=0) sodium and albumin concentrations and osmolality (Table 5.1) suggest that the choice of perfusate composition was appropriate. However, variation was found to occur with time:- In vivo there was a decline of albumin, total protein, and osmolality but no decline in sodium concentration. This was not seen ex vivo. An explanation for this may be that excessive crystalloid solution containing sodium chloride (Plasmalyte B) was administered ex vivo. The difference in the total protein concentrations in vivo and ex vivo is difficult to explain.

It would have been expected that the concentration of most plasma constituents including haemoglobin and red cells in the perfusate diluted with Plasmalyte B would have been less than in vivo.

That this was not the case may have been due to the fact that the blood collected from the abbatoir was from animals that were considerably older than the ($\pm$ 3 month old) animals used for this study. The abbatoir animals weighed from 75-150 kg. These older animals may have higher blood haemoglobin and total protein concentrations as is found in guinea pigs (Biisk, 1976).

With respect to the total protein and albumin concentrations and osmolality the ex vivo preparation was stable; however, here a rise in sodium concentration occurred with time. This may be due to water uptake by the perfused liver, evaporation of warmed perfusate, or more interestingly as a feature of liver function:- Potassium uptake by the perfused liver is regarded as an indicator of good function (Hickman and Terblanche, 1985), the decline in potassium ex vivo correlated with an increase in sodium ($r=-0.54$) suggesting that there may be a relationship with the sodium/potassium-ATPase system.

Although the rise in urea concentration ex vivo was not statistically significant there was a strong correlation with hepatic ATP. This may support the use by Jablonski et al (1971) of urea concentration as an indicator of hepatic viability.

In summary, the plasma and perfusate composition (as determined in the investigations performed here) was largely similar, the ex vivo composition varying less with time than was the case in vivo. This situation may be improved, in future in vivo experiments, by better control of in vivo fluid balance.

5.4.5 PARAMETERS OF LIVER FUNCTION AND INJURY

5.4.5.A. Oxygen Consumption

Unit hepatic oxygen consumption was less but more stable ex vivo than in vivo (Figure 5.2). Hepatic oxygen consumption is dependent on the substrate load presented to the liver (Hems et al, 1966), as well as on the metabolic effects of hormones as found with glucagon stimulation (Van Dyke et al, 1983). Thus a decrease in oxygen consumption could be expected in the ex vivo preparation due to the decreased substrate load and absence of significant hormonal effects. This fact was confirmed by others (Tygstrup et al,1971). Thus the decrease in oxygen consumption ex vivo when compared with the in vivo state may not necessarily indicate that liver function is impaired. The values for the oxygen consumption ex vivo in this study were similar to those of others (Abouna et al, 1969)(Jablonski et al, 1971).

5.4.5.B. Bile Flow

Bile flow was significantly decreased ex vivo (Figure 5.3).

Many of the stimuli for bile flow viz. secretin, glucagon, insulin (Jablonski et al, 1971) and bile salt load, as well as neurogenic effects (Gordon et al, 1972)(Hickman et al, 1971) and blood flow (Gordon et al, 1972) are absent or diminished in the isolated perfused state. Since addition of some of these stimuli in other studies resulted in an increase in bile production (Hardison and Norman, 1967) (Gordon et al, 1972)(Ruwart et al, 1979) it becomes apparent that bile flow per se cannot be regarded as an accurate indicator of liver function unless these factors are standardized as can be done in the in the ex vivo model. Tygstrup et al (1971) found a similar decrease in bile production when comparing the two models. Thus the marked decrease in oxygen consumption and bile flow ex vivo may be due to a number of factors apart from a decrease in hepatic function per se. These tests may however be of more use to compare ex vivo perfusions, where conditions are largely standardized, than to evaluate the extent of impairment ex vivo with respect to the in vivo state.

5.4.5.C. Adenine Nucleotide Status

(i) ATP, ADP, AMP and TAN

Free energy is required for a cell to remain viable and is used for the active transport of molecules and ions as well as the synthesis of biomolecules and macromolecules from simple precursors. This free energy is derived from the oxidation of foodstuffs and carried by adenosine triphosphate (ATP). ATP is an energy-rich molecule because the triphosphate unit contains two phosphoanhydride bonds. Energy is liberated when ATP is hydrolysed to adenosine diphosphate (ADP) and orthophosphate or adenosine monophosphate (AMP) and pyrophosphate (Stryer, 1975). ATP is thus fundamental to cell and so organ function and an assessment of its status will give an indication of viability and the potential for metabolic

function of the liver as "almost all liver functions are dependent on the continuous supply of energy in a form of ATP" (Kono et al, 1982).

A number of investigators have used the status of intracellular adenine nucleotides to assess the viability of the liver in the isolated state (Hems et al, 1966)(Hardison et al, 1967)(Gores et al, 1986) while Tygstrup et al, (1971) used this to compare in vivo and ex vivo function using different pig livers.

Kamiike et al (1988) have shown that the maintenance of ATP and total adenine nucleotide (TAN) levels in stored livers could be used to predict the viability of human transplanted liver grafts. The recovery levels of these indicators at the end of the operation for transplantation was a further predictor of function. They stress that the TAN level was related to the viability of the graft as the recovery of ATP after revascularisation was limited by the TAN level maintained during ischaemia.

In the present study ATP was significantly lower but more stable ex vivo than in vivo where ATP tended to rise while ADP and AMP were no different (Figure 5.4). TAN was non-significantly lower at 2 hours ex vivo (Figure 5.5). Tygstrup et al (1971) showed a non-significant decline in the individual adenine nucleotides (ATP, ADP, AMP) as well as in total adenine nucleotides ex vivo when comparing them with the in vivo state. Hardison et al (1967) showed that hepatectomy alone caused a 64% decrease in mean ATP concentration compared with biopsies taken at laparotomy. However after 4 hours of perfusion (using different livers to the hepatectomy group) the mean hepatic intracellular ATP concentration was 81% compared to the biopsy taken at laparotomy. A quantitative comparison of the adenine nucleotide levels with the present study was not possible as these investigators determined the quantity of nucleotides per wet gram of liver whilst in this study this was determined per dry gram of liver.

(ii) The Energy Charge

The determination of energy charge was intended to furnish a quantitative estimate of the energy state of the cell (Atkinson, 1968). Many metabolic reactions are controlled by the energy charge which is a measure of the high energy phosphate state of the cell. As ATP contains two anhydride bonds and ADP one, the energy stored in the ATP-ADP system is proportional to the mole fraction of ATP and half the mole fraction of ADP (Stryer, 1975).

$$\textbf{The energy charge} = \frac{[ATP] + 1/2\,[ADP]}{[ATP] + [ADP] + [AMP]}$$

(Atkinson, 1968)

As the energy charge of most cells is buffered this ratio is kept within a narrow range; as further ATP generation by the cell is inhibited by a high energy charge whilst ATP utilisation is stimulated by a high energy charge. (Stryer, 1975). Intracellular ATP is maintained at a constant level ranging from 0.85 to 0.90 of energy charge. The energy charge is regarded as being of central importance in maintaining the metabolic stability necessary to life as a rise in energy expenditure would result in a drop in energy charge

unless accompanied by a concomitant increase in phosphorylation of ADP to ATP (Kono et al, 1982). However, in experiments where livers were isolated from their blood supply for increasing periods (30, 60, 90 minutes) Kono et al, (1982) demonstrated a drop in energy charge which however was not indicative of the irreversibility of the liver viability. Viability was dependent on the ability of the liver to restore the energy charge which in turn was dependent on the TAN level. The work of Kamiike et al (1988) cited above supports this.

In isolated pig liver preparations energy charge has also been used as an indicator of the metabolic stability of the liver (Winkler et al, 1986)(Hickman et al, 1988). Winkler et al,(1986) found the mean energy charge to range from 0.76 to 0.70 in a number of control perfusions. In the present study the mean EC taken over the whole study period ex vivo was 0.574 ± 0.031 whilst which was significantly less than that in vivo (0.646 ± 0.014). The difference between the values obtained by Winkler et al, (1986) and the present ex vivo study is not easily explained. This may reflect the enhanced blood flow in their liver perfusions of $1.14 ml g^{-1} min^{-1}$ compared to $0.84 ml g^{-1} min^{-1}$ in the present study as these investigators showed that a decrease in hepatic blood flow to 32% of the initial flow resulted in a decrease in energy charge to 81% of the baseline energy charge of 0.70. Finally Winkler et al (1986) used Danish Landrace pigs. It is possible that hepatic energy charge varies not only between species [in anesthetised dogs Kono et al (1982) found an energy charge of 0.85 ± 0.01] but also between strains of animals. It is noteworthy that in neither study the energy charge was below 0.5 which is reported to lead to a lethal disintegration of cellular economy (Ozawa et al, 1974)(Kono et al, 1982).

In summary, The significantly lower hepatic ATP content was reflected in a lower energy charge ex vivo indicating that less free energy was available to the cell. The constant TAN level ex vivo which was similar to the in vivo level suggests that the liver maintained the potential for metabolic viability and remained stable over the two hour study period.

5.4.5.D. Potassium

There was a rise in plasma potassium in vivo and a decline ex vivo (Figure 5.6). The initial high concentration of K+ ex vivo is a feature of the abbatoir blood and has also been noted in other reports (Hickman et al, 1971)(Jablonski et al, 1971). The decline in potassium concentration ex vivo has been shown to correlate with good function (Hickman and Terblanche, 1985) however this study was not strictly comparable with the present as in the former a continuous infusion of insulin and secretin was administered to the perfused liver. Stewart et al (1953), have shown that a rise in hepatic vein K+ concentration is an early indicator of hepatic insufficiency in association with inadequate perfusion, or lack of oxygen or of glucose.

In vivo there was a rise in plasma potassium concentration (measured in the carotid artery) with time. This trend is difficult to explain; if this can be attributed solely to the liver this might suggest early liver

impairment which was reversible as there was subsequent uptake of potassium ex vivo. There was however no correlation of any other index of liver function, blood flow or oxygenation with this potassium rise in vivo.

5.4.5.E. Aspartate Aminotransferase

The AST levels in vivo were in the normal range (Hickman et al, 1970) whilst the AST ex vivo was significantly higher and increased with time (Figure 5.7). This was not related to macroscopic haemolysis, which could have been caused by the roller pumps, and thus is likely to be of liver origin. [Section 8.3.2, indicates that, over the standard perfusion period, in the absence of a liver there is no increase in plasma (free) haemoglobin as a result of roller pump induced haemolysis and no increase in perfusate AST concentration.] Initially this was probably due to the damage caused by removal of the liver but the continuing rise as shown at the one and two hour sampling times suggests further on going damage to liver cells as a function of the isolated perfused state. Hickman et al (1971) showed that the AST concentration in the perfusate continued to rise over a study period of six hours. Jablonski et al (1971) also noted a rise of AST with time but this rise could not be related to the success of the perfusion according to their above mentioned criteria (Section 5.1.2). The significance of this steady rise in terms of determining the function of the isolated perfused liver is not clear.

It was decided that in future studies plasma and perfusate haemolysis would be assessed quantitatively by spectrophotometry while Alanine Aminotransferase (ALT) would also be determined in conjunction with AST as this is regarded as a more specific and sensitive indicator of liver damage (Price and Alberti, 1985).

5.4.5.F. Hepatic Lactate Utilisation

As far as is known hepatic lactate utilisation has not been compared in vivo and ex vivo but has been assessed in vivo.

(i) In Vivo

Goldstein et al, (1971) studied hepatic lactate utilisation in 10 dogs and found that there was "negligible hepatic metabolism of lactate in the resting anaesthetized state". In this study they determined that lactate utilisation averaged -1.0 ± 1.3umol $min^{-1}kg^{-1}$ body weight and increased progressively to 4.2 ± 2.4umol $min^{-1}kg^{-1}$ body weight during a three hour undisturbed study period. These statistics suggest that, as was the case in the present in vivo study, hepatic lactate release [as denoted by a (-) sign] occurred throughout the study period; livers alternated at times between uptake and release. This study was similar to the present study in that the animals were left undisturbed. As other investigators perturbed their models, only their base line data can be compared with that of the present study. The baseline uptake of lactate (4.65 mg $min^{-1}100g^{-1}$) determined by Macdonald et al (1979) in dogs was similar to that in the present study as were the values in pigs (Nagano et al, 1990a). However, despite perturbing their models no control studies were performed to allow a further direct comparison with our findings over time. Macdonald et al (1979)

and Nagano et al, (1990a) showed that hypoxia and ischaemia resulted in decreased lactate utilisation. They determined independently that the liver starts to release lactate when the hepatic oxygen uptake is less than 2-3ml $O_2min^{-1}100g^{-1}$ whilst Nagano et al (1990a) determined that lactate release also occurs when the hepatic venous PO_2 ($HVPO_2$) is 10-13 mmhg. In the present in vivo study, hepatic oxygen consumption was never less than these quoted values. In the one study where the $HVPO_2$ was in this range [Study F at 2 hours (Figure 5.8 in vivo)] lactate uptake (rather than release) was in fact occurring. Lactate uptake in the ex vivo study correlated inversely with $HVPO_2$ whilst there was no correlation with hepatic blood flow in vivo. Clearly there must be other factors apart from hypoxia or ischaemia which determine hepatic lactate uptake or release.

Lactate release by the liver may occur as a feature of either alkalaemia or acidaemia (Johnson et al, 1969)(Cohen and Woods, 1983) neither of which were a feature of the present study (Table 5.1).

In the isolated perfused rat liver Lang et al, (1989) have shown that lactate production is decreased by exposure to hypotonic perfusates and enhanced following exposure to hypertonic perfusates.

It has been shown in the rat liver perfused in situ that "stimulation of the nerve plexus around the hepatic artery and portal vein caused a shift from lactate uptake to output" (Balle et al, 1987)(Gardemann et al, 1987). It is possible (but unlikely) that the perivascular placed probes in the present study could provide such a stimulus. It would have to be postulated that this is on an intermittent basis. Further, this may imply that in experimental liver models where the hepatic artery and/or portal vein are transected (Nangano et al, 1990a) with concomitant damage to these plexuses the absence of this stimulus may favour hepatic lactate uptake.

Haussinger and Lang (1990) have suggested that lactate uptake or release may be dependent on the liver glycogen content which is likely to be less in starved than in fed animals. Lactate release is less in glycogen depleted livers as little lactic acid can be produced from hepatic glycogen under these circumstances (Halperin and Fields, 1985). This is supported by the paper of Schimassek (1965).

It is possible that the variable lactate utilisation reported by Goldstein et al (1971) and seen in the present study is a result of variable effects on liver glycogen stores of preoperative starvation of the animals used. Goldstein et al (1971) did not communicate the nature of the maintenance fluid administered in their study. In the present study Dextrose 2.5% in Plasmalyte B was administered and it is interesting to postulate that this may have affected lactate release by some livers over the study period. This hypothesis gains some support from Halperin and Fields (1985) who argue that glucose administration serves as a substrate for the further formation of lactic acid in patients with lactic acidosis. To elucidate this problem further liver glycogen stores would have to be correlated with lactate utilisation and the effects on both determined after the administration of intravenous glucose.

Hyperlactatemia is also seen in association with hypermetabolic disease (Siegel et al, 1979) which may raise lactate and glucose levels by increased flux through the glycolytic cycle as stimulated by

catecholamines (Mizock, 1987)(Johnson et al, 1969). On this basis it could be postulated that lactate release in vivo in the present study was due to hypermetabolism induced by a stressor such as inappropriate fluid balance or inadequate anaesthesia. The development of malignant hyperthermia (MH) must also be considered. However, Hall et al, (1980) have shown (using malignant hyperthermia susceptible Pietrain pigs) that despite a rise in systemic lactate concentrations, hepatic lactate uptake (rather than release) was a feature of malignant hyperthermia.

In summary, the occasional hepatic lactate release seen in vivo in this study cannot be explained as due to consistent hepatic hypoxia, inadequate blood flow or acid base changes. Lactate utilisation by the liver is complex and is also dependent on nervous impulses to the liver parenchyma, as well as liver glycogen stores and may be affected by an induced hypermetabolic state. This study did not allow a determination of which of these latter factors were operative in this model. However, in an attempt to establish as metabolically "stable" a model as possible for the in vivo study of lignocaine extraction and clearance, only studies where lactate uptake occurred were accepted.

(ii) Ex Vivo

A number of investigators have used the clearance of an administered dose of lactate as a measure of liver function (Abouna et al, 1969)(Drapanas et al, 1966) in the isolated perfused pig liver preparation. They showed rapid decay of lactate concentration when plotted against time but did not assess transhepatic extraction. Johnson et al, (1969) showed using the isolated perfused pig liver that a lactate load is no longer efficiently cleared at hepatic flows of less than 150 ml min^{-1} when the perfusate was less than 50% saturated. The interpretation of the net uptake or release of lactate by the liver as seen in this study in the absence of such a loading dose is more complex:- The high hepatic venous PO_2 (Table 5.1) [which correlated inversely with lactate uptake (Table 5.2.A)], the normal pH (Table 5.1) and the consistent hepatic blood flow of 0.84ml gm^{-1} per minute which represents an absolute flow of 532 ± 4.8 ml min^{-1} suggest that neither hepatic hypoxia, pH changes or inadequate blood flow could be invoked as an explanation for this. This was similar to the conclusion drawn from data on the in vivo study. It is thus not apparent from this study why variable hepatic uptake and release should occur ex vivo whilst other parameters of liver function such as oxygen consumption, bile flow, liver ATP, energy charge and TAN are constant. Energy charge correlated inversely with lactate uptake (Table 5.2.A.) implying that the energy state of the hepatic cell may in some way determine the need for lactate uptake perhaps for the purposes of gluconeogenesis in the Cori cycle.

(iii) Conclusion

As the hepatic utilisation of lactate has been used as an indicator of hepatic ischaemia and hypoxia by investigators and the clearance of administered lactate by the isolated liver as an indicator of hepatic function it was decided to determine whether lactate utilisation might serve as a comparative indicator of

the function of the pig liver in vivo and ex vivo. No statistically significant difference in lactate utilisation in vivo when compared with ex vivo was found. There was intermittent lactate release in vivo in this study which confirmed the work of others. Ex vivo this occurred despite stable parameters of liver function. In the absence of hepatic hypoxia and ischaemia it must be concluded that other factors are responsible for this phenomenon which cannot be elucidated further in this study but may relate to the energy stores in the particular liver. Thus in this study lactate uptake was not a useful indicator of hepatic function in vivo and ex vivo.

5.4.5.G. Parameters of Liver Function and Injury: Conclusions

It is clear from the above and the work of others (Gores et al (1986) that in the isolated state there is ongoing liver cell dysfunction. Although the exact aetiology of this impaired hepatic function is not known, it is reasonable to suggest that this may be, in part, as a result of ischaemic hypoxia (inadequate perfusion) and hypoxic hypoxia (inadequate perfusate oxygen content)(Ganong, 1983) incurred as a result of the process necessary for harvesting, establishing and maintaining the isolated perfused state (Lemasters et al, 1983). Thus as the process of harvesting and establishing of stable perfusion takes in the order of 15-20 minutes it is likely from the work of Misra (1972) and Bradford (1986), that the isolated livers used in these studies have suffered some hypoxic injury.

5.4.6 CORRELATION ANALYSIS

Correlation analysis has been used as a tool for the generation of hypotheses regarding the "functional integrity" of the isolated perfused liver. "A high degree of correlation between a set of observations indicates that they are determined by a common factor" (Ramsoe et al, 1971). With a view to correlating lignocaine extraction and clearance in future studies with parameters of hepatic function this correlation analysis endeavoured to establish the relationship, if any, between parameters of hepatic function in the absence of lignocaine both in vivo and ex vivo. To this end a correlation matrix of all relevant parameters investigated so far was made. These are reported in Table 5.2.A & B, or in the text where appropriate.

As may be expected more significant relationships were evident ex vivo where parameters largely reflect changes in the liver whilst in vivo parameters may be perturbed by many other factors not related to liver function. It was decided to establish the relationships between the parameters chosen to assess hepatic function namely, oxygen consumption, bile flow, adenine nucleotide status, potassium uptake, AST release and lactate uptake. With respect to these ex vivo relationships not already highlighted above the following:-

Oxygen consumption correlated negatively with bile flow, pH and potassium concentration, ATP and TAN. Oxygen consumption would appear to be central to the function of the liver as was bile flow, the amount of energy in the form of ATP and the viability as measured by TAN (Kamiike et al, 1988). This analysis suggests that increased hepatic oxygen consumption tended to decrease ATP and TAN whilst releasing metabolic products and so decreasing perfusate pH. The decline in K+ concentration may reflect the enhanced oxygen consumption associated with the activity of the Na+/K+ ATPase pump mechanism. The decreased bile flow although significantly correlated with increased oxygen consumption was probably biologically unrelated and due to the absence of choleretic factors.
Further correlations such as the relationships between AST, urea and pH, and pH and ATP and EC to name a few were less readily explained.

In conclusion the low degree of correlation between the parameters investigated may have a number of causes (Ramsoe et al, 1971). Each parameter may have been determined by a separate factor i.e. the liver functions were dissociated. Alternatively, the material may have been too uniform as the livers had not been damaged or stressed sufficiently and thus functions were within the "normal" range. Therefore small changes may have been obscured because of the analytical error of the tests (employed to determine these changes) and the low number of observations.
This analysis has served to indicate that in order to investigate whether lignocaine elimination can serve as a useful indicator of impaired hepatic function in this model, there is a need to impair the function of the isolated liver further. This should be done to establish a spectrum of impaired livers and associated indices of liver function so that an appropriate correlation analysis can be performed with hepatic lignocaine elimination. As it is clear that the isolated liver is already impaired to a certain degree and that this may be contributed to by hypoxia (Section 5.4.5.G), it was decided to damage livers further in the isolated state by administering a hypoxic perfusate (Chapter 8) for this investigation.

5.5 RESULTS (EXPERIMENT B)

The six liver perfusions fulfilled the criteria for successful perfusion (Section 2.5.3) and made up this study which was performed to determine whether there was any difference between livers perfused after a prior in vivo study (Experiment A) and those resected immediately for perfusion (Experiment B).
All results reported here will therefore be contrasted with those from Experiment A (Section 5.3) above which will be reproduced here for convenience of comparison.

5.5.1 HEPATIC BLOOD FLOW AND PERFUSATE COMPOSITION

The mean liver weights in Experiment (B) and (A) were similar (679 ± 30g and 634 ± 25g respectively) as was the mean hepatic blood flow over the two hour study period (0.82 ± 0.04ml $100g^{-1}min^{-1}$ and 0.84 ± 0.03ml $100g^{-1}min^{-1}$).

The perfusate composition is compared in Table 5.3. The perfusate composition remained stable with respect to the parameters reported over the two hour study period except for sodium which varied with time in both experiments in a similar fashion and glucose which decreased in experiment (B) and was less than in experiment (A) at the two hour sampling time.

The osmolality was higher in the group of perfusions comprising experiment (B). This may have been largely due to the higher initial urea values in this group which reached statistical significance at the two hour sampling time.

Table 5.3

Perfusate Compostion Ex Vivo: in Experiments Without Prior In Vivo Study (B) and in Experiments with prior in vivo study (A)

Parameter	Experiment	Time (Hrs) 0	1	2
Sodium	B	136 ± 2	142 ± 1[a]	144 ± 1[a]
(mmol L^{-1})	A	136 ± 1	139 ± 1	143 ± 3[ab]
Total Protein	B	53 ± 5	53 ± 2	54 ± 2
(g-100ml^{-1})	A	54 ± 3	58 ± 3	57 ± 2
Albumin	B	34 ± 2	34 ± 1	34 ± 1
(g−100ml^{-1})	A	36 ± 1	36 ± 1	36 ± 1
Osmolality	B	* 284 ± 5	* 284 ± 9	283 ± 6
(mosmol L^{-1})	A	* 260 ± 6	* 256 ± 7	264 ± 6
Haemoglobin	B	11.3 ± 0.9	11.7 ± 0.4	11.8 ± 0.5
(g%)	A	11.5 ± 0.5	11.9 ± 0.4	12.1 ± 0.3
Glucose	B	154 ± 9.5	112 ± 11[a]	* 101 ± 21.6[a]
(mg 100ml^{-1})	A	351 ± 125	296 ± 96	* 314 ± 86
Lactate	B	14.6 ± 2.9	10.1 ± 1.6	16.5 ± 3.5
(mg 100ml^{-1})	A	24.7 ± 4.5	16.3 ± 7.7	18.0 ± 7.8
Urea	B	26 ± 4.9	31 ± 5.7	* 36 ± 6.5
(mg 100ml^{-1})	A	16.5 ± 2.4	19.0 ± 2.3	* 21.3 ± 4.4
pH	B	7.41 ± 0.03	7.40 ± 0.02	7.4 ± 0.02
	A	7.41 ± 0.02	7.44 ± 0.01	7.42 ± 0.02
Temperature	B	38.4 ± 0.1	38.2 ± 0.2	38.2 ± 0.1
(degree celsius)	A	38.5 ± 0.2	38.3 ± 0.1	38 ± 0.1
Hepatic Venous	B	136 ± 3.2	137 ± 7.9	141 ± 6.4
PO_2	A	103.4 ± 3.7	104.9 ± 6.1	103.4 ± 11.4

Mean ± (SEM) Experiment B (n=6) ex vivo liver perfusion in absence of prior in vivo study. Experiment A (n=6) ex vivo liver perfusion after prior in vivo study. * = significant difference between experiments. (a) = significant difference with time 0. (b) = significant difference between time 1 and 2 hrs. $p = < 0.05$.

5.5.2 PARAMETERS OF LIVER FUNCTION AND INJURY

The indices of liver function (Table 5.4) were generally stable in both experiments in that they did not vary with time. Potassium concentration however fell with time over the two hour study period and was different between the two groups, again (as with urea above [5.2.1]) due to differing initial values.

The values for aspartate aminotransferase concentration in experiment B showed wider variation (minimum=43 and maximum=386) compared with experiment A (minimum=56 and maximum=130) and this was reflected in higher but not significantly different mean values which may have been contributed to by more haemolysis evident in this group on macroscopic evaluation.

The adenine nucleotide values and energy charge did not vary with time in either experiment (Table 5.4) and so the data over the two hour study period was pooled for ease of comparison (Table 5.5). Mean hepatic ATP content and energy charge in experiment B were significantly higher than in Experiment A. Whilst ADP, AMP and TAN content were significantly lower in those livers perfused immediately (Experiment B) compared with those who had an prior in vivo study (Experiment A) (Table 5.5).

Table 5.4

Liver Function Indices Ex Vivo: in Experiments without Prior In Vivo Study (B) and in Experiments with Prior In Vivo Study (A)

Parameter	Experiment	Time (hrs) 0	1	2
Oxygen Cons.	B	2.8 ± 0.2	2.5 ± 0.2	2.5 ± 0.2
($ml0_2$ 100mgliv.$^{-1}$)	A	2.6 ± 0.2	2.16 ± 0.2	2.6 ± 0.3
Bile	B	-	5.6 ± 0.7	3.0 ± 0.6
Flow (ml hr^{-1})	A	-	6.0 ± 0.9	6.5 ± 2.7
K+ (mmol L^{-1})	B	* 5.1 ± 0.4	* 4.0 ± 0.3[a]	* 3.4 ± 0.3[a]
	A	* 6.4 ± 0.3	* 6.0 ± 0.6	* 4.8 ± 0.1[ab]
AST	B	111 ± 31	155 ± 49	173 ± 49
(UL^{-1})	A	76 ± 5	82 ± 3	99 ± 10[a]
ATP	B	5.04 ± 0.44	5.16 ± 0.26	* 6.76 ± 0.44[1]
(uM gm-$liver^{-1}$)	A	4.14 ± 0.71	4.63 ± 0.75	* 4.04 ± 0.81
ADP	B	* 2.62 ± 0.37	* 2.45 ± 0.30	*2.56 ± 0.29
(uM gm-$liver^{-1}$)	A	* 4.64 ± 0.34	* 4.74 ± 0.76	* 4.49 ± 0.71
AMP	B	* 1.02 ± 0.24	0.99 ± 0.20	* 0.99 ± 0.14
(uM gm-$liver^{-1}$)	A	* 2.83 ± 0.75	1.91 ± 0.31	* 2.73 ± 0.88
Energy Charge	B	* 0.737 ± 0.033	0.745 ± 0.025	* 0.783 ± 0.026
	A	* 0.551 ± 0.066	0.622 ± 0.032	* 0.549 ± 0.070
TAN	B	* 8.73 ± 0.81	* 8.61 ± 0.40	10.27 ± 0.31
(uM gm-$liver^{-1}$)	A	* 11.66 ± 0.61	* 11.29 ± 0.12	11.72 ± 0.96

Mean ± (SEM) Experiment B (n=6) ex vivo liver perfusion in absence of prior in vivo study. Experiment A (n=6) ex vivo liver perfusion after prior in vivo study. * = significant difference between experiments. (a) = significant difference between time 0 and 1 or 2 hours. (b) = significant difference between time 1 and 2 hrs. $p = < 0.05$. (1) p value 0.057 when compared with ATP at time zero. Oxygen Cons = Hepatic oxygen consumption. Liv. = liver. AST= Aspartate aminotransferase.

Table 5.5

Adenine Nucleotide Status Ex vivo: in Experiments Without Prior In Vivo Study (B) and in Experiments with prior in vivo study (A)

Parameter	Experiment B	Experiment A	P value
ATP	5.65 ± 0.50	4.27 ± 0.71	0.0102
ADP	2.55 ± 0.30	4.77 ± 0.58	0.0001
AMP	0.98 ± 0.18	2.49 ± 0.66	0.0014
TAN	9.20 ± 0.61	11.56 ± 0.91	0.0014
EC	0.755 ± 0.027	0.574 ± 0.031	0.0001

Adenine nucleotide data means Experiment B (n=18) and Experiment B (n=18) pooled as no variation of these parameters with time. Quoted as mean and standard error of the least square mean. Significant difference between pooled means given by P value. Adenine nucleotides in uM-gm^{-1} dry liver.

5.6 DISCUSSION (EXPERIMENT B)

This study once again confirms the stability of the ex vivo preparation over the two hour study period in terms of perfusate composition as well as oxygen consumption, bile flow, adenine nucleotide status and energy charge. The decline in potassium as an indicator of continued liver viability (Hickman and Terblanche, 1985) is again noted here as is the possibly related rise in perfusate sodium concentration. The objective of this study was however to determine whether the prior in vivo study performed as described was detrimental to the function of the perfused liver. If this were so it would mitigate against using the same liver to compare in vivo versus ex vivo function.

The major difference between the two studies was shown to be the adenine nucleotide status and the energy charge (Table 5.5).

These two parameters are discussed at some length in Section (5.4.5.C.). The 14% higher hepatic cellular ATP and the 31% higher energy charge in perfused livers not studied earlier in vivo indicate a higher energy state of these livers. The mean energy charge of 0.76 in this group is also within the range of a control group of perfused pig livers studied by Winkler et al (1986). Ozawa et al (1974) have stated that "It is recognised that the maintenance of liver function depends on a continuous supply of ATP" while Lanir et al (1988) have shown that donor livers with higher ATP content and energy charge achieve better results after hepatic transplantation and speculate that this might have been due to these livers being in a better metabolic state. This might suggest that isolated liver preparations with higher values for these parameters are preferable.

In contrast to these higher parameters for livers in Experiment B the hepatic cellular ADP and AMP content was lower, reflected in a 26% lower TAN value for this group of livers. Kamiike et al (1988) have suggested that after an insult (liver transplantation) the recovery of a low ATP was dependent on the TAN level. However in this case where ATP was already high the implications of these findings are not clear.
In addition it is possible that the higher potassium concentration of the perfusate in Experiment A (Table 5.4) reflects damage to the liver as a result of the prior in vivo experiment.
Although the exact importance of these findings remain to be determined it is clear that the prior in vivo study has important effects on the energy state of the isolated perfused liver which may well be detrimental to liver function and thus would mitigate against using the same liver for an in vivo versus ex vivo comparison of lignocaine metabolism.

5.7 CONCLUSION

This pilot study has shown a significant difference in adenine nucleotide status and energy charge when liver function in ex vivo livers studied only in isolation were compared with those that had undergone a prior in vivo study. This suggests that the latter method is not appropriate for the envisaged in vivo versus ex vivo comparison of lignocaine metabolism.
This study also established that the in vivo preparation was less stable than the ex vivo preparation with regard to:
(a) hepatic blood flow
(b) plasma composition:- possibly due to injudicious fluid replacement
(c) some liver function tests:-namely, hepatic ATP content and oxygen consumption.
The in vivo and ex vivo preparations differed in that the plasma and perfusate composition, initially similar, changed with time, and the mean arterial percentage of total hepatic blood flow, oxygen consumption, bile flow and hepatic ATP content and energy charge were less ex vivo. Further, both AST and plasma K+ were higher ex vivo, potassium declining and AST rising over the two hour study period.
TAN values were similar suggesting that the ex vivo liver remained metabolically viable while the stability of this parameter together with the stability of oxygen consumption, bile flow and hepatic adenine nucleotide status suggest that the ex vivo liver did not deteriorate significantly over the two hour study period.

Therefore in order to compare more accurately lignocaine extraction ratio and clearance in vivo and ex vivo this study indicated that the comparison should be made using different livers and that there was also a need to improve the stability of the in vivo model.

This was attempted by:

(1) Instituting invasive monitoring of haemodynamic parameters in an attempt to administer appropriate fluid therapy.

(2) Standardizing the depth of anaesthesia using a measure of expired volatile anaesthetic concentration.

(3) Paralysing all animals with the use of a muscle relaxant to avoid any potential instability due to muscle movement.

(4) Using a more sophisticated ventilator allowing better control of pulmonary ventilation.

(5) Minimising surgical trauma and the duration of anaesthesia (time for surgical preparation).

Finally, although the reason for the in vivo release of lactate could not be determined, this might indicate impaired hepatic function or a varying metabolic state. Thus it was decided that in order to study livers of uniform metabolic state, only livers in which hepatic uptake of lactate occurred would be accepted for further analysis in vivo.

CHAPTER 6: HEPATIC EXTRACTION AND CLEARANCE OF LIGNOCAINE IN VIVO

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CHAPTER 6: HEPATIC EXTRACTION AND CLEARANCE OF LIGNOCAINE IN VIVO

SUMMARY

This study was performed to establish whether hepatic lignocaine extraction ratio and clearance in the pig was similar to that determined by others in man. Derived pharmacokinetic parameters were employed to achieve constant lignocaine concentrations during the second hour of a two stage infusion of lignocaine hydrochloride. Hepatic extraction and clearance of lignocaine were determined during this period by transhepatic sampling and measurement of hepatic arterial and portal venous blood flow using perivascular ultrasonic flow probes placed at laparotomy. This data was compared with similar studies from other laboratories performed in man as well as in the sheep, dog, monkey and cat. The lignocaine extraction ratio of 0.61 in the pig was found to be similar to that in man supporting the hypothesis that the pig liver may be used as a model for hepatic lignocaine extraction in humans.

6.1 INTRODUCTION

6.1.1 OBJECTIVES

The two major objectives of this investigation were:

(a) To investigate and to compare the hepatic extraction of lignocaine in the pig with that of reported studies on humans to assess whether the pig can serve as a model of hepatic extraction in man.

(b) To compare the hepatic extraction and clearance of lignocaine in vivo with that in the isolated perfused pig liver.

The methods used for these experiments were chosen to serve both objectives: the transhepatic catheterisation technique and method of blood flow measurement could be used to compare with hepatic lignocaine extraction and clearance found in humans (Stenson et al,1971)(Wiklund et al,1977) and was directly comparable to the methodology used in the isolated perfused pig liver preparation to be described (Chapter 8).

In addition, the in vivo experimental design employed allowed assessment of the effects of lignocaine on hepatic blood flow and function. As no studies of the effects of lignocaine on hepatic function appear to have been reported it was decided to extend this study to address this by comparing the group receiving

lignocaine with a group of control animals. The methodology for the hepatic blood flow and function study will be discussed more fully in chapter 7.

6.1.2 MOTIVATION

6.1.2.A. Hepatic Extraction and Clearance

To determine the hepatic clearance of lignocaine in vivo (and ex vivo) by measuring hepatic extraction and blood flow both constant lignocaine concentration (Pang, 1980) and blood flow (Zierler, 1961) must be achieved. The methodology of Mitenko and Ogilvie (1972) and Wagner (1974) was employed here to achieve constant plasma lignocaine concentrations as described in chapter 4.

6.1.2.B. Departure from Earlier Methodology

In an attempt to achieve constant hepatic blood flow through a haemodynamically stable in vivo preparation a number of modifications were made to the methods employed in the earlier study discussed in section 5.6.1. Emphasis was placed on:

(1) The anaesthetic technique (monitoring of anaesthetic depth and addition of muscle paralysis).

(2) Invasive monitoring of haemodynamic variables and determination of cardiac output (CO), and pulmonary capillary wedge pressure (PCWP), in order to guide fluid administration in an attempt to maintain cardiovascular stability.

(3) Optimising fluid balance by immediately compensating for sampling losses.

(4) The use of a more sophisticated ventilator allowing standardisation of inspiratory flow rate.

6.2 MATERIALS AND METHODS

6.2.1 PROTOCOL

Seven male Landrace x Large White pigs 22-25kg in weight were studied. The animals were anaesthetized and prepared surgically for transhepatic sampling and hepatic blood flow measurement. Lignocaine was administered as a two stage infusion and hepatic extraction and clearance determined during the second hour of lignocaine administration. The animals were sacrificed under general anaesthesia and the livers resected for weighing.

6.2.2 ANAESTHESIA

The animals were anaesthetized with intravenous (iv) thiopentone (2.5%) till loss of the eyelash reflex (500-750mg) and in addition muscle paralysis (which had not been employed in earlier studies) was instituted using pancuronium 6mg iv. Anaesthesia was administered by means of a circle system with an in line carbon dioxide absorber. Isoflurane (vaporiser setting 1.5%) was administered in nitrous oxide and oxygen (30%) at an initial fresh gas flow rate (FGFR) of 6 litres per minute for 30 minutes (timed by a stop watch with an audible alarm). The FGFR was then decreased to 1 liters per minute. Three preliminary experiments and 5 of the described experiments in which end tidal isoflurane concentration could be measured using a volatile agent monitor (Engstrom Emma) indicated that the endtidal isoflurane concentration ranged from 0.8-1% during the experimental period (second hour of lignocaine infusion).[1] (For calibration of agent monitor see appendix A.2.1.).

6.2.3 MECHANICAL VENTILATION

The animals were ventilated with an Ohio Anaesthesia Ventilator (Wisconsin, USA) using standardized settings (section 7.1.2). Normocapnia was monitored using an end-tidal CO_2 monitor (Gould, Capnograph Mark 111, Netherlands) and normocarbia (35-40mmHg) confirmed by regular blood gas analysis and maintained by adjusting deadspace only.

6.2.4 SURGICAL PREPARATION

6.2.4.A. CATHETER PLACEMENT

Catheters were placed at the following sites:

(1) The right (R) internal carotid artery for blood sampling and monitoring of arterial pressure.

(2) The R internal jugular vein (pulmonary artery catheter sheath, Baxter) for the administration of drugs and a maintenance infusion.

(3) A dedicated catheter was placed into the left internal jugular vein for lignocaine infusion (vide infra 6.2.6).

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1 Note: At the time of these experiments this institution (Groote Schuur Hospital) had only one volatile anaesthetic agent monitor. As it was logistically impossible to measure the endtidal isoflurane concentration in all experiments, the standardized anaesthetic technique described above had to be adopted.

(4) A balloon tipped catheter was passed from the left external jugular vein and threaded into the median lobe of the liver till a wedge position was achieved and then pulled back 3 cm. Its intrahepatic position was confirmed at laparotomy and again at the end of the study period prior to liver resection.
(5) A pulmonary artery catheter (Baxter) for CO (Chapter 7.1) and PCWP measurement was passed via catheter (2) through the R internal jugular vein and floated into the pulmonary artery. Its position was confirmed by the characteristic changes in the transduced pressure wave form observed using a Hellige Servomed SMK154-3 Monitor (Freiburg, Germany). Care was taken that the catheter was inserted to the 40cm mark to ensure that the injectate outlet orifice for cardiac output measurements was clear of the pulmonary artery catheter sheath which had been previously shortened to 9cm in length. Post mortem dissection in preliminary experiments confirmed that this orifice lay just outside the right atrium in pigs of this size.
(6) A laparotomy was performed and a catheter passed via the splenic vein and threaded to lie in the portal vein at the porta hepatis.
Thus catheters were placed for fluid (2) and drug (2,3) administration as well as monitoring of cardiovascular variables (1,5). Catheters (4) and (6) permitted transhepatic sampling whilst the carotid artery catheter (1) was regarded as a hepatic artery equivalent (Rowland, 1972a).

6.2.4.B. BILE DUCT CANNULATION

The common bile duct was cannulated and allowed to drain to a measuring receptacle for hourly bile volume measurement. The cystic duct was tied.

6.2.4.C. SURGICAL BIOPSIES

(1) Biopsies for determination of adenine nucleotide status were taken from standardized sites on the free edge of the right lateral lobe of the liver using precooled modified Wollenberger clamps at the times indicated in Figure 6.1. These biopsies were stored under liquid nitrogen.
(2) Two further biopsies for light and electronmicroscopic evaluation (Chapter 7) were taken at the same times. The biopsy sites were sutured with 3-0 chromic.

6.2.4.D. ULTRASONIC PROBE PLACEMENT

Mobilisation of the hepatic artery and portal vein was performed taking care to maintain as far as possible the nervous plexus on the hepatic artery. Precalibrated perivascular ultrasonic flow probes were placed round the hepatic artery (2mm) and portal vein (6mm) at the porta hepatis taking care that the portal probe was proximal to the gastric tributary of the portal vein so that all the portal venous blood supplying the liver

was measured. The electronic leads to the probes were aligned along the axis of the vessel to minimise the potential for probe displacement and thus blood flow obstruction. Hepatic blood flow was measured using the ultrasonic flow meter described earlier (Section 2.3.1.B). This incorporates a non-occlusive zero facility. This was confirmed at the start of an experiment by briefly occluding the vessels manually to confirm a zero reading. Probe placement occurred as the final part of the surgical preparation and the abdomen was then carefully closed with towel clips whilst continuously observing the flow meters to exclude probe movement with potential vessel obstruction. The preparation was left undisturbed for the duration of the study. (See appendix A.1.1 for details of the flow meter calibration.)

6.2.5 FLUID ADMINISTRATION AND MAINTENANCE OF BODY TEMPERATURE

Balanced salt solution (Plasmalyte B) to which was added dextrose to achieve a 2.5% solution was administered as a continuous infusion at a rate of $10ml kg^{-1} kg^{-1}$ using an infusion pump (IVAC 631, USA). All volumes of blood aspirated for analysis were replaced with 3 times their volume of Plasmalyte B without dextrose. When necessary, in order to maintain cardiovascular stability more Plasmalyte B was administered to maintain the PCWP at baseline levels.

To maintain body temperature the same devices as described in section (2.2.4) were employed and core temperature was monitored using the thermistor incorporated in the pulmonary artery catheter and measured by a 9520A Cardiac Output Monitor (Edwards Laboratories, California USA).

6.2.6 LIGNOCAINE ADMINISTRATION AND SAMPLING FOR ANALYSIS

6.2.6.A. Motivation and Method

To achieve a constant plasma concentration at the earliest possible time without developing a potentially toxic concentration, a loading infusion at a certain rate (Q1) was administered for a time (t) followed by a continuous infusion (Q2) for the duration of the study.

The method of adminstration of this infusion to reproducibly achieve and maintain a constant concentration is critical.

If this infusion was simply attached to an established intravenous line through which intravenous maintenance fluid via a volumetric pump was administered, certain errors would result:

(1) The time when administered lignocaine reached the pig's circulation would be dependent on the rates of both infusion pumps.

(2) The amount of lignocaine reaching the systemic circulation would initially be decreased by dilution in the second medium but would eventually become constant, but only if both infusions were maintained at the same rate. To avoid these problems as well as the potential error involved in using two infusion pumps the following method was devised.

A single standard fr8 feeding catheter cut to 50 cm (a template catheter was used for preparation of all subsequent catheters) was inserted into the left internal jugular vein solely for the purpose of lignocaine administration. The four potential rates of infusion (Q1) required for pigs 22-25 kg were determined. Using a standard template catheter the time required for lignocaine to traverse the catheter was determined at these rates in triplicate (See appendix A.3.2). In the actual experiment the infusion and a countdown timer were started simultaneously. This timer was set for the (predetermined) time required for lignocaine to traverse the catheter. Time zero was taken after completion of this infusion period, when a second timer was started, to time exactly a period of 10 minutes for infusion of lignocaine at the initial infusion rate Q1. After this the infusion was slowed to rate Q2 for the remainder of the study.

Lignocaine hydrochloride (10%) was administered using a calibrated (Appendix A.3.1) syringe pump (Vial Medical SE 200) at an initial infusion rate of 1.41 $mgkg^{-1}$ per minute (Q1) for 10 minutes and then decreased to a rate of 0.165 $mgkg^{-1}$ per minute (Q2) for the rest of the experiment.

6.2.6.B. Sampling Schedule for Lignocaine Analysis

A 10cc blood sample was taken prior to lignocaine administration to be used to to determine a standard curve for the analysis of lignocaine and its metabolites as well as to confirm the absence of lignocaine.

Samples were then taken at 5, 10 15, 30, 45, 60, 75, 90, 105 and 120 minutes after the start of the lignocaine infusion (Figure 6.1).

6.2.6.C. Sampling for Lignocaine Analysis

Samples (2cc) for lignocaine analysis were taken in standard fashion by means of a three way tap after aspiration of a suitable volume for the deadspace of the particular catheter thus ascertaining a mainstream sample. Samples were taken in rapid succession from the HA, PV and then from the HV catheter. At the time of sampling from a particular catheter the hepatic arterial and portal vein flows were read off the ultrasonic flow meter and noted down. Hepatic venous blood sampling was performed over ± 30 seconds via the balloon tipped catheter after inflation of the balloon to wedge position. The balloon was immediately deflated after this to avoid damming of hepatic venous blood at the catheter tip.

6.2.7 CONDUCT OF EXPERIMENT

After completion of the surgical preparation the animal was allowed to stabilize for 30 minutes. After this, base line measurements of cardiovascular variables, hepatic blood flow and temperature were made and sampling for biochemical analysis of liver function and blood composition was performed as follows: (See Figure 6.1.)

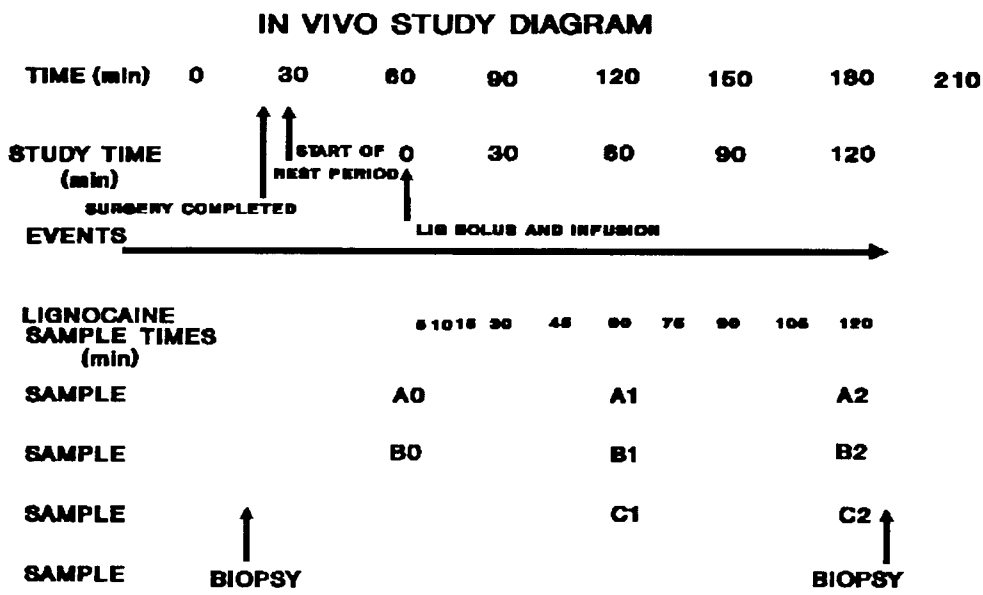

Figure 6.1 For Symbols see text.

Transhepatic samples (carotid artery, PV and HV) were taken to determine oxygen and lactate uptake at this time (AO) and then hourly (A1) and (A2). Carotid arterial samples were drawn to determine haemoglobin concentration, plasma total protein, albumin, urea, ALT, glucose, sodium, and potassium concentrations, plasma osmolality and for the determination of arterial bloodgases and pH (BO) and then hourly (B1) and (B2). The collecting receptacle for bile volume measurement was emptied for hourly volume determination as indicated by C1 and C2 on the accompanying diagram. Lignocaine administration was then started. Physical parameter measurements were repeated half hourly (reported in chapter 7). In addition hepatic blood flow was measured at the same times as sampling for lignocaine analysis was done. At the end of the two hour experimental period a second set of biopsies were taken. The position of the hepatic vein and portal vein catheters were again verified afterwhich the liver was resected and allowed to drain of blood. The gallbladder was removed prior to weighing the liver.

6.2.8 CALCULATIONS:

For convenience all calculations pertinent to this chapter are again repeated here.

6.2.8.A. Lignocaine Administration and Hepatic Extraction

Loading infusion **(Q1) = C1 x V1 x K1O/(1-$e^{-\beta t}$). (1)** (Mitenko and Ogilvie, 1972)(Wagner, 1974)

Maintenance infusion **(Q2) = C1 x V1 x K1O (2)** (Mitenko and Ogilvie, 1972) (Where C1 = the desired plasma concentration of lignocaine, V1 is the volume of the central compartment in $mlkg^{-1}$, K1O is the elimination constant and β the slow phase hybrid disposition rate constant).

Hepatic lignocaine extraction was determined from the carotid artery (HA), portal vein (PV), and hepatic vein (HV) lignocaine blood concentrations at each individual time point during the last hour of the study period (60, 75, 90, 105, 120 minutes after the start of the infusion).

Hepatic extraction ratio (E) was determined from:

$$E = \frac{HAQ \times [HA] + PVQ \times [PV] - THBF \times [HV]}{HAQ \times [HA] + PVQ \times [PV]} \quad (3)$$

Where HAQ, PVQ, THBF are the hepatic arterial, portal and total blood flow (HAQ + PVQ) respectively at the time of lignocaine sampling and [HA], [PV], and [HV] the lignocaine whole blood concentration in the respective vessels.

Hepatic clearance (CL) of lignocaine was determined from the quotient of total hepatic blood flow (Q) and the extraction ratio (E). **CL = QE (4)**

Hepatic intrinsic clearance (CLintrinsic) according to the Venous Equilibrum model

CLintrinsic = **QE ÷ (1-E) (5)**

6.2.8.B. Liver Oxygen Consumption and Lactate Uptake

Liver oxygen consumption and lactate uptake were calculated according to the Fick principle.

(HA concentration - HV concentration) x HA Flow min^{-1} + (PV concentration - HV concentration) x PV Flow min^{-1}, and expressed per 100g of liver wet weight. Oxygen Content (C): (ml $100ml^{-1}$ blood): C = 1.34 x Hb x % saturation + 0.0225 x PaO2 (Kpa) (where 0.0225 is the solubility factor for oxygen in blood, [Leigh, 1982] and 1.34ml of O_2 saturates 1 gram of haemoglobin [Ganong, 1983]).

6.2.9 EXCLUSION CRITERIA

As there is a low (3.4%) but recognised incidence of Malignant Hyperthermia susceptibility in the Landrace x Large White strain of pig (Harrison et al, 1969) and these animals are prone to stress, the following physiological exclusion criteria were established: [The number of animals excluded on these criteria (and therefore not included in this presentation) in both the study of in vivo lignocaine elimination and its effects on hepatic blood flow and function (Chapter 7) are indicated in brackets behind each criterion].

(a) Persistent temperature over $39^{o}C$ (1).

(b) Persistent heart rate over 150 bpm (2).

(c) Metabolic acidosis requiring more than 10 meq of sodium bicarbonate for correction (2)[2].

(d) Inability to maintain a PaO_2 of > 100mmhg at the FGFR and ventilatory parameters set (2).

Technical exclusion criteria were:

(a) Portal vein flow probe not sited proximal to the splenic vein inlet (2).

(b) Suspected mechanical obstruction of portal vein or hepatic artery due to flow probes (1).

(c) Excessive haemmorhage during surgery (1).

Biochemical Exclusion Criteria:

(a) Hepatic lactate release (4).

6.2.10 STATISTICAL ANALYSIS

Statistical analysis of the data was performed at the Medical Research Council Biostatistical Unit. Repeated measures analysis of variance was used to determine within and between group differences (Crowder and Hand, 1990). $P<0.05$ was regarded as significant. Results are presented as mean ± 1 standard error of the mean.

6.3 RESULTS

6.3.1 LIGNOCAINE ELIMINATION

Seven pigs were studied.

Whole blood lignocaine concentrations in the HA, PV and HV did not vary significantly with time during the 60-120 minute study period (Figure 6.2) and the mean concentrations over this period were (5.1 ± 0.4ug ml^{-1}), (4.9 ± 0.5ug ml^{-1}) and (2.0 ± 0.3 ug ml^{-1}) respectively. (The HA and PV concentrations were not significantly different.) Similarly the mean hepatic extraction ratio (0.61 ± 0.04),

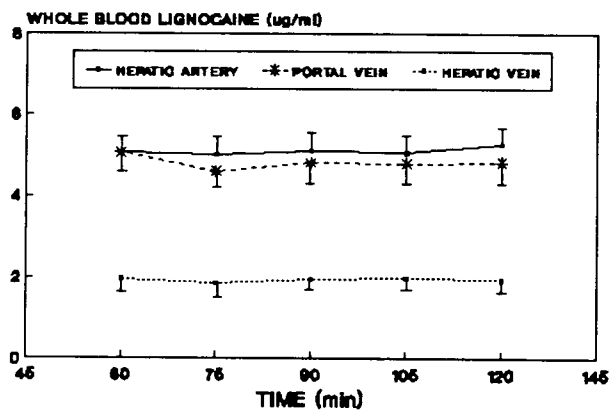

Figure 6.2 Mean (± SEM) whole blood lignocaine concentration in the hepatic vessels in vivo in 7 pigs during study period

2 In addition one of these animals manifested hepatic lactate release

blood clearance (381 ± 70 ml min-1), unit blood clearance (54.8 ± 11.2 ml $min^{-1}100gm^{-1}$) and intrinsic clearance (1132 ± 280 ml min^{-1}) as well as unit intrinsic clearance (166.7 ± 45.0 ml $min^{-1}100gm^{-1}$) did not vary with time (table 6.1).

Table 6.1

Hepatic Lignocaine Extraction Ratio and Clearance in the Pig

Parameter Time (mins):	**60**	**75**	**90**	**105**	**120**
Extraction Ratio	0.61±0.04	0.61±0.06	0.61±0.03	0.60±0.03	0.62±0.02
Hepatic Clear. (ml min^{-1})	372±74	403±84	380±72	386±73	367±54
Unit Hepatic Clear. (ml $min^{-1}100gm^{-1}$)	54±11	59±14	55±12	56±12	53±9
Intrinsic Clear. (ml min^{-1})	1200±390	1259±293	1070±266	1060±257	1090±232
Unit Intrinsic Clear.(ml $min^{-1}100gm^{-1}$)	189.1±61.9	185.5±57.0	157.2±43.1	154.5±40.4	158.8±35.2

Values = mean ± SEM. No variation with time of parameters over study period. Clear=clearance.

6.3.2 HEPATIC BLOOD FLOW

The hepatic blood flow (HAQ and PVQ) remained stable and did not vary significantly with time over the 60-120minute study period (Figure 6.3). The mean unit HA, and PV blood flows over this period were (35.7 ± 8.9 and 52.6 ± 7.1 ml 100gm-$liver^{-1}$) respectively. Although the HAQ throughout the study did not differ from the baseline measurement (time=0) taken just prior to the start of the lignocaine infusion the PVQ was significantly less from baseline throughout the study period.

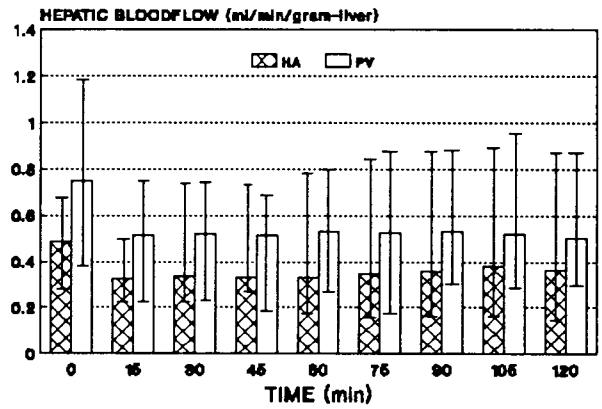

Figure 6.3 Mean (± SEM) hepatic artery and portal vein and total hepatic blood flow (HA + PV) over two hour experimental period in vivo. Baseline = 0 prior to lignocaine administration. PV flow different but HA flow no different from baseline over study period. From 15-120 minutes no variation with time of HA, PV or THBF.

6.3.3 HEPATIC FUNCTION

Liver function indices and plasma composition were stable and did not vary with time over the second hour after the start of the lignocaine infusion (Table 6.2).

Table 6.2

Liver Function Indices and Plasma Composition in Anaesthetized Pigs

Parameter **Time (hrs)**	**0 = baseline**	**1**	**2**
ATP (uM gm-liver^{-1})	7.10 ± 0.63	-	8.00 ± 0.39
ADP (uM gm-liver^{-1})	4.01 ± 0.21	-	4.43 ± 0.15
AMP (uM gm-liver^{-1})	1.81 ± 0.21	-	1.29 ± 0.23
ATP/ADP	1.79 ± 0.18	-	1.80 ± 0.09
Energy Charge	0.702 ± 0.019	-	0.744 ± 0.017
TAN(uM gm-liver^{-1})	12.92 ± 0.67	-	13.72 ± 0.46
Oxygen consumption (ml $O_2$100g^{-1})	4.9 ± 0.68	4.9 ± 0.9	3.4 ± 0.6
Bile Flow (ml hour^{-1})	-	-	6.5 ± 1.9
ALT (U/L)	24 ± 4	21 ± 2	22 ± 4
Albumin(g 100ml^{-1})	34 ± 2	28 ± 2[a]	28 ± 2[a]
Total Protein (g 100ml^{-1})	42 ± 3	36 ± 2	31 ± 1[a]
Osmolality (mosmols L^{-1})	257 ± 4	254 ± 5	253 ± 5
Hepatic venous PO_2 (mmHg)	60 ± 2	46 ± 3[a]	53 ± 3
Arterial PO_2 (mmHg)	243 ± 17	215 ± 7	211 ± 7
Haemoglobin (g%)	10.4 ± 0.2	10.8 ± 0.3	11.4 ± 0.4
Urea (mg 100ml^{-1})	27 ± 6	23 ± 5	19 ± 3
Glucose (mg 100ml^{-1})	88 ± 10	67 ± 11	78 ± 16
Lactate utilisation (mg min^{-1} 100 g^{-1})	5.2 ± 1.5	5.3 ± 1.8	3.5 ± 1.1
Lactate (HA) (mg 100ml^{-1})	16.1 ± 1.7	16.2 ± 1.8	15.8 ± 2.8
Lactate (PV) (mg 100ml^{-1})	16.8 ± 1.7	17.9 ± 2.2	15.6 ± 2.2
Lactate (HV) (mg 100ml^{-1})	9.0 ± 1.4	12.0 ± 2.3	11.3 ± 1.8
Temp. degrees Celsius	37.0 ± 0.2	36.8 ± 0.2	37.0 ± 0.1

Mean ± (SEM) (n=7) 0= baseline values before the administration of Lignocaine. (a) = significant difference from baseline (b) = significant difference over study period ie. between 1 and 2 hours. $P < 0.05$.

6.4 DISCUSSION

The implementation of an anaesthetic technique incorporating muscle relaxation and fluid replacement guided by determination of the PCWP using a pulmonary artery catheter appears to have resulted in a more stable preparation with regard to hepatic blood flow than achieved in the experiments described in chapter 5. The stability of hepatic blood flow is a prerequisite for the achievement of a constant concentration (Zierler, 1961).

The objective to achieve a constant lignocaine concentration in the region of 5ug ml^{-1} in this preparation for eventual comparison with studies in the isolated perfused pig liver (Chapter 8) was also achieved.

6.4.1 METHODS OF COMPARING DRUG CLEARANCE AND EXTRACTION

In designing this experiment one of the objectives was to compare the hepatic extraction ratio and clearance of lignocaine with data from studies in humans. There are essentially 3 methods of measuring blood clearance that can be adopted.

6.4.1.A. Determination of Lignocaine Clearance after a Bolus dose

After a bolus injection, the decay of a drug is subjected to pharmacokinetic analysis to determine the area under the drug concentration time graph (AUC)[3]. The mean systemic total drug clearance (Cls) can then be determined from:

Cls = Dose / AUC (1) (Tucker et al, 1977)

Mean hepatic drug clearance CL will then be determined from:

CL = Cls - Clr (2)

where Clr is an estimate of the renal clearance of a drug.

Finally the extraction ratio of the drug can be determined from:

E = Clh/Q (3)

where Q is the mean hepatic blood flow.

This methodology when applied to the data of 6 seperate experiments [Lalka et al, (1976); Rowland et al, (1971); Tucker and Mather, (1975)] in a review by Tucker et al, (1977) found a mean extraction ratio for lignocaine of 0.65.

However, this methodology requires a number of assumptions.

(1) That the hepatic blood flow (Q) chosen of 1500 ml is appropriate.

(2) That lignocaine is only cleared by the liver and kidney.

---

3 AUC = $A/\alpha + B/\beta$ for a two compartment model (Greenblatt and Koch-Weser, 1975), or can be calculated using the trapezoidal rule.

(3) That renal clearance remains constant. [Renal clearance was calculated as CLr = fu x Cls where fu is the fraction of the parenterally injected dose excreted unchanged under normal conditions of urine pH and taken to be 0.01 from the work of (Adjepon-Yamoah and Prescott, 1973) and (Lalka et al, 1976)] .

The authors (Tucker et al, 1977), aware that lignocaine itself may increase hepatic blood flow (Wiklund, 1977) have adjusted the calculation of E for their own data (Tucker and Mather, 1975) & (Rowland et al, 1971) using a hepatic blood flow of 2000ml determined by Wiklund (1977). This resulted in a decrease in the value of E in their data from 0.69->0.63.

The assumptions which have been made would make comparison with an animal study difficult.

6.4.1.B. Direct Measurement of Hepatic Extraction at Constant Drug Concentrations.

A second method for determining the parameters in question involves achieving (or approximating) a constant systemic concentration (Wiklund, 1977) and determining hepatic extraction by means of direct sampling from a hepatic vein, a systemic artery (as hepatic artery equivalent) and if possible (in animals) the portal vein. Hepatic blood flow must be determined. Such a study design was used by Stenson et al (1971) and Wiklund (1977). The estimated hepatic blood flow (EHBF) was determined in both studies by the ICG infusion method described by Bradley (1945) and modified by Caesar (1961).

This method is likely to be more accurate in determining the hepatic extraction and clearance of lignocaine as direct measurements of hepatic extraction ratio (E) (calculated as:

$$E = (Ca - Chv)/ Ca \qquad (4)$$

and an indirect measurement of hepatic blood flow were made. (where Ca and Chv = concentration in arterial blood and hepatic venous blood respectively)

Assumptions made here are:

(1) That hepatic arterial concentrations and portal vein concentrations are similar. This has been confirmed in the present study in pigs, where the HA whole blood concentration of (5.1 ± 0.4 ug ml^{-1}) was not statistically different from the portal vein concentration of 4.9 ± 0.5 ug ml^{-1}), as well as in dogs (Difazio and Brown, 1972).

(2) That the hepatic venous sample adequately reflects global hepatic effluent. (See section 2.6.3.)

(3) That estimated hepatic blood flow determined using ICG extraction is an accurate means of assessing hepatic blood flow. Although this has been accepted (Wiklund et al, 1975) others regard this as inaccurate (Skak and Keiding, 1987).

6.4.1.C. Determination of Total Body Clearance at Constant Drug Concentrations

This method for determining hepatic clearance assumes that all drug is hepatically cleared. Thus lignocaine clearance at constant plasma concentrations is equal to:

Lignocaine infusion rate / constant whole blood concentration

and extraction ratio (E) would be this clearance divided by hepatic blood flow.

However, this method if used to determine lignocaine hepatic clearance in man or the pig would be in error as Stenson et al (1971) in man and this study in pigs shows that lignocaine is metabolised extrahepatically (Section 6.4.5).

6.4.2 ESSENTIAL DETAILS OF HUMAN STUDIES

In order to compare the data from pigs with those in humans a brief summary of the essential points of importance in the two human studies mentioned will follow here.

6.4.2.A. Study of Wiklund (1977) reported by Tucker et al (1977)

The data used to determine the hepatic extraction ratio for lignocaine of E = 0.72 (SD=0.10) were derived from 4 healthy males (20-30 years of age) who had received a continuous lignocaine infusion at a rate of 4mg min^{-1} for a period of 150 minutes. At that time the extraction ratio of lignocaine was determined as in (4) and the EHBF shown to be a mean of 2000ml min^{-1}.

6.4.2.B. Study of Stenson et al (1971)

The data presented here for comparison (Table 6.2) are from a group of 17 patients (16-58 years of age) with cardiac disease who were undergoing cardiac catheterisation. Only the data from patients with normal liver function tests were used for the present comparison. (This excluded five patients included by Stenson et al, 1971.) After a bolus dose of lignocaine (50mg) and infusion of 40ug $kg^{-1}min^{-1}$ arterial and hepatic venous samples were taken at 60, 80, 90 and 100 minutes for determination of the extraction ratio. During this period four estimations of hepatic blood flow were also made.

6.4.3 HEPATIC LIGNOCAINE EXTRACTION IN MAN AND THE PIG

The methodology used by Stenson et al (1971) to determine hepatic extraction was similar to that adopted in the present study in pigs. This is true for the study of Wiklund (1977) as well, however, in the latter study only 4 data points were used, one per patient.

Table: 6.3

Lignocaine Extraction Ratio and Clearance in the Pig and Man

Author		Stenson 1971	Tucker 1977
	Pig	Man	
Number	7	12	4
Infusion Rate ($ug\ kg^{-1}min^{-1}$)	165	40	4.0[a]
Extraction Ratio	.61	.66	.72
Hepatic Clear.($ml\ min^{-1}$)	381	-	1430
Unit Hepatic Clearance. ($ml\ min^{-1}\ kg^{-1}$)	15.8	-	18.5[b]
Hepatic Blood Flow ($ml\ min^{-1}$)	614	860[c]	2000
Blood Concentration: ($ug\ ml^{-1}$)			
Arterial	5.1	2.0	2.0
Hepatic Vein	2.0	0.7	-

(a) = milligrams per minute, (b) = calculated from presented data, (c) = ml per m^2 body surface area.

The extraction ratios for lignocaine determined from the data of Stenson et al (1971) of 0.66 (SD=0.10) (n=12)[4] was statistically similar to that determined in pigs in this study (p=0.169) (Comparison performed using an unpaired Student's T-test).

6.4.4 EXTRACTION RATIO AS A COMPARATIVE INDEX OF DRUG METABOLISM

The question arises whether extraction ratio can be used to compare the metabolism of a drug in different species. The major objection would be that extraction ratio for most drugs is dependent on hepatic blood flow (Wilkinson and Shand, 1975). There is however experimental evidence that the extraction ratio of lignocaine does not change with hepatic blood flow in contrast to some other drugs. Administration of dl-propranolol to dogs reduced hepatic blood flow and resulted in an increased oxyphenbutazone extraction

4 The value for the extraction ratio of lignocaine determined from all of the data presented by Stenson et al is 0.64 (SD=0.04) (this includes 5 patients with deranged liver function left out of the comparison above) This value was also statistically similar to that determined in pigs

(from 0.13 to 0.23) (Branch et al, 1973a) whereas the extraction ratio (0.79) of lignocaine was unaffected[5] (Branch, et al, 1973b). Lautt and Skelton (1977) have shown the same lack of effect of flow (and concentration) on the extraction of lignocaine in cats. In these animals lignocaine is a poorly extracted drug (extraction ratio = 0.28 see table 6.4). The investigators speculate that this lack of effect is due to some specific property of lignocaine. In the present study in pigs there was no correlation of extraction ratio (n=34) with either hepatic arterial (r=0.23), portal venous (r=0.05) or total hepatic blood flow (r=0.17).

These considerations suggest that for lignocaine, extraction ratio is independent of flow and as such the comparison with the data of Stenson et al (1971) in humans is valid.

6.4.5 HEPATIC LIGNOCAINE METABOLISM AS A PERCENTAGE OF OVERALL METABOLISM

The percentage contribution of hepatic metabolism of lignocaine to its overall metabolism can be determined if the assumption is made that at steady state the rate of metabolism equals the rate of infusion. Stenson et al (1971) determined this to be 70% (SD=16%) in humans whilst in pigs in the present study this was 43% (SD=14%).

6.4.6 SUMMARY OF HUMAN AND PIG DATA

In summary the presented studies in humans indicate that the hepatic extraction ratio for lignocaine can be regarded as being of the order of 0.65. (based on a mean value of 0.64 (SD=0.04) found by Stenson et al, (1971) in 17 patients and the mean value of 0.65 from the 6 separate studies reviewed by Tucker and Mather (1977) involving 41 patients. The study of Wiklund et al (1977) (N=4) however, found an extraction ratio of 0.72 (SD=0.1) but is only based on single parameter estimates in 4 patients). A statistical comparison of the study of Stenson et al (1971) in humans with the present study in pigs found the hepatic extraction ratio to be similar.

This supports the hypothesis that the pig may serve as a useful model for the hepatic metabolism of lignocaine in man.

6.4.7. HEPATIC LIGNOCAINE EXTRACTION AND CLEARANCE IN THE PIG AND OTHER ANIMALS

The question arises whether the similarity in the hepatic extraction of lignocaine in man and the pig is unique or whether other animals would be equally suitable as human models in this regard. Table 6.4 shows the limited data in the literature that allowed a comparison of lignocaine extraction and clearance at

5 A recent study (Al-Asady et al, 1989) shows that β-blockers can directly inhibit lignocaine metabolism thus this may be a possible explanation for this phenomenon. This requires further investigation.

constant drug concentrations in studies that were more or less comparable to that performed in this study in pigs. The studies reported in the sheep, (anaesthetized and awake) dog, and cat, all used direct hepatic venous and arterial sampling for lignocaine concentration determination. In addition in the cat and sheep study, portal venous sampling was also performed so allowing the determination of hepatic extraction using formula (3)(Section 6.2.8.A.). In the monkey study, the method described under section 6.4.1.C. above was used. All studies were performed between 45mins - 120 minutes after starting a lignocaine infusion.

Table: 6.4

Lignocaine Extraction Ratio and Clearance in Animals

Author		**Mather 1986**		**Lelorier 1977**	**Benowitz 1974**	**Lautt 1977**
Animal	**Pig**	**Sheep Awake**	**Anaes**	**Dog Anaes**	**Monkey Awake**	**Cat Anaes**
Number	7	4	4	6	7	9
Infusion Rate (ug $kg^{-1}min^{-1}$)	165	4.7[a]	4.7[a]	140	100	200
Extraction Ratio	.61	.86[b]	.92[b]	.79	.66	.28
Hepatic Clearance (ml min^{-1})	381	1265[b]	952[b]	342	-	-
Unit Hepatic Clearance. (ml min^{-1} kg^{-1})	15.8	-	-	-	64	8.1
Hepatic Blood Flow (ml min^{-1})	614	1400[b]	1022[b]	-	-	-
Blood Concentration (ug ml^{-1}):						
Arterial	5.1	3.3[b]	4.12[b]	4.9	-	6.1
Hepatic Vein	2.0	-	-	1.0	-	-

(a) = milligrams per minute, (b) = calculated from presented data, (c) = ml per m^2 body surface area.
Anaes= Anaesthetized.

A comparison of this nature is subject to the considerations discussed in section 4.5.1.

Using the extraction ratio of lignocaine as a method of comparison it can be seen that the extraction ratio determined in sheep (anaesthetized and awake) of 0.86-0.92 and cat (0.28) are very different from that of 0.66 determined by Stenson et al (1971) in man. The extraction ratio in the dog is 0.79 but it would appear that values in the monkey (0.66) and the pig are closest to the human value for this parameter. The extraction ratio in the monkey may be falsley elevated as this was not determined using a direct method but by assuming that the total body clearance of lignocaine equals hepatic clearance and dividing this value by the hepatic blood flow (determined by microsphere injection). This assumption may not be valid

for the monkey as both the present study in the pig and that of Stenson et al (1971) in man have shown that only 43% and 70%, respectively, of total body lignocaine metabolism was accounted for by hepatic clearance. Mather et al (1986) have confirmed that some renal clearance of lignocaine occurs in sheep and it has been shown in vitro that lignocaine is metabolized in the hind quarters of this animal (Upton, 1991).

6.4.8. CONCLUSION

This study has shown that target constant concentrations of lignocaine can be achieved in the pig using the methodology described. The hepatic extraction ratio for lignocaine in the pig was similar to that determined for man and in this regard, the pig, with the possible exception of the monkey is closer than the other animals reported on.

This, together with the suggestion that the isolated pig liver yielded the same lignocaine metabolite profile as that reported for man (Chapter 3), supports the hypothesis that the pig liver may serve as an appropriate model for human hepatic lignocaine metabolism.

CHAPTER 7: THE EFFECT OF LIGNOCAINE ON IN VIVO HEPATIC FUNCTION AND BLOOD FLOW

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CHAPTER 7: THE EFFECT OF LIGNOCAINE ON IN VIVO HEPATIC FUNCTION AND BLOOD FLOW

SUMMARY

This study was performed to determine whether lignocaine administered at a constant concentration affected hepatic blood flow and function or had demonstrable effects on hepatocellular ultrastructure. Fourteen pigs were randomly allocated to receive either a two stage infusion of lignocaine hydrochloride or of saline. The animals were anaesthetized, appropriately catheterised and surgically prepared for measurement of haemodynamic parameters, hepatic blood flow and for transhepatic sampling. Liver biopsies were taken prior to and after 2 hours of the two stage infusion in both groups of animals for histological analysis and determination of adenine nucleotide status. A mean systemic constant whole blood lignocaine concentration of 5.1ug ml^{-1} was achieved during the second hour of infusion. Despite a significantly lower heart rate during this period in the lignocaine treated group as compared with the saline group there was no difference in hepatic blood flow or any other measured haemodynamic parameter. There was also no difference between the two groups in the indices used to measure hepatic function and plasma composition prior to and during the second hour of the respective infusions. On histological examination there were no electronmicroscopic changes that could be specifically attributed to the administration of lignocaine. This suggests that in anaesthetized pigs a constant lignocaine concentration of 5ug ml^{-1} has no detrimental effect on either hepatic function or blood flow and does not result in ultrastructural changes after two hours of lignocaine administration.

7.1 INTRODUCTION

7.1.1 MOTIVATION

A drug that is to be used as an indicator of hepatic function should (amongst other criteria) be non toxic (Branch, 1982) and lack (adverse) pharmacological effects (Barstow and Small, 1990). The central nervous (Rutten et al, 1989)(Liu et al, 1983) and cardiovascular effects (Reiz and Nath, 1986) of lignocaine have been extensively studied in animals and man. However, no reports on a study of the effects of lignocaine

on normal hepatic function could be found. Wiklund (1977) has shown that there is an increase in the estimated hepatic blood flow in man with continued infusion of lignocaine to a concentration in the lower therapeutic range, whilst Mather et al, (1986) could not demonstrate this effect in sheep. As far as known there have been no studies of the effect on hepatic blood flow of lignocaine administered specifically to achieve a concentration in the upper therapeutic range.

As stated in section 6.1.1 the study of lignocaine extraction and clearance provided an opportunity to assess the effects of lignocaine on hepatic function and blood flow by comparing the lignocaine treated group with a group which was in all respects similar but received only an infusion of saline. Tarba and Cracium, (1990) have recently reported an increase in mitochondrial basal respiratory rate as well as ultramicroscopic hepatocellular changes associated with the in vitro administration of lignocaine (and other local anaesthetics). This prompted the investigation of whether there were any detectable hepatocellular electronmicroscopic changes after administration of lignocaine in vivo.

7.1.2 METHODOLOGY

In order to determine the effects of lignocaine on hepatic blood flow all factors that were likely to affect hepatic blood flow had to be standardized in the saline and lignocaine treated group. The chief factors that may independently affect hepatic blood flow are (a) the method of ventilation as this may affect airway pressure and so intrathoracic pressure, (b) the blood carbon dioxide concentration, (c) the type and depth of anaesthesia employed as well as (d) the surgery performed (Gelman, 1987b)(Gelman, 1989). In order to address these issues the methodology described below (Section 7.2.2) was employed.

7.2 MATERIALS AND METHODS

To compare the effect of lignocaine on hepatic blood flow and function 14 pigs were studied. The study of the seven pigs receiving lignocaine has been described in section 6.2. A further seven standard pigs were studied concurrently using exactly the same methodology but substituting a saline infusion for the lignocaine infusion. Only aspects of the methodology not elaborated on earlier or pertinent to the above factors (Section, 7.1.2) will be highlighted here.

7.2.1 PROTOCOL

Fourteen standard pigs (seven in each group) were randomly allocated to receive either a two stage lignocaine (Experiment L) or saline (Experiment S) infusion to assess the effects of lignocaine administration on hepatic blood flow and function. Animals were anaesthetized and ventilated in a standard fashion and prepared for the measurement of mean arterial pressure (MAP), heart rate (HR),

cardiac output (CO), pulmonary cappilary wedge pressure (PCWP), as well as hepatic arterial flow (HAQ) and portal venous flow (PVQ). Further, catheters were placed to assess hepatic oxygen consumption and lactate utilisation as well as to determine biochemical parameters of liver function and blood composition while bile was collected hourly after ligating the cystic duct. Liver biopsies were taken prior to and two hours after the infusion of lignocaine was started for analysis of adenine nucleotide status as well as for evaluation by light and electronmicroscopy.

After surgical preparation baseline measurements were obtained in each group after a ½ hour rest period. The two stage infusion (of lignocaine or saline) was then started and hepatic blood flow and function studied during the second hour of the infusion period when constant lignocaine concentrations had been established as described in sections (4.4.5.B) and (6.2.6).

Calculation of oxygen and lactate utilisation were performed as described in section 6.2.8.B. Exclusion criteria were described in section 6.2.9, and statistical analysis was performed as described in section (6.2.10).

7.2.2 CONTROL OF FACTORS AFFECTING HEPATIC BLOOD FLOW

7.2.2.A. Mechanical Ventilation and Normocarbia

The respiratory rate of awake pigs in this study population was 25 breaths per minute (bpm). This rate was used as the standard rate for mechanical ventilation. In preliminary experiments using the standard anaesthetic technique (Section 6.2.2) and this respiratory frequency, normocarbia (35-40 mmHg) was established with a tidal volume of 10ml kg^{-1} measured using a Draeger Volumeter (USA) in the expiratory limb of the circle system. Using the Ohio Anaesthesia Ventilator (Wisconsin, USA) the appropriate tidal volume was set according to the weight of the study animal. The inspiratory and expiratory flow rates were fixed for all experiments whilst 'expiratory time' was adjusted to achieve a respiratory rate of 25 bpm. The 'inspiratory pressure limit' and 'trigger effort dials' were set at maximum to avoid any interference of these functions with set respiratory parameters. Thus in all experiments the same ventilator settings were maintained. Normocarbia was maintained by interposing tubes of varying length between the y-piece of the circle system and the endotracheal tube so increasing and decreasing deadspace as appropriate. This method avoided the need for altering tidal volume or respiratory rate to maintain normocarbia and was adopted in an attempt to maintain constant airway pressure within and as far as possible between experiments.

7.2.2.B. Anaesthesia and Surgery

Anaesthesia was standardized as described in section (6.2.2). Muscle relaxation was maintained using regular doses of pancuronium (4mg every 45 minutes) to avoid shivering and variations in muscle tone

which might provoke changes in airway pressure in ventilated animals. Muscle paralysis has the further advantage that it avoids non ventilator cycled respiratory efforts by the animal. This allows better control of normocarbia. Pancuronium was chosen to maintain muscle relaxation as it does not appear to affect hepatic blood flow (Varma et al, 1977) and did not interfere with the HPLC method used for lignocaine and metabolite determination. All surgery was performed by a single operator (Professor R Hickman).

7.2.3. MONITORING OF PHYSICAL PARAMETERS

7.2.3.A. Pressure and Ecg

Transduced pressure was monitored using a Hellige Servomed SMK154-3 Monitor which has 2 pressure channels. Mean arterial pressure was monitored continuously using channel (1) whilst PCWP was monitored using channel (2). The accuracy of this system was verified. (Appendix A.3.3). The pressure transducers were set to zero at the start of each study at a standard level approximating the mid-cardiac level. For this the height of the angle of the mouth of the recumbent pig was chosen. The Ecg incorporated in this monitor was used to determine heart rate.

7.2.3.B. Cardiac Output and Temperature

Cardiac output and core temperature were monitored using a 9520A Cardiac Output Monitor (Edwards Laboratories, California USA). Cardiac outputs were determined as follows: The appropriate calibration factors for the particular pulmonary artery catheter (93A 131 7F) were set for 5cc of injectate and the self test mode was run. Cardiac output measurements (in triplicate) were performed by injection of 5cc of normal saline[1] at a temperature less than $5^{o}C$. Saline was cooled using an in line coil submerged in crushed ice (Coset Cooling Container Model 93520, Edwards American Laboratories) and injected in under 4 seconds by a single investigator at end expiration (Runciman et al, 1981). Runciman et al (1981) have determined that the maximum variation between cardiac output measurements attributable to thermistor or computer error is less than 1% and that the average cardiac output measured in triplicate is within 10% of a simultaneously performed dye dilution measurement. The coefficient of variation for CO determination in triplicate in the present study was: 4.8% ± SEM = 0.9 (n=30) over the range 2.10 - 5.10 L min^{-1}.

7.2.4 HISTOLOGICAL EVALUATION OF LIVER BIOPSIES

Liver biopsies for histological assessment by light and electron microscopy were taken from 4 animals in which lignocaine was administered and from one which received only saline as a control. These were

1 Saline was substituted for the more conventional dextrose 5% to avoid any potential effect of varying glucose loads on metabolism. The product of the specific gravity and specific heat of these two indicators are equal (Ganz and Swan,1972)

taken at the same time as biopsies for adenine nucleotide status, namely, immediately after surgical preparation and two hours after the start of a lignocaine or saline infusion. (See appendix F for details of processing and equipment used for this analysis.)

7.3 RESULTS

7.3.1 HEPATIC FUNCTION

The pigs studied in Experiment (L) (n=7) and Experiment (S)(n=7) were of similar weight, namely 24.0 ± 0.43 kg and 23.7 ± 0.42 kg respectively as were the mean weights of the livers resected at the end of the study 702 ± 31g and 684 ± 27g.

The pigs in each group were similar with respect to the baseline measurements made of the indices of hepatic function and plasma composition prior to the administration of lignocaine or saline.

The mean systemic whole blood lignocaine concentration in Group L over the second hour after the start of the lignocaine infusion was 5.1 ± 0.4 ug ml^{-1}.

In this group the adenine nucleotide status, hepatic oxygen consumption, and lactate utilisation[2] after two hours of lignocaine administration was no different from that in the saline group whilst the bile volume produced over this period was also similar (Table 7.1). Similarly the plasma composition including the ALT concentration was no different between the two groups over the second hour after the start of the lignocaine infusion save for the PaO_2 and hepatic venous O_2 at the times indicated in Table 7.1. It should be noted that although some parameters differed from baseline values (Experiment L: albumin, total protein, hepatic venous O_2; Experiment S: hepatic ATP, TAN, oxygen consumption, total protein) after one hour of lignocaine administration, plasma composition remained stable over the chosen period of study (second hour after start of lignocaine infusion) and remained within physiological limits, while adenine nucleotide status appeared to improve.

2 Hepatic lactate release was an exclusion criterion (section 6.2.9). 5 Pigs were not included for analysis on this basis, 2 of these received saline and 3 lignocaine. This was not significant (Fischers exact Test).

Table 7.1

Liver Function Indices and Plasma Compostion: in Lignocaine Treated Group (L) vs Saline Group (S)

Parameter	Group..Time(hrs):	0	1	2
ATP (uM gm-liver^{-1})	(L)	7.10 ± 0.63	-	8.00 ± 0.39
	(S)	6.78 ± 0.49	-	8.38 ± 0.45[a]
ADP (uM gm-liver^{-1})	(L)	4.01 ± 0.21	-	4.43 ± 0.15
	(S)	3.55 ± 0.24	-	3.95 ± 0.20
AMP (uM gm-liver^{-1})	(L)	1.81 ± 0.21	-	1.29 ± 0.23
	(S)	1.45 ± 0.22	-	1.37 ± 0.19
ATP/ADP	(L)	1.79 ± 0.18	-	1.80 ± 0.09
	(S)	1.98 ± 0.24	-	2.13 ± 0.10
Energy Charge	(L)	0.702 ± 0.019	-	0.744 ± 0.017
	(S)	0.724 ± 0.026	-	0.755 ± 0.010
TAN(uM gm-liver^{-1})	(L)	12.92 ± 0.67	-	13.72 ± 0.46
	(S)	11.78 ± 0.42	-	13.70 ± 0.61[a]
Oxygen(ml O_2 100g^{-1})	(L)	4.9 ± 0.68	4.9 ± 0.9	3.4 ± 0.6
	(S)	4.5 ± 0.37	3.4 ± 0.3	2.7 ± 0.3[a]
Bile	(L)	-	-	6.5 ± 1.9
Volume (ml hr^{-1})	(S)	-	-	10.3 ± 2.3
ALT (U/L)	(L)	24 ± 4	21 ± 2	22 ± 4
	(S)	22 ± 2	22 ± 2	25 ± 2
Albumin(g 100ml^{-1})	(L)	34 ± 2	28 ± 2[a]	28 ± 2[a]
	(S)	29 ± 1	29 ± 1	26 ± 1
Total Protein	(L)	42 ± 3	36 ± 2	31 ± 1[a]
(g 100ml^{-1})	(S)	47 ± 3	36 ± 1[a]	39 ± 2[a]
Osmolality	(L)	257 ± 4	254 ± 5	253 ± 5
(mosmols L^{-1})	(S)	269 ± 6	261 ± 7	260 ± 6
Hepatic venous	(L)	60 ± 2	* 46 ± 3[a]	53 ± 3
PO_2 (mmHg)	(S)	61 ± 3	* 59 ± 4	58 ± 4
Arterial	(L)	243 ± 17	* 215 ± 7	* 211 ± 7
PO_2 (mmHg)	(S)	271 ± 9	* 270 ± 7	* 249 ± 12
Haemoglobin (g%)	(L)	10.4 ± 0.2	10.8 ± 0.3	11.4 ± 0.4
	(S)	10.7 ± 0.4	10.4 ± 0.4	10.5 ± 0.3
Urea (mg 100ml^{-1})	(L)	27 ± 6	23 ± 5	19 ± 3
	(S)	24 ± 7	18 ± 4	29 ± 8
Glucose(mg 100ml^{-1})	(L)	88 ± 10	67 ± 11	78 ± 16
	(S)	64 ± 7	59 ± 9	61 ± 9
Lac.Uptake	(L)	5.2 ± 1.5	5.3 ± 1.8	3.5 ± 1.1
(mg min^{-1}100g^{-1})	(S)	4.8 ± 0.8	3.0 ± 0.3	2.6 ± 0.4
Lactate (HA)	(L)	16.1 ± 1.7	16.2 ± 1.8	15.8 ± 2.8
(mg 100ml^{-1})	(S)	13.9 ± 1.1	14.1 ± 1.5	15.5 ± 1.6
Lactate (PV)	(L)	16.8 ± 1.7	17.9 ± 2.2	15.6 ± 2.2
(mg 100ml^{-1})	(S)	11.7 ± 0.9	14.8 ± 1.1	13.6 ± 2.0
Lactate (HV)	(L)	9.0 ± 1.4	12.0 ± 2.3	11.3 ± 1.8
(mg 100ml^{-1})	(S)	9.2 ± 1.4	11.8 ± 1.3	10.8 ± 2.2
Temp. degrees	(L)	37.0 ± 0.2	36.8 ± 0.2	37 ± 0.1
Celsius	(S)	37.2 ± 0.2	36.9 ± 0.3	37.1 ± 0.3

Mean ± (SEM) 0 = baseline values before the administration of Lignocaine (group L) or Saline (group S).* = significant difference between groups.(a) significant difference from baseline = time 0. (b) significant difference over study period ie. between 1 and 2 hours. $P < 0.05$ Lac= Lactate, Temp = temperature.

7.3.2 CARDIOVASCULAR PARAMETERS AND HEPATIC BLOOD FLOW

7.3.2.A. Cardiovascular Parameters

The baseline measurements (time=0) taken prior to the administration of lignocaine or saline are compared in Table 7.2. The baseline heart rate (HR), mean arterial pressure (MAP), cardiac output (CO), pulmonary cappilary wedge pressure (PCWP) and $PaCO_2$ were similar in the lignocaine and saline group respectively. The infusion of lignocaine resulted in a significant decrease in heart rate within that study group after one hour of administration. This resulted in a significantly lower heart rate in Group (L) with respect to Group (S) during the subsequent one hour study period. No other parameters varied with time or were different between the two groups.

Table 7.2

Cardiovascular Parameters during Lignocaine (L) and Saline (S) Infusion.

Parameter	Study	Time 0	60	90	120
Heart Rate	(L)	129 ± 2	* 106 ± 4[a]	* 105± 4[a]	* 106 ± 4[a]
(bpm)	(S)	139 ± 8	* 124 ± 7	* 129 ± 8	* 126 ± 8
MAP	(L)	83 ± 7	85 ± 9	86 ± 9	90 ± 9
(mmHg)	(S)	95 ± 7	92 ± 7	94 ± 7	94 ± 8
Cardiac Output	(L)	131 ± 14	105 ± 12	109 ± 12	105 ± 11
ml kg^{-1}	(S)	156 ± 13	134 ± 12	133 ± 10	130 ± 12
PCWP	(L)	5.8 ± 0.5	7.8 ± 1.1	8.1 ± 0.7	9.0 ± 0.7
(mmHg)	(S)	7.1 ± 0.7	6.6 ± 0.7	7.1 ± 0.7	7.2 ± 0.8
$PaCO_2$	(L)	38 ± 1	38 ± 1	38 ± 1	37 ± 1
(mmHg)	(S)	38 ± 1	38 ± 1	37 ± 1	37 ± 1

Mean ± (SEM) (L) = Pigs receiving Lignocaine infusion (n=7) (S) = pigs receiving saline infusion (n=7) both administered after baseline readings determined (Time = O). * = significant difference between (L) and (S) group. (a) = significant difference between time and time = 0 minutes.p = < 0.05. For abbreviations refer to accompanying text. bpm = beats per minute.

7.3.2.B Hepatic Blood flow

The baseline hepatic arterial flow, (48.6 ± 5.9 ml.100gm-liver^{-1}) and portal venous flow, (77.9 ± 11.2 ml.100gm-liver^{-1}) in the lignocaine group was no different from that in the saline group where the values were (65.4 ± 9.7 and 60.3 ± 5.1 ml.100gm-liver^{-1}) respectively, [(p=0.13) and (p=0.062) respectively] (Figure 7.1). These parameters remained stable over the subsequent study period in both groups.

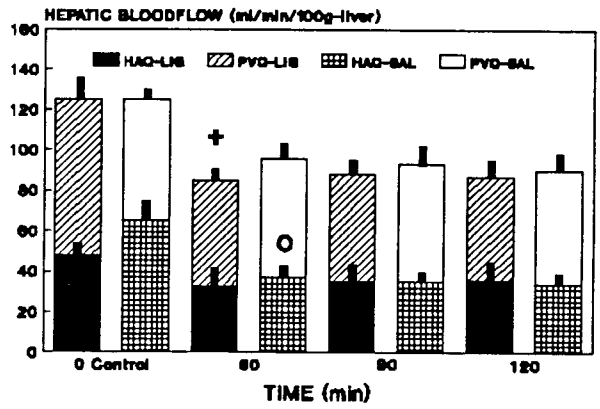

Figure 7.1 Mean (± SEM) hepatic blood flow in the hepatic artery (HAQ) and portal vein (PVQ) in pigs receiving lignocaine (Lig) (n=7) or saline (Sal) (n=7) administered after time 0 when baseline values were taken. Total hepatic blood flow = HAQ + PVQ. + = significant difference from baseline PVQ and total hepatic blood flow. o = significant difference from baseline HAQ.

There was a significant decrease in PVQ in Group (L) after the administration of lignocaine which resulted in significant decrease in total hepatic blood flow (THBF) at 60 minutes. In Group (S) there was a significant decline in HAQ after 60 minutes and so a decrease in THBF which did not reach significance. However there was no difference between the lignocaine and saline treated group with regard to HAQ, PVQ or THBF during the 60 minute study period despite a constant lignocaine concentration of 5.1 ug ml^{-1} in the lignocaine treated group.

7.3.3 HEPATIC HISTOLOGY

Samples were taken from 4 animals to which lignocaine was administered as well as one to which saline was administered as a control. Initial biopsies were taken prior to the administration of these agents and final biopsies after two hours of administration.

7.3.3.A. Light Microscopy

On light microscopy some swelling of hepatocytes was noted in all the final biopsies taken when compared with the initial biopsies (see Table F.1, Appendix F, section F.2.3.).

7.3.3.B Electronmicroscopy

All initial biopsies showed nuclear chromatin to have a normal distribution. There was fine vesiculation of the smooth and rough endoplasmic reticulum, the sinusoids were normal in appearance whilst occasional intracytoplasmic lipid droplets were present. The mitochondria were occasionally lucent, were variable in

shape, their membranes were intact and the cristae and matrix were preserved (see Table F.2, Appendix F, section F.2.3).

All the final biopsies showed clumping of the nuclear chromatin, pronounced vesiculation of both smooth and rough ER and occasionally dilated sinusoids, as well as intracytoplasmic lipid droplets. The mitochondria were swollen, had lost their variability in shape and there was loss of cristae and matrix lucency. The membranes were ruptured with extrusion of contents [see (arrow) Figure 7.2]. Myelin figures were present.

In the final biopsies no differences could be detected between those from animals to which lignocaine had been administered and the animal which only received saline.

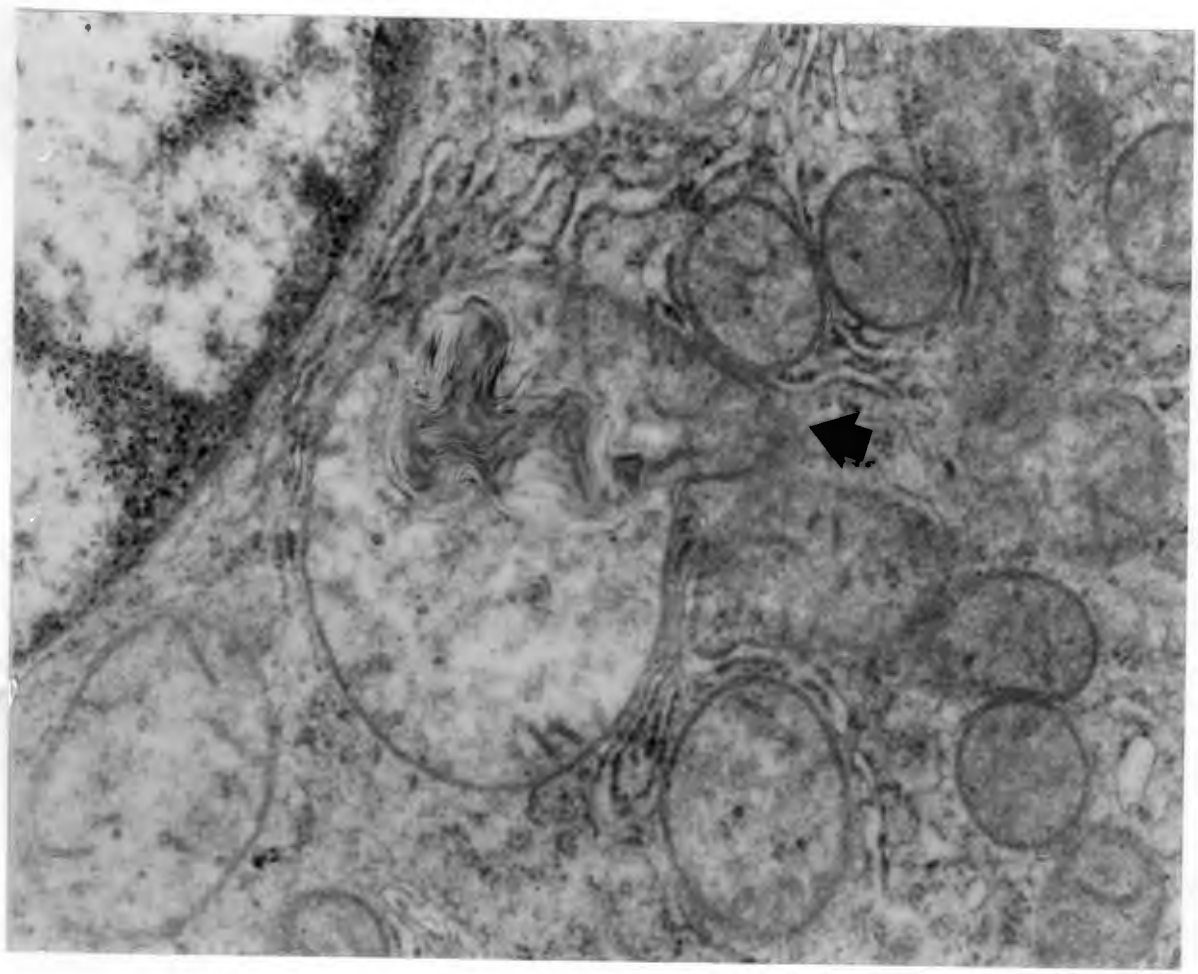

Figure 7.2 Electronmicrograph of liver biopsy (Group L) taken at the end of the in vivo study period showing (arrow) ruptured mitochondria with myelin figure. Magnification x39487.

7.4 DISCUSSION

This study was performed primarily to assess whether lignocaine infused to a constant concentration in the upper therapeutic range resulted in any detrimental effects on liver function or hepatic blood flow. The former was thought unlikely as there are no reports in the literature linking lignocaine with hepatic damage. However, there is a single report implicating Tocainide a so-called oral analogue of lignocaine in the development of reversible liver damage in association with the use of amiodarone (Nauta et al, 1984). The mean systemic whole blood concentration during the study period was 5.1 ug ml^{-1} (Plasma concentration = 5.9 ug ml^{-1}). This is in the upper therapeutic range for humans variably quoted as: between 1.5-5.5 ug ml^{-1} (Salzer et al, 1981) and 1.4-6 ug ml^{-1} (Collinsworth et al, 1974) in plasma. As higher concentrations are unlikely to be used clinically this would appear to be an appropriate concentration to assess potential detrimental effects on hepatic blood flow and function.

7.4.1. LIVER FUNCTION AND HISTOLOGY

With respect to the indices of liver function determined here, this study suggests that lignocaine has no detrimental effect on hepatic function in vivo. Further, there was no electron microscopic evidence that a two hour infusion of lignocaine hydrochloride resulted in histological changes in the liver which differed from those found in the absence of lignocaine administration. Histological changes did however occur with time and these were present in all livers studied. It is thus possible that, if there were changes induced by lignocaine, as has been found by Tarbia and Cracium (1990) in vitro, that these may have been obscured. It should be noted that the baseline biopsies taken prior to infusion of lignocaine or saline were taken after preparatory surgery as well as after approximately an hour and a half of anaesthesia. Despite this, biopsies taken approximately two hours later demonstrated obvious changes suggesting progressive damage. These changes consisted principally of damage to mitochondria. These nonspecific liver changes may occur as a result of differing insults to the liver. The final common pathway for cell injury has been postulated to be the formation of free radicals which may be the result of the exogenous metabolism of chemicals or drugs or hypoxia (Cotran et al, 1989). Free radical formation has also been implicated as a mechanism for the hepatic toxicity of volatile anaesthetic agents. (See Brown, 1988 for a review). Thus it is possible that these changes are related to the volatile anaesthetic (isoflurane) used, or alternatively due to the delayed effects of the surgery performed or on the basis of some as yet unrecognised mechanism. It is tempting to speculate that these findings may relate to the impaired adenine nucleotide status found in the isolated perfused pig livers studied after a prior in vivo study (Chapter 5).

Finally it should be noted that the histological changes observed may not be irreversible (Cotran et al, 1989).

7.4.2. HEPATIC BLOOD FLOW

When assessing the effects of lignocaine on hepatic blood flow due consideration should be given to the fact that these animals had recently undergone surgery and were anaesthetized and mechanically ventilated. Mechanical ventilation, anaesthesia, and the $PaCO_2$ may significantly affect hepatic blood flow (Gelman,1987b)(Gelman, 1989) and thus were standardized within and between experiments as described (Section 7.2.2).

Wiklund (1977) has shown in awake humans (n=4) that the infusion of lignocaine at a dose rate of 4mg per minute, resulting in a whole blood concentration of 2.0 ug ml^{-1} after 150 minutes, produced an increase in heart rate, cardiac output and mean arterial pressure as well as a 37% increase in estimated hepatic blood flow (EHBF). These changes did not occur in a placebo group.

The present study observed a similar time interval after the start of a lignocaine infusion but the concentration of lignocaine was at least twice that of those in the study of Wiklund et al (1977). There was no difference in the measured cardiovascular parameters save for a decrease in heart rate and also no difference in hepatic blood flow between the two groups. This suggests that at these concentrations there was no detrimental effect on hepatic blood flow. An increase in hepatic blood flow associated with administration of lignocaine might have been expected from the study by Wiklund (1977)-(Mather et al, 1986). The fact that this did not occur might be explained as follows:

The stimulatory effect of lignocaine on cardiovascular haemodynamics has been shown to be mediated through the autonomic nervous system (Kao and Jalar, 1959) while a direct negatively inotropic effect occurs on the myocardium (Nayler et al, 1969) and variable effects (vasoconstriction and vasodilation) occur on vascular smooth muscle (Blair, 1975). The degree of predominance of one over the other appears to be a function of the blood level of the agent (McWhirter et al, 1973).

It is conceivable, but speculative, that at the lignocaine concentration achieved in the present study in pigs, the direct cardiodepressant effects are more predominant than the autonomically mediated effects of lignocaine, resulting in a transition from increased hepatic blood flow to an ultimately diminished hepatic blood flow at increasing concentrations. The presence of central nervous system depressants in the form of anaesthetic agents with cardiovacular depressant effects of their own may enhance this (McWhirter et al, 1973). This theory gains support from the finding of Bromage and Robson (1961) in anaesthetized humans where acute toxicity (manifested by a falling blood pressure) occurred at a plasma lignocaine concentration of around 10ug ml^{-1}.

This theory would have to be tested by infusing lignocaine to a higher and lower constant concentration in groups of subjects for comparison of the effects on hepatic blood flow.

Mather et al, (1986) studied the effects of steady state lignocaine concentration (using a single infusion regime as was done in the present study) on hepatic blood flow in awake and anaesthetized sheep serving

as their own controls. In the awake sheep (n=4), lignocaine infused to a mean blood concentration of 3.3ug ml^{-1} appeared not to affect hepatic blood flow. In respect of the anaesthetized sheep (n=4), the authors speculate that the depressant effect of halothane on hepatic blood flow may have been opposed by an effect of lignocaine.

Fettman et al (1984) infused lignocaine (2mg $kg^{-1}hr^{-1}$ = 33ug $kg^{-1}min^{-1}$) in anaesthetized endotoxaemic Yucatan minipigs (n=4) and found no increase in hepatic blood flow despite an increase in MAP when compared to control endotoxemic pigs.

Thus to date, the increased hepatic blood flow demonstrated in man at low lignocaine concentrations has not been conclusively shown in animal experiments. However, this work in pigs adds to the work of others in showing that there is no detrimental effect of lignocaine on hepatic blood flow when this is maintained within the human therapeutic range. If lignocaine administered to the upper therapeutic range had depressed hepatic blood flow instead, it would decrease its own clearance. This would set in motion a positive feed back loop with a resultant rise in lignocaine concentration and the potential for toxicity.

7.5 CONCLUSION

This study assessed the effects of lignocaine on hepatic function, ultrastructure and blood flow over a period of one hour when a constant whole blood lignocaine concentration of 5.1ug ml^{-1} had been achieved after an hour of continuous lignocaine administration. When compared with subjects in which saline was infused in place of lignocaine no difference in the parameters of hepatic function or hepatic blood flow were found. Electron microscopic indications of cellular damage were found but these could not be attributed to the effect of lignocaine. This study suggests that in the anaesthetized pig lignocaine is not detrimental to the liver after two hours of infusion. If these findings can be extrapolated to man then lignocaine would fulfill one of the prerequisites of an indicator of hepatic function, namely that it be non-toxic to the liver.

CHAPTER 8: HEPATIC EXTRACTION AND CLEARANCE OF LIGNOCAINE IN THE NORMOXIC AND HYPOXIC ISOLATED PERFUSED PIG LIVER

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CHAPTER:8 HEPATIC EXTRACTION AND CLEARANCE OF LIGNOCAINE IN THE NORMOXIC AND HYPOXIC ISOLATED PERFUSED PIG LIVER

SUMMARY

A number of investigations are described in this chapter. In the first instance lignocaine uptake by the perfusion system and the effects of hypoxia on perfusate composition were investigated in the absence of a liver to establish whether measured changes in indices of liver function, perfusate composition or lignocaine elimination in later experiments could be attributed to the effects of hypoxia. Lignocaine elimination and hepatic function were studied in the normoxic isolated perfused pig liver and compared with data determined in vivo (Chapter 6) to determine whether this was similar, thus supporting the use of the isolated liver model for the study of the effects of hypoxia. Finally, the effects of hypoxia on the elimination of lignocaine and the formation of MEGX as well as on indices of hepatic function were investigated using the isolated perfused pig liver preparation. In vivo and ex vivo hepatic lignocaine elimination was found to be similar while hepatic lignocaine elimination and MEGX formation were found to be highly significantly impaired by hypoxia.

8.1 INTRODUCTION

8.1.1 THE ISOLATED PERFUSED PIG LIVER AS A MODEL

The purpose of this work was to investigate whether lignocaine extraction ratio and clearance could be used as potential indicators of hepatic function. In order to determine this it was decided to compare a group of standard isolated perfused livers with a group of livers, in all respects similar, which were however impaired by using a hypoxic perfusate. This method of further injuring the isolated liver was chosen as hypoxia is likely to increase the injury already sustained by a standard perfused liver (Bradford et al, 1986)(Lemasters et al, 1983) thus generating a spectrum of livers with near normal to impaired hepatic function for this assessment (See also discussion in sections 5.4.5.G and 5.4.6). In addition the effect of hypoxia on hepatic lignocaine elimination had not been investigated.

Hypoxia is defined as O_2 deficiency at the tissue level. **Hypoxic hypoxia** is where the PO_2 of arterial blood is reduced and **ischaemic hypoxia** is where the blood flow to a tissue is so low that adequate O_2 is not delivered (Ganong, 1983). In order to render the liver hypoxic (rather than ischaemic) in vivo, global hypoxia would have to be induced in the pig. This was likely to result in an unstable preparation (Larsen et

al, 1976)(Tashkin et al, 1972)(Jones, 1981) with a number of variables which would be difficult to control. It was thus decided to study this using the isolated perfused pig liver (IPPL). This preparation permitted a study of the effects of hypoxia in this regard whilst maintaining all other variables constant. In addition to this objective it was decided to establish whether the normoxic isolated perfused pig liver preparation extracted and cleared lignocaine to the same degree as the in vivo liver. If this was found to be the case, this would lend further justification to the use of the IPPL for the investigation described above.

8.1.2 LIGNOCAINE EXTRACTION RATIO AND CLEARANCE IN VIVO AND IN THE NORMOXIC AND HYPOXIC IPPL

In order to make a comparison between in vivo and ex vivo lignocaine extraction ratio and clearance it was decided to study this in the normoxic IPPL at similar constant lignocaine concentrations using similar sized livers, parameters of unit hepatic blood flow, temperature, perfusate composition, and acid base status to that established in vivo (Chapter 6). In turn, to assess the effects of hypoxia on the IPPL these same parameters would be reproduced. Thus, the group of normoxic livers studied here would be used to draw a comparison with both the group of livers studied in vivo (Chapter 6) as well as with the isolated livers subjected to hypoxia (Study C) described in this chapter. By studying hepatic lignocaine elimination at similar temperatures (Larsen, 1971), hepatic blood flow rates (Lautt and Greenway, 1987), and HA to PV flow ratios (Ahmad et al, 1984), these potentially important variables were standardized. Further, although preliminary experiments (Appendix E) had indicated that lignocaine extraction by the pig liver was independent of concentration over the experimental range, it was decided to exclude this as a potential variable by performing this investigation at similar hepatic affluent lignocaine concentrations.

8.1.3 LIGNOCAINE UPTAKE BY THE PERFUSION CIRCUIT IN THE ABSENCE OF THE LIVER (STUDY A)

In order to make an accurate assessment of the extraction of lignocaine by the liver using the IPPL, the extent to which lignocaine was lost from the perfusate due to other factors had to be assessed. The perfusion circuit and the bubble oxygenator used in these experiments were made of PVC and other plastics whilst the oxygenator surface was coated with silicone antifoam. It was conceivable that lignocaine might bind to these. It was thus necessary to ascertain whether any lignocaine was lost in the absence of the liver and if so at what point in time a steady state was achieved (Study A).

8.1.4 PERFUSATE COMPOSITION IN THE ABSENCE OF A LIVER SUBJECTED TO NORMOXIA AND HYPOXIA (STUDY B)

Blood is a living tissue and as such may undergo changes during the course of perfusion which could be erroneously attributed to changes in liver function. It was thus necessary to study the effects of a standard period of perfusion on all the parameters routinely measured in these experiments both during a normoxic perfusion and a hypoxic perfusion again without a liver being incorporated in the circuit (Study B).

8.2 MATERIALS AND METHODS

8.2.1 PROTOCOL: LIGNOCAINE METABOLISM AND HEPATIC FUNCTION IN THE NORMOXIC AND HYPOXIC ISOLATED PERFUSED PIG LIVER (STUDY C)

Fifteen pig livers, harvested from 22-25kg male Landrace x Large White pigs were isolated and perfused as described in sections (2.3.2) and (2.5). The livers were randomly allocated to the normoxic group (n=7) or hypoxic group (n=8). Hepatic arterial and portal venous blood flow were set at 35ml and 53ml per 100 gram liver weight (see later) respectively and the perfusate temperature maintained at 37^{o}C. Thirty percent oxygen was administered in the oxygenator. After the preparation was stable (½ hour) the liver was perfused with oxygenated blood for a further ½ hour before the administration of a bolus and infusion of lignocaine to achieve a constant lignocaine concentration as described in section 4.4.5.C. In the normoxic group, lignocaine hydrochloride was administered as a 2% solution; an initial bolus of 40mg was followed by an infusion of 2.8mg per minute. To achieve a similar lignocaine concentration in the hypoxic liver experiments, equivalent volumes of lignocaine in saline were administered as a 0.5% solution in the hypoxic group. Thus a bolus dose of 10mg and an infusion of 0.7mg per minute was administered. Lignocaine extraction and clearance as well as hepatic function were studied over the next two hours in the normoxic group of livers for comparison with the in vivo study (Chapter 6) as well as for comparison with the hypoxic isolated perfused pig liver group. In the latter study 2% oxygen was fed into the oxygenator (vide infra).

8.2.2 CONDUCT OF ISOLATED PERFUSED PIG LIVER STUDIES

8.2.2.A. Liver Harvesting

At laparotomy prior to liver resection, baseline liver biopsies for the determination of adenine nucleotide status as well as liver water content were taken. Further an arterial sample was drawn from the pig for analysis of alanine aminotransferase (ALT) concentration in order to exclude animals with subclinical hepatic disease. The liver was resected and cannulated with cannulae of known mass and then weighed

prior to perfusion. The mass of the cannula and an assumed weight of 30 grams for the gall bladder was then subtracted to arrive at an assumed liver mass. This assumed mass was used to determine the perfusate flows to the HA and PV so as to give a flow of 35 and 53ml $100gram^{-1}$ liver respectively. These values were the mean flows determined in vivo (Chapter 6). The true liver mass representing functional parenchymal mass was calculated by establishing the true weight of the gall bladder after dissection at the end of the study. Thus, **True Liver Mass** = Weight of resected liver and attached cannulae - (weight of cannulae + true weight of gall bladder). This value of liver mass was used in all subsequent calculations.

8.2.2.B. Liver Perfusion

All livers were perfused immediately after weighing. The oxygenator used to oxygenate the perfusate was initially supplied with a fractional oxygen percentage (FO_2) of 30% monitored with a polarographic oxygen monitor (Ohio Oxygen Monitor 201,USA). This was connected between the outflow of the rotameter block and the inflow of the oxygenator [Section 2.4.2 and figure 2.2 (b)-M] and was calibrated daily in 100% oxygen and 100% nitrogen to set the zero reading. The rotameter flows per minute were; nitrogen = 2 litres, oxygen = 450ml and carbon dioxide 75 ml. These were adjusted to maintain the $PaCO_2$ between 35-40mmHg whilst keeping the (FO_2) at 30%. In the experiments where the livers were subjected to hypoxia; after initial normoxic perfusion, a hypoxic gas mixture was administered by decreasing the administered oxygen from 450ml per minute to just enough to show a 2% reading on the oxygen monitor. In order to assess only the effect of oxygen induced hepatic damage, independent of pH changes, the perfusate pH was monitored continuously using a pH electrode (Radiometer, Copenhagen)[1] suspended in the perfusate of the portal venous reservoir. The volume of perfusate in the reservoir was held constant by adjusting the height of the reservoir above the liver surface. The perfusate pH was held above 7.43 by intermittent or continuous (in the hypoxic study) infusion of 4.2% sodium bicarbonate. This was infused using a calibrated infusion pump (B.Braun, Perfusor Secura, West Germany).

The perfusate temperature, monitored continuously with a calibrated (see appendix A.3.4) mercury in glass thermometer suspended in the portal venous reservoir, was held at 37°C, which was the mean core temperature determined from the in vivo study (Chapter 6).

8.2.2.C. Conduct of Study

After the usual ½ hour stabilisation period (section 2.5.1) during which the (FO_2) was kept at 30% in both the normoxic and hypoxic study group a baseline biopsy for adenine nucleotide status and transhepatic sampling for hepatic oxygen consumption and lactate uptake were performed (A baseline) (See figure 8.1, page 8.9). In addition, a sample was drawn from the hepatic artery cannula for determination of perfusate glucose, haematocrit, haemoglobin, total protein, albumin, AST, ALT, urea, sodium and potassium concentration as well as for plasma osmolality and arterial blood gases and pH (B baseline). This sample

1 Calibrated daily using pH indicator solutions.

was also analysed for haemolysis. Immediately after this, in the hypoxic study only the (FO_2) was decreased to 2%. The alarm limits on the oxygen monitor were appropriately adjusted to ensure that this concentration was maintained. In all other respects the normoxic and hypoxic studies were similar and were performed as follows.

One half hour after taking the baseline samples mentioned above a further sample (B0) was again drawn for the corresponding analyses (as for $B_{baseline}$ above) this specifically and solely for comparison with the in vivo study[2] where a sample for this analysis was taken at this time just prior to the administration of lignocaine. Samples A and B were repeated as indicated in Figure 8.1.

The lignocaine bolus and infusion was begun 60 minutes after the start of the perfusion as indicated on the accompanying diagram and transhepatic samples for lignocaine analysis were drawn at 5, 10, 15, 30 min, as well as at (50, 55, 60, 65, 70 min) and (110, 115, 120, 125, 130 min) thereafter. The bracketed times constituted the experimental periods when parameters of lignocaine elimination were determined for correlation with other indices of hepatic function (See section 8.4.1, page 8.31). The samples were analysed in duplicate for lignocaine and MEGX concentration. The volume of bile collected over the previous hour was noted at 60 and 120 minutes (C1 and C2) (Figure 8.1). At the end of the study a further biopsy for liver water content was performed. The liver was then decannulated and left to drain for 15 minutes after which the gallbladder was removed and both organs were weighed. The final flow settings of the Sarns' roller pumps (which had been checked every 15 minutes during the course of the experiment) were confirmed and the volume of flow in one minute collected in an Erlenmeyer flask to determine (in triplicate) the mean HA and PV flow for the particular study.

8.2.2.D. Determination of Liver Tissue Water Content

The biopsies taken in vivo (initial) and at the end of the ex vivo study (final) to determine liver H_2O uptake were immediately wrapped in tin foil for transport to the laboratory. Here they were weighed (W1) and then placed in a dessicator for 48 hours when the weight was again determined (W2) using a fine balance. The percentage change of tissue water content was determined as follows: (W1-W2)/W1 was determined for both the initial and final specimens taken. The difference between these was divided by the ratio (W1-W2)/W1 for the initial specimen and then multiplied by 100.

8.2.2.E. Histological Examination of Biopsies

In one normoxic study and in 3 hypoxic studies, biopsies were taken in vivo and at the end of the study period ex vivo to determine the changes due to hypoxia by light microscopy (LM) and electron microscopy (See appendix F.2 for methodology). In addition, in one further hypoxic liver, one biopsy was taken from each lobe and assessed by LM to establish whether hypoxic changes were diffuse.

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2 Chapter 6. Sham samples of equivalent volume were also drawn from the hypoxic livers but they were discarded.

8.2.3 COMPARISON OF IN VIVO AND EX VIVO HEPATIC LIGNOCAINE EXTRACTION RATIO AND CLEARANCE AND LIVER FUNCTION USING DIFFERENT LIVERS

This comparison is presented here, at this stage, in the thesis for two reasons: **(a)** It would be difficult to study lignocaine extraction and clearance under severely hypoxic circumstances in vivo. A demonstration that there is no difference in hepatic lignocaine extraction and clearance in vivo and ex vivo would justify studying the effects of hypoxia in the IPPL. **(b)** In order to minimise repetition.

8.2.3.A. Introduction

The in vivo study investigating lignocaine extraction ratio and clearance (Chapter 6) and the normoxic isolated perfused pig liver study just described were designed specifically to compare in vivo and ex vivo lignocaine extraction and clearance as well as hepatic function under (as far as possible) standardized conditions of liver weight, hepatic blood flow, lignocaine concentration (and duration of administration) plasma vs perfusate composition and temperature. Thus, seven livers were studied in vivo and the mean unit hepatic arterial, and unit portal venous flows and temperature determined over the one hour study period (second hour of a two stage infusion of lignocaine). Seven livers were then studied ex vivo, the mean unit flows determined in vivo could be administered and the temperature controlled to be similar to the in vivo value. Lignocaine extraction and clearance and hepatic function were studied for comparison with the in vivo study over the same time period after the start of a lignocaine infusion at similar mean (HA and PV) plasma lignocaine concentrations. By using pigs over a similar weight range (22-25kg) it was hoped that the mean liver weights between the two studies would be comparable.

8.2.3.B. Material and Methods

The methodology used for the assessment of lignocaine metabolism, liver function and plasma composition in vivo is described in section 6.2. The methodology used for the study ex vivo is described above (Section 8.2.2). Only the salient points necessary to the comparison will be highlighted here. See figure 8.1 and 8.2. In both studies the animals were anaesthetized using the standard technique adopted in section 6.2, and the respective livers were prepared for transhepatic sampling and hepatic blood flow measurement. The time to the start of the study period in vivo and ex vivo after induction was the same. Liver function and plasma/perfusate composition were assessed at similar times within the respective study periods (See figures 8.1 & 8.2). An exception to this was that the baseline lactate uptake and oxygen consumption were determined one half hour prior to the administration of lignocaine in the IPPL ($A_{baseline}$) and just before this time in the in vivo preparation (Ao). Subsequently A1 and A2 were drawn at similar times during the study period. Samples (BO, B1, B2) were drawn from the hepatic artery cannula for determination of perfusate glucose, haematocrit, haemoglobin, total protein, albumin, AST, ALT, urea, sodium and potassium concentration as well as for plasma osmolality and arterial blood gases and pH. In addition bile was collected for determination of hourly bile flow (C1, C2).

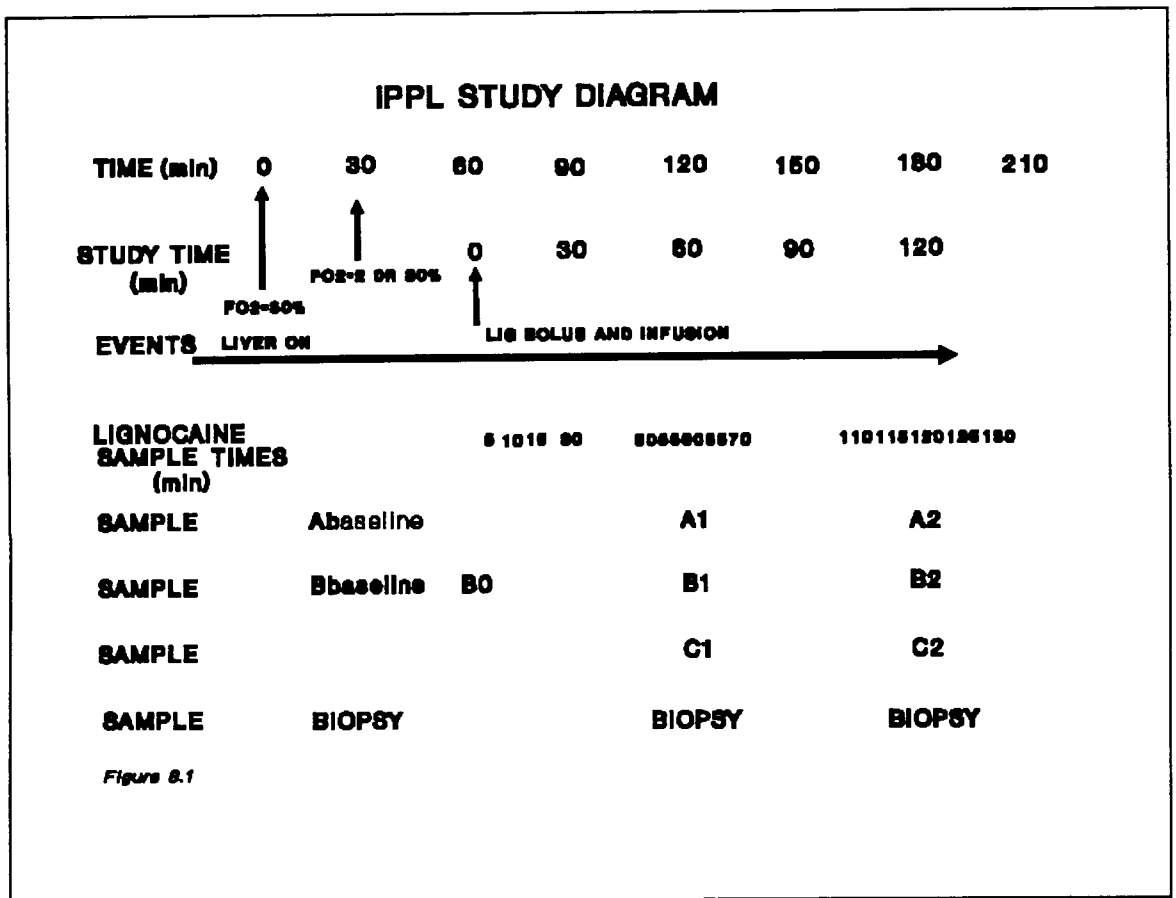


Figure 8.1

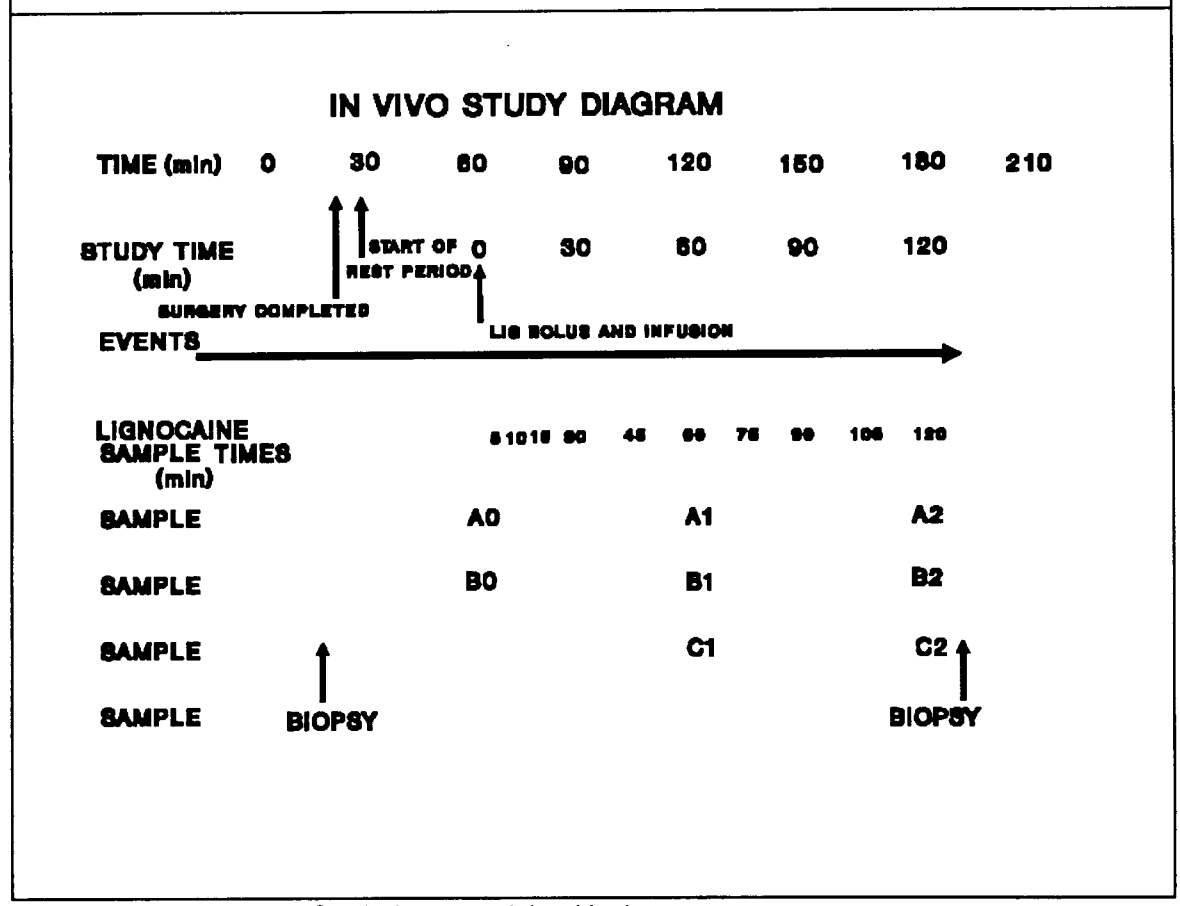


Figure 8.1 (top) and 8.2. Symbols are explained in the text.

Lignocaine was administered in both preparations to achieve comparable lignocaine concentrations as described in sections (4.4.5.C.) and (6.2.6). The samples used to determine lignocaine extraction and clearance at each individual time point were taken over similar time periods after the start of lignocaine administration, namely in vivo (60-120 mins) and ex vivo (50-130 mins) but were taken more frequently ex vivo (n=10) than in vivo (n=5).

Only two biopsies for the determination of adenine nucleotide status were taken in vivo (so as not to disturb the preparation within the study period) and these were used for comparison with those taken at similar times ex vivo namely one half hour prior to lignocaine administration and after two hours of lignocaine administration.

8.2.4 EXCLUSION CRITERIA

In vivo studies were excluded as documented in section 6.2.9. Exclusion criteria for isolated liver perfusions were as established in section (2.5.3). In addition, in this study two normoxic isolated liver experiments were excluded (and are not presented here) as lignocaine concentrations[3] exceeded the range for which first order metabolism had been confirmed in preliminary experiments (Appendix E).

8.2.5 LIGNOCAINE UPTAKE STUDY (STUDY A) AND PERFUSATE COMPOSITION STUDY (STUDY B) PERFORMED IN THE ABSENCE OF A LIVER

Three experiments were performed in study (A) and 5 for each group (normoxic and hypoxic) in Study (B). These three groups of experiments were all closely similar as they were intended to mimic exactly a standard perfusion incorporating a liver. Therefore, sham sampling was performed (for lignocaine in Study (B) and for metabolic parameters in Study (A) as outlined in detail above (Section 8.2.2.C). Infusions of saline were administered in Study (B) to mimic the lignocaine administration in standard perfusions.

Briefly, fresh heparinised (5000u/liter) abbatoir blood from at least 5 donor pigs was collected and all experiments were started within 1½ hours after this time. The perfusate consisted of exactly 1600 ml of this blood diluted with 600ml Plasmalyte B + 10ml of sodium bicarbonate (4.2%). Instead of the liver completing the circuit the hepatic venous drainage " Y " tube (Figure 2.1) was used, the portal vein cannulae feeding into one limb and the arterial cannula into the other and so draining to the hepatic venous reservoir. All samples were drawn from a three way tap in the "hepatic artery line".

The perfusate was warmed to 37^{O}C over 3/4 of an hour, comparable to the period prior to liver insertion. This was followed by a further ½ hour during which the liver would normally be perfused prior to the start of the study proper, after which, the FO_2 was diminished only in the hypoxic perfusate group but not in the

3 These higher values suggested an error of lignocaine administration as the calculated extraction ratios for these experiments were within the normal range.

other two groups. During this period sampling for perfusate composition was started as outlined above. A lignocaine hydrochloride bolus (Study A) of 12mg (calculated to achieve a concentration ± 5ug ml^{-1} of lignocaine base) or a sham bolus and infusion (Study B) was administered ½ hour later and all sampling (as well as sham sampling) was done at standard times as for a standard liver perfusion described above.

8.2.6 STATISTICAL ANALYSIS

The statistical analysis of the data was performed at the Medical Research Council Biostatistics Unit. Repeated measures analysis of variance was used to determine within and between group differences. Pearsons correlation coefficient was used to generate the correlation matrices presented. $p < 0.05$ was regarded as significant and $p < 0.01$ as highly significant. Data are presented as mean ± 1 standard error of the mean.

8.3 RESULTS

8.3.1 LIGNOCAINE UPTAKE BY THE PERFUSION CIRCUIT IN THE ABSENCE OF THE LIVER STUDY (A)

There appeared to be a decline in lignocaine concentration for the first 15 minutes after injection of the bolus dose (Figure 8.3) after which a constant concentration was achieved which did not vary over the period 30-120 minutes indicating that there was no further loss of lignocaine to the circuit.

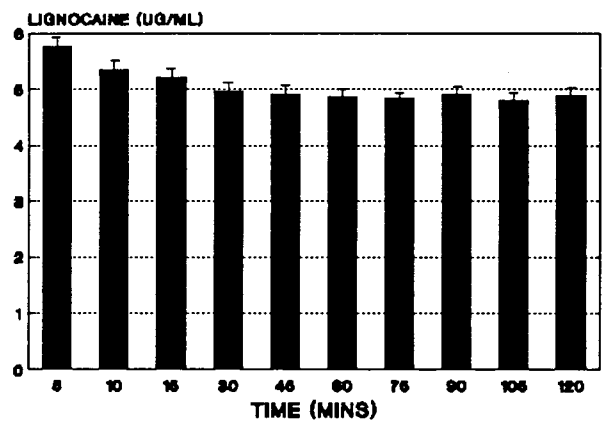


Figure 8.3 Mean (+ SEM)(n=3) perfusate plasma lignocaine concentration in the circuit after a bolus dose in the absence of a liver.

8.3.2 PERFUSATE COMPOSITION IN THE ABSENCE OF A LIVER WHEN SUBJECTED TO NORMOXIA AND HYPOXIA STUDY (B)

In general the perfusate composition did not vary with time and was no different under hypoxic conditions (Table 8.1). Notable exceptions were that in both studies there was a similar statistically significant decrease in glucose concentration over the two hour study period as well as a rise in potassium concentration of approximately 0.7 mmol which reached significance in the normoxic study. There was a tendency for the perfusate pH to decline in blood rendered hypoxic ($p=0.054$).

Table 8.1

Perfusate Composition under Normoxic and Hypoxic Conditions During 2 Hour Study Period

Parameter	Study	Time:	(Baseline)	1hr	2hr
PaO_2	(N)		144 ± 17	* 140 ± 12	* 144 ± 14
	(H)		157 ± 10	* 28 ± 3[a]	* 25 ± 4[a]
Sodium (mmol L^{-1})	(N)		136 ± 2	138 ± 3	140 ± 4
	(H)		137 ± 2	139 ± 3	140 ± 2
Total Protein	(N)		55 ± 4	56 ± 4	57 ± 4
(g $100ml^{-1}$)	(H)		51 ± 5	47 ± 5	54 ± 5
Albumin	(N)		33 ± 2	33 ± 1	33 ± 1
(g $100ml^{-1}$)	(H)		31 ± 1	33 ± 1	33 ± 1
Osmolality	(N)		* 272 ± 6	267 ± 7	266 ± 4
(mosmol L^{-1})	(H)		* 244 ± 5	253 ± 3	153 ± 6
Haematocrit	(N)		34 ± 2	36 ± 2	36 ± 1
	(H)		34 ± 1	36 ± 1	36 ± 1
Haemoglobin (g%)	(N)		11.9 ± 0.5	12.0 ± 0.4	12.0 ± 0.5
	(H)		11.3 ± 0.5	11.9 ± 0.6	12.0 ± 0.6
Plasma Hb	(N)		42 ± 6	41 ± 14	38 ± 13
(mg $100ml^{-1}$)	(H)		56 ± 14	52 ± 15	43 ± 14
Glucose	(N)		58 ± 12	40 ± 7	29 ± 10[a]
(mg $100ml^{-1}$)	(H)		63 ± 6	36 ± 7[a]	28 ± 8[a]
Lactate	(N)		41.2 ± 4.4	45.6 ± 4.6	45.7 ± 4.6
(mg $100ml^{-1}$)	(H)		29.7 ± 8.4	43.9 ± 4.6	42.9 ± 4.1
ALT (U/L)	(N)		44 ± 5	40 ± 7	45 ± 4
	(H)		48 ± 5	47 ± 3	50 ± 2
AST (U/L)	(N)		44 ± 6	51 ± 7	61 ± 5
	(H)		63 ± 9	91 ± 11	81 ± 16
Potassium	(N)		7.0 ± 0.2	7.4 ± 0.2	7.7 ± 0.2[a]
(mmol L^{-1})	(H)		7.0 ± 0.3	7.6 ± 0.3	7.6 ± 0.3
Urea	(N)		21 ± 4	30 ± 7	22 ± 5
(mg $100ml^{-1}$)	(H)		19 ± 5	21 ± 6	18 ± 4
pH	(N)		7.49 ± 0.03	7.42 ± 0.03	7.42 ± 0.02
	(H)		7.45 ± 0.03	7.39 ± 0.03	7.37 ± 0.04

Mean ± (SEM) parameter values of perfusate composition in the absence of a liver subjected to normoxia (N)(n=5) or hypoxia (H)(n=5) * = significant difference between groups (a) = significant difference with baseline time. P = < 0.05 AST = Aspartate aminotransferase and ALT = Alanine aminotransferase.

8.3.3 COMPARISON OF IN VIVO AND EX VIVO HEPATIC LIGNOCAINE EXTRACTION AND CLEARANCE AND LIVER FUNCTION

The mean weights of the livers in vivo (n=7) and ex vivo (n=7) were 695 ± 26g and 632 ± 22g respectively which were not statistically different (P=0.09), whilst the mean unit HA and PV flows were also similar (35.7 ± 8.9 and 52.6 ± 7.1 ml gram^{-1}) in vivo and (35.8 ± 0.7 and 54.4 ± 0.8 ml gram^{-1}) ex vivo respectively.

8.3.3.A. Lignocaine Metabolism

The whole blood lignocaine concentration in the HA, PV and HV both in vivo and ex vivo did not vary with time over the study period. Similarly, the derived parameters of lignocaine extraction ratio and clearance did not vary with time and were thus pooled. The mean values of these concentrations and derived parameters are compared in Table 8.2. showing them to be similar in vivo and ex vivo.

Table 8.2

Comparison of In Vivo and Ex Vivo Hepatic Lignocaine Extraction Ratio and Clearance

Parameter	In Vivo	Ex Vivo	P Value
Whole Blood Conc:			
Hepatic Artery	5.1 ± 0.4	4.8 ± 0.3	0.51
Portal Vein	4.9 ± 0.5	4.8 ± 0.4	0.85
Hepatic Vein	2.0 ± 0.3	1.8 ± 0.2	0.63
Extraction Ratio	0.61 ± 0.038	0.63 ± 0.021	0.67
Hepatic Clear. (ml min^{-1})	381 ± 70	363 ± 16	0.85
Unit Hepatic Clear.(ml min^{-1} 100g^{-1})	54.8 ± 11.2	57.1 ± 2.1	0.84
Intrinsic Clear. (ml min^{-1})	1132 ± 280	1069 ± 109	0.87
Unit Intrinsic Clear.(ml min^{-1} 100g^{-1})	166.7 ± 45.0	170.6 ± 18.2	0.89

Mean ± (SEM) Clear=Clearance. This comparison was performed using an unpaired Student's T-test.

8.3.3.B. Hepatic function and Plasma and Perfusate Composition

(1) Plasma versus Perfusate Composition

At the start of the in vivo and ex vivo studies the plasma and perfusate were similar in respect of most of the determined values (Table 8.3) except for the haemoglobin, total protein and glucose concentrations, pH and potassium which were lower in vivo and the PaO_2 which was higher in vivo. Despite the latter the hepatic venous PaO_2 was similar. Over the study period there was a significant rise in sodium ex vivo which resulted in a difference between the two groups at the one and 2 hour sampling times. This change

was evident too when the same livers were compared in vivo and ex vivo (Chapter 5, Table 5.1). However, in that study there was also a difference in the albumin concentration between perfusate and plasma which was not encountered here over the study period. Plasma and perfusate osmolality again differed during the study period at the one and two hour sampling time and urea rose ex vivo resulting in a significant difference between the groups as found during the earlier in vivo and ex vivo comparison.

Table 8.3

Plasma and Perfusate Composition In Vivo and Ex Vivo

Parameter	Study	Time: 0hr	1hr	2hr
Sodium	in vivo	136 ± 1	* 135 ± 1	* 133 ± 1
(mmol L^{-1})	ex vivo	141 ± 2	* 144 ± 2	* 147 ± 1^{a}
Total Protein	in vivo	*42 ± 3	*36 ± 2	*31 ± 1^{ba}
(g $100ml^{-1}$)	ex vivo	*59 ± 2	*57 ± 2	*59 ± 3
Albumin	in vivo	34 ± 2	28 ± 2^{a}	28 ± 2^{a}
(g $100ml^{-1}$)	ex vivo	32 ± 2	32 ± 1	32 ± 6
Osmolality	in vivo	257 ± 4	* 254 ± 5	* 253 ± 5
(mosmol L^{-1})	ex vivo	267 ± 5	* 271 ± 4	* 268 ± 7
Haemoglobin	in vivo	* 10.4 ± 0.2	* 10.8 ± 0.3^{a}	11.4 ± 0.4
(mg%)	ex vivo	* 12.7 ± 1.0	* 13.2 ± 1	12.6 ± 0.6
Plasma Haemoglobin	in vivo	37 ± 16	56 ± 14	31 ± 11
(mg $100ml^{-1}$)	ex vivo	53 ± 8	54 ± 9	56 ± 11
Glucose	in vivo	* 88 ± 10	67 ± 11	78 ± 16
(mg $100ml^{-1}$)	ex vivo	* 148 ± 15	98 ± 10^{a}	80 ± 10^{a}
Lactate (HA)	in vivo	16.1 ± 1.7	16.2 ± 1.8	15.8 ± 2.8
(mg $100ml^{-1}$)	ex vivo	10.1 ± 2.7	9.1 ± 2.7	21.4 ± 4.5^{ab}
Lactate (PV)	in vivo	16.8 ± 1.7	* 17.9 ± 2.2	15.6 ± 2.2
(mg $100ml^{-1}$)	ex vivo	10.3 ± 2.7	* 9.3 ± 2.7	20.8 ± 4.2^{ab}
Lactate (HV)	in vivo	9.0 ± 1.4	12.0 ± 2.3	11.3 ± 1.8
(mg $100ml^{-1}$)	ex vivo	14.7 ± 3.7	9.9 ± 2.6	20.3 ± 4.6
Hepatic venous	in vivo	60 ± 2	46 ± 3^{b}	53 ± 3
PO_2 (mmHg)	ex vivo	56 ± 2	58 ± 2	60 ± 2
PaO_2 (mmHg)	in vivo	* 243 ± 17	* 215 ± 7	* 211 ± 7^{b}
	ex vivo	* 127 ± 10	* 122 ± 7	* 120 ± 4
Urea	in vivo	27 ± 6	* 25 ± 5	* 19 ± 3
(mg $100ml^{-1}$)	ex vivo	33 ± 4	* 41 ± 4	* 44 ± 4
Temperature	in vivo	37.0 ± 0.2	36.8 ± 0.2	37 ± 0.1
Degrees Celsius	ex vivo	37	37	37
pH	in vivo	* 7.43 ± 0.02	* 7.44 ± 0.02	* 7.45 ± 0.02
	ex vivo	* 7.50 ± 0.01	* 7.48 ± 0.01	* 7.45 ± 0.01^{ab}
Potassium	in vivo	* 3.9 ± 0.2	*4.1 ± 0.1	4.3 ± 0.1
mmol L^{-1}	ex vivo	* 5.4 ± 0.2	*4.8 ± 0.2	4.8 ± 0.3

Mean ± (SEM) in vivo (n=7) ex vivo (n=7). * = significant difference between groups. (a) = significant difference with time = 0. (b) = signicant difference between time 1 and 2 hours.

(2) Parameters of Liver Function and Injury

(i) Oxygen Consumption and Bile Flow

Despite an initially higher hepatic oxygen consumption in vivo (4.9 ± 0.7 vs 2.6 ± 0.3 ml $min^{-1}100g^{-1}$) (Figure 8.4) this declined in vivo to 3.5 ± 0.7 ml $min^{-1}100g^{-1}$ which was no different from the value of 2.5 ± 0.2 ml $min^{-1}100g^{-1}$ ex vivo at the two hour sampling time. The bile flow ex vivo, 5.6 ± 0.4 ml $hour^{-1}$ at one hour and 4.4 ± 0.6 ml $hour^{-1}$ at 2 hours although less than in vivo (9.31 ± 2.3 vs 6.6 ± 0.2 ml $hour^{-1}$ respectively) was not significantly so during the two hour study period (Figure 8.4b) whilst neither varied with time.

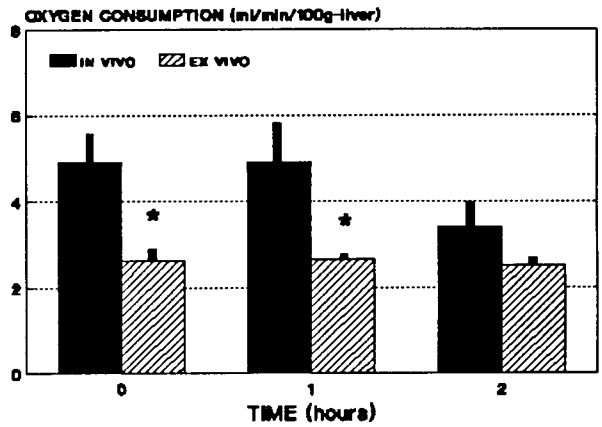
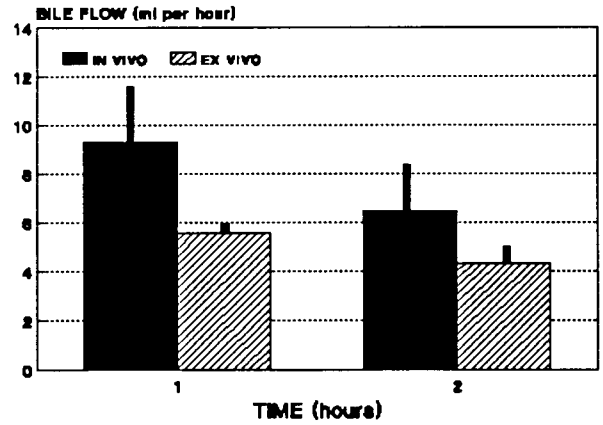


*Figure 8.4 Hepatic oxygen consumption and bile flow in vivo and ex vivo * = difference between in vivo and ex vivo values $p<0.05$.*



(ii) Adenine Nucleotide Status

Although the hepatic ATP content ex vivo appeared lower at the two sampling times (5.8 ± 0.3) and 6.6 ± 0.3) than in vivo (7.1 ± 0.6) and (8.0 ± 0.4) uM gram-liver^{-1} respectively this was not significant (Figure 8.5) whilst the hepatic ADP content was equivalent and there was a significantly lower AMP content ex vivo of the initial biopsies taken. These differences resulted in a statistically lower TAN content ex vivo at both sampling times (Figure 8.6)(10.2 ± 0.3 and 11.5 ± 0.8 ex vivo and 12.9 ± 0.9 and 13.7 ± 0.5 uM gram-liver^{-1} respectively in vivo).

However, both the hepatic energy charge and the ATP/ADP ratio (Figure 8.6) were no different in the two preparations. (Hepatic energy charge: 0.702 ± 0.013 and 0.744 ± 0.17 in vivo at the 0 and 2 hour sampling times versus 0.746 ± 0.020 and 0.748 ± 0.020 respectively ex vivo. ATP/ADP ratio respectively: 1.79 ± 0.18 and 1.80 ± 0.09 in vivo and 1.85 ± 0.14 and 1.80 ± 0.18 ex vivo.

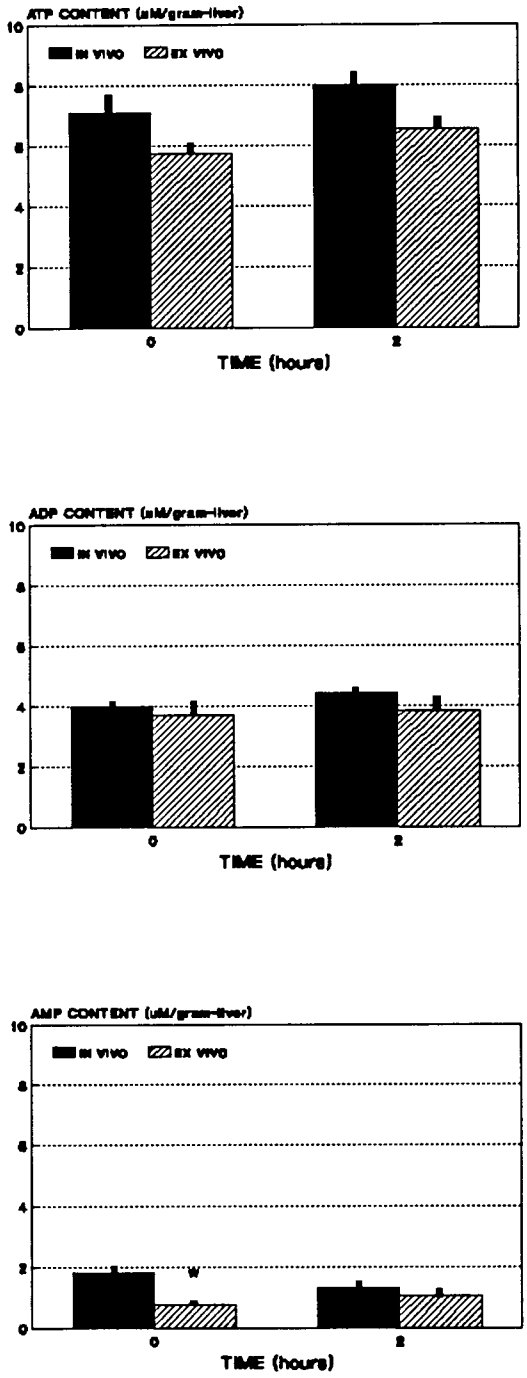
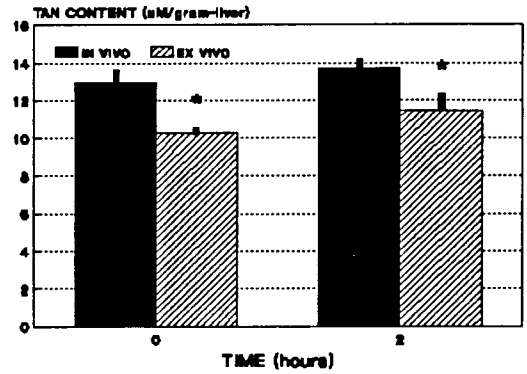
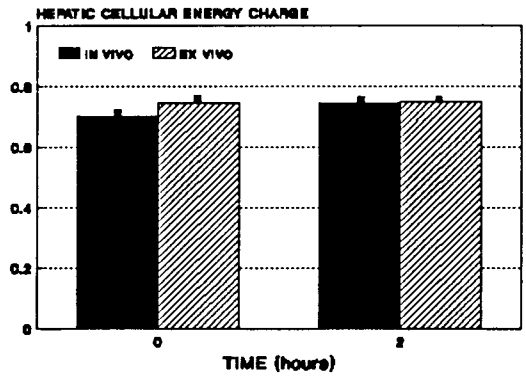


Figure 8.5. Hepatic adeninine nucleotide content in vivo and ex vivo.





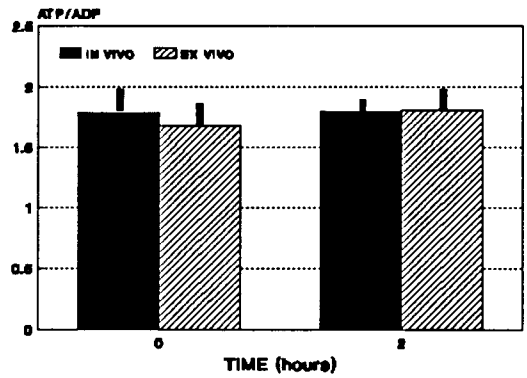


*Figure 8.6 Hepatic TAN content, energy charge and ATP/ADP ratio in vivo and ex vivo. * = difference between in vivo and ex vivo values $p<0.05$.*

(iii) Alanine Aminotransferase.

The alanine aminotransferase concentration was initially significantly higher ex vivo (44 ± 4 UL^{-1}) when compared with in vivo (25 ± 4 UL^{-1})(Figure 8.7) however, at the two hour sampling time this was no longer the case: ex vivo 35 ± 8 UL^{-1} and in vivo 22 ± 4 UL^{-1}). The decrease in concentration ex vivo with time was not statistically significant.

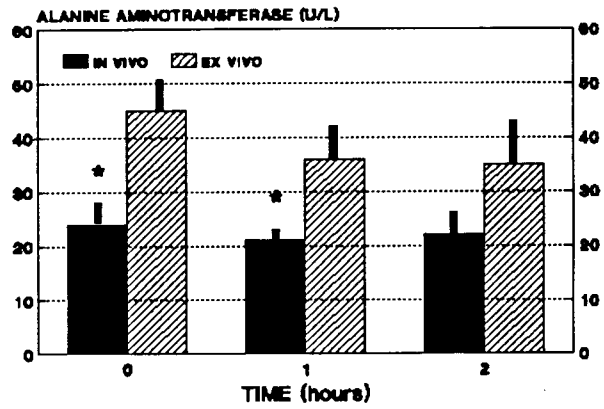


*Figure 8.7 Plasma and perfusate alanine aminotransferase concentrations in vivo and ex vivo. * = difference between in vivo and ex vivo value P < 0.05.*

(3) Summary of Findings of In Vivo versus Ex Vivo Comparison

Lignocaine extraction and clearance as well as hepatic function were compared in vivo (n=7) and ex vivo (n=7) using different livers. Liver weight and hepatic blood flow as well as hepatic affluent lignocaine concentrations were similar in each group.

Lignocaine extraction ratio and clearance as well as intrinsic clearance were similar in both groups.

Plasma and perfusate were different in respect of the haemoglobin, urea, sodium and potassium concentrations as well as the osmolality and the pH. Nevertheless these values remained within the normal physiological range. Oxygen consumption and ALT concentration were only different during the initial part of the study period whilst bile flow was nonsignificantly less ex vivo. Although TAN was consistently less ex vivo the energy charge and ATP/ADP ratio were similar.

Hepatic lactate uptake was measured in both studies but this was not compared as lactate release in vivo during the study period was regarded as an exclusion criterion.

8.3.4 LIGNOCAINE METABOLISM AND HEPATIC FUNCTION IN NORMOXIC AND HYPOXIC ISOLATED PERFUSED PIG LIVERS (STUDY C)

The livers harvested for the normoxic (n=7) and hypoxic studies (n=8) were similar in weight, 632 ± 22g and 700g ± 28g (p=0.09) respectively and had similar hepatic adenine nucleotide status in vivo before resection (Figure 8.8) whilst the plasma ALT concentrations in the donor pigs prior to liver harvesting were also similar [normoxic (36 ± 2.4 UL^{-1}) and hypoxic group of livers (30 ± 3.5 UL^{-1})] . The mean unit HA and PV flows ex vivo were 35.8 ± 0.7 ml $min^{-1}100g^{-1}$ and 54.4 ± 0.8 ml $min^{-1}100g^{-1}$ in the normoxic group and 34.2 ± 0.9 ml $min^{-1}100g^{-1}$ and 52.1 ± 1.0 ml $min^{-1}100g^{-1}$ respectively in the hypoxic group of livers which was
similar.

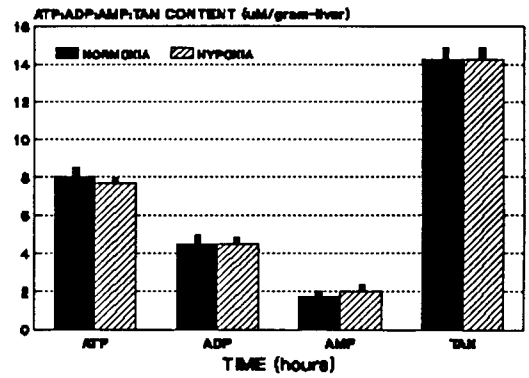


Figure 8.8 In vivo adenine nucleotide status of livers harvested for investigation of the effects of normoxia and hypoxia.

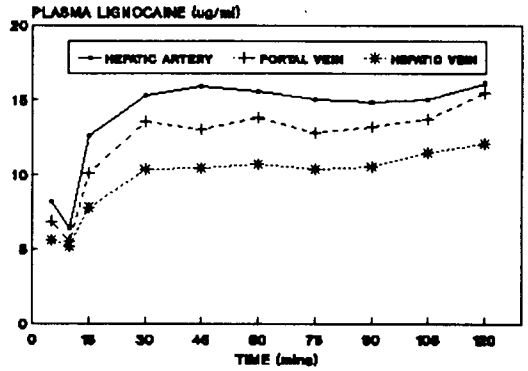
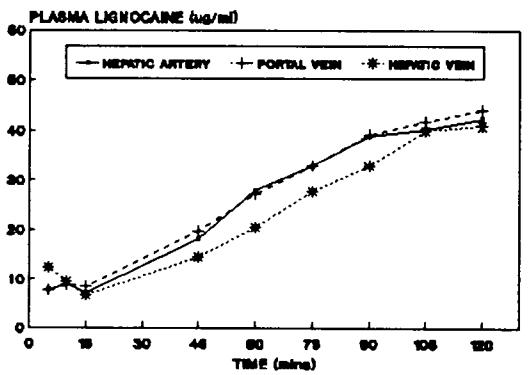


Figure 8.9 Two hypoxic isolated perfused pig liver experiments in which doses of lignocaine as given to normoxic livers were administered.

8.3.4.A. LIGNOCAINE METABOLISM

(i) Preliminary Experiments

In a number of preliminary experiments it was found that the standard lignocaine bolus dose and infusion rate determined to achieve a hepatic affluent target concentration of ± 5ug ml^{-1} in the normoxic IPPL (Section 4.4.5.C.) resulted in much higher concentrations of lignocaine when administered to individual hypoxic livers (See figure 8.9). This suggested that the elimination of lignocaine was impaired by hypoxia. It was found that a constant lignocaine concentration within the expected



range could be achieved in hypoxic perfusions when a ¼ bolus of lignocaine and an infusion rate ¼ of that administered in the normoxic study were used (Figure 8.10).

(ii) Definitive Experiments

Lignocaine

In the definitive experiments, mean whole blood lignocaine concentrations in the HA, PV and HV in the normoxic and hypoxic studies did not vary significantly with time for the duration of the total study period (50-130 minutes). Figure 8.10 shows that during the two experimental periods (50-70 min and 110-130 min) hepatic arterial and portal venous lignocaine concentrations were similar but that the HV concentrations were highly significantly different when the groups of normoxic and hypoxic livers were compared. This reflects the decreased lignocaine elimination by the hypoxic livers.

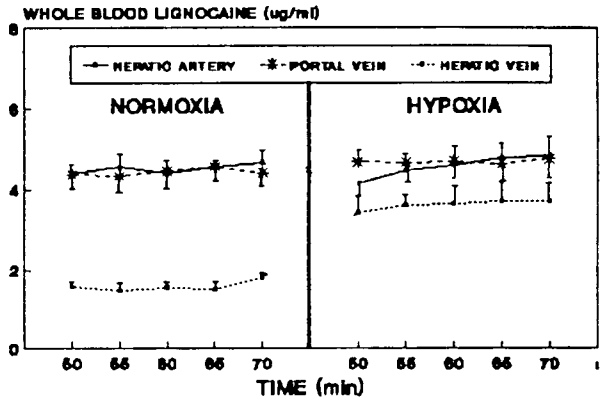
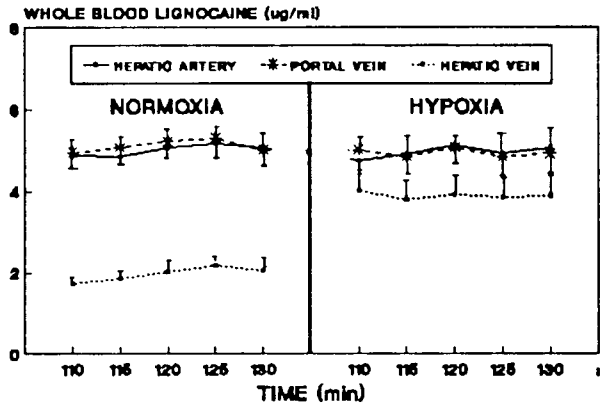


Figure 8.10 Mean (± SEM) whole blood lignocaine concentrations in the hepatic vessels in normoxic (n=7) and hypoxic (n=8) livers over the two study periods (50-70 min-top)(110-130 min-bottom).



Although not statistically significant, inspection of figure 8.10 suggests that there was a tendency for the mean lignocaine concentration to increase over the total study period (50-130 min) in both groups.

As there was no variation with time in the derived parameters of lignocaine metabolism calculated at each individual time point (n=10), the pooled mean values of these parameters are compared in Table 8.4.

Lignocaine extraction ratio in hypoxic livers was 64% less than in normoxic livers. Lignocaine hepatic clearance and unit hepatic clearances were 61% and 64% less, while hepatic intrinsic clearance and unit

Intrinsic clearance were 82% and 83% less respectively in hypoxic livers when compared with normoxic livers.

MEGX

MEGX was not invariably detected in the hepatic venous effluent of all studies and chromatographic interference with plasma constituents made its determination in some samples unreliable. These sample values were ignored. Mean hepatic vein MEGX concentrations in the normoxic group (n=53) and the hypoxic group (n=43) were significantly different as were the mean MEGX/lignocaine ratios in the hepatic venous effluent (Table 8.4).

Table 8.4

Comparison of Lignocaine Metabolism in Normoxic and Hypoxic Pig Livers

Parameter	Normoxia	Hypoxia	P Value
Whole Blood Lignocaine Concentration (ug ml^{-1}):			
Hepatic Artery	4.8 ± 0.3	4.8 ± 0.5	0.97
Portal Vein	4.8 ± 0.4	4.8 ± 0.5	0.83
Hepatic Vein	1.8 ± 0.2	3.8 ± 0.5	<0.01
Lignocaine			
Extraction Ratio	0.63 ± 0.021	0.23 ± 0.034	<0.01
Hepatic Clearance(ml min^{-1})	363 ± 16	141 ± 19	<0.01
Unit Hepatic Clear.(ml min^{-1} $100g^{-1}$)	57.1 ± 2.1	20.3 ± 3.1	<0.01
Intrinsic Clearance (ml min^{-1})	1069 ± 109	195 ± 37	<0.01
Unit Intrinsic Clear.(ml min^{-1} $100g^{-1}$)	170.6 ± 18.2	28.4 ± 5.3	<0.01
Hepatic Vein			
MEGX Plasma Concentration (ug ml^{-1})	0.85 ± 0.06	0.34 ± 0.04	<0.01
MEGX/Lignocaine ratio	0.379 ± 0.061	0.073 ± 0.014	<0.01

Pooled mean ± (SEM) values in normoxic and hypoxic group of livers over the whole study period.

8.3.4.B. Hepatic Function and Perfusate Composition

(1) Perfusate Composition

The perfusate composition (baseline values) prior to decreasing the FO_2 in the hypoxic group of livers were similar to those in the normoxic group (Table 8.5). The hypoxic gas mixture administered resulted in a hepatic affluent PaO_2 of ± 18 mmHg in the hypoxic group of livers with a significantly lower hepatic venous PO_2.

Table 8.5

Perfusate Composition of Normoxic and Hypoxic Pig Livers Ex Vivo

Parameter	Study	Time: (baseline)	1hr	2hr
PaO_2(mmHg)	(N)	128 ± 13	* 122 ± 7	* 120 ± 4
	(H)	134 ± 5	* 18 ± 2[a]	* 16 ± 2[a]
Urea	(N)	31 ± 8	41 ± 4[a]	44 ± 4[a]
	(H)	29 ± 4	40 ± 4[a]	44 ± 4[a]
Sodium (mmol L^{-1})	(N)	141 ± 2	144 ± 2	147 ± 1[a]
	(H)	143 ± 2	142 ± 2	147 ± 4
Total Protein	(N)	63 ± 2	57 ± 2	59 ± 3
(g $100ml^{-1}$)	(H)	57 ± 2	52 ± 2	56 ± 1
Albumin	(N)	33 ± 1	32 ± 1	32 ± 6
(g $100ml^{-1}$)	(H)	34 ± 2	33 ± 1	32 ± 1
Osmolality	(N)	269 ± 4	271 ± 4	* 268 ± 7
(mosmol L^{-1})	(H)	263 ± 5	285 ± 10	* 291 ± 11[a]
Haematocrit	(N)	35 ± 1	37 ± 1	36 ± 2
	(H)	34 ± 1	34 ± 1	33 ± 1
Haemoglobin (g%)	(N)	12.1 ± 0.6	13.2 ± 1.0	12.6 ± 0.6
	(H)	12.4 ± 0.4	12.6 ± 0.3	12.5 ± 0.3
Plasma Haemoglobin	(N)	63 ± 8	54 ± 9	56 ± 11
(mg $100ml^{-1}$)	(H)	102 ± 27	81 ± 29	69 ± 27
Glucose	(N)	59 ± 4	98 ± 10	80 ± 10
(mg $100ml^{-1}$)	(H)	62 ± 6	106 ± 10	52 ± 11
Lactate (HA)	(N)	10.1 ± 2.6	* 9.1 ± 2.7	* 21.4 ± 4.5[ab]
(mg $100ml^{-1}$)	(H)	15.7 ± 4	* 45.7 ± 7.1[a]	* 43.7 ± 6.1[a]
Lactate (PV)	(N)	10.3 ± 3.1	* 9.3 ± 2.7	* 20.8 ± 4.2[ab]
(mg $100ml^{-1}$)	(H)	8.2 ± 1.9	* 36.2 ± 5.1[a]	* 44.3 ± 6.1[a]
Lactate (HV)	(N)	14.7 ± 6.9	* 9.9 ± 2.6	* 20.3 ± 4.5
(mg 100 ml^{-1})	(H)	9.4 ± 1.9	* 35.9 ± 4.8[a]	* 54.4 ± 5.4[ab]
Hepatic Venous PO_2	(N)	56 ± 2	* 58 ± 2	* 60 ± 2
(mm Hg)	(H)	52 ± 3	* 10 ± 1[a]	* 7 ± 1[a]
Hep. O_2 extraction	(N)	0.18 ± 0.016	* 0.17 ± 0.013	* 0.16 ± 0.014
Ratio	(H)	0.20 ± 0.013	* 0.57 ± 0.037	* 0.59 ± 0.026

Mean ± (SEM) values in normoxic isolated livers (N)(n=7) and hypoxic livers (H)(n=8) * = significant difference between normoxic and hypoxic values (a) = significant difference between value and baseline value. (b) = significant difference between 2 hour value and 1 hour value. P = <0.05.(c) Hep O_2 Extraction Ratio = Hepatic oxygen extraction ratio= (Oxygen supply - hepatic effluent oxygen content)/ Oxygen supply.

The perfusate composition remained stable and similar during the course of the study in respect of the total protein, albumin, haematocrit, haemoglobin and plasma haemoglobin concentrations as well as the glucose concentration in both groups. With respect to the latter, the results of study (B) above which showed a decline of perfusate glucose in the absence of a liver (Table 8.1) suggest that there was a net release of glucose by these livers.

The sodium concentration rose in both studies but this was only significant in the normoxic group of livers whilst the osmolality rose significantly in the hypoxic group of livers.

In order to maintain perfusate pH at or above 7.43 in the hypoxic liver group, 128ml ± 16 ml of 4.2% sodium bicarbonate was required whilst only one perfusion received 10ml of 4.2% sodium bicarbonate in the normoxic group.

Hepatic water uptake of the livers (Determined from the difference between pre and post perfusion liver weight)(See also section 8.3.4.B.vi) was 64 ± 16ml in the normoxic group and 118 ± 41 ml in the hypoxic group but was not statistically significant.

There were significantly higher lactate concentrations in the hypoxic group of livers. Further there was a significantly higher oxygen extraction ratio in hypoxic livers.

(2) Parameters of Liver Function and Injury

The baseline values for oxygen consumption, adenine nucleotide status, potassium and aminotransferse concentrations as well as lactate uptake taken prior to the decrease in FO_2 were similar for both groups of livers examined.

(i) Oxygen Consumption and Bile Flow

Oxygen consumption (Figure 8.11) remained constant in the normoxic livers but was highly significantly decreased in livers subjected to hypoxia with respect to baseline values and the normoxic group, decreasing from 3.10 ± 0.16 ml $min^{-1}100g\text{-}liver^{-1}$ to 0.85 ± 0.07 ml $min^{-1}100g\text{-}liver^{-1}$ after 1½ hours of hypoxia but not deteriorating further over the hour, when the oxygen consumption was 0.88 ± 0.08 ml $min^{-1}100g\text{-}liver^{-1}$.

Hepatic oxygen consumption correlated significantly with most other indices of hepatic function (Table 8.6. page 8.27) but not with perfusate ALT concentration and hepatic lactate uptake.

Bile flow (Figure 8.11) decreased with time in the normoxic group from 5.5 ± 0.4 to 4.3 ± 0.6 ml $hour^{-1}$ and was highly significantly less in the hypoxic group after 1½ hours of hypoxia 0.9 ± 0.3 ml $hour^{-1}$ but again there was no further deterioration with time[4].

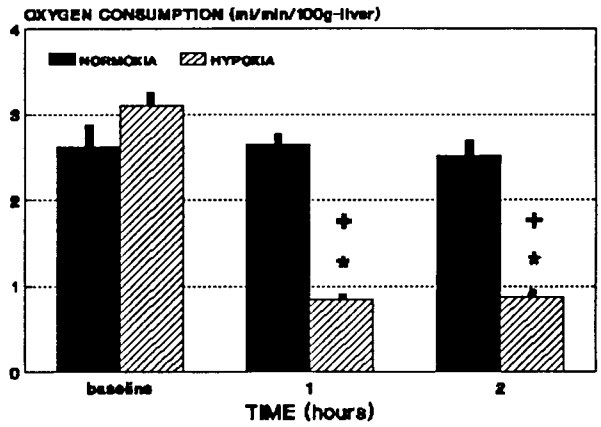
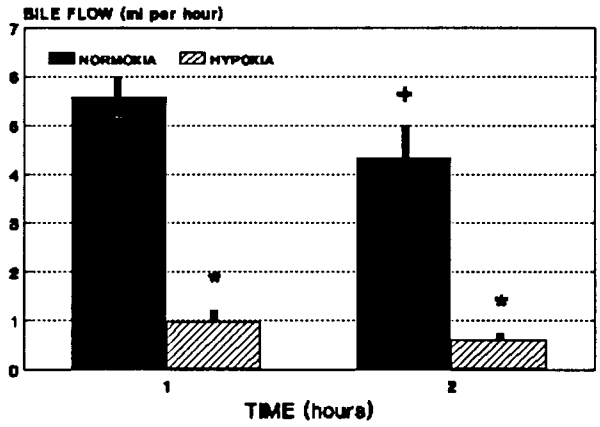


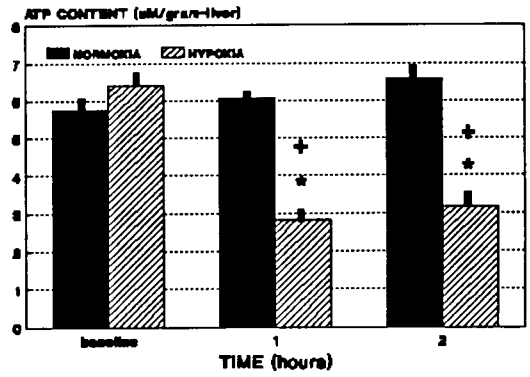
*Figure 8.11 Hepatic oxygen consumption and bile flow in normoxic and hypoxic livers. * = difference between hypoxic and normoxic livers. + = difference with time from baseline. $p<0.05$.*

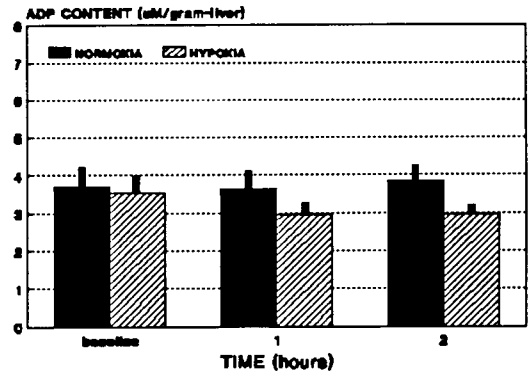


4 Although the volume of bile was collected with graduated 1ml tuberculin syringes the margin of error here compared to the absolute value must be great, thus a further deterioration in flow may well have occurred which could not be established using these methods. Further, the scatter of data points was such that correlation analysis of this data with other indices of hepatic function would have been inappropriate.

(ii) Adenine Nucleotide Status

Hepatic ATP, ADP and AMP (Figure 8.12), and thus TAN content, energy charge and ATP/ADP (Figure 8.13) were stable throughout the normoxic study. Hepatic ATP and TAN content[5], energy charge and ATP/ADP ratio decreased highly significantly with respect to baseline values and normoxic liver values whilst hepatic AMP was significantly higher under hypoxic conditions. These changes occurred after one and a half hours of hypoxia without further deterioration over the next hour. ATP, TAN, EC and ATP/ADP correlated highly significantly with oxygen consumption and potassium release (Table 8.6), the correlations being the strongest for hepatic ATP content. There was no correlation of these parameters with hepatic lactate uptake nor with perfusate ALT concentration while only ATP and TAN correlated with perfusate AST concentration.





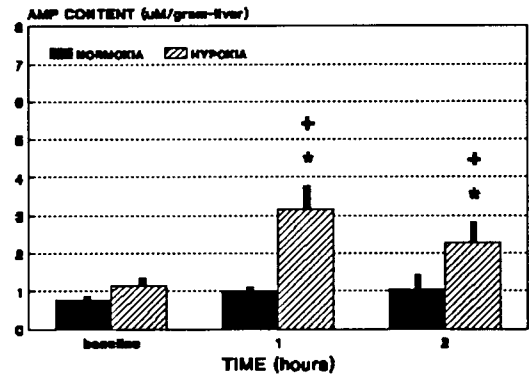
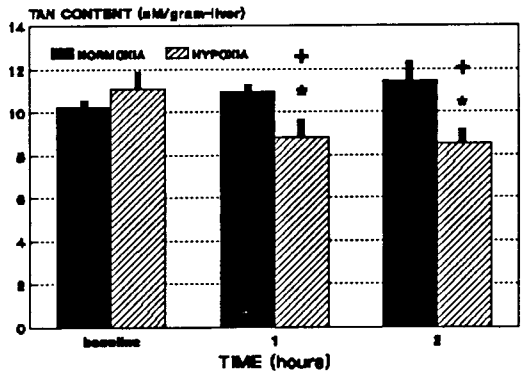
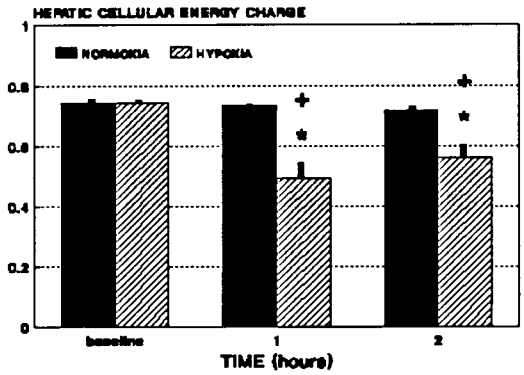


Figure 8.12 Hepatic adenine nucleotide content in normoxic and hypoxic livers. = difference between normoxic and hypoxic livers. + = difference with time from baseline. $p<0.05$.*

5 TAN significant, but not highly significant at 1 hour





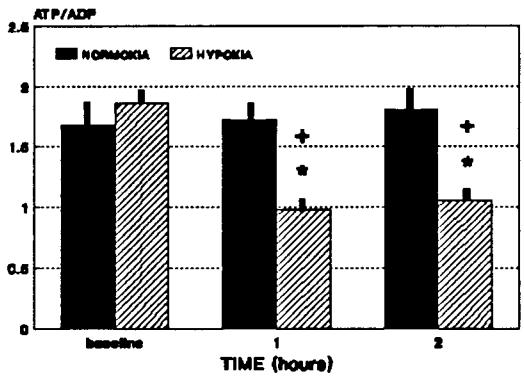


*Figure 8.13 Hepatic TAN content, energy charge and ATP/ADP ratio in normoxic and hypoxic livers. * = difference between normoxic and hypoxic values. + = difference with time from baseline. $p<0.05$.*

Table 8.6

Correlation Matrix of Combined Data from Normoxic and Hypoxic Pig Livers

	O_2	ATP	ATP/ADP	TAN	EC	AST	ALT	K+	LU
0_2		.89 .0001	.80 .0001	.60 .001	.72 .0001	-.47 .008	-.21 .256	-.83 .0001	-.18 .32
ATP						-.55 .003	-.01 .92	-.91 .0001	-.32 .11
ATP/ADP						-.38 .054	.069 .734	-.80 .0001	-.30 .1315
TAN						-.59 .0012	-.13 .52	-.57 .0025	-.30 .129
EC						-.28 .16	.187 .358	-.80 .0001	-.251 .215
AST							.40 .026	.588 .0008	.115 .544
ALT								.04 .85	-.33 .07
K+									.133 .48

Data presented as r value/p value for n= 30 points*.

O_2= hepatic oxygen consumption, EC= energy charge, AST Aspartate aminotransaminase, ALT Alanine aminotransaminase, K+ = perfusate potassium, LU= hepatic lactate uptake. Bile flow, hepatic venous PO_2 and the PO_2 in HA and PV were not correlated with the other indices of hepatic function as the scatter of the data points made a regression analysis inappropriate. *Missing values were: for adenine nucleotides n=4 and for K+ n=1.

(iii) Potassium

There was a highly significant rise in potassium concentration (Figure 8.14) in the hypoxic group which was in excess of the 0.7mmolL^{-1} that occurred in study (B) above (section 8.3.2)(Perfusate composition in the absence of a liver).

Hepatic potassium release as indicated by a rise in perfusate potassium correlated inversely with all indices of hepatic function and oxygenation save perfusate ALT concentration (Table 8.6).

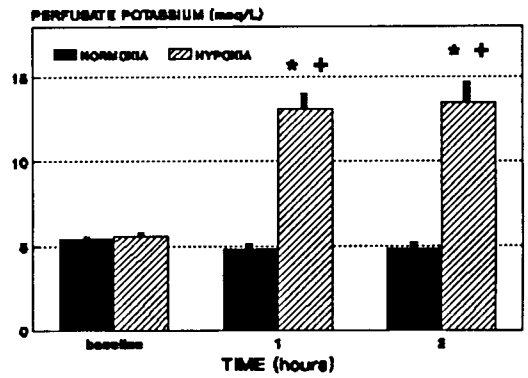


*Figure 8.14 Perfusate potassium concentration in normoxic and hypoxic liver studies. * = difference between normoxic and hypoxic values. + = difference with time from baseline. $p<0.05$.*

(iv) Aminotransferases

There was no significant difference in ALT perfusate concentrations between the two groups studied (Figure 8.15) however, there was a marked but variable increase in AST concentrations which achieved significance at the 2 hour sampling time (2½ hours after instituting hypoxia). This was thus later than all the other indicators of hepatic function mentioned above. AST concentrations correlated with most indices of hepatic function whilst ALT concentrations correlated with none. Nevertheless there was a weak correlation between AST and ALT perfusate concentrations (Table 8.6).

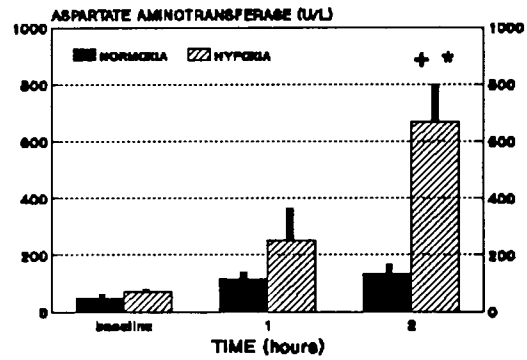
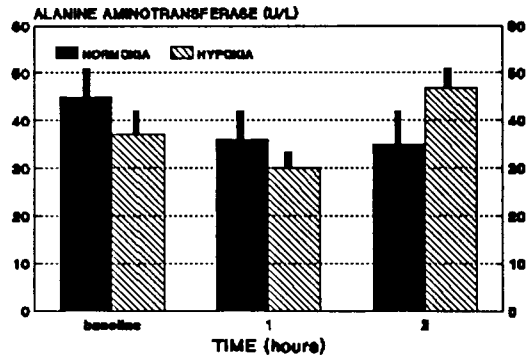


*Figure 8.15 Perfusate aminotransferase concentration (AST above/ALT below) in normoxic and hypoxic liver studies. * = difference between normoxic and hypoxic values. + = difference with time from baseline. $p<0.05$.*



(v) Hepatic Lactate Utilisation

Despite significantly higher HA and PV lactate concentrations (Table 8.5) in the hypoxic livers there was no difference in lactate utilisation between the two groups. As before (section 5.3.3 F) there was no consistent uptake of lactate; both uptake and release occurred during the course of the study period. As the mean values of lactate uptake may not clearly reflect this, Figure 8.16 has again been constructed to reflect this point.

Further there was no correlation between lactate concentration in the HA or PV and lactate utilisation. Lactate utilisation did not correlate with any other index of hepatic function (Table 8.6).

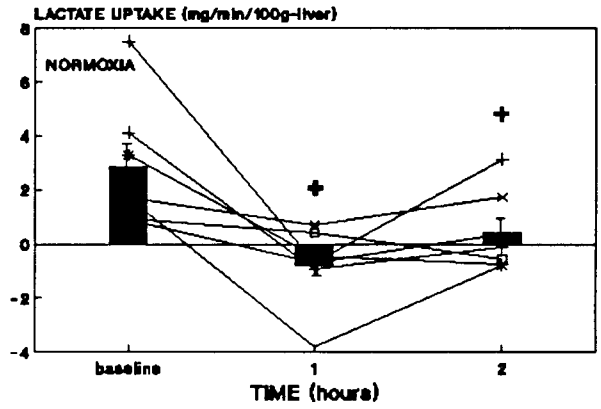
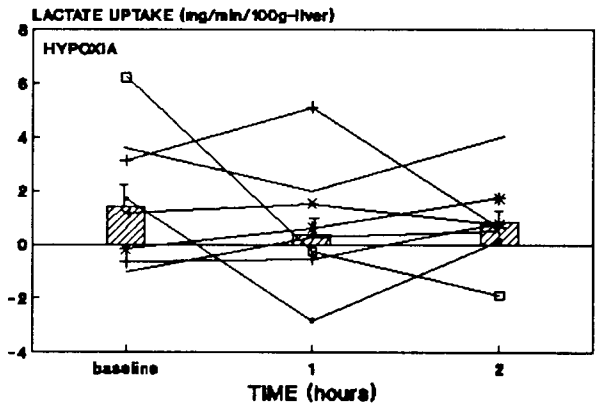


Figure 8.16 Lactate uptake (+) and release (-) by normoxic and hypoxic livers. Presented as individual liver utilization as well as group means. No difference between normoxic and hypoxic livers. + = difference with time from baseline. $p<0.05$.



(vi) Liver Tissue Water Uptake

There was a significantly greater increase in liver tissue water content (8.2.2.C.) in hypoxic livers compared with normoxic livers (2.8 ± 0.9% vs 1.2 ± 0.5%)($p=0.048$).

8.3.4.C. Light and Electron Microscopic Analysis of Liver Biopsies

Analysis of biopsies by light microscopy taken at the end of the isolated liver perfusion in 3 hypoxic experiments and 1 normoxic experiment showed few differences. Hepatocytes were swollen, with mild dilatation of the sinusoids in the hypoxic tissue, whilst the portal tracts, central veins and bile canaliculi appeared similar. (Table F.3 Appendix F, Page F.4)

Electron microscopy however, showed that in the hypoxic studies, hepatocytes were more swollen, with focal widening of the intercellular spaces, and also showed more pronounced mitochondrial changes:

Mitochondria were more swollen and lucent, and there was more mitochondrial matrix loss, cristae loss and membrane rupture as well as the presence of myelin figures. The hepatocyte nuclei showed a decrease in enchromatin and both smooth and rough endoplasmic reticulum showed vesiculation. There were also focal cytoplasmic vacuoles and swelling of the Kupffer cells. (Figure F.1, Table F.3, Appendix F, Pages F.4, F.5)

Three biopsies taken from different lobes in the same hypoxic liver all showed by light microscopic evaluation, swollen hepatocytes with dilatation of sinusoids (most pronounced in the pericentral region) and dilatation of the central veins, while the portal tracts appeared unremarkable. If these biopsies can be regarded as representative of the whole liver this would indicate that hypoxia resulted in diffuse changes in the liver.

8.3.4.E. Summary of Findings in Normoxic versus Hypoxic Isolated Perfused Pig Livers

The two groups of livers used for comparison of the effect of hypoxia on lignocaine extraction ratio and clearance were similar in terms of their adenine nucleotide status, prior to liver resection for isolated liver perfusion. The livers were similar in weight and ex vivo were perfused at equivalent unit HA and PV flows by means of a perfusate which was similar in composition and which remained within physiological limits throughout the course of the study period. Indices of liver function (hepatic oxygen consumption, adenine nucleotide status and lactate uptake, as well as perfusate potassium and aminotransferase concentrations) were no different between the two groups when normally oxygenated during a control period prior to hypoxia. The decrease in oxygen supply resulted in a mean perfusate PaO_2 of 18 mmHg in the hypoxic group compared with a mean PaO_2 of 122 mmHg in the normoxic group of livers. One and a half hours after instituting hypoxia there was a highly significant decrease in hypoxic livers in hepatic oxygen consumption as well as in ATP, TAN, ATP/ADP and energy charge and an increase in perfusate potassium when compared with baseline values and the values obtained in the normoxic liver group at corresponding times. Bile flow was also highly significantly less in the hypoxic group of livers. ALT and lactate uptake were no different between the groups whilst AST increased, but this only achieved statistical significance 2½ hours after the onset of hypoxia.

In the hypoxic group of livers there was a significantly lower hepatic lignocaine extraction ratio, absolute clearance and unit clearance as well as absolute intrinsic clearance and unit intrinsic clearance when compared with the normoxic group of livers.

At similar hepatic affluent lignocaine concentrations in the two groups, hepatic venous MEGX and MEGX/lignocaine concentrations were significantly less in the hypoxic group of livers.

Two and one half hours of hypoxia in the IPPL resulted in important ultrastructural changes when compared with a normoxic liver.

8.4 DISCUSSION

8.4.1 DESIGN OF THE STUDY

The major objective of the three studies (A)(B)(C) described in this chapter was to determine whether there was a significant impairment of lignocaine extraction and clearance (study C) as well as other indices of hepatic function in the hypoxic pig liver. If this were the case then the next objective was to determine whether lignocaine extraction and clearance correlated with specific indices of hepatic function in order to establish whether lignocaine metabolism per se could be used as an indicator of hepatic function.

As the isolated liver preparation "must be considered a dying organ" (Gores et al, 1986) it was anticipated that subjecting the liver to hypoxia was likely to hasten this process. Thus it was predicted that constant lignocaine concentrations might not be achieved over a prolonged period (one hour). Therefore it was decided to attempt to study lignocaine extraction during a "quasi" steady state by sampling for lignocaine determination five times at 5 minute intervals around the two time points mentioned (60 min and 120 min after the start of lignocaine infusion). Parameters for lignocaine elimination were determined during these two experimental periods for each individual time point (n=5), the mean values of which were then used to test the relationship of hepatic lignocaine elimination with other indices of hepatic function determined at 60 and 120 minutes after the start of lignocaine infusion respectively. This correlation analysis and the discussion pertaining thereto is presented in chapter 9.

Studies (A) and (B) were performed in the absence of a liver in the circuit using exactly the same study design to ascertain whether lignocaine extraction (study A) and changes in indices of hepatic function (study B) could indeed be attributed to the liver.

8.4.2 DISCUSSION OF STUDY (A) AND (B)

Study (A) showed that there was no significant uptake or loss of lignocaine 30 minutes after the administration of a bolus dose to a standard perfusion in the absence of a liver. Thus it may be assumed that lignocaine elimination can be attributed to the isolated perfused liver after this time period.

Study (B) showed that the perfusate per se, or more specifically the blood constituents were altered very little by hypoxia and that there were few time dependent changes. There was only a tendency for the pH to decrease in both studies while potassium rose no more than 0.7mmolL $^{-1}$ over the study period. However, perfusate glucose decreased by approximately 50% over the two hour study period. Thus, with these exceptions, all changes in measured indices of hepatic function in perfusions incorporating a liver can be attributed to the perfused liver.

8.4.3 DISCUSSION OF THE COMPARISON OF IN VIVO AND EX VIVO HEPATIC LIGNOCAINE EXTRACTION AND CLEARANCE AND LIVER FUNCTION

The pilot study performed in chapter 5 showed that the use of the same liver in a sequential experiment for this comparison would be inappropriate and thus different livers were used. Although other authors have also used pigs to draw a comparison between in vivo and ex vivo galactose elimination (Tygstrup et al, 1971)(Welch and Parbhoo, 1973), BSP and alcohol elimination (Elmslie et al, 1971)(Keiding et al, 1979), as well as ICG elimination (Tygstrup et al, 1971)(Winkler et al, 1970) there are no reports of a study comparing hepatic lignocaine elimination. The present study was designed to achieve similar hepatic affluent concentrations of lignocaine, unit hepatic blood flows, liver weights and perfusate composition ex vivo to that found in vivo to minimise potential confounding variables for this comparison.

The lignocaine extraction ratio by in vivo (0.61) and ex vivo (0.63) livers was similar during the second hour of a lignocaine infusion and as hepatic blood flow and perfusate flows were equivalent, the derived parameters of lignocaine clearance were also similar.

Winkler et al, (1970) studied hepatic ICG clearance, at similar plasma concentrations during ICG infusion, and found this to be similar in vivo and ex vivo. Tygstrup et al (1971) found ICG clearance to be "almost two times greater" ex vivo[6]. In contrast in the same study Tygstrup et al (1971) found galactose elimination to be significantly less ex vivo which was confirmed by Welch and Parbhoo (1973). Elmslie et al, (1971) and Keiding et al (1979) found alcohol eliminination to be significantly less ex vivo.

The question arises whether the differences found were solely due to different methodologies adopted by these investigators or whether these differences were due to the individual properties of the administered substances, all of which have been used as indicators of hepatic function.

Both lignocaine (Shand et al, 1975) and ICG (Zito and Reid, 1978) are substances with high hepatic extractions. The elimination of lignocaine can probably best be described using the venous equilibrium model (Pang and Rowland,1977b)(Ahmad et al, 1983) also known as the "well stirred model" which envisages the liver as a single well mixed compartment, such that the concentration of substance in the hepatic venous effluent is in equilibrium with that throughout the liver (Rowland et al, 1973).

In contrast ethanol (Keiding et al, 1979) and galactose elmination (Keiding and Chiaranti, 1978)(Keiding et al, 1976) follow Michaelis Menten or saturation kinetics and can probably be best analysed by the parallel tube model. This model represents the sinusoids as single tubes arranged in parallel and thus substrate concentration is assumed to decline exponentially along the sinusoid. The mean sinusoidal concentration

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6 The enhanced elimination of ICG ex vivo reported by Tygstrup et al, (1971) is difficult to explain. The authors speculated that this might be due to in vivo inhibition of ICG elimination which can be supported by later findings in this regard by Gelman et al, (1984) who showed that ICG half-life significantly increased during halothane anaesthesia (the anaesthetic used for the study of Tygstrup et al, 1971.)

is the logarithmic average (Chat) of the hepatic inflow (Cin) and hepatic outflow concentrations (Cout) (Smallwood et al, 1990).

ICG unlike lignocaine is not metabolised. In contrast the hepatic conversion of galactose (ultimately to UDPglucose) involves the enzyme galactokinase and requires ATP. In the pig liver this appears to be the rate-limiting step (Keiding et al, 1976). Alcohol in turn is metabolised by alcohol-dehydrogenase in both pig and man (Keiding et al, 1979). Thus the metabolism of these substances can be regarded as fundamentally different.

Returning to the comparison of in vivo and ex vivo function it is clear that the elimination of the two substances that are highly extracted, namely lignocaine and ICG were no different in vivo and ex vivo as shown in the present study for lignocaine and that of Winkler et al, (1970). In contrast in those studies where substrates exhibiting saturation kinetics [namely, alchohol (Keiding et al, 1979)(Elmslie et al, 1971) and galactose (Tygstrup et al, 1971)(Welch and Parbhoo, 1973)] were employed there was a significant decrease in elimination of these substances ex vivo.

Thus these two groups of drugs are differently affected by the isolated perfused liver; the metabolism of those substrates exhibiting saturation kinetics was clearly impaired whilst this was not the case for the highly extracted substrates.

The study of Tygstrup et al (1971) is the only study in which some other aspects of hepatic function can be compared with those performed in the present work. Although in the latter study perfusate composition was not described in detail, oxygen consumption, bile flow and adenine nucleotide status could be compared. In agreement with the present study, hepatic oxygen consumption and TAN content were less ex vivo while the ATP/ADP ratio was similar in vivo and ex vivo. Bile flow was significantly less ex vivo in the study of Tygstrup et al, (1971) while this was not statistically so in the present study. Bearing in mind especially the similarities in adenine nucleotide status it is indeed interesting that galactose elimination was significantly impaired ex vivo in the study of Tygstrup et al (1971). This impairment might be mainly due to the absence of a substrate or hormone necessary for galactose elimination in the isolated preparation.

In Summary and Conclusion

In vivo and ex vivo hepatic lignocaine extraction ratio and clearance were similar in the two models described above. Thus in this respect the IPPL can indeed be regarded as representative of the in vivo model.

Although bile flow and oxygen consumption were less ex vivo this may not necessarily indicate that hepatic function was impaired, as supported by the fact that in vivo and ex vivo energy charge and ATP/ADP ratio were similar.

When the present study is compared with similar studies involving the pig liver performed by others it is not clear why these investigators found a decrease in galactose and alcohol elimination ex vivo, while this was

not the case for lignocaine extraction. This is unlikely to be due only to methodological differences between the studies and is probably due to different mechanisms (and sites) of hepatic metabolism of these substrates. This suggests that when these substrates are used as indicators of hepatic function they may be measuring differing aspects of liver function.

8.4.4 DISCUSSION OF LIGNOCAINE METABOLISM AND HEPATIC FUNCTION IN NORMOXIC AND HYPOXIC ISOLATED PERFUSED PIG LIVERS (STUDY C)

This study has indicated that hypoxic hepatic injury in the isolated perfused pig liver significantly impairs hepatic lignocaine extraction ratio and clearance as well as several indices of liver function. Severe hypoxia (2% O_2 in the oxygenator inflow) had been chosen as Rabol et al, (1974) had shown that this was necessary to achieve impaired hepatic function different from normally oxygenated isolated perfused pig livers. Rabol showed that the effects of hypoxia appeared to be limited to circumstances where the hepatic venous PO_2 was less than 30 mmHg (2-19 mmHg in the present study) as above this no changes in hepatic oxygen consumption, adenine nucleotide status and galactose elimination occurred.

8.4.4.A. Hepatic Lignocaine Extraction and Clearance

Hepatic lignocaine extraction ratio and clearance were compared in hypoxic and normoxic livers at similar affluent lignocaine concentrations over the same time period after the start of a lignocaine infusion so avoiding any potential concentration and time dependent effects on these parameters.

Constant hepatic affluent lignocaine concentrations which did not vary with time over the experimental period were achieved in the normoxic IPPL studies when lignocaine was administered as described earlier (Section 4.4.4.C. and 4.4.5.C.). However, this lignocaine administration regimen, as determined for normoxic perfusions, resulted in significantly higher lignocaine concentrations when administered to hypoxic livers (Figure 8.9, Page 8.19). This indicated that hepatic lignocaine elimination was impaired by hypoxia. Although a plateau concentration appeared to be achieved in the first experiment, albeit at a higher concentration than in normoxic livers (Figure 8.9), in the second experiment shown, where the concentration of lignocaine exceeded 20ug ml^{-1}, the possibility that zero order metabolism was occurring appeared likely, as a constant lignocaine concentration was not established. At concentrations of lignocaine at which hepatic lignocaine metabolism becomes saturated (zero order metabolism) the apparent decrease in extraction ratio of lignocaine might be falsely attributed to an hypoxic effect. Thus, in order to compare hepatic lignocaine elimination in normoxic and hypoxic livers it was preferred to do this at similar constant concentrations. Preliminary experiments suggested that the extraction ratio of lignocaine in hypoxic livers was around 1/3-1/4 of that in normoxic livers. Therefore only one quarter of the bolus dose and infusion rate of lignocaine administered to normoxic livers was administered in the hypoxic group. This resulted in comparable constant hepatic affluent lignocaine concentrations in the

hypoxic group of livers which did not vary with time over the experimental period (Figure 8.10). The similar profile of lignocaine concentrations in the hypoxic experiments compared with those in normoxic experiments confirmed the assumption that first order metabolism had also been maintained at these concentrations in hypoxic experiments. This had been established for normoxic livers in seperate preliminary experiments (Appendix E) which indicated that lignocaine elimination by the normoxic isolated perfused pig liver was independent of concentration over the range encountered here.

The non-significant tendency for lignocaine concentration to increase over time seen in figure 8.10 may be the result of a slow deterioration of hepatic function of the ex vivo liver (Gores et al, 1986). Alternatively, this may be an early manifestation of lidocaine induced enzyme inactivation (Saville et al, 1989). As this phenomenon had been anticipated this study had been designed to determine lignocaine extraction ratio and clearance at two time points (60 and 120 minutes after the start of lignocaine administration) by repetitive sampling (n=5) over a short time period. This permitted an assessment of the relationship of lignocaine elimination with other indices of hepatic function determined at these time points and discussed further in chapter 9.

An alternative method that could have been adopted to achieve predictable concentrations in hypoxic experiments was to study the decay of lignocaine after a bolus dose in IPPL preparations subjected to hypoxia as described in chapter 4. However, it was not clear from the literature whether this approach would have been useful as Jones et al, (1984) using an isolated perfused rat liver preparation showed no difference in the decay of propranolol after a bolus dose in normoxic livers compared with those subjected to acute hypoxia, whilst the same group (Webster et al, 1985) showed a significant difference when studying omeprazole.

The ± 60% decrease in lignocaine extraction ratio and hepatic clearance and the ± 80% decrease in intrinsic clearance in the hypoxic liver group can be attributed to the effects of hypoxia as the livers used in the normoxic and hypoxic groups were similar with regard to the measured indices of liver function prior to the experimental period and during the experimental period this was evaluated at similar affluent lignocaine concentrations, temperature, and unit hepatic blood flow using livers of similar weight, whilst the perfusate composition was broadly similar and remained within physiological limits.

8.4.4.B. MEGX Formation

The direct measurement of the hepatic clearance of MEGX as described in this thesis for lignocaine, cannot be used to assess the effects of hypoxia as the hepatic clearance of a metabolite is complicated by sequential first pass metabolism, i.e. the metabolism of MEGX itself, after its formation from lignocaine, prior to its exit from the liver (Pang, 1983).

With respect to the lower MEGX concentration in the HV effluent in hypoxic experiments when compared to normoxic experiments, it must be born in mind that in the hypoxic experiments, despite similar hepatic affluent concentrations of lignocaine, a lower dose of lignocaine was administered and thus in a recirculating system the lower MEGX concentration may be a reflection of this. This consideration is complicated by the fact that MEGX is further broken down by the liver (Figure 1.1, Page 1.10) and it is likely that this process would also be impaired by hypoxia thus tending to raise (rather than lower) the level of MEGX in hypoxic liver experiments.

This study design does however permit the comparison of the ratio of MEGX to lignocaine in the hepatic venous effluent to assess the effects of hypoxia on the formation of MEGX. This ratio can be affected by changes in hepatic blood flow and by the clearance of drug by other (non hepatic) metabolic routes (Lane and Levy, 1981). In this study, hepatic blood flow was standardized and in the isolated state the liver could be the only site of elimination. Further, both the concentration of lignocaine (Pang and Rowland, 1977b) and MEGX (Pang and Rowland, 1977c) in the hepatic venous effluent are regarded as being in equilibrium with that in the liver according to the venous equilibrium model.

This data however, should be treated with due circumspection as the concentrations of MEGX were low, and the HPLC method used for this quantitation had a coefficient of variation of ± 14%. Further, interfering peaks occurred and thus in some experiments these values for MEGX had to be ignored while in others MEGX was not detected at all, probably because the concentrations were below the limit of sensitivity of the assay. Nevertheless the data suggests that hypoxia significantly decreases the formation of MEGX from lignocaine at equivalent hepatic affluent lignocaine concentrations.

8.4.4.C The Effects of Hypoxia on Drug Metabolism

Hypoxia may affect hepatic drug elimination in two principal ways. Oxygen may act as a terminal electron acceptor in the generation of high energy bonds (bioenergetic hypoxia) or may be required as a direct substrate for oxidases or oxidation (metabolic hypoxia).

Oxidases that have been studied extensively in this regard are the group of enzymes known as cytochrome P450. The effect of hypoxia on these systems and so on the metabolism of specific drugs will depend on the affinity of the particular enzyme for oxygen relative to the oxygen concentration to which it is exposed (Jones, 1981).

In the rat, lignocaine is aromatically hydroxylated as well as deethylated (to MEGX) by two separate entities of cytochrome P450 enzymes (Von Bahr et al, 1977). These enzymes are distributed throughout the acinus with a relatively higher concentration in the pericentral hepatocytes (zone 3) (Traber et al, 1988). This appears to be the acinar zone which is most susceptible to hypoxic tissue damage (Lemasters and Thurman, 1981) due to its relative distance from the well oxygenated periportal area (Jones et al, 1984). Thus it would be expected that compounds eliminated primarily in this zone will be significantly affected by

hypoxia, although a whole range of sensitivities to hypoxia are still evident within the zone (Webster et al, 1985).

Although investigations in subcellular systems make clear that the metabolism of different drugs will be differentially affected by the severity of hypoxia (Jones, 1981) the effect of hypoxia on drug metabolism in intact organs has been little studied.

Nakatsu (1985) found that theophylline elimination was reduced by hypoxia in the isolated perfused rat liver. Jones et al, (1984) and Webster et al, (1985) have shown impaired elimination of propranolol and omeprazole respectively in the isolated perfused rat liver subjected to one hour of hypoxia. In contrast, Jones et al, (1984) showed that steady state sodium taurocholate concentrations were unaffected by hypoxia while Smith et al (1983) showed an increase in misonidasole elimination during hypoxia again in the isolated perfused rat liver.

Galactose elimination (Winkler et al, 1986) and both galactose Vmax and Km (Keiding et al, 1990) were impaired in the isolated perfused pig liver subjected to hypoxia. In the later study, galactose intrinsic clearance (Vmax/Km) was however not impaired by hypoxia.

Larsen et al, (1976) showed in anaesthetized cats that when 12.5% or 10% oxygen was administered via a ventilator there was a significant decrease in hepatic oxygen consumption, bile flow, ethanol and ICG elimination in comparison with controls where 15% oxygen was delivered.

Shorey et al, (1969) used isotope labelled bromosulphthalein, rose bengal and bilirubin in the rat and guinea pig and showed that the major effect of hypoxia on hepatic elimination of these substances was the excretory step between liver cells and canalicular bile.

8.4.4.D Hypoxic Tissue Damage

It is not possible from the present study to differentiate the effects of hypoxia per se from the secondary tissue damaging effects of hypoxia on lignocaine extraction by the liver. However, over the experimental period 1½ after the institution of severe hypoxia there was no decrease in lignocaine extaction ratio and no further deterioration in the measured indices of hepatic function, save for the continued (delayed) rise in AST (vide infra). This suggests that there was no progressive damage to the livers during this period.

The light microscopic evaluation of biopsies taken from different sites in the liver suggest that hypoxia resulted in diffuse changes. The electronmicroscopic changes in hypoxic livers compared with that in a normoxic liver indicate more extensive damage in the former (Table F.4, Appendix F, Page F.5). However, it was not possible to determine whether these changes were irreversible.

In the present study 2% hypoxia resulted in a highly significant decrease in liver ATP, TAN, EC, ATP/ADP ratio, oxygen consumption and bile flow as well as a rise in AMP, perfusate potassium and lactate concentrations but no difference in lactate uptake. This confirmed the findings of Winkler et al (1986) in the isolated pig liver subjected to 15% oxygen concentration for 80 minutes. In addition in this investigation,

Winkler et al,(1986) reoxygenated the livers at the end of the study period and found after 40 minutes that restoration of a number of parameters occurred but that this restoration was not complete for liver ATP and EC, while a raised potassium and lactate concentration also remained.

Jones et al, (1984) and Webster et al, (1985) showed that the effect of hypoxia on elimination of propranolol and omeprazole started immediately (omeprazole earlier than propranolol) and that this effect was unlikely to have been simply a nonspecific effect of tissue damage as in both studies the elevated drug concentrations due to hypoxia for one hour returned to normal upon reoxygenation.

Despite the apparent return of some aspects of liver function in isolated liver preparations Bradford et al, (1986) have shown periportal and pericentral damage as indicated by trypan blue uptake [indicative of irreversible cellular damage (Marotto et al, 1988)] after only 30 minutes of perfusion by a hypoxic buffer using the isolated perfused rat liver of fasted animals. Further, Lemasters et al, (1983) have shown by electron-microscopic analysis that this hypoxic injury was not simply reversed by reoxygenation but aggravated. They contend that relatively brief periods of centrilobular anoxia (15-45 mins) would be sufficient to produce liver injury and necrosis. This would support the contention that in standard liver perfusions where harvesting and the establishing of liver perfusion takes 15-20 minutes, a degree of anoxic liver damage may have occurred.

Winkler has stated that the time span or the degree of hypoxia necessary for irreversible hepatocellular damage to occur is unknown (Winkler et al, 1986). With respect to ischaemia, Misra et al, (1972) have determined that 60 minutes of complete hepatic ischaemia is lethal in dogs while Fischer et al, (1976) have shown in pigs that at least this period is required but that some pigs have survived 120 minutes of ischaemia. The latter is confirmed by the study of Harris et al, (1981) again in pigs, showing that massive liver necrosis occurred with ischaemia of 180 minutes duration. However, in the quoted studies of hepatic ischaemia, other factors apart from liver hypoxia may be pertinent.

The considerations outlined above suggest that in the present experiments, where the livers were subjected to severe hypoxia for a period of 90 minutes prior to the study period, structural, and probable irreversible, liver damage is likely to have occurred. Thus both the direct effect as well as tissue damaging effect of hypoxia may have been reponsible for the significant decrease in lignocaine extraction found.

The mechanism of tissue damage as a result of hypoxia is unclear (Bradford, et al, 1986). However, the effect of hypoxia on hepatic potassium release as postulated by Stewart et al, (1953) and largely supported by Lambotte's (1977) research 24 years later, may perhaps serve as a model of hypoxic damage at the cellular level. Stewart et al, (1953) noted a large and immediate increase in the hepatic vein plasma potassium concentration in the canine liver in vivo when subjected to hypoxia (as was the case in the present study in pigs) with a frequent immediate return to subnormal levels on reoxygenation. They determined that potassium loss appeared to occur in phases and postulated that the first phase represented the immediate response of the cell membrane to hypoxia, with outflow of potassium as energy

requirements were no longer met, and the second phase heralded the "disintegration of the biochemical anatomy of the cell" with the onset of irreversible protoplasmic change. Lambotte (1977) in turn showed in the isolated perfused dog liver that the partial reduction of the level of ATP as a result of early anoxia resulted in hyperpolarisation of the hepatocyte cell membrane probably due to enhanced potassium permeability with resultant potassium efflux from the cell. Prolonged hypoxia resulted in an enhanced cellular influx of sodium and water with resultant cellular distension causing further changes in membrane permeability for potassium[7].

It is tempting to speculate from the above that cellular damage as a result of hypoxia has occurred when plasma potassium does not return to normal (or subnormal) upon reoxygenation of the liver. This would suggest that cellular damage had indeed occurred in pig livers subjected to hypoxia in the study of (Winkler et al 1986) and by extension also in the present study where the livers were subjected to more severe hypoxia for a longer period.

Marotto et al, (1988) have conceded that there must be multiple mechanisms for cell death associated with hypoxia and have proposed a model of superoxide mediated hypoxic damage from their study involving the isolated perfused rat liver preparation. Briefly, low flow hypoxia results in anoxic acinar zones around the central vein contiguous with periportal normoxic zones. In these anoxic zones ATP declines and is converted to hypoxanthine, and xanthine oxidase is increased by proteolytic conversion of xanthine dehydrogenase. At the anoxic edge sufficient oxygen is present to allow the formation of superoxides by xanthine oxidase. These superoxides and their metabolites cause injury and death to midzonal cells. The anoxic edge progresses to include new cells in the oxygen dependent injury as the cells die and their metabolism stops, while further damage to other cells is caused by superoxide leaving native cells, a process that increases with time.

8.4.4.E. Effects of Hypoxia on Specific Indices of Hepatic Function

(i) Oxygen Consumption

Hepatic oxygen consumption (and bile flow) have been widely used as an indicator of hepatic function in isolated liver preparations (Gores et al, 1986) although as discussed earlier (Section 5.4.5) their use for the purpose of comparing in vivo and ex vivo function may not be appropriate (Tygstrup, 1975). As hepatic oxygen utilisation is both dependent on the perfusate haematocrit as well as the hepatic blood flow (Hardison et al, 1967) a valid comparison between the two studies requires that these parameters were similar as was the case in this study. Hepatic oxygen uptake was significantly impaired by hypoxia confirming the findings of others (Keiding et al, 1990)(Winkler et al, 1986)(Webster et al, 1985). Winkler et

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7 These findings may explain the increased tissue water uptake,the lower perfusate sodium and higher osmolality in the hypoxic liver group compared with the normoxic liver group (Section 8.3.4.B.1)

al, (1986) also showed that hepatic oxygen uptake was independent of the route of perfusion via the hepatic artery or portal vein.

The oxygen extraction ratio increased significantly in livers subjected to hypoxia but despite this oxygen was not fully extracted from the perfusate as shown by the mean hepatic venous PaO_2 of between 7-10 mmhg (Table 8.5.) This may indicate that (a) the demand for oxygen by the liver was decreased by hypoxia, (b) that the demand was in fact not decreased but that at very low intracellular oxygen tensions the function of the key respiratory enzymes became limiting for the rate of oxygen uptake (Winkler et al, 1986), thus the supply of oxygen administered could not be fully utilised, (c) That all oxygen was in fact extracted from perfusate in contact with viable hepatocytes but shunted perfusate (in contact with non-oxygen extracting hepatic tissue) returned to the hepatic venous effluent raising its PaO_2.

In support of (a) above, it can be postulated that the liver "switches off or down" when confronted with a hypoxic condition or put differently "hepatic metabolism adapts well to hypoxia" (Tygstrup, 1975) perhaps as a protective mechanism. Further, on reoxygenation after a relatively short period of hypoxia (60 minutes) hepatic metabolism as ascertained by drug metabolism in parallel with oxygen consumption returned promptly to normal (Webster et al, 1985) suggesting that very little liver damage had occurred when the period of hypoxia remained short.

In support of (b) above, Rabol et al, (1974) found, when studying graded hypoxia in the pig that only at a PaO_2 of less than 30mmHg, measured in hepatic venous blood, a gradual decrease in the functional parameters of the liver occurred. Winkler et al, (1986) stressed that the most sensitive indicator of hypoxic metabolism was the hepatic venous oxygen tension as this gave an indication of the oxygen tension within the liver cells. Jones and Kennedy (1982) emphasised that the intracellular diffusion coefficient for O_2 in the region of the mitochondria was less than the extracellular diffusion coefficient resulting in a significant O_2 gradient in the vicinity of mitochondria under hypoxic conditions which might be a critical factor for O_2 supply to mitochondria. It is thus possible that below the hepatic venous PO_2 (30mmhg) determined by Rabol et al (1974) the function of the key respiratory enzymes may become limiting for the rate of oxygen uptake and so only at this stage does a decrease in hepatic oxygen consumption begin to occur.

The present study was not designed to determine the reason for the decreased hepatic oxygen consumption associated with hypoxia. However, it is postulated that although the liver extracts proportionately more oxygen (extraction ratio increases) during periods of hypoxia the decreased oxygen consumption may be explained by both a decrease in actual oxygen demand as well as a decrease in oxygen transfer.

(ii) Bile Flow

As described for the comparison of oxygen consumption, bile flow is also dependent on perfusate haematocrit (Thomsen and Larsen, 1983) and liver blood flow (Gordon et al, 1972) which was similar in the two groups.(Table 8.3). Bile flow was significantly impaired by hypoxia (Figure 8.11). In vivo many

neurohormonal factors influence bile volume and composition, however these are not present ex vivo allowing the particular influences on bile volume to be studied (Hardison and Norman, 1967). It is clear from the present study that hypoxia is very detrimental to bile flow thus the volume of bile produced was very small in comparison with normoxic livers. This may result in some error in volume determination. Shorey et al, (1969) found a decrease in bile flow as a result of hypoxia in conscious adult rats and established that the major effect of hypoxia on bile secretion is the depression of the secretory step between liver cells and bile.

(iii) Adenine Nucleotide Status

Hypoxia resulted in a significant decrease in hepatic ATP, TAN, ATP/ADP and EC as well as an increase in AMP. (Figures, 8.12 and 8.13). The stability of both the normoxic and hypoxic studies is evident from the constant values of these parameters over the experimental period. The changes in these parameters as a result of hypoxia in pig livers have been observed by others (Keiding et al, 1990)(Winkler et al, 1986).

As an adequate hepatic ATP content is fundamental to cell function (Kono et al,1982) an estimate of its quantity has been used as an indicator of hepatic function ex vivo (Gores et al, 1986) as well as to predict the viability of human transplanted liver grafts (Kamiike et al, 1988). The decrease in hepatic ATP content associated with hypoxia is thus likely to indicate that liver functions will be impaired. In contrast the energy charge gives an indication of hepatic energy state of the cell (Atkinson et al, 1968) and hepatic TAN content gives an indication of the available nucleotides for energy storage and thus the likelihood of recovery after an insult (Kamike et al, 1988).

In the hypoxic livers the hepatic energy charge 1½ and 2½ after institution of hypoxia were 0.50 ± 0.05 and 0.56 ± 0.05 respectively (Figure 8.12) which is within the range which may lead to lethal disintegration of the cell (Kono et al, 1982)(Ozawa et al, 1974).

(See Section 5.4.5 for further detail explaining the importance of the adenine nucleotide status.)

(iv) Aminotransaminases as Indicators of Hypoxic Hepatic Injury

In this investigation perfusate AST and ALT were studied as indicators of hepatocellular damage while plasma haemoglobin was assessed to exclude haemolysis, a potential cause of a rise in AST. There was no difference in perfusate ALT concentrations in hypoxic livers when compared with the normoxic group of livers (Figure 8.15) a finding which was also reported on by others (Jones et al, 1984)(Winkler et al, 1986). The rise in perfusate AST concentration reached significance only at the two hour sampling time in contrast to the indicators of hepatic function mentioned above which showed significant changes at one hour. This delayed rise in AST concentration [in the absence of any significant change in plasma haemoglobin values (Table 8.3)] suggests that this is a less rapid indicator of hypoxic injury than lignocaine extraction or the other indices mentioned, whilst the lack of any change in ALT concentration makes this a poor (early) indicator of hypoxic insult to the liver. Lemasters et al, (1983) have shown that early hypoxia is associated

with the formation of microvilli (blebs) on centrilobular hepatocytes which are released on reoxygenation. The authors suggest from these observations that hepatic enzymes can be released from viable tissue through shedding of cell surface blebs. They suggest that as these blebs contain mostly cytosol and exclude larger organelles such as mitochondria a shedding mechanism explains how cytosolic enzymes such as ALT and LDH can be released selectively after mild to moderate injury. In severe liver injury leading to necrosis, both mitochondrial and cytosolic enzymes appear in the blood. This could occur due to a generalised breakdown of the plasma membrane permeability leading to indiscriminate release of cellular contents.

This hypothesis does not serve to explain why in the present study a significant rise in AST (a mitochondrial and cytosolic enzyme) was found but no rise in ALT (a cytosolic enzyme). This rise in AST could not be explained as due to haemolysis as this was no different between the hypoxic and normoxic groups. Thus to explain this rise it is postulated that mitochondria which show early changes in hypoxia (Cortran et al, 1989) leak enzymes, ie AST, first into the cytoplasm which then becomes the predominant cytosolic enzyme and results in a rise in perfusate concentration. This theory gains some support from the mitochondrial changes indicative of damage shown on electromicroscopic analysis of liver biopsies from hypoxic livers in this study (Figure F.1, Appenix F, Page F.5). It must also be postulated that only at a later stage, when less oxygen dependent cellular constituents are also damaged will significant ALT be released. The question arises whether the release of these enzymes is due to focal rather than global damage from hypoxia. The evaluation of liver biopsies taken from different sites after 2½ hours of hypoxia and studied by means of light microscopy showed diffuse rather than patchy changes, suggesting that this damage is global and that the release of aminotransferases by the liver may indicate global damage.

(v) Lactate Utilisation

Hepatic lactate utilisation will be discussed only briefly here as the many factors affecting this parameter have been discussed at length in section 5.4.5.F.

Despite significantly higher perfusate lactate concentrations after hypoxia (Table 8.3) there was no difference in lactate utilisation between the normoxic and hypoxic group of livers (Figure 8.16). It is noteworthy that lactate release occurred even in the normoxic group of livers. The findings in the present study confirmed similar findings in the study of Winkler et al, (1986).

The high perfusate lactate concentrations in the hypoxic group of livers would suggest hepatic lactate release, however the mean values at times 1 and 2 hours suggest that overall hepatic uptake was occurring. This can perhaps be explained as follows; in the absence of a liver perfusate lactate concentrations were relatively high (± 40mg $100ml^{-1}$) (Table 8.1, page 8.12) and independent of whether the perfusate was oxygenated or not. In the presence of an oxygenated liver in the circuit (Table 8.3 Time = baseline) perfusate lactate concentrations were consistently lower (± 10-15mg $100ml^{-1}$) and lactate uptake occurred in both groups of livers (Figure 8.16 time= baseline). However, on instituting hypoxia,

lactate concentrations again rose to ± 40mg $100ml^{-1}$ in the hypoxic livers but remain lower in oxygenated livers. This suggests that the hyperlactatemia of the perfusate in the hypoxic livers may be contributed to by red cell lactate release (Thomsen et al, 1983) and may not necessarily be due to lactate release from the liver. There may also be a decrease in lactate uptake by the liver as a result of hypoxia (Tashkin et al, 1972).

It had been postulated that endogenous hepatic lactate uptake in vivo and in the IPPL could be used as indicators of hepatic function (Section 5.1.3) and thus the lack of lactate release by in vivo livers was used as a criterion for accepting an experiment in the experiments described in Chapter 6 and 7. This was done in an attempt to achieve as stable a metabolic model as possible. (See section 5.4.5.F.i.)

The data from this study showed no correlation between lactate uptake and any other index of hepatic function (Table 8.6). This suggests that lactate uptake per se is not a useful indicator of hepatic function. Lactate uptake did however correlate with perfusate glucose concentration ($r=-.48$, $p= 0.009$, $n=28$).

(vi) Summary of the Effects of Hypoxia on Specific Indices of Hepatic Function

This study indicates that early hypoxic hepatic injury is most rapidly indicated by changes in hepatic oxygen consumption and adenine nucleotide status as well as impaired bile flow and hepatic potassium release following which this may be indicated by a rise in perfusate AST concentration. Significant ALT release does not occur after subjecting the liver to 2½ hours of hypoxia despite evidence of significant ultra structural changes. Lactate uptake is not a useful indicator of hepatic hypoxic damage.

8.4.5. REVIEW OF POSSIBLE MECHANISMS OF IMPAIRED LIGNOCAINE EXTRACTION DUE TO HYPOXIA

The effects of hypoxia on lignocaine extraction are likely to be due to both the direct metabolic effects of hypoxia as well as impaired metabolism brought about by liver injury secondary to hypoxia as discussed in section 8.4.4.D.

The elimination of a substance by the liver may depend on a number of factors (Roberts and Schenker, 1983).

(i) Uptake and transport across the hepatocyte plasma membrane.

(ii) Intracellular transport.

(iii) The binding of drug to blood constituents

(iv) The metabolism or secretion of the drug

(v) The total blood flow to and distribution of flow (shunting) within the organ.

An examination of these factors in the light of possible effects resulting from hepatic hypoxic liver injury may thus be pertinent.

(i & ii) The uptake, and intracellular transport as well as transport across the hepatocyte membrane.

Chen et al, (1980) have shown that hepatocyte lignocaine uptake was not saturable over a wide range of lignocaine concentrations and suggest that lignocaine enters the hepatocyte by diffusion not related to a carrier system. The effects of hypoxia on hepatic uptake have not been studied for lignocaine but have been for propranolol indicating that the hepatic uptake of this drug is unimpaired by hypoxia (Jones et al, 1984). As lignocaine interacts at common binding sites with propranolol (Chen et al, (1980) it is possible that lignocaine uptake too would be unimpaired by hypoxia.

(iii) The binding of drug to blood constituents

The question arises whether hypoxic effects on the liver could alter lignocaine binding in the perfusate and if so whether this could contribute to the decrease in hepatic extraction ratio.

There are a number of blood components to which drugs can bind namely the erythrocytes as well as various plasma proteins. The binding of drug to erythrocytes or indeed whole blood drug binding in general has been very little studied as plasma drug binding is more readily determined than whole blood binding. Thus, most investigators have studied plasma binding and then estimated whole blood binding using blood-to-plasma concentration ratios (Wood, 1986). With respect to drug binding in plasma then, the three major protein groups responsible are albumin, $alpha_1$ acid glycoprotein (AAG) and the lipoproteins.

The effect of protein binding on hepatic elimination of a particular drug depends on,

(a) The free fraction (FF) of drug ie that percentage of the drug that remains unbound in plasma.

(b) The hepatic extraction ratio (ER) of the particular drug.

If the hepatic extraction ratio of the drug is high (0.7)(Blaschke, 1977) and greater than the free fraction of the drug i.e. ER > FF, then the elimination of the drug is regarded as **non-restrictive** (ie not restricted by protein binding) (Wood, 1986) and will be unaffected by changes in protein binding alone (Wood, 1986)(Williams and Mamelok, 1980). This is the case for drugs such as lignocaine and propranolol whose elimination would thus be predominantly dependent on blood flow. These drugs are regarded as demonstrating **flow limited clearance** (Williams and Mamelok, 1980) because of a high hepatic extraction ratio.

Alternatively the rate of metabolism of drugs with low extraction ratios (0.2) is not limited by the amount of drug brought to the liver and these drugs demonstrate **capacity limited clearance** (Blaschke, 1977). They may be regarded either as,

(i) **capacity limited, binding sensitive drugs** which have a high affinity for plasma proteins and at therapeutic concentrations are normally more than 85% bound to plasma proteins. (Blaschke, 1977) or as,

(ii) **capacity limited, binding insensitive drugs** which have a low affinity for plasma proteins and are less than 30% bound at therapeutic concentrations and whose elimination in contrast is unaffected by changes in protein binding alone (Blaschke, 1977).

Restrictive elimination is observed when the extraction ratio is less than the free fraction (Wood, 1986)
The free fraction of lignocaine in plasma is about 30% (Tucker and Mather, 1975)(Holley et al, 1984)(Routledge and Barchowsky et al, 1980). If this is assumed to be similar in pigs' plasma then from the above the hepatic elimination of lignocaine would appear to be independent of protein binding under normoxic conditions when the ER is 0.61.

For altered lignocaine plasma binding to be implicated as a possible reason for this drugs' decreased hepatic extraction during hypoxia, the plasma protein binding would have to be less than its extraction ratio during hypoxia as only then could it be postulated that lignocaine clearance could have been impaired by plasma binding ie that restrictive clearance was occurring.

Under hypoxic conditions assuming no change in FF (vide infra) where the extraction ratio was 23% lignocaine extraction would have to be regarded as almost capacity limited [extraction ratio less than 0.2 (Blaschke, 1977)] but still plasma protein binding insensitive (Blaschke, 1977). Thus as the extraction ratio is less than the free fraction it is unlikely that during hypoxia hepatic extraction of lignocaine would be limited by protein binding. [Despite this the elimination would have to be defined as restrictive by the above earlier stated criteria as the ER is now less than the FF, (Wood, 1986)]

This study does not investigate whether the FF of lignocaine remains constant during hypoxic and normoxic liver perfusion. However, a number of criteria to assure constancy in this regard were met. Lignocaine plasma binding is affected by changes in pH (McNamara et al, 1981) and temperature (Wood, 1986) and as with all drugs can be affected by drug concentration (Routledge and Barchowsky et al, 1980)(Tucker et al, 1970). and plasma albumin level, (Wood, 1986) all of which were similar between the normoxic and hypoxic studies. Lignocaine plasma binding is not affected by the presence of its major metabolites (McNamara et al, 1981) which was different for the two studies. Heparin has been implicated as an agent which may lower plasma lignocaine binding (Routledge and Stargel et al, 1980). However, this was administered as an anticoagulant in both studies in standardized amounts and was thus common to both.

$Alpha_1$ acid glycoprotein for which the liver is the major site of biosynthesis is possibly the major determinant of plasma protein binding for lignocaine (Barry et al, 1990). The free fraction of lignocaine has been shown to correlate inversely with AAG concentration (Barry et al, 1990)(Edwards et al, 1982). The concentrations of AAG are usually 100 times lower than albumin which in turn appears to be responsible for approximately 40% of lignocaine protein binding under therapeutic conditions (McNamara et al, 1981). Thus despite a low capacity, AAG has a high affinity for basic drugs (Barry et al, 1990). It could thus be postulated that if there was a differential change in the level of perfusate AAG as a result of hypoxia this could result in differences in protein binding. AAG is an acute phase reactive protein which rises in association with trauma over 24-48 hours (Edwards et al, 1982), but may only peak at 5-6 days (Holley et al, 1984). In contrast in hepatic ischaemia (Von Allmen et al, 1991) AAG synthesis is reduced, while in liver

disease (cirrhosis) this reduction is proportional to the severity of the disease. This reduction results in greater FF of lignocaine (Barry et al, 1990).

Thus in the present study of hepatic hypoxia it is likely (if anything) that AAG will have decreased or remained normal, thus either increasing the free fraction of lignocaine or not materially affecting this. Therefore as only a decrease in FF could potentially limit the extraction of lignocaine, AAG is unlikely to have affected lignocaine extraction under hypoxic conditions.

In summary, lignocaine is a highly extracted (flow limited) drug under normoxic conditions and as such its hepatic elimination is independent of binding to blood constituents. Under hypoxic conditions lignocaine extraction by the liver approaches capacity limitation and the question must arise whether its elimination is binding sensitive or insensitive. If lignocaine protein binding remains unaffected by the hypoxic state as the above theoretical discussion suggests and thus the FF does not decrease, then the impaired hepatic lignocaine extraction encountered during hypoxia cannot be explained as due to restrictive elimination.

(iv) The metabolism or secretion of the drug

The metabolism of lignocaine which is dependent on enzyme systems and possibly on energy supplies may be affected by metabolic and bioenergetic hypoxia respectively (Jones, 1981) as discussed in section 8.4.4.A. Apart from these direct effects of hypoxia secondary tissue damage may also be implicated. The nature and effects of such damage on lignocaine metabolism can only be speculated upon.

The effect of acute hypoxia on the hepatic excretion of lignocaine has also not been studied for this drug but has been investigated using labelled BSP, Rose Bengal and bilirubin (Shorey et al, 1969). These authors concluded "that the diminished biliary excretion of these substances was primarily due to impairment of their transport from liver to bile" and was not limited by hepatic uptake. Whether this applies to lignocaine would have to be specifically investigated.

(v) The total blood flow to and distribution of flow (shunting) within the organ.

The total and unit blood flow to the two groups of livers was similar, however it is possible that liver hypoxia affected the distribution of flow within the liver.

A model for the impaired metabolism of propranolol, a highly extracted drug like lignocaine, in liver cirrhosis has been proposed (Wood et al, 1979). This model (the intact hepatocyte theory) explains impaired drug metabolism in this condition on the basis of the development of intrahepatic shunts which perfuse nonfunctioning tissue, while the remaining blood flow is exposed to a reduced mass of normally functioning hepatocytes. It is possible that hypoxia results in a similar phenomenon through the release of vasodilator metabolites (Richardson and Withrington, 1981). Assuming that this occurs to a greater extent in hepatic tissues more susceptible to hypoxia, then these areas which are likely to have the most impaired function may be favoured with more blood resulting in the shunting described above and thus impaired extraction of lignocaine.

Summary of Possible Mechanism of Impaired Lignocaine Metabolism During Hypoxia.

This discussion has been largely speculative as this is apparently the first study investigating the effect of hypoxia on the metabolism of lignocaine and so must also be based on information from other investigations involving the few substrates that have been studied to date with respect to hypoxia in intact tissue preparations.

It may be concluded that the impaired lignocaine clearance and MEGX formation encountered during hypoxia may be attributed to both metabolic and energetic hypoxia as well as the unknown effects of tissue damage. There is also the possiblity that shunting of blood is occurring. Information gained from other studies, if true for lignocaine, suggest that its uptake into the hepatocyte is unlikely to be affected by hypoxia. Altered protein binding is unlikely to be relevant in the present study whilst secretion of lignocaine into bile may well be impaired.

8.5 CONCLUSION

This study and analysis have shown that hepatic lignocaine extraction ratio and clearance in the pig were similar in vivo and ex vivo when compared using different livers as described. Lignocaine elimination and MEGX formation were significantly impaired by hypoxia in the isolated liver, as was hepatic oxygen consumption, bile flow and adenine nucleotide status while there was a rise in potassium concentration but a delayed rise in aspartate aminotransferase and no change in alanine aminotransferase levels or hepatic lactate uptake.

These changes may be due to both the direct effects as well as the indirect tissue damaging effects of hypoxia.

CHAPTER 9: CORRELATION OF LIGNOCAINE EXTRACTION RATIO AND CLEARANCE WITH INDICATORS OF HEPATIC FUNCTION

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CHAPTER 9: CORRELATION OF LIGNOCAINE EXTRACTION RATIO AND CLEARANCE WITH INDICATORS OF HEPATIC FUNCTION

SUMMARY

This analysis using data from prior experiments showed that lignocaine extraction ratio and clearance correlated with other indices of hepatic function and dysfunction. Lignocaine extraction ratio, hepatic oxygen consumption, ATP content, bile flow and potassium release were shown to be equivalent, more highly sensitive, and earlier indicators of hypoxic hepatic injury than hepatic aspartate aminotransferase release in this isolated perfused pig liver model.

9.1 INTRODUCTION

A strong correlation between two physiological parameters suggest that that they may subtend a common factor (Ramsoe et al, 1971). Knowing that this relationship exists under certain well defined circumstances, may permit the prediction of the order of value of one such parameter from a knowledge of the second (Carlisle et al, 1979). Thus the first parameter may be used as an indicator for the second. With this in mind the aim was to establish whether lignocaine extraction ratio and clearance correlated with indices of hepatic function.

Correlation analysis of liver function indices have been performed in patients with varying hepatic diseases (Miloszewski et al, 1970)(Hamilton, 1977) as well as in the isolated perfused pig liver (Ramsoe et al, 1971), and in the studies described in Chapter 5. In the latter two studies the few significant correlations could be attributed to small disturbances of liver function with resultant small differences compared to the precision of the methods used to determine these indices. This indicated that in order to test the relationship of hepatic lignocaine elimination with indices of hepatic function a spectrum of impaired to near normal livers and hence associated indices of hepatic function should be used. As it is clear that the standard isolated liver has suffered a measure of anoxic damage (Lemasters et al, 1983)(Bradford, 1986) and that severe hypoxia is required to impair hepatic function further (Rabol et al, 1974) two groups of livers were used to attempt to engender the required spectrum of data points for a suitable correlation analysis. For this analysis the data from experiments described in chapter 8 will be used.

9.2 MATERIALS, METHODS AND RESULTS

The materials and methods used are discussed in section 8.2.2 whilst the design of this study is discussed in section 8.4.1. Briefly, mean hepatic lignocaine extraction ratio and clearance were calculated using transhepatic samples (n=5) analysed in duplicate for lignocaine concentration at 60 and 120 minutes after the start of lignocaine administration and correlated with indices of hepatic function determined at the same time points in 7 normoxic and 8 hypoxic isolated perfused pigs liver experiments. The statistical relationships between the parameters were determined using Pearsons' Correlation Coefficient. Table 9.1 indicates the correlation of lignocaine extraction and clearance with certain indices of hepatic function measured whilst figures 9.1-9.2 are scattergrams of lignocaine extraction ratio plotted against some of these indices.

Table 9.1

Correlation Matrix of Lignocaine Extraction Ratio and Clearance against Liver Function Indices using Combined Data from Normoxic and Hypoxic Livers

LIGNOCAINE:	EXTRACTION RATIO			UNIT CLEARANCE		
	(r)	(p)	(see)	(r)	(p)	(see)
(Y)		(X)			(X)	
ATP (uM gm^{-1}liver)	.94	.0001	0.64	.93	.0001	0.69
ATP/ADP	.82	.0001	0.28	.80	.0001	0.27
TAN (uM gm^{-1}liver)	.64	.0004	1.64	.64	.0004	1.66
EC	.77	.0001	0.095	.76	.0001	0.096
AST ($U L^{-1}$)	-.63	.0002	265	-.61	.0003	269
ALT ($U L^{-1}$)	-.13	.498	18	-.16	.390	18
K+(mmol L^{-1})	-.89	.0001	2.1	-.89	.0001	2.1
LU (mg min^{-1} 100gm^{-1}liver)	-.17	.367	1.8	-.14	.453	1.8

Data are presented as r value, p value standard error of the estimate (see) for n=30 points*.EC=energy charge, AST=Aspartate aminotransaminase, ALT=alanine aminotransaminase, K+=perfusate potassium, LU=lactate uptake. X = independent variable and Y = dependent variable for standard error estimate.
*missing values: adenine nucleotide status (n=4), Potassium (n=1).

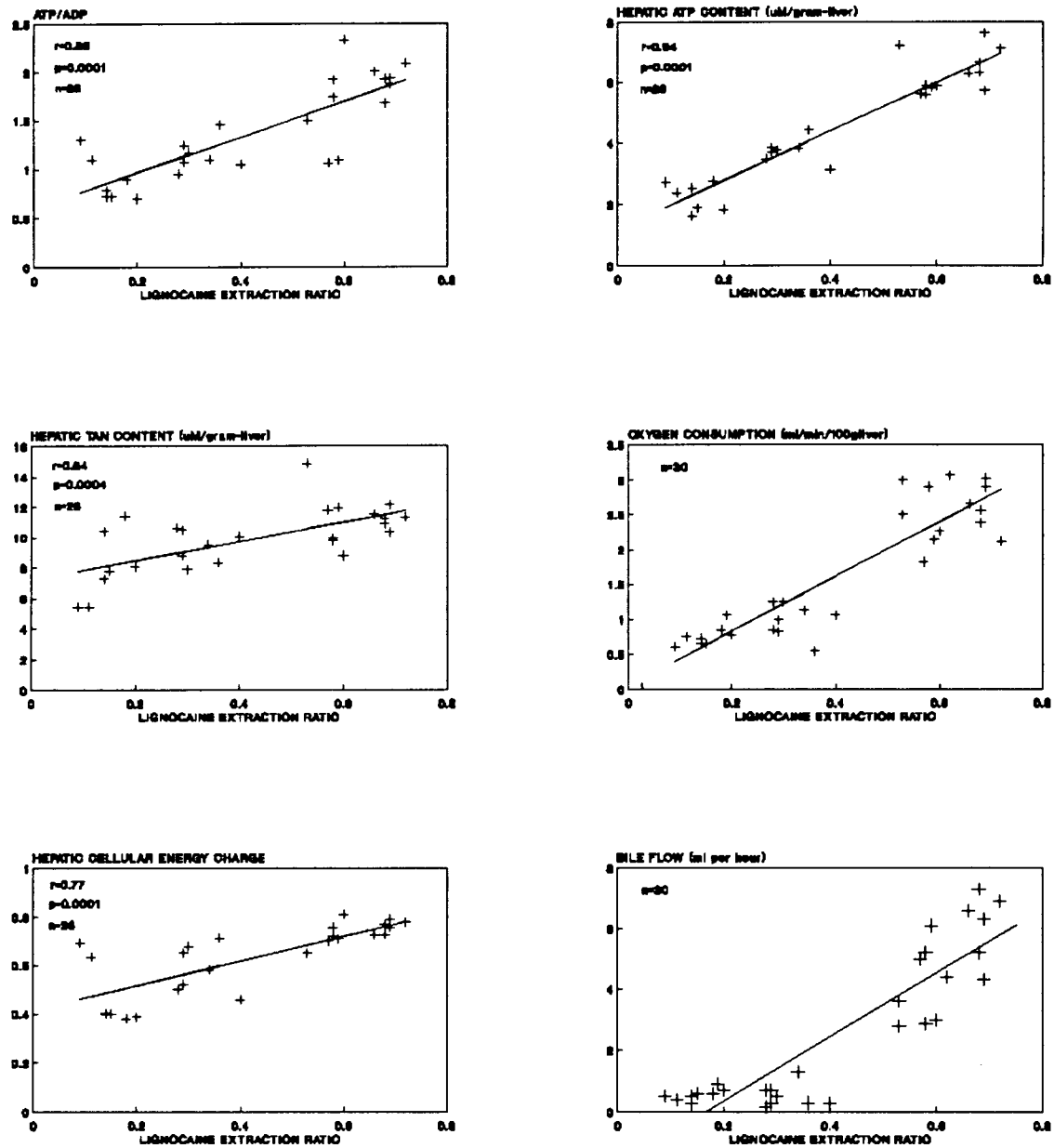

Figure 9.1 Scattergrams of hepatic lignocaine extraction ratio against hepatic ATP, ATP/ADP, TAN, energy charge, bile flow and oxygen consumption using combined data from hypoxic and normoxic livers.

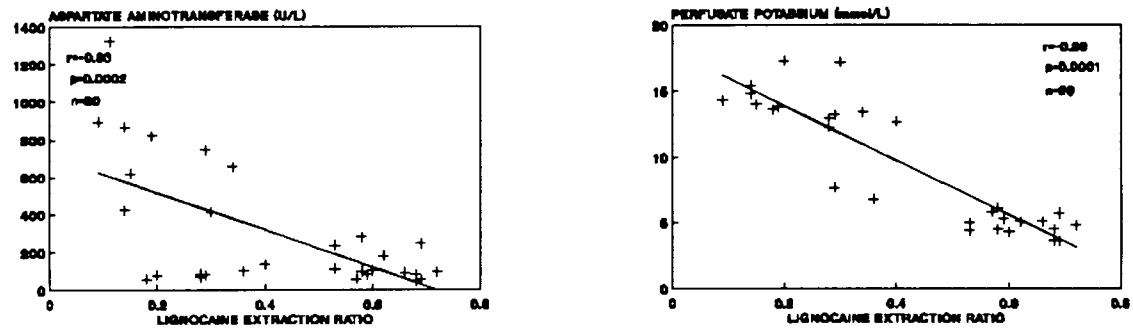

Figure 9.2 Scattergrams of hepatic lignocaine extraction ratio against perfusate aspartate aminotransferase and potassium concentration using combined data from hypoxic and normoxic livers.

9.4 DISCUSSION OF CORRELATION ANALYSIS

This analysis was not performed to assess the effects of hypoxia on lignocaine elimination per se, as for this a dose response study of the effect of different hepatic affluent oxygen partial pressures on lignocaine elimination would be required. Indeed, this may not be feasible as hypoxia induced changes occur only below a hepatic venous PO_2 of 30mmHg (Rabol, 1974) and this range was 2-19 mmHg in the livers perfused with a hypoxic perfusates in these experiments. The object was instead to generate a range of data points for both hepatic lignocaine elimination and the hepatic function indices measured, so that their relationship could be assessed. It is clear that this model has its limitations for assessing this with respect to certain indices of hepatic function: with respect to the data for lignocaine elimination and hepatic oxygen consumption, it can be seen that this relationship may not be linear and further that this relationship cannot be assessed for bile flow from the present scatter of data points (Figure 9.1)(shown here for lignocaine extraction ratio only). Thus, in the isolated perfused liver where hormonal factors and substrate load that influence hepatic oxygen consumption (Hems et al, 1966)(Van Dyke et al, 1983) and bile flow (Jablonski et al, 1971)(Gordon et al, 1972) in vivo, are absent, the relationship between lignocaine elimination and hepatic function cannnot be accurately tested. However, the correlations of lignocaine extraction ratio with hepatic ATP content, ATP/ADP ratio, TAN content and hepatocellular energy charge, as well as with perfusate aspartate aminotransferase and potassium concentration, all demonstrate a straight line relationship[1] (Figures 9.1 & 9.2), suggesting that factors that affect hepatic lignocaine extraction ratio and clearance also affect indices of hepatic function ex vivo. Figures 9.1 and 9.2 show strong ($r > 0.75$) highly significant correlations between lignocaine extraction ratio and hepatic ATP content, ATP/ADP ratio, and

1 See Hardison and Norman (1968) who established a linear realtionship using a similar scatter of data points.

energy charge as well as perfusate pottassium concentration. The correlations were less strong for hepatic TAN content and perfusate AST concentration.

Due to the fact that in this study standard unit hepatic blood flows were administered to the livers ex vivo and that hepatic clearance is a multiple of the extraction ratio it is evident that these two parameters are similarly correlated (Table 9.1). This analysis might suggest that hepatic lignocaine extraction ratio and hepatic clearance might be equivalent indicators of hepatic function. However, this may not be the case where hepatic blood flow varies as would be the case in vivo. This serves to highlight the fact that this analysis, performed using the isolated perfused liver, cannot be directly extrapolated to the clinical siuation.

In this evaluation linear regression analysis was performed of parameters of lignocaine elimination on indices of hepatic function as we wished to determine whether there was a relationship of lignocaine elimination with other indicators of hepatic function. An alternate question might have been **what** factors affect lignocaine elimination. To answer this question multiple linear regression analysis could have been performed as this is a technique used for testing the relationship between a single quantitative dependent variable and many quantitative explanatory variables (Daly et al, 1991).

9.4 SENSITIVITY AND SPECIFICITY OF LIGNOCAINE EXTRACTION RATIO AS AN INDICATOR OF SEVERE HEPATIC HYPOXIA

To further evaluate the potential for using lignocaine extraction ratio as an indicator of hypoxic hepatic injury in the present model the sensitivity and specificity of this test in relation to that of the other mentioned indices can be compared.

$$\textbf{Sensitivity} = \frac{\textbf{number of true positive results}}{\textbf{total instances of liver hypoxia}}$$

Sensitivity determines what likelihood the test has of being positive if hypoxia (defined as the liver being subjected to an oxygenator inflow of 2%) is present.

$$\textbf{Specificity} = \frac{\textbf{number of true-negative results}}{\textbf{total instances of liver normoxia}}$$

Specificity determines the likelihood the test has of being normal if hypoxia as defined above is <u>not</u> present. (Griner et al, 1981).

The cut-off point (the point used to define an abnormal result) defines a set of operating characteristics for the test in question. This point may be arbitrarily defined to assure either greater sensitivity of a test at the expense of specificity or vice versa (Griner et al, 1981). In this analysis the cut-off value has been

determined as the mean of normoxic values ± 1SD as this cut-off point has been used by others to assess liver function tests in terms of sensitivity and specificity (Jost et al, 1987)(McDonagh et al, 1991).

Table 9.2

Comparison of Sensitivity and Specificity of Lignocaine Extraction Ratio with Indices of Hepatic Function

	Sensitivity	Specificity	Cutoff Value*
Lig ER	100%	86%	0.55
O2	100%	86%	2.18 ($ml100gm^{-1}liver$)
ATP	100%	83%	5.67 ($uMgm^{-1}liver$)
ATP/ADP	91%	91%	1.37
TAN	58%	91%	9.81 ($uMgm^{-1}liver$)
EC	91%	91%	0.700
AST	57%	78%	204 (UL^{-1})
ALT	28%	71%	53 (UL^{-1})
BF	100%	78%	3.5 ($mlhour^{-1}$)
K+	100%	86%	4.8 ($mmolL^{-1}$)

* Mean (± 1SD) of normoxic liver parameter values. Lig ER = lignocaine extraction ratio, O2 = hepatic oxygen consumption, EC = energy charge, AST = Aspartate aminotransaminase, ALT = alanine aminotransaminase, BF = bile flow, K+ = perfusate potassium.

It is clear from Table 9.2 that with respect to the operating charachteristics defined above, lignocaine extraction ratio, hepatic oxygen consumption, ATP content and hourly bile flow (indicators of hepatic function) and potassium release (indicator of hepatic dysfunction) are equally sensitive indicators of hypoxic hepatic injury as defined in this model. In contrast EC and ATP/ADP ratio and TAN (indicators of hepatic function) whilst having greater specificity than the above tests are less sensitive indicators. AST and more especially ALT release (indicator of hepatic dysfunction) are the least sensitive and specific indicators in this study. It should be added that this excercise of determining sensitivity and specificity using the criteria of oxygenator inflow concentration is of course highly artificial and has been included only because it serves to highlight the methodology that can be used to evaluate a potential indicator of hepatic function.

In general when a test is to be used for screening it should be sensitive whilst when it is to be used to confirm a diagnosis it should be specific (Griner et al, 1981).

9.6 CONCLUSION

This analysis was performed to establish whether hepatic lignocaine extraction ratio and clearance could be used as an indicator of other indices of liver function when a range of livers with near normal to severely impaired hepatic function were studied in the isolated perfused state. Clearly, this analysis cannot be directly extrapolated to the clinical situation. Nevertheless, the strong correlation of lignocaine extraction ratio with indices of hepatic function and dysfunction, the equivalent sensitivity of this test to a number of these indices, and the greater sensitivity and earlier indication of hypoxic injury than hepatic AST release observed in this model, may suggest that further clinical evaluation could reveal lignocaine extraction ratio to be a useful early indicator of impaired hepatic function.

CHAPTER 10: CONCLUSIONS

10.1 **CONCLUSIONS** **10.2**

10.2 **FUTURE STUDIES** **10.3**

CHAPTER 10: CONCLUSIONS

The aim of this work was to establish whether hepatic lignocaine elimination was impaired by hypoxia and whether lignocaine extraction ratio and clearance could be used as an indicator of hepatic function. This was studied using the isolated perfused pig liver. To establish whether the pig liver could be used as a possible human model for this investigation and whether lignocaine had any detrimental effects on hepatic blood flow and function when administered in vivo, hepatic lignocaine elimination and the effects of lignocaine administration on hepatic function and blood flow were studied in the intact anaesthetized animal. Hepatic lignocaine elimination was then studied in the isolated perparation at similar hepatic affluent lignocaine concentrations to determine whether this was similar to that determined in vivo. It was postulated that this comparison could best be drawn using the same liver in a sequential experiment. Preliminary investigations were performed to establish this, as well as to determine the necessary pharmacokinetic parameters to achieve similar hepatic affluent concentrations in both preparations.

Pharmacokinetic analysis of lignocaine decay established that this could best be described by a two compartment model in both the anaesthetized pig and the isolated perfused pig liver preparation. Derived parameters were used successfully to achieve constant similar lignocaine concentrations for comparison of in vivo and ex vivo lignocaine elimination.

It was established that the comparison of in vivo and ex vivo hepatic function using the same liver in a sequential experiment was inappropriate as the prior in vivo study was shown to have significant effects on the adenine nucleotide status of the subsequently perfused livers when compared with livers perfused immediately.

Hepatic lignocaine extraction ratio in vivo in the pig was found to be similar to that reported for man. In this regard the pig, with the possible exception of the monkey, was found to be closer than other animals reported on. The pig liver can thus be regarded as an appropriate animal model for human hepatic lignocaine metabolism.

There was no detrimental effect of lignocaine on hepatic blood flow and function in vivo, nor were there ultramicroscopic changes that could be attributed to its administration. It was thus concluded that lignocaine may be regarded as an innocuous indicator.

Lignocaine extraction ratio and clearance in vivo and ex vivo were found to be similar when different livers were used for this comparison. This would support use of the isolated liver to study the effects of hypoxia on hepatic lignocaine metabolism.

Lignocaine extraction ratio, clearance and intrinsic clearance as well as monoethylglycinexylidide formation were significantly impaired in livers subjected to hypoxia. Lignocaine elimination correlated strongly with hepatic cellular ATP, energy charge and ATP/ADP ratio as well as with hepatic potassium release but less strongly with hepatic aspartate aminotransferase release when this relationship was tested using the combined data from hypoxic and normoxic livers ex vivo. These correlations were positive for hepatic adenine nucleotide status and negative for hepatic potassium and aminotransferase release.
Hepatic alanine aminotransferase release was not significantly affected by hypoxia.

Endogenous hepatic lactate utilization had been postulated as a potential indicator of hepatic function but was not significantly affected by hypoxia and showed no relationship with any other indicators of liver function ex vivo.

Lignocaine extraction ratio, hepatic oxygen consumption, ATP content, bile flow and potassium release were shown to be equivalent, more highly sensitive and earlier indicators of hypoxic hepatic injury than hepatic aspartate aminotransferase release in the isolated perfused pig liver.

This work using the pig has shown that lignocaine extraction ratio determined as described, can be used, without detrimental effects on hepatic function or blood flow, as an indicator of some aspects of hepatic function and dysfunction. Lignocaine elimination is impaired by acute severe hypoxic hepatic injury. If this data can be extrapolated to man, and the similarity in human and porcine hepatic extraction ratio for lignocaine suggests that this might be the case, then lignocaine extraction ratio may prove a potentially useful clinical indicator of hepatic function.

10.2 FURTHER STUDIES

It is proposed that lignocaine extraction ratio may be studied as an on-going monitor of hepatic function post liver transplantation along the following lines.

After hepatic transplantation patients will have been cannulated with a pulmonary artery catheter (which includes a central venous port) and a radial placed arterial catheter.

Lignocaine hydrochloride can be infused intravenously to a low systemic constant concentration (± 2ugml^{-1}) using the methodology described in this thesis. The hepatic extraction ratio for lignocaine can then be determined from an arterial sample and a venous sample drawn through a hepatic venous catheter placed at laparotomy. This sampling is performed repeatedly (to confirm that the concentration is indeed constant) and correlated with other clinically used indices of liver function as well as patient outcome.
This methodology appears justified if the safety of lignocaine and the similar portal vein and systemic arterial concentrations found at steady state in the pig, as well as the independance of lignocaine extraction ratio on hepatic blood flow (Lautt and Skelton, 1977) can be extrapolated to man.
A potential problem with this study design may be the reported time dependent decrease in lignocaine metabolism with long-term infusions (Saville et al, 1989). Whether this is important when once daily short infusions of lignocaine (± 2 hours) are used needs to be determined.
A further problem may be the use of a hepatic venous catheter for fear of graft damage. However, hepatic venous catheterisation has been used extensively in patients with liver disease (Huet and Villeneuve, 1983) and doppler microprobes have been attached to hepatic affluent vessels without incident, to assess hepatic blood flow in man after liver transplantation (Payen et al, 1990).

If this remains a concern an alternative less invasive but less accurate method would be to approximate systemic lignocaine extraction ratio by sampling from the radial artery and from the central venous catheter port (lying within the right atrium) while infusing lignocaine directly into the pulmonary artery, thus avoiding contamination of samples taken from the right atrium with infused lignocaine.

An immediate result (± 20 minutes) for interpretation can be achieved if plasma lignocaine concentrations are assessed by fluorescence polarization immunoassay (Oellerich et al, 1990), whilst in the near future continuous in vivo analysis of blood lignocaine concentration may be possible using a lignocaine sensitive electrode (Yokono et al, 1991).

It is hoped that this envisaged study will find lignocaine extraction ratio to be a useful early clinical indicator of hepatic dysfunction associated with hepatic transplantation.

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APPENDIX A: CALIBRATION EXPERIMENTS

A.1 **CALIBRATION OF FLOW METERS AND ROLLER PUMPS A.2**

A.1.1 Calibration of flow meters A.2

A.2.2 Calibration of roller pumps A.4

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APPENDIX A: CALIBRATION EXPERIMENTS

A.1. CALIBRATION OF FLOW METERS AND ROLLER PUMPS

A.1.1 CALIBRATION OF FLOW METERS

A.1.1.A. Methodology

For the in vitro calibration of the two flow meters and three perivascular flow probes used in these studies the following apparatus was devised: (Figure A.1) A rectangular water tight box was made from perspex, allowing the passage of two pieces of silastic tubing of internal diameter 6.25mm and 2.5mm through each "short" side. Thus, in all, four pieces of tubing passed into the box. Each internal end was cannulated with a perspex connector.

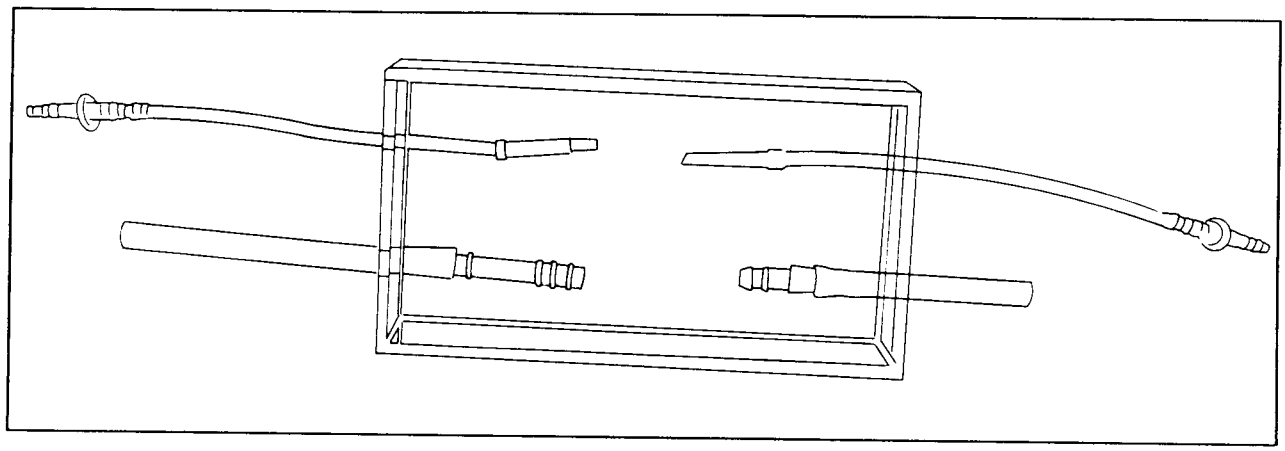

Figure A.1 Diagram of device used for calibration of flow meters

In order to calibrate the flow probes suitable lengths of vessel were harvested from a pig; for the hepatic artery flow probe, a length of internal iliac artery was used whilst for the portal venous cannula a piece of inferior vena cava (IVC) was used as the hepatic artery and portal vein of the pig are to short to be used for this purpose. The vessels were cannulated from each end by the appropriate perspex connectors mentioned and then securely tied with a silk tie leaving an expanse of non cannulated vessel between them around which the flow probe in question could be placed. The perspex container was then filled with normal saline so that the perivascular flow probes and vessels were submerged.

The "outside" ends of the silastic tubing were connected to further lengths of tubing; one side feeding into a Sarns Roller pump and ending in a container filled with fresh heparinised pig blood. The other end completed the circuit returning pumped blood to the same container. This end could be placed in an Erlenmeyer flask allowing accurate measurement of the volume of blood pumped through the vessel in question in one minute.

The method of calibration of a particular flow probe was as follows. The probe was placed around a distended vessel and in the absence of flow the flow meter was set to zero. The Sarns roller pump was then set at a certain frequency and the flow per minute into the Erlenmeyer flask was measured with a stop watch three times in succession. During the course of these measurements the flow meter readings were taken by a second observer. This procedure was then repeated at various settings of the roller pump.

A.1.1.B. SEM 275 Flowmeter and 9mm probe

Prior to the calibration described above the normal calibration routine for this particular flowmeter was performed, with the probe head immersed in alcohol, confirming that the scale reading (in percentage deflection) was within ± 2% of zero and the full scale deflection was 100%. The calibration study was then performed as described above.

Table A.1 Calibration of SEM 275 Flow Meter

Mean Blood Flow (ml min^{-1})	Mean Flow Meter Reading
125	13%
200	18%
400	40%
505	51%
580	62%
700	73%
845	85%

$Y = 9.58X + 11.1$ $r = 0.998$

A.1.1.C. T201D Ultrasonic Flow Meter Channel 2 and 6mm Flow Probe

Prior to the calibration of this system the flow probe (probe 6S203) was immersed in water for 10 minutes and the self test mode facility instituted to ensure that the analog meter voltage reading was within 15% of the factory calibrated value of 0.46Volt. (The reading: 0.44Volt) The calibration procedure was then as described above.

Table A.2 Calibration of T201D Ultrasonic Flow Meter

Mean Blood Flow (ml min^{-1})	Mean Flow Meter Reading
228	243
278	297
388	400
524	500
635	626
760	746

Y=0.930X + 37.6 r = 0.998 ± 5% variation over range.

A.1.1.D. T201D Ultrasonic Flow Meter Channel 1 and 2mm Flow Probe

As for (C) prior to the calibration of this system the flow probe (probe 2RS716) was immersed in water for 10 minutes and the self test mode facility instituted to ensure that the analog meter voltage reading was within 15% of the factory calibrated value of 0.52Volt. (The reading: 0.45Volt) The calibration procedure was then as described above.

Table A.3 Calibration of T201D Ultrasonic Flow Meter

Mean blood flow (ml min^{-1})	Mean flow meter reading
70	68
106	104
116	117
140	142
175	170
193	197
236	240
276	282
314	322

Y=1.04X - 4.5 r = 0.999 ± 1.6% variation over range.

A.1.2. CALIBRATION OF ROLLER PUMPS

Prior to the determination of the stroke volume of each pump the occlusion of the rollers was adjusted so that there was no drop greater than 2.5cm when a 90cm fluid level was maintained above the pump. The pumps were calibrated using a mixture of fresh abbatoir blood and plasmalyte B constituted in the ratio of standard perfusate ie 2.7:1. The pumps were set at a certain speed and the number of revolutions per minute (rpm) counted using a stop watch. The flow volume per minute was then measured three times using an Erlenmeyer flask. The mean volume so obtained divided by the rpm yielded the stroke volume. This procedure was repeated at a number of different pump settings.

A.1.2.A. Hepatic artery pump calibration (Tubing 2.5 mm i.d.)

Table A.4

Pump Setting (rpm)	Volume Per Minute (ml)	Stroke Volume (ml)
16	40	2.5
20.5	49	2.39
27.5	64	2.30
36	84	2.33
41	98	2.39

mean stroke volume = 2.38 (SEM=0.03)

A.1.2.B. Hepatic artery pump calibration (Tubing 6.25 mm i.d.)

Table A.5

Pump Setting (rpm)	Volume Per Minute (ml)	Stroke Volume (ml)
9.25	118	12.60
12.5	158	12.64
16.0	200	12.50
19.5	248	12.71
22.5	280	12.59
28.5	360	12.60

mean stroke volume =12.60 (SEM=0.02)

A.1.2.C. Portal vein pump calibration

Table A.6

Pump Setting (rpm)	Volume Per Minute (ml)	Stroke Volume (ml)
11.5	151	13.10
15.5	207	13.35
21.5	288	13.39
25.5	338	13.52
29.7	407	13.70
34.0	455	13.38
39.5	532	13.45

mean stroke volume = 13.41 (SEM=0.07)

A.1.2.D. Stability of pump flow over a two hour study period

To ascertain the stability of pump flow over the experimental period the number of pump revolutions per minute were counted every 15 minutes and the flow per minute measured three times in succession using an Erlenmeyer measuring cylinder.

(i) Hepatic artery pump (tubing 2.5mm i.d.)

Table A.7

TIME	0	15	30	45	60	75	90	105	120
R.P.M	41	42	42	42	42	42	42	42	42
MEAN FLOW	97.3	98	97.6	97.3	97	97.6	96.6	97.3	96.3

Range 96.3-98 ie 1.7% variation

(ii) Portal vein pump (tubing 6.25mm i.d.)

Table A.8

TIME	0	15	30	45	60	75	90	105	120
R.P.M.	34	34	33	33	34	33	34	33	33
MEAN FLOW	451	451	451	447	450	451	450	440	448

Range 440-451 ie <1% variation

A.2 CALIBRATION OF VOLATILE AGENT MONITOR FOR MEASUREMENT OF ISOFLURANE CONCENTRATION

A.2.1 MOTIVATION

The Engstrom Emma anaesthetic vapour monitor is an instrument for measuring anaesthetic vapour concentrations. The anaesthetic vapour is monitored by placing a transducer, housing a vibrating quartz crystal, in the stream of anaesthetic gas. The quartz crystal is covered by a thin layer of silicone antifoam. As anaesthetic agents are highly soluble in this substance a concentration dependent increase in mass of the crystal occurrs with a resultant decrease in frequency of vibration. The change in frequency is thus a measure of the anaesthetic concentration. The transducer which also contains a reference crystal can be mounted in the anaesthetic breathing system. The instrument is not sensitive to oxygen, carbon dioxide or nitrogen but may be affected by nitrous oxide. This problem can be circumvented by setting to zero the transducer in the presence of the administered concentration of nitrous oxide.

As this device is not an absolute measure of the anaesthetic vapour a calibration curve was performed using the methodology described below. This was necessary as calibration vapours were not available at the time of these experiments.

A.2.2. PRINCIPLE OF CALIBRATION METHOD

The principle used was to introduce known concentrations of isoflurane in air into the transducer and then to plot the corresponding Engstrom Emma readings to establish a calibration curve. Using this curve subsequent experimental Engstrom Emma readings could be determined.

Known concentrations of isoflurane were constituted using the gravimetric method.

A measured mass of liquid isoflurane was injected into an air tight glass container of known volume Figure A.2-(A) which was connected to a pressure measuring device (a mercury filled manometer). Time was allowed for the isoflurane gas to reach room temperature measured with a calibrated thermometer. The pressure of the gas was measured and from Avogadro's Hypothesis and the Universal gas constant the concentration of isoflurane was determined as follows.

Volume of Isoflurane (V1) at Standard Temperature and Pressure (STP) =

(Mass of isoflurane/ Mass of one mole of isoflurane) * 22.4 litres.

The true Volume of isoflurane (V2) at ambient temperature and pressure was determined from the relationship =

$$P1 \times V1/T1 = P2 \times V2/T2$$

Where P1 and T1 are standard pressure and temperature; 760mmHg and 273.2 degree K, and P2 was 760mmhg + the pressure measured in the manometer at the ambient temperature T2.

The concentration of isoflurane in the glass container was given by :

V2/ volume of glass container.

A.2.3 MATERIALS AND METHOD:

A.2.3.A. Equipment

(1) Vaporisation Chamber:

A round glass vaporisation flask Figure A.2-(A) with a stoppered glass top containing an injection port (B) (Rotaflow T/F 2/13, England) was used.

This had a mounted glass side-arm to which was connected a glass U tube filled with mercury. Gas flow through the side arm could be interrupted by a screw tap (F) allowing the measurement of pressure in the manometer on unscrewing the tap at the time the ambient temperature had been reached. The volume of the apparatus was determined to be 1070ml by filling the flask, stopper and side arm with water.

(2) Engstrom Emma Transducer Connector.

See Figure A.3.

In order to calibrate the Engstrom Emma the known concentration of isoflurane in the flask had to be introduced into the transducer (D) to reach an equilibrium

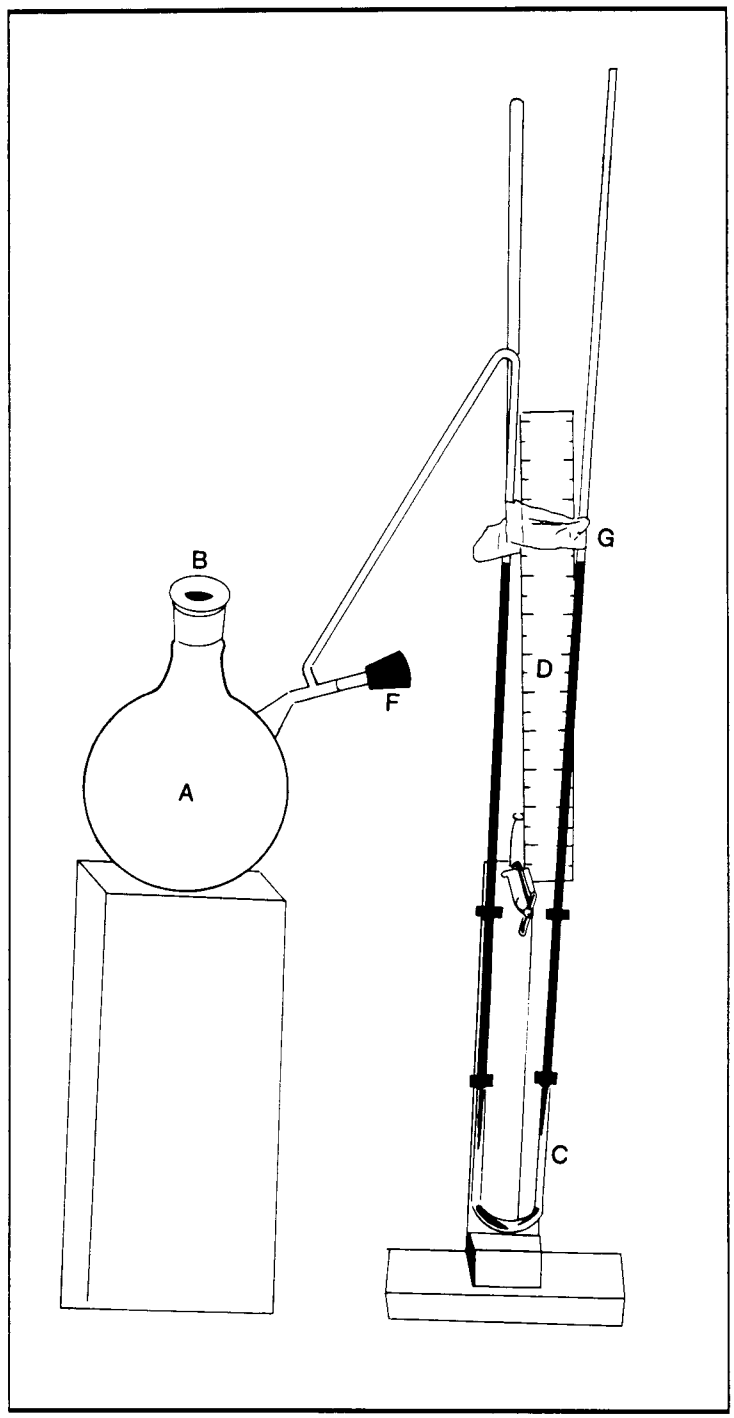

Figure A.2 Diagram of device used to produce test vapours for calibration

concentration with the silicone antifoam of the vibrating quartz crystal. In order to achieve this the openings at each end of the transducer head were covered with standard brass lined APL anaesthetic adaptors (A) one at each end. The outlets of these adaptors were rebored to fit on one, a steel injection port, and the other a steel "bleed off" screw valve (B). When these were connected to the transducer the total deadspace was 17cc. Gas containing a known concentration of isoflurane could thus be injected through one port with a 50cc glass syringe (C) while bleeding off deadspace gas through the other port.

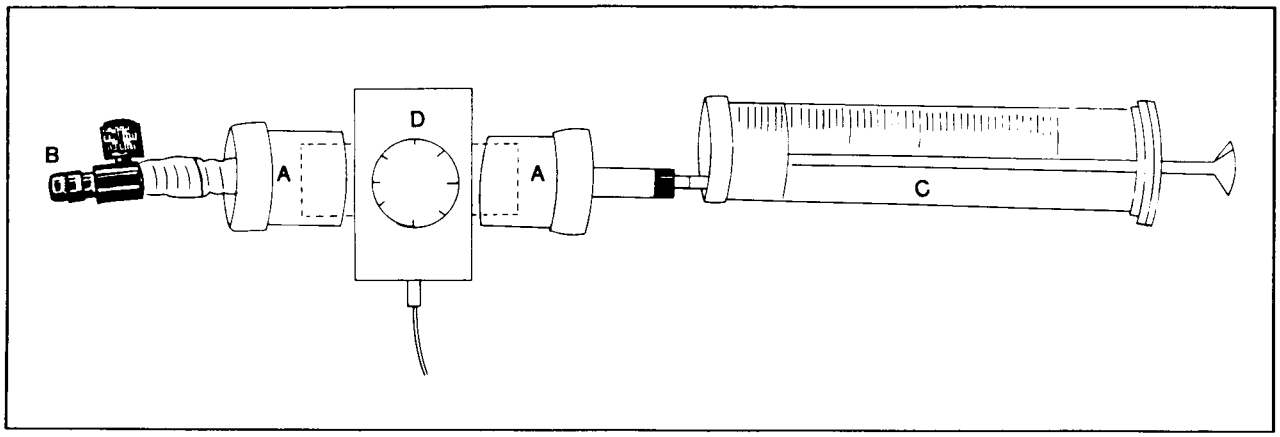

Figure A.3 Diagram of device use to calibrate a volatile agent monitor

A.2.3.B. Method

The Engstrom Emma was subjected to the standard warm up period. In the gain preset mode the transducer and meter were congruent (reading of 4 in both). After each measurement the transducer was cleared of all measureable isoflurane with a fresh air stream and was then reset to zero.

A 100ul Hamilton Syringe (#710 Switzerland) was used to inject isoflurane into the vaporisation chamber. The volume range of this syringe was calculated to be most suitable to yield a concentration range of isoflurane of 1.5 ± 1.0%.

Isoflurane was aspirated into the syringe and the weight was determined using a fine balance (Mettler H10W,England). Immediately after injection of isoflurane the empty syringe was reweighed and the mass injected was regarded as the difference between the two readings. (It had been determined earlier that over a 5 minute period an isoflurane filled syringe placed on the fine balance did not decrease in weight indicating that very little loss by vaporisation of isoflurane was occurring through the fine needle of this syringe).

Before each experiment the vaporisation flask was prepared by removing the glass stoppered top and flushing air through it for 10 minutes to clear any remaining isoflurane. The tap to the manometer was opened to reset the mercury level and then closed. The sample of isoflurane was then injected through the

injection port of the glass stopper into the flask. All connections were then tested on each occasion with soap solution to exclude any confounding leaks which would show up by the appearance of soap bubbles. After 15 minutes equilibration time with room temperature the temperature reading of a calibrated glass thermometer lying with its tip against the glass chamber was read[1]. The screw tap was briefly opened to allow measurement of the pressure rise in the manometer in mmHg. It was then closed to minimise possible dilution of the isoflurane concentration.

Fifty ml of isoflurane containing gas was then aspirated slowly into a glass syringe (Sanitex, Switzerland) via the injection port of the glass stopper and this syringe was then connected immediately to the injector port of the transducer assembly as shown in Figure A.3. Forty ml of vapour was then injected to flush out the deadspace gas in the transducer assembly afterwhich the "bleed off" valve was closed. The reading on the Emma was taken when the needle was stable. The last 10ml was then injected and the whole injection procedure repeated twice more. The maximum concentration reading achieved of the three injections on the Emma Scale was taken as the measured concentration.

A.2.4 RESULTS AND DISCUSSION

Table A.9 Calibration of Volatile Agent Monitor

Isoflurane Concentration:	**Emma Reading:**
(Y)	(X)
0.59%	0.75%
1.20%	1.10%
1.40%	1.38%
1.85%	1.65%
2.18%	1.99%
2.27%	1.89%

Subjecting these data points to least squares linear regression analysis where Y is the isoflurane concentration and X the measured concentration (Emma Reading) yielded a line $Y = 1.36X - 0.403$ with a correlation coefficient of 0.989. The true end tidal isoflurane concentration during an experiment could thus be determined by substituting the Engstrom Emma reading for X in this formula.

Potential sources of error in this method may lead to a possible underestimation of the Emma measurement readings. Potential for error is vaporisation of isoflurane from the syringe prior to chamber injection or after injection before reweighing. It was shown that this would be negligible.

A further source of error would be diffusion of isoflurane into components of the test equipment. This potential was minimised by in the main using glass equipment. Another potential site for error is due to possible dilution of isoflurane vapour on opening the tap for pressure measurement. This could not be

1 It was not possible to introduce this into the flask which would have been the preferred method.

avoided as aspirating vapour for analysis prior to this measurement as this would affect the pressure measurement by inducing a vacuum. The tap was thus opened only momentarily and the deadspace above the mercury level was minimised as far as possible.

It is also possible that the injection of the total of 150 ml of isoflurane containing gas into the transducer did not fully displace the 17ml of deadspace air already present. This would again only amount to a very small error.

A.3 MISCELLANEOUS CALIBRATION EXPERIMENTS

A.3.1 VIAL MEDICAL SE 200 INFUSION PUMP

The Vial Medical SE 200 pump (France) allows various syringe options. Terumo 50ml syringes (Tokyo, Japan) were used for the calibration experiments and in all subsequent experiments as this was the only syringe available to us for which this infusion pump could be set. The volume that the pump was required to deliver was 1.7ml per hour for the IPPL experiments and 2.2, 2.3, 2.4, and 2.5ml per hour in the in vivo experiments. For the initial rapid infusion over 10 minutes in the in vivo experiments at settings of 18.7, 19.5, 20.0,and 21.2 ml per hour the pump was required to deliver 3.1, 3.2, 3.3,and 3.5ml. The pump was therefore calibrated at a number of infusion rates. However, to confirm its continued accuracy the pump was calibrated before and at the conclusion of all the experiments for this thesis at a setting of 1.7ml per hour. The infusion pump was calibrated using an infusion volume of saline timed with a stop watch over either one hour or 10 minutes as appropriate. This was pumped via an extension tubing into a standard 10 ml measuring cylinder.

Table A.10

Experiment (Before)	Pump Setting (ml)	Zero Reading (ml)	Reading One hr (ml)	Volume (ml)
(1)	1.7	3.1	4.8	1.7
(2)	1.7	4.8	6.5	1.7
(3)	1.7	6.5	8.2	1.7
(4)	1.7	8.2	9.8	1.6
(5)	1.7	6.0	7.7	1.7
(After)				
(1)	1.7	2.3	4.0	1.7
(2)	1.7	4.0	5.7	1.7
(3)	1.7	5.7	7.4	1.7

Table A.11

Experiment	Pump Setting (ml)	Zero Reading (ml)	Reading One hr (ml)	Volume (ml)
(1)	2.2	5.8	7.0	2.2
(2)	2.2	3.6	5.8	2.2
(3)	2.3	1.2	3.6	2.4
(4)	2.3	3.6	3.9	2.3
(5)	2.4	5.2	7.6	2.4
(6)	2.4	6.4	8.8	2.4
(7)	2.5	2.6	5.1	2.5

Table A.12

Experiment	Pump Setting (ml)	Zero Reading (ml)	Reading 10 min (ml)	Volume (ml)
(1)	18.7	4.7	7.8	3.1
(2)	18.7	4.8	7.9	3.1
(3)	19.4	6.1	9.2	3.1
(4)	19.4	1.8	5.1	3.3
(5)	20.0	5.8	9.1	3.3
(6)	20.0	6.7	10.0	3.3
(7)	21.2	5.8	9.3	3.5

A.3.2 DETERMINATION OF TIME PERIOD TO TRAVERSE STANDARD 50 CM F8 FEEDING CATHETER

In order to determine the time ("dead space" time) required for lignocaine infused at a certain rate at the start of an in vivo experiment to reach the end of the standard catheter used to cannulate the external jugular vein, the following bench experiment was devised. The infusion pump was set at the approriate rate and a lignocaine filled (Terumo) syringe was connected by means of the standard extension tubing to the empty catheter. The period for the lignocaine to traverse this catheter was then determined three times.

Table A.13 Determination of Time Period to Traverse a Standard Catheter

Experiment	Pump Setting (ml)	time (sec)	time (sec)	time (sec)	mean time (sec)
(1)	18.7	125	128	125	126
(2)	19.4	129	128	127	128
(3)	20.0	126	125	127	126
(4)	21.2	121	121	120	121

A.3.3 CALIBRATION OF HELLIGE SERVOMED SMK 154-3 PRESSURE MONITOR

This monitor has two channels; Channel (1) was used to measure pulmonary artery and pulmonary capillary wedge pressures and Channel (2) was used to measure arterial pressure. The accuracy of the system was verified using the transducers used throughout the experimental period.

Table A.14 Calibration of Monitor used to detemine Arterial Blood Pressure

Sphygmomanometer Pressure (mmHg)	Channel 1 (mmHg)		Channel 2 (mmHg)	
	incrs.	decrs.	incrs.	decrs.
230	225	230	223	224
220	214	217	215	214
210	204	207	206	203
200	196	197	195	193
190	184	187	185	183
180	176	176	179	174
170	165	167	166	164
160	155	157	157	155
150	146	147	148	146
140	138	137	138	134
130	128	128	130	126
120	116	117	120	116
110	109	107	106	106
100	100	98	96	96
90	89	89	90	87
80	78	77	90	87
70	67	69	68	67
60	57	60	57	58
50	47	48	48	49
40	38	38	39	39
30	28	29	30	30
20	18	18	20	21
10	9	9	10	11

Incrs.= pressures measured during increments of increasing pressure. Decrs. = pressures measured during increments of decreasing pressure.

The respective transducers were separately connected to the inflation tubing of a Tycos Sphygmomanometer (Taylor Instrument Cooperation, Rochester USA) by means of a saline filled extension tube with a three way tap interposed. The third port of this was connected to the standard inflation bulb allowing the generation of pressure in the system or the slow decrease of pressure by bleeding off air via the inflation bulb bleed-off valve. The channel and transducer system were then set at the zero level of the mercury column and then the system was gradually pressurised in increments of 10mmHg. To detect

possible lag the bleed off valve was opened slightly once the maximum column pressure of 230 mmHg had been reached and the accuracy of the system was again verified while the pressure was decreasing.

A.3.4 CALIBRATION OF TEMPERATURE PROBE AND MERCURY IN GLASS THERMOMETERS

Two new mercury in glass thermometers and one rectal temperature probe connected to a Tele-thermometer (Yellow Springs,USA) were used throughout these experiments. These were calibrated together as follows. The bulbs and probe of the thermometers were immersed in iced water to which warm water was slowly added. Temperature readings were taken every 2 minutes.

Table A.15 Calibration of Temperature Measuring Devices

Telethermometer + probe °C	Mercury in Glass no:1 °C	Thermometers no:2 °C
2	2	2
10	10	10
11	12	12
12.5	13.5	13.5
15	15.5	15.5
20.5	21	21
25	26	26
31.5	33	33
34	36	36
34.5	36.5	36.5
36	38	38
36.5	38.5	38.5
37	39	39
39	41.2	41.2

Comment: Readings taken using the telethermometer were corrected to the values determined using the mercury in glass thermometers.

A.3.5 CALIBRATION OF SCALE USED FOR WEIGHING LIVER

Table A.16 Calibration of Scale used to Determine Liver Weight

Counter Weight Scale: Weight Placed (grams)	Weight Placed (grams)	Measuring Scale Scale Reading (grams)
0	0	0
0	100	100
50	100	50
50+30	100	20
50+30+10	100	10
30+10	100	60

This shows that the scale is accurate over the range 10-100grams and that the weights used above are accurate.

Assessment of weights to be used: 100,200,300, 500 grams.

Counter Weight Scale Weight Placed (grams)	Weight Placed (grams)	Measuring Scale Scale Reading (grams)
200+300	500+50	51
500	200+300+50	49*
100	200	100
200+100	300	<0#

* This implies that either the 500g weight is to heavy or 200g + 300g is too light. # This implies that the 300g weight is too light by ± 1g.

Conclusion scale and all weights (except 300g) accurate.

APPENDIX B: BIOCHEMICAL AND HAEMATOLOGICAL ANALYSIS

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B.2 ASPARTATE AND ALANINE AMINOTRANSFERASE B.2

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APPENDIX B: BIOCHEMICAL AND HAEMATOLOGICAL ANALYSIS

B.1 ARTERIAL BLOOD GASES AND ACID BASE ANALYSIS

Gas analysis and acid base status of blood and perfusate were determined using the ABL 300 Acid Base Laboratory (Radiometer Copenhagen, Denmark). This equipment was calibrated (one point and two point calibrations) one and three hourly respectively. The two point calibration was confirmed using standard solutions. (Qualicheck, Radiometer, Copenhagen). Samples were taken in preheparinsed 2ml syringes and either analysed immediately or after expulsion of air, the syringe was capped and stored on ice for analysis at the earliest possible time.

B.2 ASPARTATE AND ALANINE AMINOTRANSFERASE

B.2.1 ASPARTATE TRANSFERASE

Principle: A continuous monitoring assay was obtained by coupling transferase reactions to specific dehydrogenase reactions. The oxo-acids formed in the transferase reactions were measured indirectly by enzymatic reduction to the corresponding hydroxy-acids, and the accompanying change in NADH concentration was measured spectophotometrically relative to a known standard.

The formula: [Absorbance of test/absorbance of standard] x concentration of standard was used to determine the concentration of the aminotransferase.

Method: Fifty ul of plasma was added to 650ul of reagent (A phosphated buffer containing L-aspartate, NADH and malate dehydrogenase) and incubated at 20-25^{O}C for 15 minutes. Fifty ul of 2-oxoglutarate solution was added and the change in absorbance recorded every 20 seconds for 3 minutes. (Standard Method: Tietz, 1987)

B.2.2 ALANINE AMINOTRANSFERASE

The methodology was identical to that described above (B.2.1) except that in the reagent mentioned L-alanine replaced L-aspartate and lactated dehydrogenase replaced malate dehydrogenase.

B.3 ADENINE NUCLEOTIDE STATUS

B.3.1 ATP, ADP AND AMP DETERMINATION

The adenine nucleotides ATP, ADP and AMP were determined using methods adapted from Lamprecht and Trautschold, (1974) and Jaworek et al, (1974).

Briefly, samples were taken using modified Wollenberger clamps and stored under liquid nitrogen until the time of analysis. Teased freeze dried sample was weighed before and after the addition of 2ml of 5% perchloric acid (PCA). The sample was then homogenised on ice and then centrifuged at $4^{o}C$ for 20 mins at 2800G. Ten ul of universal indicator was added to 1ml of supernatant and, using Tris/KOH/KCL, titrated to a pale green colour (pH 7-7.5). The dilution factor (F3) was calculated. After standing for 20 minutes the sample was centrifuged for a further 20 minutes at $4^{o}C$. The supernatant was then decanted and used in the assay. A micro method was adapted for all 3 assays.

(a) ATP Determination

For each mole of ATP 1 mole of NADPH is formed. This product was determined by measurement of extinction at 340 nm. One hundred ul of sample was pipetted into a cuvette and 1400ul of reaction mixture [25ul 100mM glucose, 50ul 1% NADP, 500ul 0.2M Tris buffer (pH 7.5), 50ul 1M MgCl2 and 775u distilled H2O] was added. Ten ul of G6PDH ($1 mgml^{-1}$) was then added and mixed after which the sample was read at 340nm (E1) Then 10ul of hexokinase ($5 mgml^{-1}$) was added mixed and read at 340nm (E2) 5 minutes later. Together with the samples, blanks [H2O and reagent mixture] and, 100ul standards [1mM ATP + Reagent mixture] were compared at 340nm.

E_{ATP} change = $(E1 - E2)_{sample\ standard} - (E1 - E2)_{blank}$

The change in E_{ATP}1mM should be 0.414 at 340nm, if it was not a standard factor was used to correct all readings. Change in $E_{ATP}/0.414$ X F3 = mM ATPgm^{-1} liver.

(b) ADP and AMP determination

The decrease in NADH as measured by the change in extinction at 340nm is proportional to the amount fo AMP and ADP present. Two hundred and fifty microlitre of sample was pipetted into a cuvette and 1250ul of Reaction mixture [300ul TRAM buffer pH7.5, 250ul 0.5MKcl, 50ul 1M MgCl2, 50ul 0.25% NADH, 100ul PEP, 5ul 10mM ATP, 495ul distilled H2O] was added. Ten microlitre of $2.5 mgml^{-1}$ LDH was added to the sample and after mixing extinction was read at 340nm (E1). Then 10ul of pyruvate kinase ($5 mgml^{-1}$) was added and after mixing and standing for 10 minutes another reading at 340nm (E2) was taken. Together with the samples, blanks [H2O and reagent mixture] and, 100ul standards [1mM ADP + Reagent mixture] were read at 340nm.

EADP change = $(E1 - E2)_{sample\ standard} - (E1 - E2)_{blank}$ The change in $E_{ADP}1mM$ for 50ul ADP in 1500ul should be 0.207 at 340nm, if not a standard factor was used to correct all readings.

Change in $E_{ATP}/1.035$ X F3 = mM ADPgm^{-1} liver.

Ten microlitre of 5mgml^{-1} myokinase was then added and mixed for reading at 340nm (E3) The same blank as for ADP was used with 50ul of 1mM AMP added. The change in E_{AMP1mM} for 50ul AMP in 1500ul should be 0.414 at 340nm, if it was not a standard factor was used to correct all readings.

Change in $E_{AMP}/2.07$ x F3 = mM AMPgm^{-1} liver.

The results of the ATP, ADP and AMP analysis were expressed as mMgram^{-1} of dry liver.

B.3.2 ENERGY CHARGE

The energy charge (EC) (Atkinson, 1968) was determined as follows:

$$EC = \frac{ATP + [\frac{1}{2}\ ADP]}{ATP + ADP + AMP}$$

B.4.ALBUMIN, TOTAL PROTEIN AND OSMOLATLITY

B.4.1 ALBUMIN

Method: A standard albumin solution of known concentration was incubated with 0.02ml plasma in bromcresol green in acetate buffer for ten minutes at 20-25^{o}C The absorbance was then determined spectophotometrically at 630nm

Calculation: [Absorbance of test solution/absorbance of standard] x concentration of standard. (Standard Method: Tietz, 1987)

B.4.2 TOTAL PROTEIN

Biuret Method: A standard protein solution of known concentration was incubated with 0.02ml plasma in 2ml biuret reagent for twenty minutes at 20-25^{o}C The absorbance was then determined spectrophotometrically at 540nm

Calculation: [Absorbance of test solution/absorbance of standard] x concentration of standard. (Standard Method: Tietz, 1987)

B.4.3 OSMOLALITY

Method: Osmolality was determined using the method of freezing point depression using a Knauer Electronic Micro-osmometer calibrated in milliosmols kg^{-1}. Samples of 0.15ml were placed in a measuring vessel in contact with the measuring head of the osmometer. Both were supercooled in a cooling chamber and the sample stirred to induce freezing. The freezing point depression relative to H_2O was so determined. The osmolality was read directly from the meter.

B.5 GLUCOSE AND UREA

B.5.1 GLUCOSE

Method: Plasma glucose was determined using an enzymatic colorimetric kit (Boehringer Mannheim, Germany) (Trinder, 1969).

B.5.2 UREA

Method: The standard urea solution and sample were incubated with urease for 45 minutes at 37^{o}C. The sample was then deproteinised and to the supernatant fluid a 1% solution of Iodine and Nesslers reagent were added. The absorbance was determined at 480nm.
Calculation: [Absorbance of test solution/absorbance of standard] x concentration of standard. (Standard Method: Tietz, 1987)

B.6 LACTATE

Samples taken for analysis of lactate concentration were aspirated into 2ml syringes and then exactly one ml was injected into previously prepared vacutainer tubes filled with 2ml perchlorate and kept on ice. The supernatant was stored at -4^{o}C until the time of analysis. All analyses were performed in duplicate using an enzymatic method adapted from Gutman and Wahlefeld, (1974)
Briefly, the principle is based on the spectrophotometric measurement of the formation of NADH as a result of the oxidation of lactate to pyruvate in the presence of lactate dehydrogenase (LDH) and NAD.
Method: 0.1ml of supernatant was added to 1ml hydrazine/glycine buffer (pH 9.0) + 0.1ml NAD + 0.01ml LDH. After mixing this was incubated at 25^{o}C for 1 hour and then the absorbance was read against a reagent blank at 340nm. The change of absorbance so determined was multiplied by the factor 5.47 to determine the lactate concentration in $uMml^{-1}$.

The intra-assay coefficient of variation determined at 4.9uMml^{-1} was 1.5% (n=7) The inter-assay coefficient of variation determined at 0.84uMml^{-1} was 1.9% (n=10)

The coefficient of variation determined at three different concentrations was:

0.6% at 0.97uMml^{-1}

3.4% at 2.05uMml^{-1}

0.8% at 4.01uMml^{-1} (n=5).

To convert uMml^{-1} to mg100ml^{-1} multiply by 9.008.

B.7 SODIUM AND POTASSIUM

Sodium and potassium were measured using a KNAI Sodium/Potassium analyser (Radiometer, Copenhagen) which was fully calibrated daily (Cal 2) and recalibrated every 2 hours (Cal 1).

B.8 BLOOD OXYGEN SATURATION AND CONTENT

Samples were taken as in B.1 above and analysis of oxygen saturation was performed (in duplicate for hypoxic IPPL samples). Oxygen saturation was determined using the OSM3 Cooximeter (Radiometer, Copenhagen) set in the animal 3 mode for pig blood. Oxygen consumption was calculated according to the method of Selkurt and Brecher, (1956) but several normal values for pig blood were assumed to be similar to those accepted for human blood (Hickman, 1972) viz. the oxygen carrying capacity of haemoglobin- 1.34ml oxygen/gram haemoglobin, and oxygen solubility in plasma 0.0225 x $Pa0_2$ Kpa (Leigh, 1982). Haemoglobin was determined using standard methods. The final calculation was as follows:

Oxygen carried by haemoglobin:

(Portal vein O_2% saturation x flow min^{-1} plus hepatic artery O_2% saturation x flow min^{-1} minus hepatic vein O2% saturation x flow min^{-1}) x haemoglobin gram 100ml^{-1} x 1.34 **plus**

Oxygen dissolved in plasma :

(Portal vein PO_2 x flow min^{-1} + hepatic artery PO_2 x flow min^{-1} minus hepatic vein PO_2 x flow min^{-1}) x 0.0225.

The final result was expressed as millilitres of oxygen consumed per 100 gram of wet liver.

B.9 HAEMATOCRIT, HAEMOGLOBIN AND HEMOLYSIS

B.9.1 HAEMATOCRIT

The haematocrit of blood and perfusate samples were determined in triplicate. Blood was run into a haematocrit tube (75mm length with internal diameter of 1mm) until the tube was 3/4 full and then heat sealed at one end. Haematocrit tubes were centrifuged (BHG centrifuge) at 12000G for 5 minutes after which the packed cell volume was read on a haematocrit reader and expressed as a percentage.

B.9.2 HAEMOGLOBIN

Blood haemoglobin was measured using a standard method (Dacie and Lewis, 1975).

B.9.3 HAEMOLYSIS

Method: An aliquot of one quarter dilution of a sample was mixed with 10% sodium hydroxide and the optical densities were read at 580 and 560nm against a distilled water blank. The value for the ratio of the respective optical densities was then determined and the concentration of hemolysed haemoglobin read off a standard chart. This result was multiplied by the appropriate dilutional factor (Hunter, 1950).

B.10 PERFUSATE SPECIFIC GRAVITY

A sample of standard perfusate (1600ml of fresh heparinised pig blood + 600 ml of plasmalyte B) was taken.

Using a pipette 500ul of this was collected and weighed using an optical balance (Sartorius Zeis, West Germany)

This was repeated 4 times.

Weight of 500ul of perfusate = 0.5325g

0.5296g

0.5304g

0.5338g

mean= 0.5315g

The specific gravity was thus $1.063gml^{-1}$

APPENDIX C: LIGNOCAINE AND METABOLITE ANALYSIS

C.1 LIGNOCAINE AND METABOLITE STANDARD CURVE DATA C.2

C.2 DETAILED STATISTICAL ANALYSIS OF STANDARD CURVES FOR LIGNOCAINE, MEGX AND GX FOR HPLC METHOD: 2. ..... C.3

C.2.1 Lignocaine C.3
C.2.2 Monoethylglycinexylidide C.4
C.2.3 Glycinexylidide C.5
C.2.4 Daily Check of Calibration C.5

APPENDIX C: LIGNOCAINE AND METABOLITE ANALYSIS

C.1 LIGNOCAINE AND METABOLITE STANDARD CURVE DATA

This calibration data for lignocaine and its metabolite analysis was generated on consecutive days and used for the statistical analysis done to determine the precision (cv) of this determination. Data designated Curve 1-4 (below) is that for data determined on a specific day. (phr= peak height ratio).

Table C.1

Lignocaine Standard Curves (Method 1)

Lignocaine Concentration.	Curve:1 phr	Curve:2 phr	Curve:3 phr	Curve:4 phr
5.125	10.457	10.581	9.845	9.334
2.562	4.887	3.994	4.426	4.384
1.281	2.334	2.089	2.218	1.989
0.641	1.106	1.024	1.059	1.023
0.320	0.536	0.507	0.507	0.436
0.160	0.266	0.181	0.214	0.232
0.080	0.118	0.098	0.125	0.108
0.040	0.051	0.046	0.055	0.055

Table C.2

Lignocaine Standard Curves (Method 2)

Lignocaine Concentration	Curve:1 phr	Curve:2 phr	Curve:3 phr
5.0	2.645	3.190	2.959
2.5	1.337	1.561	1.580
1.25	0.647	0.638	0.698
0.625	0.301	0.360	0.296
0.312	0.140	0.159	0.158
0.156	0.065	0.686	0.069

Table C.3

Monoethylglycinexylidide Standard Curves

MEGX Concentration	Curve:1 Phr	Curve:2 Phr	Curve:3 Phr
2.64	1.571	1.643	1.785
1.32	0.718	0.847	0.934
0.661	0.314	0.357	0.394
0.331	0.223	0.260	0.182
0.165	0.075	0.093	0.089
0.082	0.048	0.053	0.036

Table C.4

Glycinexylidide Standard Curves

GX Concentration	Curve:1 Phr	Curve:2 Phr	Curve:3 Phr
0.578	0.892	1.003	1.023
0.289	0.435	0.525	0.517
0.145	0.211	0.229	0.221
0.072	0.083	0.138	0.121
0.031	0.045	0.063	0.056
0.016	0.024	0.036	0.027

phr= peak height ratio

C.2 DETAILED STATISTICAL ANALYSIS OF STANDARD CURVES FOR LIGNOCAINE, MEGX AND GX FOR HPLC METHOD: 2

C.2.1 LIGNOCAINE[1]

As discussed in section 3.3.3 in order to use a calibration line for the determination of lignocaine or metabolite concentration in a plasma sample a straight line relationship must exist between the concentration of lignocaine (C) and the peak height ratio (R) of lignocaine/internal standard. To achieve a straight line relationship for the present system a natural logarithm transformation was required. Figure C.1 shows that Y = lnR vs X = ln C gives a straight line with a slope close to one.

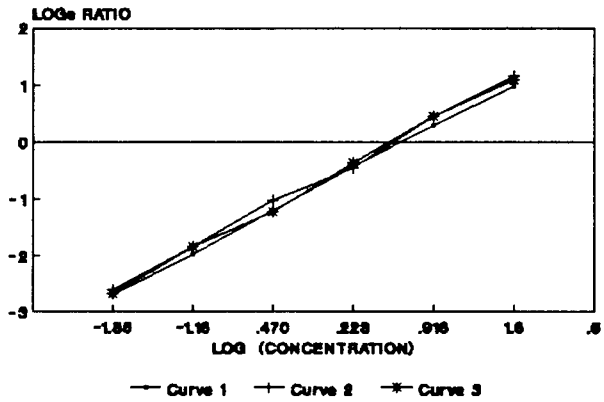

Figure C.1 Lignocaine standard curves: method 2.

Table C.5 below summarizes the results of fitting the model $y = \alpha + \beta$ (X - mean X) + error.

Test of homogeneity of the slopes and intercepts showed that the slopes can be accepted as homogeneous: $F_{2,12} = 0.34$. The mean of the three estimated slopes is 1.0875. There is significant

1 Statistical analysis performed by Professor S Maritz.

variation between the intercepts; $F_{2,14}$ = 6.13, $p<0.05$. These results are in good agreement with those found for lignocaine using method 1.

Table C.5

Lignocaine Standardization Data

Intercept=a	s.e.(a)	slope=b	s.e.(b)	error variance
-0.8440	0.0141	1.0738	0.0119	0.001195
-0.7301	0.0305	1.0975	0.0256	0.005579
-0.7598	0.0274	1.0911	0.0232	0.004521

The estimated coefficient of variation is 6.1-7% over the calibration range determined from: $var(X_{hat}) \approx 0.003184 + 0.000530 + (X+0.1239)^2 (0.000379)$.

C.2.2 MONOETHYLGLYCINEXYLIDIDE

Figure C.2 shows that Y = lnR vs X = ln C gives a straight line with a slope close to one.

Test for homogeneity of slopes: $F_{2,14}$ = 2.60; $p>0.05$.

Mean slope 1.0438. Test for homogeneity of intercepts: $F_{2,14}$ = 1.24; $p>0.10$. The coefficient of variation is 13.1%-13.9% over the calibration range.

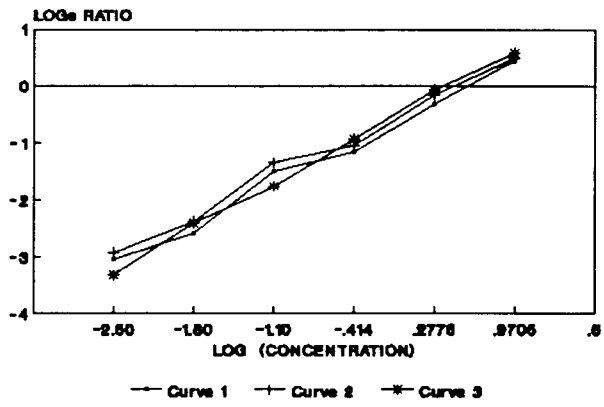

Figure C.2 Monoethylglycinexylidide standard curves: method 2.

Table C.6

Monoethylglycinexylidide Standardization Data

Intercept=a	s.e.(a)	slope=b	s.e.(b)	error variance
-1.3487	0.0613	1.0165	0.0518	0.022571
-1.2238	0.0600	0.9911	0.0507	0.021636
-1.3084	0.0255	1.1238	0.0215	0.003898

C.2.3 GLYCINEXYLIDIDE

Figure C.3 shows that Y = lnR vs X = ln C gives a straight line with a slope close to one. Test for homogeneity of slopes: $F_{2,14} = 3.78$; $p>0.05$. Mean slope 1.0260. Test for homogeneity of intercepts: $F_{2,14} = 14.85$ $p<0.01$. The coefficient of variation is 15.3-15.9% over the calibration range.

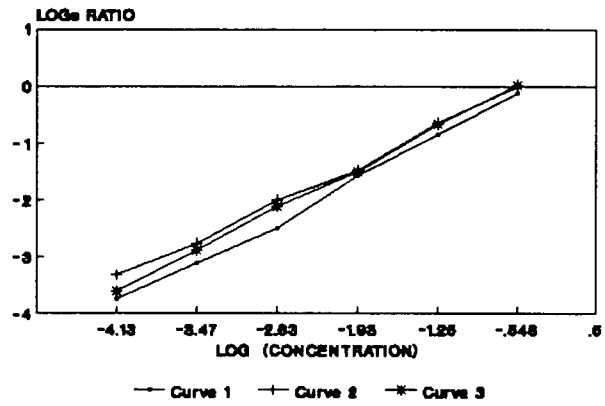

Figure C.3. Glycinexylidide standard curves: method 2.

Table C.7

Glycinexylidide Standardization Data

Intercept=a	s.e.(a)	slope=b	s.e.(b)	error variance
-1.967	0.0384	1.0620	0.0324	0.008855
-1.6950	0.0299	0.9690	0.0253	0.005370
-1.7898	0.0200	1.0470	0.0169	0.002397

C.2.4 DAILY CHECK OF CALIBRATION

The value of ln (ratio) obtained for a concentration near the center of the calibration range should fall in an interval:

$a + bX \pm 2(s^2 + s^2_\alpha)^{\frac{1}{2}}$ where s^2 is the error variance, s^2_α = the estimated variance of the true intercepts. Table C.7 summarizes the relevant numbers of the three data sets.

Table C.7

Drug Concentration Intervals for Daily Check of Calibration.

	s^2	s^2_α	$2(s^2 + s^2_\alpha)^{\frac{1}{2}}$
Lignocaine	0.003765	0.002864	0.16
MEGX	0.005541	0.009922	0.25
GX	0.016035	0.01390	0.26

C.2.5 ANCILLARY APPARATUS USED FOR LIGNOCAINE ANALYSIS

(a) pH meter: Orion Research Model 601A digital ionanalyser (Massachussets, USA)

(b) Optical Balance: Sartorius Zeiss (West Germany)

(c) Whirlimix: Hook and Tucker Ltd

APPENDIX D: PHARMACOKINETIC ANALYSIS OF LIGNOCAINE DECAY + PCNONLIN MODELS

D.1 METHOD OF RESIDUALS FOR INITIAL VALUE DETERMINATION D.2

D.2 DESCRIPTION OF PCNONLIN MODELS USED IN ANALYSIS OF PHARMACOKINETIC DATA D.3

D.3 COMPARTMENTAL ANALYSIS USING "GOODNESS OF FIT" PLOTS FOR MEAN LIGNOCAINE DECAY DATA IN VIVO D.6

D.4 ANALYSIS OF "GOODNESS OF FIT" IN 5 INDIVIDUAL STUDIES IN VIVO D.10

D.5 DERIVED PHARMACOKINETIC PARAMETERS FROM A BOLUS DOSE OF LIGNOCAINE IN 5 PIGS D.11

D.6 DETAILED PHARMACOKINETIC ANALYSIS OF LIGNOCAINE DECAY IN THE ISOLATED PERFUSED PIG LIVER D.12

- D.6.1 Choice of PCNONLIN Model D.12
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D.7 APPLICATION OF PHARMACOKINETIC PARAMETERS DETERMINED FOR THE PIG TO ACHIEVE CONSTANT CONCENTRATIONS USING AN EXPONENTIALLY DECLINING INFUSION D.20

APPENDIX D: PHARMACOKINETIC ANALYSIS OF LIGNOCAINE DECAY + PCNONLIN MODELS

D.1 METHOD OF RESIDUALS FOR INITIAL VALUE DETERMINATION

In order to run PCNONLIN (an iterative nonlinear pharmacokinetic computer program) certain "Initial Values" i.e. parameter estimates are required to start the iterative process. These can be taken from the literature where appropriate or alternatively by the process of "curve stripping" or application of the method of residuals (Gibaldi and Perrier, 1975).

The decay data is plotted on a semilogarithmic concentration versus time plot and using the method of residuals the curve can be resolved into its exponential components. Assuming a two compartment model the curve can be described as:

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

where α and β are the apparent first order fast and slow phase hybrid disposition rate constants respectively, and A and B are the corresponding zero time intercepts.

Since α is larger than β by definition the term $Ae^{-\alpha t}$ will approach zero more rapidly than will the term $Be^{-\beta t}$ and will then reduce to $C = Be^{-\beta t}$ which in terms of common logarithms is

$$\text{Log } C = \text{Log } B - \beta t/2.303.$$

This terminal linear phase of the curve resulting from a plot of the logarithm of plasma concentration versus time has a slope of $-\beta/2.303$ and when extrapolated to zero yields an intercept of log B. Thus both B and β can be determined by graphical analysis.

A and α in turn can be determined (graphically) by the method of residuals in the following fashion. The terminal linear phase of the log concentration time curve is back extrapolated to zero. By subtraction of the concentration-time values on the extrapolated line from the corresponding true concentration-time values, a series of residual concentration time values can be obtained. A line bisecting these graphically determined points will approximate the residual curve described by:

$$C = Ae^{-\alpha t}$$

and thus A and α can be determined as described above.

A similar method can be adopted if one postulates a 3 compartment model described by the equation:

$$C = Pe^{-\tau t} + Ae^{-\alpha t} + Be^{-\beta t}$$ (Gibaldi and Perrier,1975).

D.2 DESCRIPTION OF PCNONLIN MODELS USED IN ANALYSIS OF PHARMACOKINETIC DATA.

MODEL 1. One compartment with bolus input and first-order output.

$$C(T) = D/VxEXP(-K10xT)$$

Estimated parameters:
(1) V = Volume
(2) K10 - elimination rate

Secondary parameters:
(1) AUC = D/V/K10
(2) K10 half-life
(3) CMAX = D/V

MODEL 2. One compartment with constant IV input and first-order output.

$$C(T) = (D/TI)/V/K10x(EXP(-K10xT^*) - EXP(-K10xT))$$

where $T^* = T - TI$ for $T > TI$
and $T^* = 0$ for $T < TI$

Estimated parameters:
(1) V = Volume
(2) K10 = Elimination rate

Secondary parameters:
(1) AUC = D/V/K10
(2) K10 half-life
(3) CMAX = C(TI)

MODEL 7. Two compartment with bolus input and first-order output; micro-constants as primary parameters.

$$C(T) = AxEXP(-ALPHAxT) + BxEXP(-BETAxT)$$

where A = D/Vx(ALPHA - K21)/(ALPHA - BETA),
B = -D/Vx(BETA - K21)/(ALPHA - BETA),

and ALPHA and BETA (ALPHA>BETA) are positive and negative roots of the quadratic equation
$(rxr + (K12 + K21 + K10)xr + K21xK10 = 0)$.

Estimated parameters:
(1) V = Volume
(2) K10 = elimination rate
(3) K12 = transfer rate, 1 to 2
(4) K21 = transfer rate, 2 to 1

Secondary parameters:
(1) AUC = D/V/K10
(2) K10 half-life
(3) ALPHA
(4) BETA
(5) ALPHA half-life

(6) BETA half-life
(7) A
(8) B
(9) CMAX = D/V

MODEL 8. Two-compartment with bolus input and first-order output; macro-constants as primary parameters.

$$C(T) = AxEXP(-ALPHAxT) + BxEXP(-BETAxT)$$

Estimated parameters:
(1) A
(2) B
(3) alpha
(4) beta

Secondary parameters:
(1) AUC = A/alpha + B/beta
(2) K10 half-life
(3) ALPHA half-life
(4) BETA half-life
(5) K10
(6) K12
(7) K21
(8) volume
(9) CMAX = D/V

MODEL 9. Two compartment with constant IV input and First-order output; micro-constants as primary parameters.

$$C(T) = Ax(EXP(-ALPHAxT) - EXP(-ALPHAxT^*)) + Bx(EXP(-BETAxT) - EXP(-BETAxT^*))$$

where $T^* = T - TI$ for $T > TI$
and $T^* = O$ for $T <= TI$

$A = (D/TI)x(K21 - ALPHA)/V/ALPHA - BETA)/ALPHA,$
$B = -(D/TI)x(K21 - BETA)/V/(ALPHA - BETA)/BETA,$

and ALPHA and BETA (ALPHA > BETA) are positive and negative roots of the quadratic.
$rxr + (K12 + K21 + K10)xr + K21xK10 = 0$

Estimated parameters:
(1) V = Volume
(2) K10 = elimination rate
(3) K12 = transfer rate, 1 to 2
(4) K21 = transfer rate, 2 to 1

Secondary parameters:
(1) AUC = D/V/K10
(2) K10 half-life
(3) ALPHA
(4) BETA
(5) ALPHA half-life

(6) BETA half-life
(7) CMAX = C(TI)
(8) A
(9) B

(NOTE: A, B are the zero time intercepts following an IV injection)

MODEL 11. Two compartment with first-order input, first-order output, no lag time and micro-constants as primary parameters.

$$C(T) = AxEXP(-ALPHAxT) + BxEXP(-BETAxT) + CxEXP(-KO1xT).$$

$$A = D/VxKO1x(K21 - ALPHA)/(ALPHA - BETA)/(ALPHA - KO1).$$

$$B = D/VxKO1x(K21 - BETA)/(ALPHA - BETA)/(BETA - KO1).$$

and ALPHA and BETA (ALPHA>BETA) are positive and negative roots of the quadratic $rxr (K12 + K21 + K10)xr + K21xK10 = 0$.

Estimated parameters:

(1) V = Volume
(2) KO1 = absorption rate
(3) K10 = elimination rate
(4) K12 = transfer rate, 1 to 2
(5) K21 = transfer rate, 2 to 1

Secondary parameters:

(1) AUC = D/V/K10
(2) K10 half-life
(3) K01 half-life
(4) ALPHA
(5) BETA
(6) alpha half-life
(7) beta half-life
(8) A
(9) B
(10) Tmax*
(11) Cmax*

* Estimated for the compiled (internal) library only.

MODEL 18. Three-compartment with bolus input, first-order output and macro-constants as primary parameters.

$$C(T) = AxEXP(-ALPHAxT) + BxEXP(-BETAxT) + CxEXP(-GAMMAxT)$$

Estimated parameters:	(1)	A
	(2)	B
	(3)	C
	(4)	Alpha
	(5)	Beta
	(6)	Gamma
Secondary parameters:	(1)	Cmax
	(2)	Volume
	(3)	K21
	(4)	K31
	(5)	K10
	(6)	K12
	(7)	K13
	(8)	K10 half-life
	(9)	Alpha half-life
	(10)	Beta half-life
	(11)	Gamma half-life

D.3 COMPARTMENTAL ANALYSIS USING "GOODNESS OF FIT" PLOTS FOR MEAN LIGNOCAINE DECAY DATA IN VIVO.

Plots D.1, D.2, and D.3 (below) are plots generated by the PCNONLIN nonlinear regression computer program used to visually assess the "goodness of fit" of the three different compartmental models under consideration for the simultaneous analysis of the pooled in vivo decay data. These plots correspond to those illustrated in Table 4.2 and discussed in section 4.4.3.B.

Figure D.1 (Plot 1) Plots generated by PCNONLIN for observed Y vs weighted calculated Y comparing the fit of a one (top), two (middle) or three compartment model.

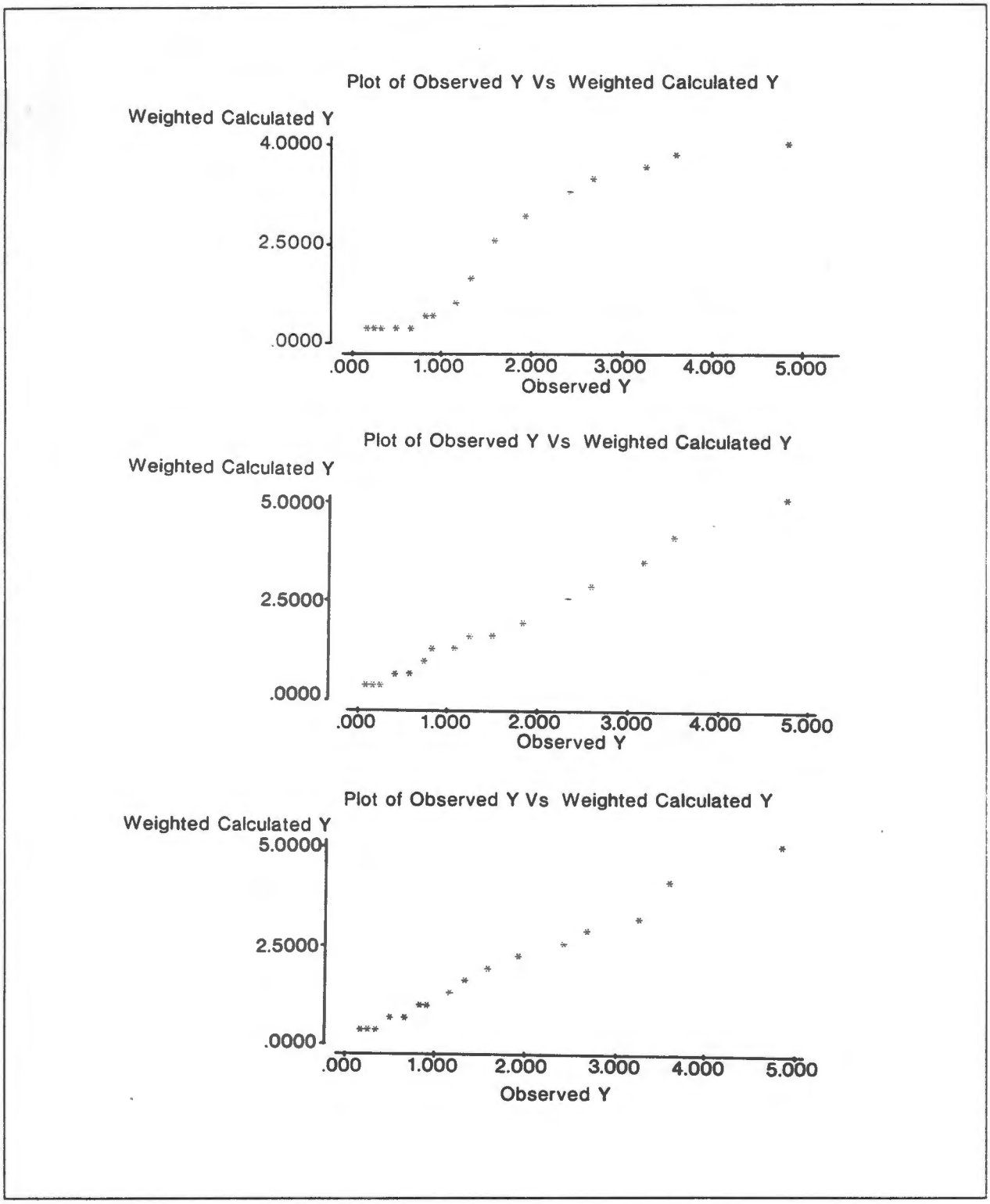


Figure D.2 (Plot 2) Plots generated by PCNONLIN for weighted calculated Y vs weighted residual comparing the fit of a one (top), two (middle) or three compartment model.

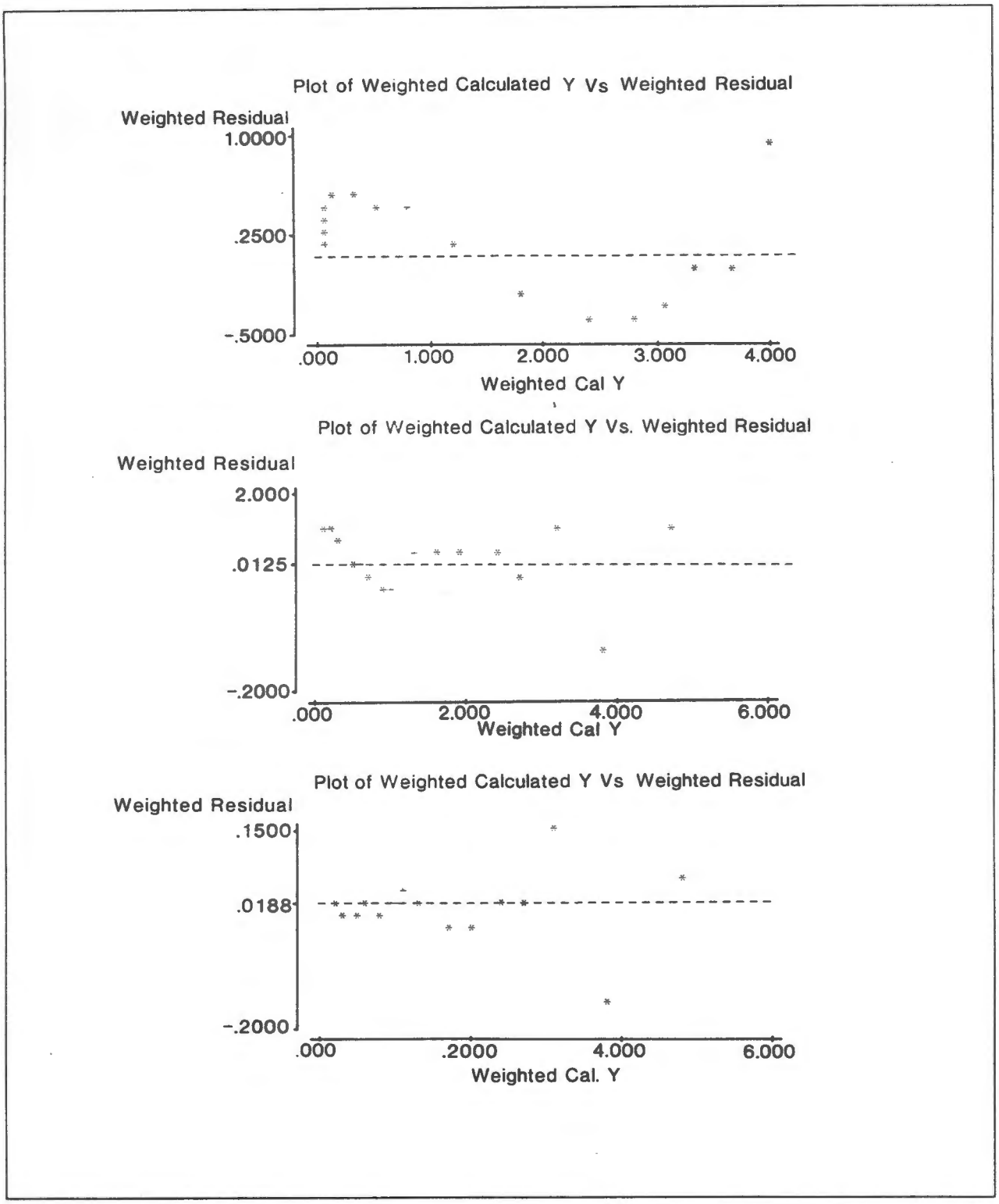
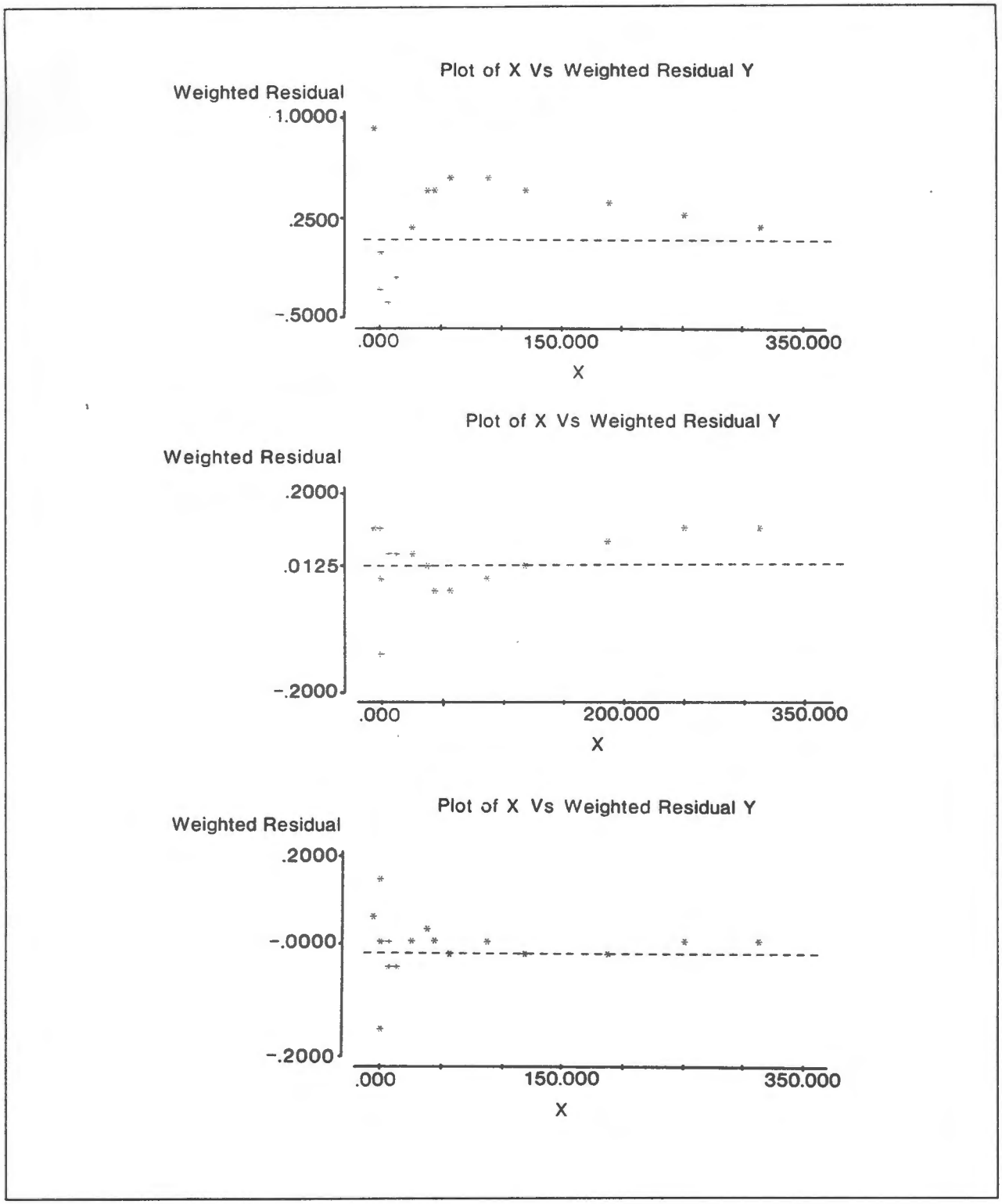


Figure D.3 (Plot 3) Plots generated by PCNONLIN for x vs weighted residual Y comparing the fit of a one (top), two (middle) or three compartment model.



D.4 ANALYSIS OF "GOODNESS OF FIT" IN 5 INDIVIDUAL STUDIES (J,L,N,P,Q) IN VIVO

Table D.1

"Goodness of fit" of Lignocaine Decay Data In Vivo to a Two Compartment Model in Five Studies

Study:	J	L	N	P	Q
Corrected sum of Squares	22.48	24.10	25.52	33.65	40.86
AIC	-27.0	-7.8	-40.8	-34.4	-18.5
Sum of Squared Residuals	0.1119	0.3723	0.047	0.072	0.1901
Correlation Coefficient	.998	.992	.999	.999	.998
Degrees of Freedom	12	12	12	12	12
Plots:					
1	L++	L++	L++	L++	L++
2	R+	R++	R++	R++	R+
3	SD	R+	SD	R++	R+

L = Linear. NL = Non linear. R = Random scatter. SD = systemic deviation. Grading: ± + or + +.
Plot 1 = Plot of observed Y versus weighted calculated Y.
Plot 2 = Plot of weighted calculated Y versus weighted residual.
Plot 3 = Plot of X versus weighted residual.
AIC = Akaike Information Criteria (Akaike, 1974).

D.5 DERIVED PHARMACOKINETIC PARAMETERS FROM A BOLUS DOSE OF LIGNOCAINE IN 5 PIGS

Table D.2

Derived Pharmacokinetic Parameters from a Bolus Dose of Lignocaine in 5 Pigs (Studies J, L, N, P, Q)

STUDY	J	L	N	P	Q	Mean	SEM
V1 (Lkg^{-1})	0.608	0.932	0.493	0.609	0.549	0.638	0.076
V2 (Lkg^{-1})	1.126	1.068	1.646	1.73	2.005	1.515	0.180
Vdarea (Lkg^{-1})	1.732	2.000	2.139	2.339	2.550	2.152	0.140
K10 (min^{-1})	0.0479±0.004	0.0183±0.0024	0.0693±0.0042	0.0353±0.002	0.0391±0.009	0.0419	0.008
K12 (min^{-1})	0.1070±0.015	0.0375±0.0116	0.1369±0.0092	0.0887±0.006	0.0877±0.0008	0.9156	0.016
K21 (min^{-1})	0.0721±0.0115	0.0483±0.0169	0.0596±0.0054	0.0468±0.004	0.0378±0.0058	0.0529	0.005
AUC ($ugml^{-1}$ min)	137.0	238.8	115.03	184.4	182.5	171.4	21.4
K10 half-life (min)	14.45±1.33	37.80±4.98	9.99±0.614	19.60±1.22	17.69±1.82	19.90	4.76
Alpha (min^{-1})	0.2106±0.0269	0.0948±0.0271	0.2493±0.0153	0.160±0.10	0.1551±0.0131	0.1786	0.029
Beta (min^{-1})	0.0164±0.0021	0.0093±0.0020	0.0165± 0.0020	0.0103±0.0010	0.0095±0.0017	0.0124	0.0016
$t_{\frac{1}{2}}\alpha$ (min)	3.29±0.42	7.306±0.209	2.77±0.171	4.31±0.276	4.46±0.379	4.42	0.78
$t_{\frac{1}{2}}\beta$ (min)	42.22±5.65	74.15±15.95	41.76±4.07	67.21±6.74	72.63±13.06	59.59	7.27
Cmax ($ugml^{-1}$)	5.52±0.248	4.49±0.240	6.77±0.27	6.44±0.151	7.125±0.237	6.06	0.47
A ($ugml^{-1}$)	4.56±0.3250	2.58±0.343	6.70±0.27	5.49±0.192	6.48±0.30	5.16	0.74
B ($ugml^{-1}$)	1.8339±0.1783	2.165±0.3400	1.52±0.10	1.76±0.109	1.56±0.169	1.76	0.114
A'($ugml^{-1}$)	5.56	2.81	8.44	6.37	7.52	6.14	0.96
B'($ugml^{-1}$)	1.868	2.184	1.544	1.776	1.574	1.789	0.115

V1 = volume of distribution of central compartment, V2 = Volume of peripheral compartment, Vdarea = dose /β(AUC), K10,K12, and K21 are first order constants (See section 4.1.4). AUC = Area under concentration time curved. $t_{\frac{1}{2}}$ = the half life, α and β are the slopes of the distribution and elimination curves respectively while A and B a their coefficients, A' and B' are recalculated to compensate for the two minute infusion period (see section 4.4.6.A.) Cn = peak concentration. J,L,N,P,Q presented as study values with 95% confidence limits.

D.6 DETAILED PHARMACOKINETIC ANALYSIS OF LIGNOCAINE DECAY IN THE ISOLATED PERFUSED PIG LIVER

The individual decay curves of lignocaine (studies K,M,O) are depicted in the top half of the composite Figure D.4 showing that:

(a) there was evidence of a wash in curve and

(b) there was evidence of recirculation as there was a slight rise in the decay curve in all three studies.

Initial graphical analysis and modelling to see whether this data fitted a one or two compartmental model was performed in the absence of these wash in curves to simplify the analysis.

Semilogarithmic plots of concentration versus time in the studies K and O and perhaps M, [Figure D.4 (bottom half)] could suggest that the decay curves may be described as being biexponential in nature.

D.6.1 CHOICE OF PCNONLIN MODEL

As explained earlier certain initial values were required by PCNONLIN to begin the pharmacokinetic analysis of data. For Model 8[1] these are A, B, a and B. This model was chosen in the first instance as these initial values could readily be determined by graphical analyis as explained above (section D.1). This modelling would then allow the determination of the necessary parameters needed as initial values for the use of model 7 (Two compartment with bolus input and first order output; microconstants as primary parameters). Model 7 would allow the determination of the parameters; Volume of distribution (V1), elimination rate constant K10, and β that are necessary to determine the Loading dose and infusion rate to achieve a constant concentration in a two compartmental model [Mitenko and Ogilvie, (1972)].

D.6.2 RESULTS AND DISCUSSION OF PHARMACOKINETIC MODELLING

Using model 8 convergence was achieved with the study K data and so the initial parameters for Model 7 were determined. The data was fitted to model 7 without weighting as weighting with $1/Y$ of $1/Y^2$ did not improve the fit. Having established that the decay data could be fitted to a two compartment model but bearing in mind that one should use the most parsimonius model (Boxenbaum et al, 1974) that is the model with the lowest number of compartments (Berman, 1966) the decay data was also fitted to Model 1 (one compartment with bolus input and first order output). Again weighting did not improve the fit of this model. The appropriateness of the fit of these two models was assessed and is summarised in table D.3

1 All models mentioned here are to be found in section D.2 of this appendix

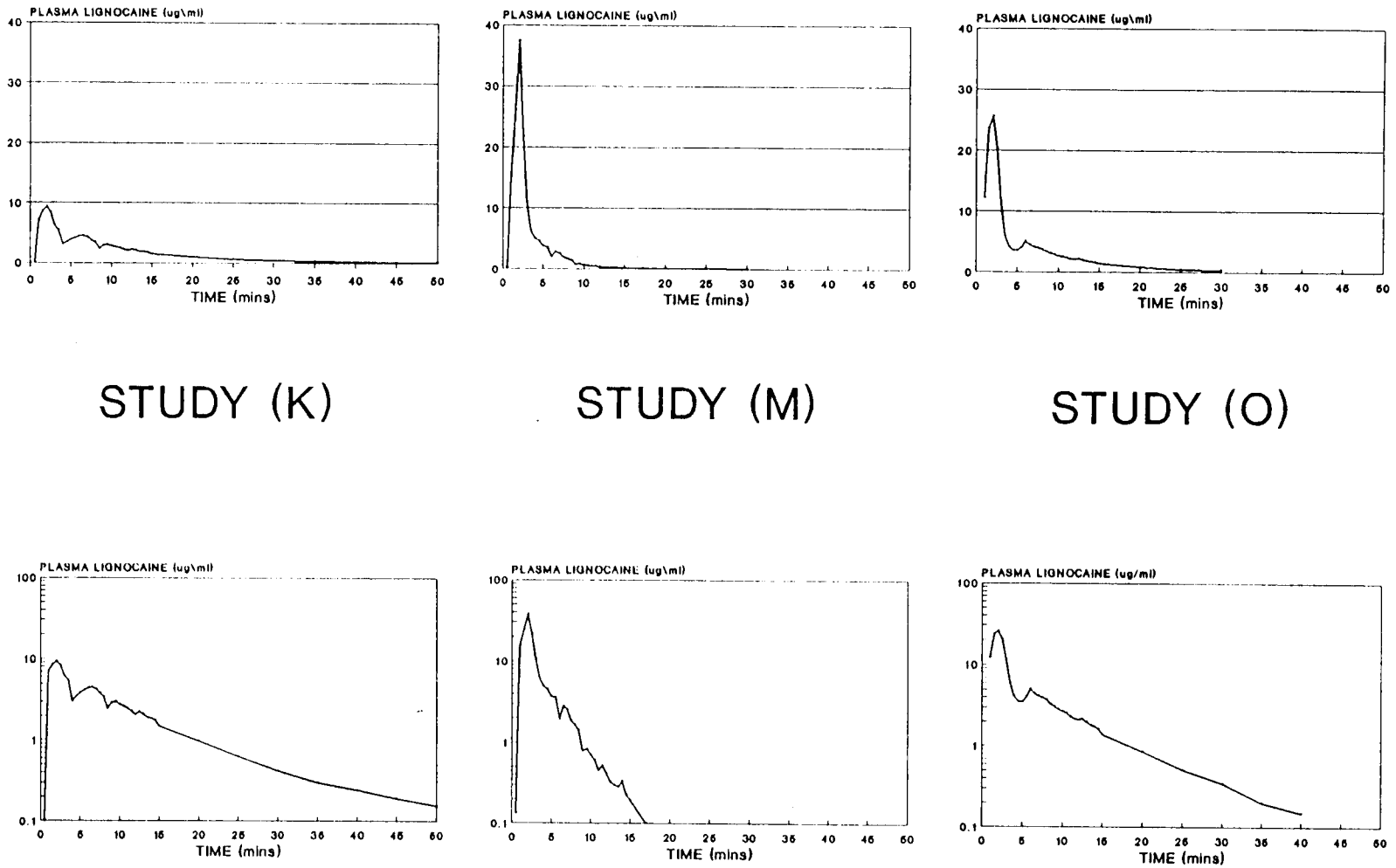


Figure D.4 Plasma lignocaine decay curves for individual isolated perfused pig liver studies plotted on arithmetic (top) and logarithmic (bottom) scales

Table D.3

Comparison of "Goodness of Fit" of Lignocaine Decay in the Isolated Perfused Pig Liver to a One and Two Compartment Model in Three Studies

	Study M		
Criteria of fit	One compartment (Model 1)	Two compartment (Model 7)	F Test
AIC	142	89	
Corrected SS	1800	1809	ns
SS residuals	47	8.1	P<.001
Correlation Coefficient	0.99	0.98	
Degrees of freedom	38	34	
	Study K		
Criteria of fit	One compartment	Two compartment	F Test
AIC	127	87	
Corrected SS	196	178	ns
SS Residuals	20.47	8.2	P<.001
Correlation Coefficient	0.948	0.977	
Degrees of freedom	39	37	
	Study O		
Criteria of fit	One compartment	Two compartment	F Test
AIC	194	141	
Corrected SS	985	996	ns
SS Residuals	151	30.5	P<0.001
Correlation Coefficient	0.949	0.985	
Degrees of freedom	36	35	

SS = Sum of squares, AIC = Akaike Information Criteria. (Akaike, 1974)

From this table it can be seen that the data better fitted a 2 compartment model when compared with a one compartment model as:

(a) the AIC was consistently lower (Akaike's information criteria) (Akaike, 1974).

(b) The correlation coefficient, although not as good an indicator of fit as in linear regression was stronger in study K and O.

(c) the sum of squared residuals was consistently lower in the two compartmental model and when submitted to an F test there was a highly significant decrease in this justifying the use of a more complex model (Boxenbaum et al, 1974)(Berman, 1966).

The initial graphical analysis on a semilogarithmic plot also tended to support a two compartmental model as did a graphical analysis of the residuals which highlighted the importance of this aspect of

pharmacokinetic analysis as stressed by Boxenbaum et al, (1974) and Berman (1966). Table D.4 gives an indication of the goodness of fit of the two compartmental models when the data was plotted as indicated.

Table D.4

Visual Assessment of "Goodness of Fit" plots comparing one and two compartment models in the isolated perfused pig liver in three studies

	Study M		Study K		Study O	
Plot	**Model**		**Model**		**Model**	
	1	7	1	7	1	7
(1)	L+	L+	NL+	L+	NL++	NL+
(2)	SD+	R	SD+	R+	SD++	SD+
(3)	SD+	R	SD+	SD	SD++	SD+

L = Linear. NL = Non linear. R = Random scatter. SD = systemic deviation. Grading: ± + or + +.

Plot 1 = Plot of observed Y versus weighted calculated Y.
Plot 2 = Plot of weighted calculated versus weighted residual.
Plot 3 = Plot of X versus weighted residual.
AIC = Akaike Information Criteria (Akaike, 1974).

There was more evidence of systematic deviation when the data was subjected to a one compartment fit. It should be stressed though that the visual comparison yielded an improvement in fit of the two compartment model with respect to a one compartment model but that the two compartment model was only a better appproximation of the true model of lignocaine decay in the isolated perfused pig liver. Table D.4 indicates that the fit of model 7 still leaves much to be desired as systematic deviations in two of the three studies were evident. This may have been due to the recirculation effect on the shape of the lignocaine decay curve. For the purposes of this study it was thus accepted that the lignocaine decay could be approximated by a two compartment model although a more complex model might yield a better fit.

D.6.3 ANALYSIS OF LIGNOCAINE WASH IN AND DECAY DATA

The analysis of the lignocaine decay data above had not taken account of the initial wash in phase. As one of the tenets of pharmacokinetic analysis is that all relevant data should be included in the analysis (Daniel and Wood, 1971) it was postulated that this could be modelled by regarding this phase as a first order input. Thus an attempt was made to fit all the data points generated in the three studies to Model 11 (Two compartment with first order input, first order output, no lag time and microconstants as primary

parameters). Convergence was achieved in only one of the studies (study K) but was inconsistent and ill-conditioned and this was not improved by weighting.

Model 9 (two compartment with constant iv input and first-order output) was then tried using the same initial values determined before for study K and with the assumption that the constant iv input was over 2 minutes. This resulted in a reasonable fit of the data. Weighting did not improve this.

As all the data (ie both wash in and decay) had now been used to fit a two compartment model it had to be established whether this model remained superior to a similar model with only one compartment i.e. PCNONLIN Model 2 (one compartment with constant iv input and first order output). Table D.5 compares these two models while table D.6 compares the visual assessment of the plots of goodness of fit.

Table D.5

Comparison of "Goodness of Fit" of Lignocaine Decay in the Isolated Perfused Pig Liver in Three Studies. PCNONLIN Model 2 vs Model 9

	Study M		
Criteria of fit	**One compartment (Model 2)**	**Two compartment (Model 9)**	**F Test**
AIC	238	241	
Corr SS	2431	2431	ns
SS Residuals	262	257	ns
Correlation	0.944	0.946	
Degrees of freedom	40	38	
	Study K		
Criteria of fit	**One compartment**	**Two compartment**	**F Test**
AIC	155	142	
Corr SS	239	260	ns
SS Residuals	40	21.45	P<0.001
Correlation	0.915	0.958	
Degrees of freedom	39	40	
	Study O		
Criteria of fit	**One compartment**	**Two compartment**	**F Test**
AIC	245	225	
Corr SS	1505	1490	ns
SS Residuals	244	159	P<0.001
Correlation	0.924	0.946	
Degrees of freedom	42	39	

AIC = Akaike Information Criteria, SS = sum of squares.

Table D.6

Visual Assessment of "Goodness of Fit" in the Isolated Perfused Pig Liver. PCNONLIN Model 2 vs Model 9.

	Model M		Model K		Model O	
	2	9	2	9	2	9
(1)	NL+	NL+	NL++	L+	NL++	NL+
(2)	SD	SD+	SD++	SD	SD++	SD+
(3)	SD	SD	SD	SD	SD++	SD+

Plot (1) = Plot of observed Y vs weighted calculated Y.
(2) = Plot of weighted calculated Y vs weighted residual.
(3) = Plot of x vs weighted residual.

Key: L = linear
NL = non linear
R = random scatter
SD = systemic deviation
graded with + or + or + +

Here again it should be noted that the analysis suggests that the two compartment model offers a better fit. In studies K and O the AIC was lower as was the sum of squared residuals and the difference was found to be highly significant when subjected to the F test. The correlation coefficient was also generally better in the two compartment model. The visual analysis of the generated plots (see table D.6) supported this only marginally in that study K was linear in plot (1)(plot of observed Y versus weighted calculated Y). The fit of all the data to model 9 is however poorer than the fit described earlier of just the decay data to model 7. This highlights further the problem stressed earlier (Section 4.4.4.A) that the disposition of lignocaine in this preparation is complex.

Although convergence was achieved in both models the corrected sum of squares as well as the weighted residuals and AIC values were high suggesting that the data might be interpreted better by a more complex model. However the possibility had not been investigated whether a better fit could be achieved with all the data ie (wash in and decay data) if fitted to Model 7 (two compartment with bolus input and first order output) and Model 1 (one compartment with bolus input and first order output). When an attempt was made to fit this data to Model 7 convergence was not achieved in any of the studies and in those that did converge the parameters were illconditioned with very wide standard errors.

A comparative analysis of the goodness of fit of models 2 and 9 with model 1 is given in table D.7.

Table D.7

Comparison of "Goodness of Fit" of Models 2, 9 and 1 of Lignocaine Decay in the Isolated Perfused Pig Liver

	Study M		
Criteria of Fit	**Model 2**	**Model 9**	**Model1**
AIC	238	241	302
Corr SS	2431	2431	2431
SS Residuals	262	257	1211*
Correlation	0.944	0.946	0.710
Degrees of freedom	40	38	40
	Study K		
Criteria of Fit			
AIC	155	142	196
Corr SS	139*	260	260
SS Residuals	40*	21.45	78*
Correlation	0.915	0.958	0.834
Degrees of freedom	39	40	42
	Study O		
Criteria of Fit			
AIC	245	255	292
Corr SS	1505	1490	1505
SS Residuals	244*	149	699*
Correlation	0.924	0.946	0.732
Degrees of Freedom	42	39	42

AIC = Akaike Information Criteria. SS = Sum of squares. * $P<0.01$ significant difference (F Test) with respect to model 9.

This shows that model 1 did not fit the data well when all the data points including the wash in data was included. The correlation coefficients of .710 and .732 indicate serious problems with this as a model (PCNONLIN Handbook). This is supported by Table D.8 where the visual assessment of goodness of fit was poor.

Table D.8

Visual Assessment of "Goodness of Fit" of Model 1 Using Wash In and Decay Data Points in the Isolated Perfused Pig Liver

Plot	**Study M**	**Study K**	**Study O**
(1)	NL++	NL++	NL++
(2)	SD++	SD+	SD++
(3)	SD+	SD	SD+

L = Linear. NL = Non linear. R = Random scatter. SD = systemic deviation. Grading: ±, + or ++.
Plot 1 = Plot of observed Y versus weighted calculated Y.
Plot 2 = Plot of weighted calculated versus weighted residual.
Plot 3 = Plot of X versus weighted residual.

D.6.4 REMARKS ON ANALYSIS

This analysis using PCNONLIN suggests that within the constraints of the models used the time course of lignocaine after a bolus injection is best described by a two compartment model with constant iv input and first order output. However, the high values for AIC as well as the corrected sum of squares and the sum of squared residuals suggest that the data might be better described by a more complex model. This was not essential to this thesis as long as the present analysis allowed the determination of a loading dose and infusion rate to achieve a constant predictable concentration of lignocaine in the isolated perfused pig liver. As one of the tenets of pharmacokinetic analysis is that all relevant data should be included in the analysis (Daniel and Wood, 1971) this was done in the analyses described above however some pharmacokineticists would object to fitting a curve through data where recirculation "humps" occur.

D.6.5 DERIVED PHARMACOKINETIC PARAMETERS

Table D.9 gives a summary of the derived pharmacokinetic parameters which were determined using PCNONLIN model 9.

Table D.9

Derived Pharmacokinetic Parameters from a Bolus Dose of Lignocaine in 3 Isolated Perfused Pig Livers. Study K, M, O.

	K	M	O	mean
V1 (litres)	2.039 ± 0.279	0.598 ± 0.064	0.764 ± 0.0815	1.128 ± 0.792
K10 (min^{-1})	0.202 ± 0.038	0.631 ± 0.273	0.375 ± 0.117	0.404 ± 0.216
K12 (min^{-1})	0.486 ± 0.204	0.105 ± 0.222	0.374 ± 0.110	0.321 ± 0.113
K21 (min^{-1})	0.403 ± 0.146	0.086 ± 0.366	0.1316 ± 0.087	0.206 ± 0.090
AUC ($ugml^{-1}$ min)	83.51	78.07	104.36	88.6 ± 8.01
K10 (min^{-1})	3.4 ± 0.64	1.09 ± 0.47	1.84 ± 0.57	2.1 ± 0.68
Alpha (min^{-1})	1.011 ± 0.353	0.748 ± 0.154	0.820 ± 0.16	0.859 ± 0.50
Beta (min^{-1})	0.080 ± 0.018	0.0730 ± 0.327	0.0601 ± 0.04	0.071 ± 0.01
Alpha $t_{\frac{1}{2}}$(min)	0.685 ± 0.239	0.924 ± 0.190	0.844 ± 1.72	0.817 ± 0.07
Beta $t_{\frac{1}{2}}$(min)	8.59 ± 1.92	9.48 ± 4.4	11.52 ± 9.32	9.8 ± 0.01
Cmax ($ugml^{-1}$)	9.54 ± 0.41	28.53 ± 1.48	23.18 ± 1.14	20.41 ± 5.65
A ($ugml^{-1}$)	10.39 ± 1.925	53.11 ± 5.21	39.36 ± 4.29	34.28 ± 12.59
B ($ugml^{-1}$)	5.51 ± 0.911	1.10 ± 3.75	4.08 ± 2.10	3.56 ± 1.29

Mean ± SEM. See Table D.2 for key to symbols.

D.7 APPLICATION OF PHARMACOKINETIC PARAMETERS DETERMINED FOR THE PIG TO ACHIEVE CONSTANT CONCENTRATIONS USING AN EXPONENTIALLY DECLINING INFUSION

This calculation (for a 25 kg pig), using the pharmacokinetic parameters determined here, was received from Professor AR Coetzee (University of Stellenbosch) and is included for the reader's interest. It is based on the technique described by Riddel et al, (1984).

To immediately achieve and maintain a target plasma concentration (Css) of 5 ug ml^{-1}:

1. Give a bolus calculated to fill the central compartment to the target concentration:

Bolus = Css x V1 where V1 = volume of the central compartment

= 5 x 25 x 0.638 x 1000 ug

= 79.8 mg

2. Administer simultaneously, the calculated maintenance infusion via a chamber containing a more concentrated solution of the drug. The dimensions, flow rates and concentrations may be calculated as follows:

(a) Choose an appropriate flow rate (F) through the system.

[A fast rate encourages mixing within the diluting chamber). Most pumps can deliver 100 ml hr^{-1} (1.66 ml min^{-1})]

(b) Calculate the concentration of the infusate (Ci).

Ci = (Css x CL) / F where Cl = clearance

= (Css x V1 x k10) / F

= 2.01 mg ml^{-1}

(c) Calculate the size of the "diluting chamber" (Va).

Va = (Css x V1 x k10) / (Ci x k21)

= 31.4 ml

(d) Calculate the mass of lignocaine (Xa) to be placed in the chamber of volume Va.

Xa = [Css x Va x V1 x (k12 + k10)] / F

= 201 mg [i.e. a concentration of 6.4 mg ml^{-1} (0.64%)]

The maintenance infusion is thus administered as a 0.2% solution at a flow rate of 100 ml hr^{-1} (4 ml kg^{-1} hr^{-1}) through a "diluting chamber" of volume 31 ml containing a 0.64% solution of lignocaine.

APPENDIX E: LIGNOCAINE ELIMINATION AT VARYING CONCENTRATIONS.

E.I LIGNOCAINE ELIMINATION AT VARYING CONCENTRATIONS E.2

E.1.1 Motivation..... E.2

E.1.2 Methods..... E.2

E.1.3 Results and Discussion..... E.2

APPENDIX E: LIGNOCAINE ELIMINATION AT VARYING CONCENTRATIONS.

E.1 LIGNOCAINE ELIMINATION AT VARYING CONCENTRATIONS

E.1.1 MOTIVATION

Winkler et al (1979) have stated that before a substance is chosen for clearance measurements, its removal kinetics has to be examined to ensure that this is independent of concentration. In preliminary experiments (vide infra) the constancy of lignocaine extraction ratio and clearance at different concentrations was established and is reported on here. The principle used to ascertain this was "to verify the constancy of clearance at substantial increases of the amounts given" (Winkler et al, 1979).
This data and methodology is briefly presented in support of the assertion that lignocaine elimination by the isolated perfused pig liver remains independent of hepatic affluent concentration over the range of lignocaine concentrations studied in this thesis.

E.1.2 METHODS:

Eight isolated pig liver perfusions (IPPL) were performed. In all perfusions a bolus dose of 26 mg of lignocaine hydrochloride was administered after which in Experiment A, (n=4) an infusion of lignocaine administered at a rate of $5mgmin^{-1}$, and in experiment B (n=4) at a rate of $2.5mgmin^{-1}$, was commenced and continued for a two hour study period. Lignocaine concentrations in the hepatic artery (HA) portal vein (PV) and hepatic vein (HV) were determined every 15 minutes from 45-120 minutes of lignocaine infusion as at this time a constant lignocaine concentration had been established (Section 4.4.5). Lignocaine extraction ratio and clearance was calculated as before (Section 6.2.8). Liver function (Table E.2) was assessed over this period by sampling hourly as described in section 2.5.1.

E.1.2 RESULTS AND DISCUSSION

The two different infusion rates of lignocaine hydrochloride administered to the IPPL preparations resulted in significantly different hepatic affluent plasma lignocaine concentrations (Table E.1) in the two similar (Table E.2) groups of livers studied.
There was no difference in hepatic lignocaine extraction ratio and clearance between the two groups, thus indicating that lignocaine elimination remained independent of concentration over this range.

Table E.1

Lignocaine Hepatic Extraction Ratio and Clearance in Relation to Concentration in the Isolated Perfused Pig Liver

	Experiment A	Experiment B	P Value
Plasma Concentration			
HA(ug ml^{-1})	10.0±1.3	3.4±0.6	<0.05
PV(ug ml^{-1})	8.8±1.1	2.6±0.4	<0.01
HV(ug ml^{-1})	2.1±0.1	0.7±0.1	<0.01
Extraction Ratio	0.75±0.02	0.72±0.03	ns
Hepatic Clearance (ml min^{-1})	396±13	386±16	ns
Intrinsic Clearance (L min^{-1})	1.69±0.18	1.51±0.24	ns
HA Flow ((ml min^{-1})	91±4	86±2	ns
PV Flow ((ml min^{-1})	434±3	441±3	ns
HV Flow ((ml min^{-1})	525±7	529±2	ns
Liver Weight (gm)	658±24	752±34	ns

Mean (±SEM) values (n=4) Comparison between experiment A and B using Unpaired Student's T-Test P <0.05 is significant, ns = non-significant.

Table E.2

Comparison of Liver Function Indices and Perfusate Composition of Experiment A (High Lignocaine Concentration) and Experiment B (Low lignocaine Concentration)

	Experiment A	Experiment B
Oxygen Consumption (ml O_2 $100g^{-1}$)	2.46±0.24	2.36±0.16
Bile Volume (ml $hour^{-1}$)	3.72±0.8	5.33±2.2
Haemoglobin (g%)	10.4±0.2	10.3±1.0
Total Protein (mg $100ml^{-1}$)	55±2	56±2
Albumin (mg $100ml^{-1}$)	27±1	29±1
PaO_2 (mmHg)	260±21	234±32
CO_2 (mmHg)	35±2	39±1
Glucose (mg $100ml^{-1}$)		
0 hour	64±7	83±6
1 hour	124±14	143±16
2 hour	94±17	96±12
AST (U L^{-1})		
0 hour	54±2	98±44
1 hour	126±26	192±57
2 hour	228±67	344±72
Potassium (mmol L^{-1})		
0 hour	5.0±0.6	4.2±0.6
1 hour	3.3±0.4	3.9±0.6
2 hour	2.9±0.2	3.3±0.3

Mean (±SEM) values (n=4 in each group) over two hour study period or at time of sampling as indicated. No significant difference between groups in any value (Unpaired Student's T-Test, P < 0.05).
AST = Aspartate aminotransaminase.

Table E2 shows that the liver function indices and perfusate composition was similar in the two groups. The lignocaine extraction ratio determined in these preliminary experiments appears higher than that determined in later experiments (Chapter 8). This may be due to small differences in methodology and could have been the effect on hepatic metabolism of the higher ($38^{o}C$) perfusate temperature (Larsen, 1971) or the different HA to PV flow ratio (Ahmad et al, 1984) used here. Alternatively the absence of isoflurane from the standard anaesthetic technique (Wood and Wood, 1982) used for liver resection, or the administration of insulin, infused to the IPPL to maintain perfusate glucose between 60-160 $mg100ml^{-1}$ may have had an effect as Thomsen and Larsen, (1983) have shown in perfused rat livers that an insulin infusion of 0.05 $U\ kg^{-1}min^{-1}$ significantly increases bile flow. However, these factors were common to both groups of livers studied [the amount of insulin administered was no different between the two groups (A: 0.10 SD=0.02 $U\ kg^{-1}min^{-1}$ and B: 0.09 SD=0.01 $Ukg^{-1}min^{-1}$)] and thus allow a valid comparison of the effect of hepatic affluent concentration on lignocaine elimination. The reasons for the apparent difference in lignocaine extraction ratio can thus only be speculative as this was not investigated further, but highlights the importance of standardization in experimentation.

A further study was performed in the IPPL in the absence of insulin administration, but with the standard anaesthetic technique used for liver procurement, and standard perfusate temperature and HA and PV flows which were constant (Chapter 8). The methodology of Pang and Rowland (1977 b) was adopted: intially a high hepatic affluent lignocaine concentration was administered and then the quantity of lignocaine infused was decreased to achieve a lower concentration, afterwhich the stability of the system was checked by returning to a higher concentration. Sampling for lignocaine analysis (n=4) was performed at the end of each 30 minute infusion period prior to the step change in infusion rate.

Table E.3

Constant Extraction Ratio of Lignocaine with Varying Hepatic Affluent Concentrations

	Mean Plasma Lignocaine Concentration ($ug\ ml^{-1}$)			
Time*	HA	PV	HV	Extraction Ratio
60 min	10.36 (0.36)	9.95 (0.39)	4.48 (0.13)	0.55 (0.024)
90 min	3.00 (0.09)	3.06 (0.10)	1.58 (0.07)	0.51 (0.035)
120 min	12.86 (0.35)	12.42 (0.37)	5.33 (0.11)	0.55 (0.023)

Mean ± (SEM) n=4. Hepatic affluent lignocaine concentrations significantly different at (*) times indicated after start of lignocaine administration. Extraction ratio similar at these times. Paired Student's T-Test. P <0.05.

Table E.3 shows that lignocaine extraction ratio did not vary when input concentrations were changed at thirty minute intervals over the standard study period. This indicates that lignocaine elimination was independent of the hepatic affluent concentration over the range studied.

APPENDIX F: MISCELLANEOUS

F.1 FLUIDS AND FOODSTUFFS F.2

F.1.1 Plasmalyte B F.2
F.1.2 Higro 16 ..... F.2

F.2 HISTOLOGICAL EXAMINATION OF BIOPSY SPECIMENS F.2

F.2.1 Light Microscopy F.2
F.2.2 Electron Microscopy F.2
F.2.3 Histological Analysis

APPENDIX F: MISCELLANEOUS

F.1 FLUIDS AND FOODSTUFFS

F.1.1 PLASMALYTE B

Plasmalyte B (Baxter, Johannesburg). One liter contains:

sodium:	130mekw
potassium	4mekw
magnesium	3mekw
chloride	109mekw
sodium bicarbonate	28 mekw
with pH	7.4

F.1.2 HIGRO 16

Hi-gro 16 (Meadow Feeds Johnnesburg) contains

Ingredient	Quantity (g/Kg)
Protein	160.0 Min
Fibre	80.0 Max
Moisture	120.0 Max
Calcium	10.0 Max
Phosphorous	6.0 Min
Total Lysine	8.5 Min

F.2 HISTOLOGICAL EXAMINATION OF BIOPSY SPECIMENS

F.2.1 LIGHT MICROSCOPY

Liver tisssue was fixed in 10% buffered formalin for a period of at least 24 hours and then processed and embedded in paraffin wax blocks. Sections of 3um thickness were cut from each block and stained with Haematoxylin and Eosin for examination by light microscopy (Nikon, Japan).

F.2.2. ELECTRON MICROSCOPY

Liver tissue was fixed in 3% phosphate buffered (0.1M, pH=7.2) gluteraldehyde. This was then washed in buffer and post fixed in 1% collidine buffered osmium tetroxide and then rinsed in buffer and dehydrated in graded alcohols. After which it was embedded in Spurrs epoxy resin. Semi thin (1um) sections were stained with toluidine blue to select suitable areas by light microsocpy. Ultrathin sections (80nm) were cut on a Richard Jung ultracut E microtome and were stained with uranyl acetate and lead citrate and viewed by means of a Phillips 201 transmission electronmicroscope.

F.2.3 HISTOLOGICAL ANALYSIS

All ultrastructural changes observed electronmicroscopically were listed using a semi-quantitative scoring system. The changes were graded according to the degree of severity of these changes.

Table F.1

Evaluation of Light Microscopic Changes Associated with Lignocaine (L:1-4) or Saline Administration in vivo

Time of Biopsy			**Baseline**			**After 2 hours of Lignocaine (L) or Saline administration**				
Sample	**Saline**	**L1**	**L2**	**L3**	**L4**	**Saline**	**L1**	**L2**	**L3**	**L4**
Hepatocyte Swelling	-	-	-	-	-	+	+	+	+	+
Liver Cell Necrosis	-	-	-	-	-	-	-	-	-	-
Portal Tracts	N	N	N	N	N	N	N	N	N	N
Central Veins	N	N	N	N	N	N	N	N	N	N
Sinusoids	N	N	N	N	N	N	N	N	N	D
Bile Ducts	N	N	N	N	N	N	N	N	N	N

(-) = absent, (+) = present, (N) = within normal limits, (D) = Dilated.

Table F.2

Semi-quantitative Evaluation of Ultrastructural Changes Associated with Lignocaine (L:1-4) or Saline Administration in Vivo

Time of Biopsy			**Baseline**			**After 2 hours of Lignocaine (L) or Saline administration**				
Sample	**Saline**	**L1**	**L2**	**L3**	**L4**	**Saline**	**L1**	**L2**	**L3**	**L4**
Nuclear Chromatin Clumping	-	-	-	-	-	+	+	+	+	+
Mitochondrial:										
Swelling	+	+	+	+	+	++	++	++	++	++
Matrix Lucency	+	-	-	+	+	++	++	++	++	++
Loss of Cristae	-	-	+	+	+	++	++	++	++	++
Amorphous Densities	-	-	-	-	-	++	++	++	++	++
Membrane Rupture	-	-	-	-	-	+	+	+	+	++
Myelin Figures	-	-	-	-	+	++	++	++	++	+++
Sarcoplasmic Reticulum Vesiculation	+	+	+	+	+	++	++	++	+++	+++
Intracytopl. Lipid Droplets	+	+	+	+	+	+	++	+	++	++

(-) = Absent, (+) = Mild, (++) = Moderate, (+++) = severe, Intracytop. = Intracytoplasmic.

Table F.3

Evaluation of Light Microscopic Changes Associated with Hypoxia (H:1-3) or Normoxia in the isolated perfused pig liver

Time of Biopsy		**Baseline**			**After 2½ hours of Hypoxia or normoxic liver perfusion**			
Sample	**Normoxia**	**H1**	**H2**	**H3**	**Normoxia**	**H1**	**H2**	**H3**
Hepatocyte Swelling	-	-	-	-	+	+	+	+
Liver Cell Necrosis	-	-	-	-	-	-	-	-
Portal tracts	N	N	N	N	N	N	N	N
Central Veins	N	N	N	N	N	N	N	N
Sinusoids	N	N	N	N	D	D	D	D
Bile Ducts	N	N	N	N	N	N	N	N

(-) = absent, (+) = present, (N) = within normal limits, (D) = Dilated.

Table F.4

Semi-quantitative Evaluation of Ultrastructural Changes Associated with Hypoxia (H:1-3) or Normoxia in the Isolated Perfused Pig Liver.

Time of Biopsy		**Baseline**			**After 2½ hours of Hypoxia or normoxic liver perfusion**			
Sample	**Normoxia**	**H1**	**H2**	**H3**	**Normoxia**	**H1**	**H2**	**H3**
Nuclear Chromatin Clumping	-	-	-	NO	-	+	+	+
Mitochondrial:								
Swelling	+	+	+	S	+	++	++	++
Matrix Lucency	-	+	+	P	+	++	++	++
Loss of Cristae	-	+	+	E	+	++	++	++
Amorphous. Densities	-	-	-	C	-	+	+	+
Membrane Rupture	-	-	-	I	-	+	+	+
Myelin Figures	-	-	-	M	-	-	++	++
Sarcoplasmic Reticulum Vesiculation	+	+	+	EN	+	++	++	+++
Intracytopl. Lipid Droplets	+	+	+		+	-	+	+

(-) = Absent, (+) = Mild, (++) = Moderate, (+++) = severe, Amorph. Dens. = amorphous densities, Membr. Rupt. = membrane rupture, Fig. = figure, Intracytop. = Intracytoplasmic.

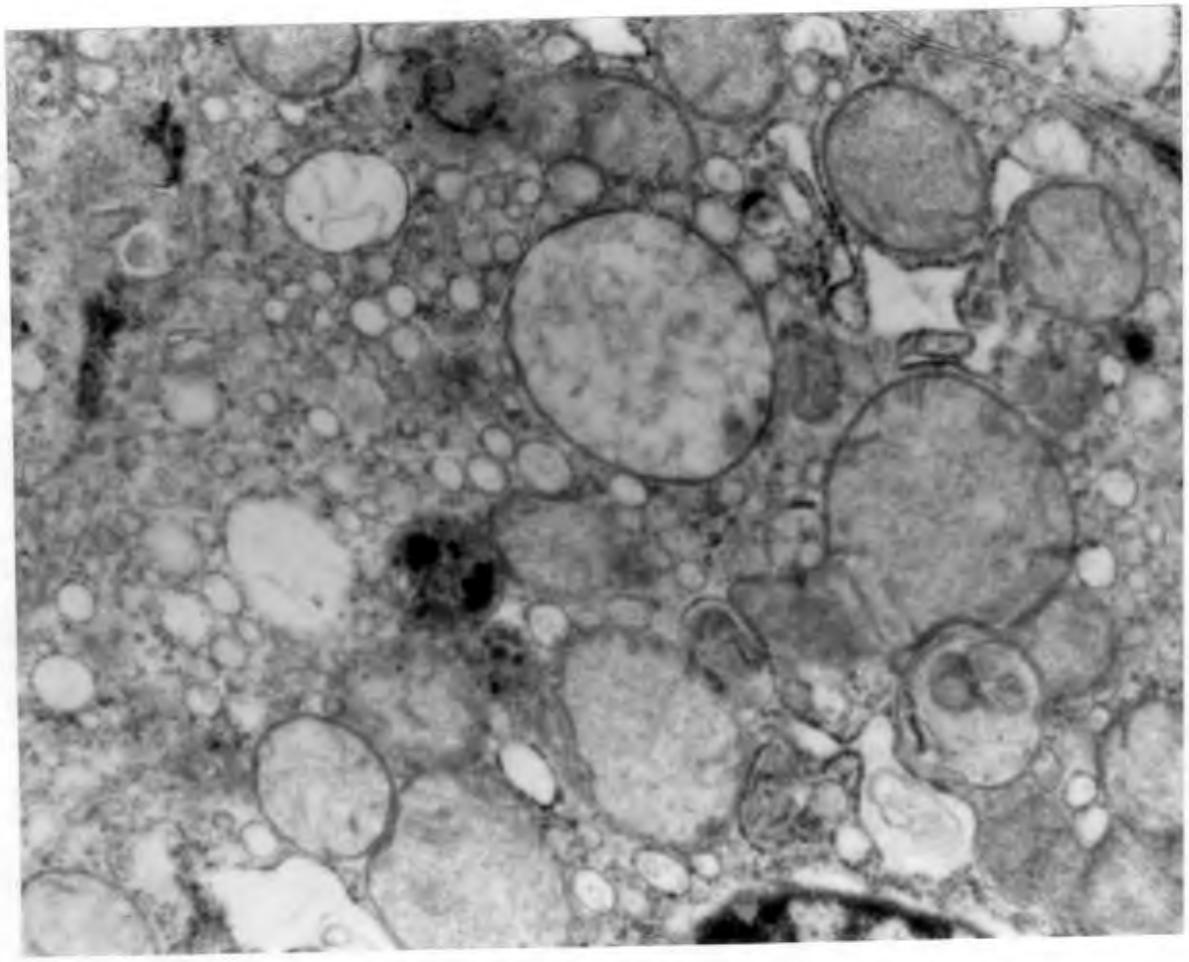


Figure F.1 Electronmicrograph of a liver biopsy taken from an isolated perfused pig liver subjected to two and one half hours of hypoxia, Magnification x 14560.