LIGNOCaine METABOLISM IN MAN

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SUMMARY

LIGNOCAINE METABOLISM IN MAN

A gas-liquid chromatographic (GLC) method has been developed for the simultaneous assay of lignocaine and its two active metabolites, ethylglycylxylidide (EGX) and glycylxylidide (GX), using a nitrogen-sensitive flame ionisation detector and single column temperature programming. A GLC method has also been developed for the assay of 4-hydroxyxylidine, a major metabolite of lignocaine.

Lignocaine metabolism was studied in healthy volunteers following oral and intramuscular administration. The metabolism of the drug was also studied in hospital patients (a) undergoing anaesthesia and a variety of gynaecological surgical procedures, (b) with chronic liver disease and (c) with acute myocardial infarction and/or cardiac failure. The plasma antipyrine half-life was measured in some of these patients as an independent measure of hepatic drug metabolising activity.

In healthy volunteers lignocaine is extensively and rapidly metabolised to EGX, GX and 4-hydroxyxylidine. 2,6 xylidine and 4-hydroxy lignocaine and other unidentified metabolites were also present in the urine after lignocaine administration.

The absorption of lignocaine was delayed in laparoscopy patients. The elimination of the drug from plasma was slow in patients undergoing anaesthesia and other minor gynaecological procedures. The elimination of antipyrine was not markedly altered by anaesthesia and/or surgery.

Lignocaine /
Lignocaine absorption, metabolism and elimination were abnormal in four patients with chronic liver disease and the plasma antipyrine half-life was abnormally prolonged in two.

During a constant intravenous infusion of lignocaine in patients with acute myocardial infarction or cardiac failure no 'steady state' plasma concentrations of the drug were reached and the concentrations rose progressively. The concentrations of lignocaine were high and potentially toxic levels were reached in the presence of cardiac failure and cardiogenic shock. There was a slow cumulation of EGX and GX in plasma during prolonged lignocaine infusion. The metabolism of lignocaine was impaired especially in patients with cardiac failure or cardiogenic shock. Antipyrine metabolism was abnormal in the patients with acute cardiac failure.
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PLANN OF THE THESIS

The thesis is divided into seven sections.

Section I consists of a general introduction and historical review.

Sections II and III cover the development of analytical methods.

Sections IV to VII concern lignocaine metabolism in healthy volunteers and lignocaine and antipyrine metabolism in hospital patients.

Sections II to VII have their own introduction, methods, results, discussion and summary.
INTRODUCTION AND HISTORICAL REVIEW
Lignocaine (diethylamino-2-6-acetoxyxylidide, diethylglycylxylidide) exists as yellow crystals with a melting point of 69°C (Lofgren, 1948; Bullock and Grundy, 1955). It is an organic base with a pKa value of 7.85 (Eriksson, 1966) and a molecular weight of 234.3.

Many local anaesthetics are esters of benzoic acid but lignocaine has an amide bond linking a lipophilic aromatic group (2, 6 xylyl) to a hydrophilic tertiary amino group (diethylamine) (Fig. 1).

Unlike other local anaesthetics, lignocaine is extremely stable in aqueous solutions and is resistant to acid or alkali hydrolysis (Lofgren, 1948; Bullock and Grundy, 1955).

B Pharmacology
(a) Action on Nerves
(i) Peripheral nerves

Like other local anaesthetics, lignocaine blocks the propagation of nerve impulses without causing depolarisation of the membrane (Ritchie and Greengard, 1966). The block in the conduction of nerve impulses may be explained by inhibition of the permeability of cellular membranes to Na⁺ and K⁺ (Shanes, Freygang, Grundfast and Amatniek, 1959; Taylor, 1959). The exact mechanism is unknown.

In addition to the blocking actions on peripheral nerve lignocaine /
The Structure of Lignocaine

\[ \text{CH}_3 - \text{O} - \text{C}_2\text{H}_5 \]
\[ \text{NH} - \text{C} - \text{CH}_2 - \text{N} - \text{C}_2\text{H}_5 \]

Aromatic group (xylyl)  Tertiary amino group (diethyl amine)

Figure 1.
lignocaine interferes with the function of all tissues in which there is transmission of electrical impulses.

(ii) Central nervous system effects

Lignocaine and other local anaesthetics are highly lipid soluble at physiological pH and cross the blood-brain barrier readily to produce direct effects on the brain (Usubiaga, Moya, Wikinski, Wikinski and Usubiaga, 1967).

Small doses of lignocaine produce a variety of subjective symptoms, such as dizziness, drowsiness, lightheadedness, headaches and numbness of the tongue and lips (Foldes, Davidson, Duncalf and Kuwabara, 1965: Eriksson and Persson, 1966: Foldes et al., 1960: Boyes and Keenaghan, 1971).

In toxic doses lignocaine produces central excitation in man with tremor and convulsions.

(b) Cardiovascular effects of lignocaine

Lignocaine given intravenously may cause a small transient rise in arterial pressure. Doses of 1 to 3.5 mg/kg infused intravenously do not usually produce any significant cardiovascular depression (Jewitt, 1971). In large doses lignocaine may produce a fall in blood pressure as a result of depression of the myocardium and dilatation of peripheral vascular beds.

Lignocaine has been found to abolish a variety of experimentally produced ventricular arrhythmias (Harris, Aguiré y Guerra, Liplak and Brigham, 1956: Katz and Zitnik, 1966: Fearon, 1968).
It is effective in the treatment of ventricular arrhythmias during surgery (Frieden, 1965) and after myocardial infarction (Gianelly, von der Groeben, Spivack and Harrison, 1967; Jewitt et al., 1968). Its effects on atrial arrhythmias are inconstant (Jewitt et al., 1968).

C TOXIC EFFECTS

Allergy to lignocaine is rare (Adriani, 1970; Alderete and Johnson, 1971) and toxic effects are usually related to excessive dosage. These effects are mainly of cardiovascular depression and central nervous excitation or depression.
CHAPTER 2  THE METABOLISM OF LIGNOCAINE

Although lignocaine has been in clinical use for over 23 years, little is known of its metabolic fate in man. Most of the information on the disposition of the drug has come from animal studies. The results of these animal studies are sometimes conflicting.

Lignocaine is very well absorbed after parenteral and oral administration and it is rapidly distributed (McMahon and Woods, 1951; Sung and Truant, 1954; Akerman, Astrom, Ross and Telò, 1966; Scott, 1971; Keenaghan and Boyes, 1972).

A  ANIMAL STUDIES

McMahon and Woods (1951 a) recovered 10 and 20% of a dose of lignocaine unchanged in the urine of mongrel dogs 24 h after intravenous and subcutaneous administration respectively. There was a marked increase in organic sulphate excretion in the urine following administration of lignocaine subcutaneously, but this was more than could be accounted for by the dose of lignocaine given. On the basis of this indirect evidence it was concluded that the aromatic ring in lignocaine was oxidised to a phenolic compound and subsequently conjugated with sulphate (McMahon and Woods, 1951 b). There was no evidence for hydrolysis of the amide bond or oxidation of the ortho-methyl groups of lignocaine. Lignocaine was assayed by the non-specific colorimetric method of Woods et al. (1951) and metabolites of lignocaine could also have been measured.

Ther (1953) /
Ther (1953) incubated lignocaine with liver homogenates and found only an insignificant increase in the acidity of the incubation medium. If hydrolysis occurred at all it was considered a very slow process.

The absorption, distribution, metabolism and excretion of lignocaine in mice, rats, monkeys, dogs and man were studied in detail by Sung and Truant (1954). Lignocaine disappeared rapidly from the blood into all tissues. The highest concentrations were found in the kidneys, brain and lungs. Following intramuscular injection there was negligible excretion of lignocaine in the faeces. In rats and monkeys 2 to 6% and less than 1% respectively were recovered as unchanged drug in the urine in 24 h. There was a marked increase in the urinary excretion of phenolic compounds.

Following partial hepatectomy in rats there was prolongation of pharmacological activity of lignocaine and in addition blood and tissue concentrations of the drug were increased. On incubation of lignocaine with the different rat tissues the liver was found to be the most active organ for metabolising the drug and oxygen was necessary for the breakdown of lignocaine. Phenolic compounds similar to those found in the urine were detected and the amounts were markedly increased when the urine or the tissue incubation medium was hydrolysed with acid.

Geddes and Douglas (1956) incubated rat liver, cerebral cortex and kidney with C\textsuperscript{14} labelled lignocaine for 5 h. Only the liver was capable of appreciable biotransformation of lignocaine.
On a paper chromatogram a large spot was noted with the same Rf value as diethylaminoacetic acid. A number of unidentified smaller spots were also noted. They concluded from these studies that the hydrolysis of lignocaine at the amide bond occurred slowly and that although oxygen catalysed the hydrolysis it could also occur under anaerobic conditions.

Using rabbit tissues, Hollunger (1960 a, b, c, d) confirmed that the liver was the major organ responsible for lignocaine metabolism and that the lignocaine metabolising enzymes required oxygen and NADPH and were localised in the liver microsomes. Lignocaine was metabolised slowly under anaerobic conditions. The identity of the products of anaerobic metabolism was not stated (Hollunger, 1960 a).

During the incubation, 0.9 mole of acetyldehyde was formed per mole of lignocaine and ethylglycylxylidide (EGX) was also detected in the medium (Hollunger, 1960 b). Ethylglycylxylidide disappeared at the same rate aerobically and anaerobically to form 2,6, xylidine. Xylidine was further oxidised but Hollunger did not isolate the oxidation products. No glycylxylidide or hydroxy metabolites of lignocaine, ethylglycylxylidide or 2, 6 xylidine were detected in the incubation medium. In addition glycylxylidide was found to be very resistant to hydrolysis by rabbit liver.

Hollunger (1960 c) succeeded in solubilising an amidase from rabbit liver microsomes which had a high specificity for ethylglycylxylidide but was not active against lignocaine or glycylxylidide when purified.

On /
On the basis of the similarities of the Rf values of monoethylaminoacetic acid and diethylaminoacetic acid, Hollunger concluded that the spot noted chromatographically by Geddes and Douglas was monoethylaminoacetic acid produced by the hydrolysis of ethylglyclyxylxylidide (Hollunger, 1960 b). This does not exclude the possibility of both diethyl and monoethylaminoacetic acid being formed. In this case the diethyl derivative could come from the amide hydrolysis of lignocaine. The evidence of Sung and Truant for hydroxylation of lignocaine was criticised by Hollunger on the basis that 2,6, xylidine gave the same colour reaction by the colorimetric method used. He therefore emphasised that the phenolic compounds of McMahon and Woods (1951) and Sung and Truant (1954) were in fact 2,6, xylidine. Whilst this is a valid criticism, these 'phenolic' compounds could have been derived from lignocaine itself. Moreover, Hollunger was unable to account for all the lignocaine that disappeared from the incubation medium on the basis of ethylglyclyxylxylidide and 2,6 xylidine alone. Hollunger himself used the non-specific methyl orange colorimetric method of Sung and Truant (1951). Hollunger proposed the scheme in Fig. 2.

The in vivo metabolism of 2,6, xylidine was studied by Lindstrom (1961) who showed that after an intragastric dose of 150 mg/kg of xylidine, large amounts of 4, hydroxy, 2, 6 xylidine and small amounts of 2 amino-3 methyl benzoic acid were recovered in the urine of rats.

In the cat, Ackerman, Astrom, Ross and Telc (1966) have demonstrated /
Fate of Lignocaine

(Modified from Hollunger, 1956b)

Lignocaine
(Diethylglycylxylidide)

Oxidation

Monoethylglycylxylidide (EGX)

Amide hydrolysis

2, 6, xyline

Oxidation

Figure 2.
demonstrated that the lungs and kidneys are also capable of metabolising lignocaine.

More recent studies have disclosed marked species variation in lignocaine metabolism. Thus, conjugates of m-hydroxy metabolites of lignocaine and ethylglycylxylidide were detected in large amounts in rat urine, in moderate amounts in dog urine, but none was detected in human urine (Boyce and Keenaghan, 1971). 2,6, xylidine was detected in the urine of dogs and man but not in rats. All species formed ethylglycylxylidide. On the basis of these animal studies the scheme on Fig. 3 is proposed.

B STUDIES IN MAN

According to Sung and Truant (1954) two medical students excreted 3 and 11% of the drug unchanged in the urine 24 h after intramuscular administration of lignocaine. Eriksson and Granberg (1965) subsequently showed that renal clearance of lignocaine was higher in acidic urine and was not significantly affected by urine flow rate. Beckett, Boyce and Appleton (1966) studied the fate of intravenous lignocaine and ethylglycylxylidide (EGX) in 3 volunteers while the urine was acidified. Even under acidic urine conditions only about 6% of the dose of lignocaine and 12% of the dose of EGX were recovered in the urine in 12 h. Furthermore, only about 4% of the administered lignocaine was recovered as EGX. 2,6 xylidine was detected in the urine but not glycylxylidide (GX). The biological half-life of lignocaine was estimated as 90 min.

Important /
Possible routes of Lignocaine metabolism in animals
(Different species may differ in detail)

\[
\begin{align*}
\text{Lignocaine} & \rightarrow \text{Ethylglycylxylidide} \\
& \rightarrow \text{Hydroxy lignocaine} \\
& \rightarrow \text{Conjugates} \\
\text{Hydroxy lignocaine} & \rightarrow \text{Hydroxy EGX} \\
& \rightarrow \text{Hydroxy GX} \\
\end{align*}
\]

\[
\begin{align*}
\text{2, 6, xylidine} & \rightarrow \text{2-Amino, 3-ethyl benzoic acid} \\
& \rightarrow \text{Hydroxyxylidine} \\
& \rightarrow \text{Conjugates} \\
\end{align*}
\]

Figure 3.
Important conclusions to be drawn from the study of Beckett et al. (1966) are (a) that lignocaine is extensively metabolised and (b) that EGX is either extensively metabolised or slowly excreted in the urine.

C ACTIVITY OF LIGNOCAINE METABOLITES

Animal studies have demonstrated that ethylglycylxylidide (EGX), 2,6 xylidine and glycylxylidide (GX) are pharmacologically active.

EGX and GX both have local anaesthetic properties (Ehrenberg, 1948: Boyes, R.N., 1972 personal communication).

2, 6 xylidine, EGX and GX have antiarrhythmic properties but are less potent than lignocaine. (Astrom, 1971: Smith and Duce, 1971: Boyes, 1972, personal communication).

The toxicity of EGX and GX is similar to that of lignocaine in that both can cause emesis and convulsions (Smith and Duce, 1971: Boyes, 1972, personal communication). 2,6 xylidine may be hepatotoxic (Boyes and Keenaghan, 1971).

Since EGX and GX possess most of the clinically important actions of lignocaine, elucidation of the metabolic pathways of lignocaine is essential for the assessment of its pharmacological and toxic actions. In particular, knowledge of the plasma concentrations and kinetics of these active metabolites is essential for the correct management of patients receiving lignocaine. Patients may receive the drug continuously for several hours or days. Because of analytical difficulties very little information is available concerning plasma concentrations of these metabolites in man.
A SUMMARY OF SECTION I

1. Lignocaine is a stable local anaesthetic of the amide type. It has antiarrhythmic properties and can cause both central nervous system excitation and depression.

2. Its metabolism is little understood, but there is some information from animal studies. The drug is extensively metabolised, probably largely by the liver.

3. In animals the following metabolites may be formed: -
   Ethylglycerylxyldide, 2,6, xylidine, 4-hydroxyxylidine, 2-amino-3-benzoic acid and hydroxy metabolites of lignocaine and EGX.

4. In man lignocaine appears to be extensively metabolised and under conditions of acidic urine only about 6% and 4% of the administered dose was excreted as unchanged drug and ethylglycerylxyldide (EGX) respectively.

5. EGX is either extensively metabolised or slowly excreted as only 12% of the administered dose of EGX appeared in the urine in 12 h.

6. 2,6, xylidine was detected in human urine after lignocaine administration. It was unstable.

7. 2,6, xylidine, EGX and GX are pharmacologically active and may account for some of the actions of lignocaine in man.

8. Specific and sensitive methods for the assay of lignocaine metabolites in human plasma and urine were not available at the start of the present study.
B THE PRESENT STUDY

(a) Introduction

Studies which have been carried out on lignocaine metabolism in man are limited in that they do not provide the following vital information:

(i) The fate of 90% of the administered dose of lignocaine.

(ii) The plasma concentrations and the kinetics of lignocaine metabolites in man. This information is essential since animal studies indicate that EGX and GX are pharmacologically active.

(iii) The rate of excretion of lignocaine metabolites.

(iv) The effect of route of administration on lignocaine metabolism.

(v) The effect of factors which modify the normal physiological state of the individual such as disease, anaesthesia, surgery and drugs on the metabolism of lignocaine in man. The importance of such a study lies in the fact that in real life situations lignocaine is usually administered to patients under abnormal physiological conditions.

The absence of specific and sensitive methods for the assay of lignocaine metabolites in plasma and in urine has prevented the study of lignocaine metabolism in man.

(b) The scope of the present study

(i) Methods have been developed for the qualitative and quantitative study of lignocaine metabolites, EGX, GX and 4-hydroxy-xylidine in plasma and in urine.

(ii) /
(ii) Detailed pharmacokinetic and metabolic studies of lignocaine have been carried out in healthy volunteers after oral and intramuscular administration of the drug.

(iii) The metabolism of lignocaine has been studied in hospital patients undergoing anaesthesia and a variety of gynaecological surgical procedures, patients with chronic liver disease and in patients admitted to the Coronary Care Unit with acute myocardial infarction or acute cardiac failure.

(iv) Antipyrine metabolism was also studied as an independent measure of changes in drug metabolism under a variety of clinical conditions.

(v) A rapid-gas-liquid chromatographic method has been developed for antipyrine assay in plasma.

(c) Recent studies on lignocaine metabolism in man

Since the present study was started (1970) the following reports have appeared in the literature on the metabolism of lignocaine in man. These have provided very little additional information.

(i) The detection of cyclic metabolites (Breck and Trager, 1971, and personal communication to L.F. Prescott).

(ii) The quantitation of lignocaine metabolites in the urine after administration of the drug to volunteers (Keenaghan and Boyes, 1972; Thomas and Meffin, 1972).

Discussion of these recent reports in relation to the present study are to be found in Sections II to IV.
METHODS FOR THE ESTIMATION OF LIDOCAINE

AND ITS METABOLITES IN BIOLOGICAL FLUIDS.
CHAPTER 1

INTRODUCTION

Measurement of the optical density of drug complexes with dyes such as bromocresol purple and methyl orange in organic solvents has been employed for the estimation of lignocaine (Woods, Cochin, Ferrifield, McMahon and Seevers, 1951; Sung and Truant, 1954). These dyes will react with many organic amines including lignocaine metabolites and such methods are therefore non-specific.

Many investigators have described gas-liquid chromatographic (GLC) methods for the assay of lignocaine (Beckett, Boyes and Parker, 1965; Svinhuvud, Ortengren and Jacobsson, 1965; Katz, 1966; Keenaghan, 1968; Prescott and Nimmo, 1971). The lignocaine is extracted from alkaline blood, plasma or urine into an organic solvent and either chromatographed directly (Svinhuvud et al., 1965; Katz, 1966) or evaporated, reconstituted in a small volume of organic solvent and chromatographed using internal standards. The internal standard is added either to the urine or plasma or to the organic solvent used for extraction. A calibration graph is prepared by plotting the ratio of the peak heights or peak areas of lignocaine to internal standard against the concentration of lignocaine. The use of an internal standard greatly improves the accuracy and precision of the assay as errors introduced during extraction, evaporation of organic solvent and sample injection are minimised.

GLC/
GLC methods have also been described for the assay of lignocaine metabolites in aqueous solution, plasma and urine (Di Fazio and Brown, 1971; Keenaghan and Boyes, 1972; Strong and Atkinson, 1972; Strong, Parker and Atkinson, 1973).

The main advantage of the GLC methods are high specificity and sensitivity. A mixture containing many compounds can be separated, identified and measured in one operation. Specificity is of particular importance in clinical studies, since some hospital patients receive an average of 5 to 6 drugs simultaneously, and one patient in 10 is given 10 drugs at the same time.
Gas-liquid chromatography was considered to be the most suitable method for the assay of lignocaine and its metabolites for the above reasons.

Initially, a Pye series 104 Model 14 GLC equipped with flame ionisation detectors was used with a Leeds & Northrup Speedomax 'W' recorder. Coiled glass columns 3 ft. x ⅛ in. o.d. were used with this instrument. Most of the studies were performed using a Hewlett Packard Model 402 gas-liquid chromatograph with a nitrogen sensitive flame ionisation detector (Model 15161A) and a dual channel 1 mv Moseley model 7128A strip chart recorder. Unless stated otherwise, 4 ft. "U"-shaped glass columns ¼ in. o.d. were used with the Hewlett Packard instrument. The columns were conditioned for at least 48 h at 10°C above the operating temperature with a slow flow of nitrogen carrier gas (15 - 25 ml/min). All gas chromatographic column packings were obtained from Applied Science Laboratories, Inc., P.O. Box 440, State College, Pennsylvania, U.S.A.

Nitrogen sensitive flame ionisation detector

This detector is a modified flame ionisation detector consisting of a cylindrical collector electrode containing a rubidium bromide crystal above the flame jet (Fig. 4). The alkali metal salt vaporises in the flame and the ionisation produces a high standing current. Organic compounds containing nitrogen are ionised to a much higher degree than those not containing nitrogen. The extent of ionisation is proportional to the nitrogen equivalent in the molecule.
Figure 4.  

A = Nitrogen detector model 15161A

B = Flow diagram of the nitrogen detector connected in a circuit as a modified flame ionisation detector.

(Modified from Hewlett-Packard's Instruction Manual)
The mechanism of the enhanced ionisation is not understood, but it is probably due to an interaction between rubidium ions and nitrogen. The ionisation current depends critically on the hydrogen and helium flow rates, the position of the crystal above the flame and the temperature of the detector. A selectivity ratio of nitrogen to carbon of 5000 : 1 can be obtained.

The main advantage of this detector is its selective sensitivity to nitrogen containing compounds. The response from the solvent front and column bleed are greatly reduced and there is much less interference from unwanted compounds in biological extracts. Chromatography can be completed more quickly and there is very little base-line drift during single column temperature programming at high sensitivity. The main disadvantage is that performance depends critically on the hydrogen and helium flow rates. The establishment of optimal operating conditions is time-consuming.
Figure 5. The oven temperature was $190^\circ\text{C}$ and the nitrogen carrier flow was 25 ml/min.
Gas-Liquid chromatogram of 1 μg each of Lignocaine (LIG), EGX and GX on a 4 ft x 1⁄4 in o.d. glass column packed with 80/100 mesh on chromosorb G coated with 5% SE 52.

Figure 6. The oven temperature was 188°C and the nitrogen carrier flow was 25 ml/min.
CHAPTER 2.
SIMULTANEOUS ASSAY OF LIGNOCAINE, ETHYLCYCLOXYLIDIDE (EGX)
AND GLYCXYLIDIDE (GX)

A Preliminary studies
(a) Chromatographic columns
   After extensive investigation of a large number of columns using liquid phases of varying polarity, no column was found on which GX and EGX could be chromatographed directly without significant tailing and adsorption losses. Acceptable chromatograms were obtained only with 3% XE 60 on Gas Chrom Q 80/100 mesh and 5% SE 52 on Chromosorb G 80/100 mesh (Figs. 5 and 6). The three compounds were eluted from the XE 60 column on the basis of polarity, while the SE 52 column separated the compounds according to molecular weight. However on these columns the GX peaks were relatively small because of absorption and there were prohibitive losses when submicrogram amounts were chromatographed. Therefore, derivative formation was considered necessary for accurate quantitative analysis.

(b) Derivative formation
   The polarity of EGX and GX was reduced by substitution of the hydrogen on the terminal nitrogen atom. The reactions examined were Schiff-base formation with benzaldehyde and acetone, silylation with N,0-bis(trimethylsilyl) acetamide (BSA) and acylation with trifluoroacetic anhydride or acetic anhydride. Acetylation gave the most consistent results, the derivatives were stable and symmetrical peaks were obtained with columns packed with 3% HI EFF 8 BP (cyclohexanediol/methanol/
(cyclohexanediol succinate) on Gas Chrom Q 100/120 mesh.

(c) Extraction of lignocaine EGX and GX from aqueous solutions

An essential step in the development of a GLC method was to find suitable solvents for extraction of the compounds of interest from biological fluids. The three compounds are bases and the aqueous solutions were made strongly alkaline before extraction with solvents of differing polarity.

Procedure

To 1 ml of an aqueous solution containing 500 µg/ml each of lignocaine, EGX and GX in 15 ml round bottomed tubes was added 0.5 ml of 5NaOH and 1 ml of the organic solvent. The tubes were stoppered, shaken mechanically for 10 min and centrifuged for 10 min. Exactly 3 µl of the organic phase was injected directly into the GLC. Three separate injections were made with each solvent.

Chromatographic conditions:

Column 5% SE 52 on Chromosorb G.

Temperatures: Oven - 180°C, detector - 300°C and flash heater - 260°C.

Gas flows: Nitrogen 25 ml/min, hydrogen 50 ml/min and air 200 ml/min.

Results:

The mean peak heights of each of the 3 compounds extracted by each of the nine solvents are shown in Fig. 7. Lignocaine was extracted by all the solvents studied, EGX by all except cyclohexane and n-hexane, but only dichloromethane, ethylacetate and chloroform extracted /
Figure 7. The relative gas liquid chromatographic peak heights of aqueous extracts containing lignocaine, EGX and GX into different organic solvents. For conditions see text.
extracted GX. Dichloromethane was the best solvent for GX and since it also extracted lignocaine and EGX it was chosen as the solvent for this study.

(d) Temperature programming

The acetyl derivatives of EGX and GX eluted very slowly relative to lignocaine and it was necessary to use temperature programming. Dual column compensation with standard flame ionisation detectors was tried initially, but a single column with the nitrogen sensitive flame ionisation detector was eventually used because it produced a much more stable base line.
THE SIMULTANEOUS ESTIMATION OF LIDOCAINE, 

EGX AND GX

Glassware

In all experiments the water cleaned glassware was washed with hydrochloric acid - methanol (1 : 5), methanol, and finally with acetone and oven dried before use.

Procedure

To 2 ml of plasma or urine in a 15 ml round bottomed stoppered glass tube was added 0.5 ml of 5 NaOH and 5 ml of freshly distilled dichloromethane containing acet-p-toluidide (APT) as the internal standard. The concentrations of APT were 0.125 μg/ml, 1.25 μg/ml or 2.5 μg/ml for samples containing less than 2.0 μg/ml, 1 to 10 μg/ml and 5 to 40 μg/ml of the 3 drugs respectively. The stoppered tubes were shaken mechanically for 20 min. Emulsion formation was avoided by gentle shaking. The tubes were centrifuged for 10 min and the upper aqueous layer was aspirated and discarded. The lower dichloromethane layer was carefully decanted into 15 ml centrifuge tubes. 25 μl of acetic anhydride and 5 μl of anhydrous pyridine were added to the dichloromethane extract and mixed. The tubes were incubated in a water bath at 40°C for 20 min. The excess dichloromethane, acetic anhydride and pyridine were removed with a Büchi rotary evaporator. In order to avoid boiling of the dichloromethane during evaporation, the water bath temperature and vacuum were kept at 30°C and 400 mm Hg respectively until the dichloromethane was removed. The temperature and vacuum were then increased to 45°C and 700 mm Hg /
700 mm Hg until evaporation of the acetic anhydride and pyridine was complete. The residue was dissolved in 20 µl of ethanol with the aid of a Vortex mixer and 1 to 4 µl aliquots injected into the gas-liquid chromatograph.

Chromatographic conditions:

- Column packing: 3% HI EFF 8BP on Gas Chrom. Q
- Gas flow rates: Helium 50 ml/min.
  - Hydrogen 30 ml/min.
  - Air 210 ml/min.
- Operating temperatures:
  - Detector 350°C
  - Flash heater 320°C
  - Oven temperature: Programmed at 3°C/min from 200°C to 245°C after an initial delay of 2 min.
  - Detector: Nitrogen-sensitive flame ionisation.
- Attenuation: $10^4 x 32 - 10^2 x 16$
- Recorder chart speed: 0.25 in/min.

At the end of each run, the oven was cooled rapidly to 150°C and then allowed to stabilise at 200°C for at least 3 min before the next injection. Figs. 8(a) and 8(b) show chromatograms of extracts of plasma and urine standard solutions.

Calibration

The peak height ratios of lignocaine, EGX or GX to APT plotted/
Figure 8a. Gas liquid chromatograms of extracts of plasma containing 0.1, 0.5 and 1 µg/ml of lignocaine (Lig), ethylglycylxylidide (EGX) and glycylxylidide (GX). EGX and GX were chromatographed as the acetyl derivates APT (acet p-toluidide) was used as the internal standard. For conditions see text.
Figure 8b. Gas liquid chromatograms of extracts of urine blank and urine containing 10 μg/ml, 20 μg/ml and 40 μg/ml each of lignocaine (Lig), ethylglycylxylidide (EGX) and glycylxylidide (GX). EGX and GX were chromatographed as the acetyl derivatives. Acet-p-toluidide (APT) was used as the internal standard.

For conditions see text.
plotted against the concentrations of the three drugs in water, plasma or urine, gave linear plots passing through the origin. Relative recovery, specificity, limits of detection and reproducibility of the assay were determined.

**Estimation of the three compounds in unknown urine or plasma samples**

Appropriate standards of the three compounds in plasma or urine were run with each set of samples, thus eliminating minor day to day variations in the peak height ratios. For plasma samples 0.5 μg/ml and 1 μg/ml each of the three drugs in blank plasma were used as standards. For urine samples, a standard containing 10 μg/ml of each of the three compounds in blank urine was used.

The concentration of each of the three compounds in plasma or urine U was calculated from the following formula $U = \frac{SY}{X}$ where $Y$ is the peak height ratio of lignocaine, ECX or GX to APT of the unknown sample, $X$ the corresponding ratio for plasma or urine standards and $S$ the concentration in μg/ml of lignocaine, ECX or GX in plasma or urine standard.
RESULTS

(a) Stability of lignocaine, EGX and GX in biological fluids

Lignocaine, EGX and GX added to plasma (1 μg/ml) and urine and plasma (10 μg/ml) were stored at 4°C and -20°C for 1 month. The samples were adjusted to pH 5.0 before storage. The recovery was complete for all 3 compounds in all samples apart from the urine stored at 4°C when there was about 36% loss of GX after 1 month.

When urine standards (10 μg/ml) were stored at -20°C for 1, 3 and 6 months at pH 5.0, 7.0 and 9.0 the recovery of all three compounds was unchanged in samples stored at pH 7.0 and pH 5.0. There was a loss of about 15% of EGX and 30% of GX after 6 months with urine at pH 9.0 (Table 1 (a)).

(b) Standard curves

Linear calibration curves passing through the origin were obtained for aqueous, plasma and urine standards. Figs. 9 (a) and 9 (b) show calibration curves for plasma standards 0.1 μg/ml to 2 μg/ml and urine standards 10 to 40 μg/ml. In plasma the range of linearity was from 0.05 to 2 μg/ml and 1 to 10 μg/ml and in urine from 0.1 to 10 μg/ml and 10 to 40 μg/ml.

(c) Reproducibility and sensitivity

The method was sensitive and samples containing 0.05 μg/ml could easily be measured. The reproducibility of the assay was good. Tables 1 (b), 1 (c) and 1 (d) show standard deviations of the method at different concentrations in plasma and urine run on consecutive days.
<table>
<thead>
<tr>
<th>Urine pH</th>
<th>Duration of storage (months)</th>
<th>Lignocaine recovery %</th>
<th>EGX recovery %</th>
<th>GX recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>1</td>
<td>101</td>
<td>105</td>
<td>105</td>
</tr>
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<td>7.0</td>
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<td>98</td>
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<td>97</td>
</tr>
<tr>
<td>9.0</td>
<td>1</td>
<td>96</td>
<td>97</td>
<td>85</td>
</tr>
<tr>
<td>5.0</td>
<td>3</td>
<td>97</td>
<td>103</td>
<td>106</td>
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<td>7.0</td>
<td>3</td>
<td>106</td>
<td>100</td>
<td>109</td>
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<td>9.0</td>
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</tr>
<tr>
<td>9.0</td>
<td>6</td>
<td>94</td>
<td>85</td>
<td>68</td>
</tr>
</tbody>
</table>

Table 1 (a). Stability of lignocaine, EGX and GX added to urine (10 µg/ml) at pH 5.0, 7.0 and 9.0 and stored at -20°C for 1, 3 and 6 months.
Figure 9a. Standard curves of the peak height ratios of lignocaine/APT, EGX/APT and GX/APT plotted against the concentrations of lignocaine, EGX and GX extracted from plasma.
Figure 9b. Standard curves of the peak height ratios of lignocaine/APT, EGX/APT and GX/APT plotted against the concentrations of lignocaine, EGX and GX extracted from urine.
Table 1(b). Results of replicate simultaneous analyses of lignocaine, EGX and GX in human plasma.
<table>
<thead>
<tr>
<th>Lignocaine concentrations (µg/ml)</th>
<th>Mean peak height ratio</th>
<th>± SD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>3.5</td>
</tr>
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<td>2</td>
<td>0.55</td>
<td>3.6</td>
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<tr>
<td>5</td>
<td>1.34</td>
<td>2.2</td>
</tr>
<tr>
<td>8</td>
<td>2.07</td>
<td>1.0</td>
</tr>
<tr>
<td>10</td>
<td>2.62</td>
<td>1.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EGX concentrations (µg/ml)</th>
<th>Mean peak height ratio</th>
<th>± SD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.22</td>
<td>4.6</td>
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<td>1.06</td>
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<td>8</td>
<td>1.68</td>
<td>4.8</td>
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<tr>
<td>10</td>
<td>1.97</td>
<td>3.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GX concentrations (µg/ml)</th>
<th>Mean peak height ratio</th>
<th>± SD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.13</td>
<td>7.7</td>
</tr>
<tr>
<td>2</td>
<td>0.25</td>
<td>8.0</td>
</tr>
<tr>
<td>5</td>
<td>0.62</td>
<td>6.5</td>
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<tr>
<td>8</td>
<td>1.01</td>
<td>2.0</td>
</tr>
<tr>
<td>10</td>
<td>1.19</td>
<td>2.5</td>
</tr>
</tbody>
</table>

6 urine samples were analysed at each different concentration.

Table 1 (c). Results of replicate simultaneous analyses of lignocaine, EGX and GX in human urine.
<table>
<thead>
<tr>
<th>Lignocaine concentrations (μg/ml)</th>
<th>Mean peak / height ratio</th>
<th>± SD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1.11</td>
<td>8.0</td>
</tr>
<tr>
<td>20</td>
<td>2.00</td>
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<td>3.7</td>
</tr>
<tr>
<td>40</td>
<td>4.05</td>
<td>4.0</td>
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</table>

<table>
<thead>
<tr>
<th>EGX concentrations (μg/ml)</th>
<th>Mean peak / height ratio</th>
<th>± SD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
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<td>2.2</td>
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<tr>
<td>20</td>
<td>1.59</td>
<td>3.1</td>
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<td>30</td>
<td>2.36</td>
<td>1.7</td>
</tr>
<tr>
<td>40</td>
<td>3.27</td>
<td>3.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GX concentrations (μg/ml)</th>
<th>Mean peak / height ratio</th>
<th>± SD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.62</td>
<td>4.8</td>
</tr>
<tr>
<td>20</td>
<td>1.17</td>
<td>6.8</td>
</tr>
<tr>
<td>30</td>
<td>1.63</td>
<td>3.7</td>
</tr>
<tr>
<td>40</td>
<td>2.25</td>
<td>2.2</td>
</tr>
</tbody>
</table>

5 urine samples were analysed at each different concentration.

Table 1 (d). Urine results of replicate simultaneous analyses of lignocaine, EGX and GX in human urine.
days. For example the standard deviation for a plasma sample containing 1 µg/ml each of lignocaine, EGX and GX were 2.3%, 4.6% and 6.5% respectively. The reproducibility was less good for concentrations of EGX and GX below 0.05 µg/ml. Thus the standard deviations for EGX and GX 0.05 µg/ml in plasma were 16.7 and 20% respectively.

(d) Specificity

Drugs which are likely to be given to patients with acute myocardial infarction receiving lignocaine include atropine, cyclizine, diazepam, nitrazepam, warfarin, practolol, procainamide and morphine. None of these drugs produced interfering peaks on the chromatogram. However, mexiletine (a new anti-arrhythmic drug) gave a peak with the same retention time as lignocaine when it was acetylated. When samples containing mexiletine were assayed without acetylation there was no interference with lignocaine. Samples containing mexiletine were assayed for lignocaine first and then acetylated for the assay of EGX and GX. Antipyrine, thiopentone, procaine and mepivacaine did not interfere with the assays.

(e) Interference

Normal human plasma and urine samples (n = 16) run through the procedure did not show interfering peaks at the attenuations used for the lowest concentrations.

(f) Recovery studies

These were performed by running aqueous standards of different concentrations of the three drugs with plasma or urine standards. The percentage recovery was calculated taking the peak height ratios of /
of the aqueous standards at each concentration as 100%. The results of 24 determinations for plasma and 30 for urine are shown in Tables 2 (a) and 2 (b). In plasma the mean percentage recovery for lignocaine was 96.6 (S.D. 6.4) for EGX 86.3 (S.D. 6.1) and for GX 54.6 (S.D. 9.2). The recovery of lignocaine, EGX and GX from urine was the same as from aqueous solutions.

(g) Recovery from plasma and urine obtained from different individuals

The results of recovery studies of lignocaine, EGX and GX in plasma (1.0 μg/ml) from 12 healthy volunteers and urine (10 μg/ml) from 8 healthy volunteers are shown in Tables 3 (a) and 3 (b). There was little variation in the amounts of the three drugs recovered from different plasma samples and the standard deviations were 5.1%, 3.4% and 3.6% for lignocaine, EGX and GX respectively.

Recovery from urine was complete for all 3 compounds.

(h) Limits of detection

The limits of detection for the three compounds added to plasma were about 0.01 μg/ml for EGX and lignocaine and 0.03 μg/ml for GX. In urine and water 0.01 μg/ml of lignocaine and EGX and 0.02 μg/ml of GX could be detected.
### Table 2 (a). Recovery of lignocaine, EGX and GX from plasma relative to aqueous standards.

<table>
<thead>
<tr>
<th>Lignocaine concentrations (µg/ml)</th>
<th>Mean Recovery %</th>
<th>± SD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>93.3</td>
<td>6.8</td>
</tr>
<tr>
<td>0.5</td>
<td>97.7</td>
<td>8.2</td>
</tr>
<tr>
<td>1.0</td>
<td>98.1</td>
<td>5.9</td>
</tr>
<tr>
<td>2.0</td>
<td>97.2</td>
<td>1.9</td>
</tr>
<tr>
<td>Mean</td>
<td>96.6</td>
<td>± 6.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EGX concentrations (µg/ml)</th>
<th>Mean Recovery %</th>
<th>± SD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>91.9</td>
<td>6.2</td>
</tr>
<tr>
<td>0.5</td>
<td>84.4</td>
<td>3.8</td>
</tr>
<tr>
<td>1.0</td>
<td>86.0</td>
<td>6.5</td>
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<td>2.0</td>
<td>83.0</td>
<td>4.5</td>
</tr>
<tr>
<td>Mean</td>
<td>86.3</td>
<td>± 6.1</td>
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</table>

<table>
<thead>
<tr>
<th>GX concentrations (µg/ml)</th>
<th>Mean Recovery %</th>
<th>± SD %</th>
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</thead>
<tbody>
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<td>0.1</td>
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</tr>
<tr>
<td>Mean</td>
<td>54.6</td>
<td>4.6</td>
</tr>
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</table>

6 plasma samples and standards were analysed at each different concentrations.
<table>
<thead>
<tr>
<th>Lignocaine concentration (µg/ml)</th>
<th>Mean Recovery %</th>
<th>± SD %</th>
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<tbody>
<tr>
<td>0.5</td>
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<tr>
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<td>99.0</td>
<td>3.6</td>
</tr>
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<td>4.5</td>
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<th>EGX concentration (µg/ml)</th>
<th>Mean Recovery %</th>
<th>± SD %</th>
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</thead>
<tbody>
<tr>
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<td>6.5</td>
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<td>1.0</td>
<td>98.9</td>
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<td>4.6</td>
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<tr>
<td>Mean</td>
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<td>6.1</td>
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<th>GX concentration (µg/ml)</th>
<th>Mean Recovery %</th>
<th>± SD %</th>
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</thead>
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<td>4.9</td>
</tr>
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<td>1.0</td>
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<td>40.0</td>
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</tr>
<tr>
<td>Mean</td>
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<td>8.2</td>
</tr>
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</table>

5 urine samples and standards were analysed at each different concentration.

Table 2 (b). Recovery of lignocaine, EGX and GX from urine relative to aqueous standards.
<table>
<thead>
<tr>
<th>Sample number</th>
<th>Lignocaine recovery %</th>
<th>EGX Recovery %</th>
<th>GX Recovery %</th>
</tr>
</thead>
<tbody>
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<td>78.9</td>
<td>54.2</td>
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<td>4</td>
<td>96.7</td>
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<td>83.5</td>
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<td>53.2</td>
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<td>87.4</td>
<td>57.0</td>
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<td>12</td>
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<td>53.2</td>
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<td>Mean % recovery</td>
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<td>81.7</td>
<td>55.4</td>
</tr>
<tr>
<td>± SD %</td>
<td>5.1</td>
<td>3.4</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Table 3 (a). Recovery of lignocaine, EGX and GX (1 µg/ml) added to plasma from 12 healthy volunteers relative to an aqueous standard containing 1 µg/ml of each drug.
<table>
<thead>
<tr>
<th>Urine sample</th>
<th>Lignocaine recovery %</th>
<th>EGX recovery %</th>
<th>GX recovery %</th>
</tr>
</thead>
<tbody>
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<td>99.3</td>
<td>107.2</td>
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<td>3</td>
<td>109.0</td>
<td>103.3</td>
<td>101.4</td>
</tr>
<tr>
<td>4</td>
<td>96.0</td>
<td>94.1</td>
<td>94.3</td>
</tr>
<tr>
<td>5</td>
<td>93.0</td>
<td>101.1</td>
<td>91.4</td>
</tr>
<tr>
<td>6</td>
<td>100.0</td>
<td>98.9</td>
<td>107.2</td>
</tr>
<tr>
<td>7</td>
<td>105.1</td>
<td>99.3</td>
<td>100.0</td>
</tr>
<tr>
<td>8</td>
<td>91.4</td>
<td>103.3</td>
<td>98.6</td>
</tr>
<tr>
<td>Mean % recovery</td>
<td>99.64</td>
<td>99.1</td>
<td>100.0</td>
</tr>
<tr>
<td>± SD %</td>
<td>6.14</td>
<td>3.69</td>
<td>5.53</td>
</tr>
</tbody>
</table>

Table 3 (b). Recovery of lignocaine, EGX and GX added to urine obtained from 8 healthy subjects relative to an aqueous standard containing 10 µg/ml of each drug.
CHAPTER 3

ESTIMATION OF 4-HYDROXYXYLIDINE

A

Preliminary studies

Difficulties were encountered because 4-hydroxyxylidine was found to be extremely labile, readily adsorbed onto glass surfaces and difficult to extract into organic solvents.

(a) Stability

Acid, neutral and alkaline solutions of 4-hydroxyxylidine (500 \( \mu \text{g/ml} \)) turned deep yellow when stored at 4\(^\circ\)C for 5 days. The yellow solution contained a black precipitate and 4-hydroxyxylidine could not be extracted into organic solvents. About 10\% of the original compound could be extracted if solutions were stored in the dark at 4\(^\circ\)C. About 80\% of the compound could be recovered if stored in the dark at -20\(^\circ\)C for 5 days at pH 6. At room temperature about 80\% of the 4-hydroxyxylidine (10 \( \mu \text{g/ml} \)) disappeared in 4 h at pH 6.

(b) Solvent extraction

4-hydroxyxylidine could only be extracted into 'polar' solvents such as ethylacetate and dichloromethane.

(c) Optimum pH for extraction of 4-hydroxyxylidine, 4-amino, 3-methyl, 1-phenol and 4-amino, 3-methyl, 5-phenol

4-amino, 3-methyl, 5-phenol and 4-amino, 3-methyl, 1-phenol were studied as potential internal standards.

To 1 ml of 1 M phosphate or 0.2 M acetate buffer containing 500 \( \mu \text{g/ml} \) /
500 µg/ml each of the three aminophenols in 15 ml tubes was added 1 ml of freshly distilled dichloromethane. The pH of the buffers were 3.0, 5.3, 7.2, 7.4, 8.0 and 10.0. The tubes were shaken mechanically for 10 min and centrifuged. 3 µl aliquots of the dichloromethane extracts were injected in triplicate into the GLC. The column was packed with 3% HI EFF 8BP on Gas Chrom. Q 100/120 mesh and the oven temperature and nitrogen flow rates were 200°C and 30 ml/min respectively. The mean peak heights were plotted against pH as shown in Fig. 10.

The optimum pH for the extraction of all 3 compounds was 7.2. Compared to 4-amino, 3-methyl, 5-phenol, 4-hydroxyxylidine and 4-amino, 3-methyl, 1-phenol were poorly extracted even at the optimal pH.

(d) Chromatographic columns

A glass column packed with 3% HI EFF 8BP on Gas Chrom Q 100/120 mesh was conditioned for 48 h at 220°C and active sites removed by several 3 µl injections of BSA. 4-hydroxyxylidine adsorption on this column was minimal with direct injection of 0.1 to 1 µg. A linear plot passing through the origin was obtained when the peak height was plotted against the concentration of 4-hydroxyxylidine injected into the column (Fig. 11). The oven temperature was 200°C and the nitrogen carrier flow rate was 50 ml/min. A flame ionisation detector was used.

Prohibitive peak tailing was observed when 4-hydroxyxylidine was chromatographed directly on other columns.

(e) /
Search for the optimum pH for the extraction of 4-amino 3-methyl 1-phenol, 4-hydroxyxylidine (4-OH xylidine) and 4-amino 3-methyl 5-phenol from aqueous solutions. Dichloromethane was the organic solvent used for the extraction. The mean GLC peak height was plotted against the pH.
Figure 11. A plot of the peak height of a dichloromethane solution of 4-hydroxyxylidine against the amount (µg) of the compound injected directly into a well conditioned 3% HI EFF 8BP column which had been previously treated with BSA.
(e) Derivative formation

Initial attempts to develop an assay for 4-hydroxy-
xyridine were unsuccessful because the compound was unstable and
adsorption losses occurred. Stable derivative formation was there¬
fore tried. Acetylation with acetic anhydride, silylation with
trimethylsilylimidazole (TMSI) and methylation with trimethylanilinium
hydroxide were studied. Only acetylation and silylation gave consis¬
tent results.

(i) Acetylation

A stable derivative was produced in the presence of excess
acetic anhydride and pyridine.

(ii) Silylation

Glass vials, 36 x 14 mm diameter with capillary end-bulbs
of about 30 µl capacity (Glass Appliances Limited, Holburn Street,
Aberdeen) were flushed with nitrogen and closed with silicone rubber
caps. 10 µl of a pyridine solution of 4-hydroxyxyridine was injected
through the cap, followed by 10 µl of TMSI. The contents were mixed
and the tube was allowed to stand at room temperature for 20 min.
2 to 3 aliquots were injected into the GLC. The reaction was
apparently complete as judged by appearance of a single symmetrical
peak. The column was 3% HI EFF BP on Gas Chrom Q 100/120 mesh.
Carrier flow rate and oven temperature were 50 ml/min and 160 °C
respectively. A flame ionisation detector was used. The solvent
front was however very broad (Fig. 12). TMSI selectively silylates
-OH groups (Pierce, 1968 : Prescott, 1974).
Gas-Liquid chromatogram of 20 μg 4-hydroxyxylidine chromatographed as the trimethysilyl derivative on 3% HIEFP 8BP on Gas Chrom Q 100/120 mesh.

Figure 12. For conditions see text.
ASSAY OF UNCONJUGATED 4-HYDROXYXYLIDINE

Urine and aqueous standards containing 10, 20, 50, 80 and 100 µg/ml of 4-hydroxyxylidine were prepared. To 1 ml of standard solution in 15 ml round bottomed glass tubes was added 1 ml of 0.2 M sodium acetate buffer pH 5 and 0.1 ml of 2 M phosphate buffer pH 10.7.* The pH of this solution was adjusted to 7.2.

5 ml of freshly distilled dichloromethane containing 5 µg/ml of n-butyryl-p-aminophenol (NBA) as internal standard was added. The tubes were shaken mechanically for 15 min and centrifuged. The top aqueous layer was transferred to round bottomed tubes with pasteur pipettes. The aqueous phase was extracted again with a further 3 ml of dichloromethane containing no internal standard. After centrifuging and discarding the aqueous layer, the organic extracts were combined in a 15 ml centrifuge tube. 70 µl of acetic anhydride and 5 µl anhydrous pyridine were added to the dichloromethane extract, mixed and incubated at 45°C for 20 min in a water bath. The dichloromethane and excess acetic anhydride and pyridine were evaporated as described for lignocaine, EGX and GX. The residue was reconstituted in 25 µl of ethanol and 1 to 4 µl aliquots injected into the GLC.

Operating /

Phosphate buffer pH 10.7 was prepared by adding 2.6 ml of 5N NaOH to 50 ml of 2 molar aqueous solution of potassium hydrogen phosphate.
Operating conditions:

Glass column packed with 1% Carbowax 20M on Gas Chrom Q 80/100 mesh.

Temperatures:
- Detector 300°C
- Flash heater 280°C
- Oven 205°C

Gas flow rates:
- Nitrogen carrier 25 ml/min
- Hydrogen 50 ml/min
- Air 200 ml/min

Detector flame ionisation
Attenuation $10^2 \times 32$ to $10^2 \times 8$

The peak heights were measured and the ratio of acetylated 4-hydroxyxylidine to acetylated NBA plotted against the concentration of 4-hydroxyxylidine. Fig. 13 shows chromatograms of extracts of 4-hydroxyxylidine obtained from urine and aqueous standards.

Acetylation of 4-hydroxyxylidine was preferred to silylation because the acetyl derivatives were very stable and it was not possible to find a suitable internal standard for the silylated derivative of 4-hydroxyxylidine. Furthermore, special precautions are required for quantitative silylation (Pierce, 1968).
Figure 13. Gas liquid chromatograms of extracts of urine blank urine and aqueous standard solutions containing 20 μg/ml, 50 μg/ml and 80 μg/ml of 4-hydroxyxylidine (4-OH xyl). N-butyryl p-aminophenol (NBA) was used as the internal standard. Both 4-hydroxyxylidine and NBA were chromatographed as the acetyl derivatives.

For conditions see text.
HYDROLYSIS OF CONJUGATED 4-HYDROXYXYLIDINE

Preliminary studies showed that conjugated 4-hydroxyxylidine in urine could be hydrolysed by hydrochloric acid or a mixture of β glucuronidase and aryl sulphatase (glusulase).

(a) Acid hydrolysis

An 8 h collection of urine was made from a subject who had ingested 400 mg lignocaine hydrochloride. To 1 ml of the urine was added 1 ml 1 N hydrochloric acid. The tubes were incubated at 100° C in a water bath for ¼ h, ½ h, ⅛ h, 1 h, 2 h, 3 h, 4 h and 6 h. At the end of the incubation period each tube was cooled quickly to 4° C. 0.1 ml of 1 N NaOH was added and the pH of each tube was carefully adjusted to 7.2 with phosphate buffer pH 10.7. The free 4-hydroxyxylidine was extracted into dichloromethane containing NBA (internal standard) and chromatographed using the method described for the assay of free 4-hydroxyxylidine (Chapter 2 B). The concentration of 4-hydroxyxylidine was determined from a calibration curve and relating peak height ratio of acetyl derivatives of 4-hydroxyxylidine/NBA to the concentration of 4-hydroxyxylidine in the urine. The concentrations of 4-hydroxyxylidine were plotted against the incubation time.

(b) Enzyme hydrolysis of urine

1 ml aliquots of the same urine sample were pipetted into round bottomed tubes. To each tube was added 1 ml 0.2 M sodium acetate buffer pH 5 and 0.05 ml glusulase (Endo Laboratories, Garden City, New York, U.S.A. 1 ml contained 100,000 units of β glucuronidase and 50,000 units of aryl sulphatase). The tubes were incubated /
incubated at 37°C for 0 h, \( \frac{1}{2} \) h, \( \frac{1}{2} \) h, \( \frac{1}{2} \) h, 1 h, 2 h, 3 h, 4 h, 6 h, 10 h and 16 h. At the end of the incubation period the tubes were cooled rapidly to 4°C. The pH of each tube was adjusted to 7.2 with 0.12 ml 2 M phosphate buffer pH 10.7. After mixing the free 4-hydroxyxylidine was extracted into dichloromethane containing NBA, acetylated and chromatographed as described for the assay of free 4-hydroxyxylidine. The 4-hydroxyxylidine concentrations were determined from a calibration curve relating the peak height ratio to concentration of 4-hydroxyxylidine to urine. The 4-hydroxyxylidine concentration in each tube was plotted against the duration of enzyme hydrolysis.

(c) Results

Fig. 14 shows the results of acid and enzyme hydrolysis of urine. With acid hydrolysis, maximum 4-hydroxyxylidine concentrations were observed after two hours of incubation but the concentration decreased with longer periods of acid hydrolysis. Thus the concentration of free 4-hydroxyxylidine fell from a maximum of 20.5 \( \mu \)g/ml at 2 h to 10 \( \mu \)g/ml after 6 h.

With enzyme hydrolysis however the concentration of free 4-hydroxyxylidine formed after the optimum incubation period of \( \frac{1}{2} \) h was much higher than that after acid hydrolysis at the optimum incubation period of 2 h.

The concentration of 4-hydroxyxylidine fell from 50 \( \mu \)g/ml at \( \frac{1}{2} \) h to 5 \( \mu \)g/ml at 4 h with a disappearance half-life of about 1 h. After 6 h no free 4-hydroxyxylidine could be detected in the incubation medium.

It /
Figure 14. The hydrolysis of conjugated 4-hydroxyxylidine in urine.
It was subsequently found that the loss of 4-hydroxyxylidine could be reduced if an antioxidant such as ascorbic acid or sodium bisulphite was added.

(a) Estimation of total conjugated 4-hydroxyxylidine in urine

Enzyme hydrolysis of urine was preferred to acid hydrolysis since the amount of 4-hydroxyxylidine formed was greater. To 1 ml urine sample in round bottomed tubes was added 1 ml 2 M acetate buffer pH 5.0 and 0.05 ml glucoamylase. After mixing the tubes were incubated for 1/2 h at 37°C. In later studies a few crystals of sodium bisulphite were added to the urine before hydrolysis. Urine standards containing 50 µg/ml of 4-hydroxyxylidine were always uncubated and run with the urine samples under the same conditions. The 4-hydroxyxylidine concentration in the urine sample Cu was calculated from the following formula.

\[ Cu = \frac{50x}{y} \mu g/ml \]

where \( x \) = the peak height ratio of acetyl derivatives of 4-hydroxyxylidine to NBA, \( y \) = the peak height ratio of the urine standard (50 µg/ml).
RESULTS OF 4-HYDROXYXYLIDINE ASSAY

(a) Standard curves

A linear calibration curve passing through the origin was obtained with samples containing 10 to 100 µg/ml when the peak height ratios of acetylated 4-hydroxyxylidine and N-butyryl-p-aminophenol (NBA) were plotted against the concentration of 4-hydroxyxylidine (Fig. 15).

When 4-hydroxyxylidine standards in urine were incubated for 1/2 h at 37°C with gluconase, the range of linearity was from 10 to 80 µg/ml.

(b) Reproducibility

The reproducibility was acceptable down to 10 µg/ml but care had to be taken to ensure that the pH of all the samples was 7.2 prior to extraction. The standard deviations of replicate analyses of different concentrations of 4-hydroxyxylidine in urine are shown in Table 4. In all cases freshly prepared urine standards were extracted immediately into dichloromethane after adjusting the pH to 7.2.

(c) Sensitivity

This was limited at concentrations below 3 µg/ml by interfering peaks from the urine extracts. However, this was adequate as the urine samples usually contained much higher concentrations of 4-hydroxyxylidine.

(d) Interfering substances

At the attenuation used for the assay of samples containing
Figure 15. A standard curve of the peak height ratio of acetylated 4-hydroxyxylidine to acetylated NBA plotted against the concentration of 4-hydroxyxylidine.
5 urine samples were assayed at each different concentration.

Table 4. Results of replicate analyses of 4-hydroxyxylidine in human urine.

<table>
<thead>
<tr>
<th>4-hydroxyxylidine concentration (μg/ml)</th>
<th>Mean peak height ratio</th>
<th>± SD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.72</td>
<td>11.1</td>
</tr>
<tr>
<td>20</td>
<td>1.45</td>
<td>6.2</td>
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<tr>
<td>50</td>
<td>3.45</td>
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<tr>
<td>80</td>
<td>5.66</td>
<td>2.8</td>
</tr>
<tr>
<td>100</td>
<td>7.33</td>
<td>5.5</td>
</tr>
</tbody>
</table>
5 to 30 μg/ml, interfering peaks were not usually seen with extracts of urine samples from healthy volunteers.

(e) Specificity

The previously mentioned drugs which may be given to patients receiving lignocaine did not interfere with the assay. However acetylated paracetamol eluted very close to the 4-hydroxyxylidine peak and interfered with the assay.

(f) Recovery studies

The results of the analysis of 25 samples of urine containing known amounts of 4-hydroxyxylidine are shown in Table 5 (a). Aqueous standards were run at the same time as the urine samples. The mean recovery relative to the aqueous standards was 127% with a standard deviation of 11.2%. The reason for the higher recovery from urine is not clear.

The relative recovery of samples containing urine 50 μg/ml of 4-hydroxyxylidine obtained from 8 healthy individuals was 124% with a standard deviation of 6.7% (Table 5 (b)).
<table>
<thead>
<tr>
<th>Urine concentration (µg/ml)</th>
<th>Mean recovery %</th>
<th>± SD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>133.3</td>
<td>17.7</td>
</tr>
<tr>
<td>20</td>
<td>124.7</td>
<td>11.6</td>
</tr>
<tr>
<td>50</td>
<td>123.4</td>
<td>10.9</td>
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<tr>
<td>80</td>
<td>126.8</td>
<td>4.2</td>
</tr>
<tr>
<td>100</td>
<td>132.2</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Mean 127.6 11.2

5 urine samples and standards were assayed at each different concentration.

Table 5 (a). Recovery of 4-hydroxyxylidine added to urine relative to aqueous standards.
<table>
<thead>
<tr>
<th>Sample number</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>115.0</td>
</tr>
<tr>
<td>2</td>
<td>120.2</td>
</tr>
<tr>
<td>3</td>
<td>123.1</td>
</tr>
<tr>
<td>4</td>
<td>135.3</td>
</tr>
<tr>
<td>5</td>
<td>125.1</td>
</tr>
<tr>
<td>6</td>
<td>117.0</td>
</tr>
<tr>
<td>7</td>
<td>131.1</td>
</tr>
<tr>
<td>8</td>
<td>124.4</td>
</tr>
<tr>
<td>Mean</td>
<td>123.9 ± 6.7%</td>
</tr>
</tbody>
</table>

Table 5 (b). Recovery of 4-hydroxyxylidine (50 µg/ml) added to urine from 8 healthy volunteers relative to an aqueous standard containing 50 µg/ml of the compound.
CHAPTER 4

ESTIMATION OF ANTIPYRINE IN PLASMA

A

(a) Introduction

Antipyrine in plasma is invariably estimated by measuring the change in optical density following conversion to 4-nitroso antipyrine with nitrous acid (Brodie, Axelrod, Soberman and Levy, 1949). This method suffers from the disadvantage that readings must be accurately timed to avoid error since the absorption increases to a maximum about 20 min after the addition of nitrite and subsequently decreases. A simple gas-liquid chromatographic method was developed. The antipyrine was measured directly and the assay could be completed in a shorter time than that required for the spectrophotometric method.

(b) Procedure

To 1.0 ml of plasma containing 5 to 50 μg/ml of antipyrine in round-bottomed stoppered glass tubes was added 0.2 ml of 5 N NaOH and 1.0 ml of chloroform containing 12.5 μg of phenacetin as the internal standard. The tubes were shaken mechanically for 10 min, centrifuged and the upper aqueous phase and interface removed by aspiration. The chloroform extract was carefully decanted into a tapered centrifuge tube and the organic phase removed by placing the tubes in a water bath at 90°C for 10 min. The residue was dissolved in 20 μl of chloroform with the aid of a vortex mixer and 1 to 3 μl aliquots injected into the gas chromatograph.

Chromatographic /
Chromatographic conditions:

Column: 6 ft x $\frac{1}{4}$ in. o.d. glass "U" tube
packed with 80/100 mesh
Gas Chrom Q coated with 0.5% SE 30 plus 0.5%
Carbowax 20 M.

Temperatures:
- Column: 220°C
- Injection port: 240°C
- Flame ionisation detector: 250°C
- Nitrogen carrier gas flow rate: 50 ml/min

(c) Results

GLC method

A straight line plot passing through the origin was obtained when the peak height ratios of antipyrine to phenacetin were plotted against antipyrine concentration range 5 to 50 μg/ml.

(i) Interference

No interfering peaks were encountered with plasma extracts.

Chromatograms of plasma extracts are shown in Fig. 16.

(ii) Recovery

The recovery of antipyrine from aqueous solutions and plasma was the same and the mean standard deviation of the method for human plasma samples containing 10 to 50 μg/ml was 2.8 (Table 6). The recovery from plasma was consistently lower than that from water if extraction was carried out without the addition of sodium hydroxide.

(iii) Antipyrine in unknown samples

An aqueous standard solution containing 25 μg/ml of anti-
pyrine /
Figure 16. Gas liquid chromatograms of extracts of blank plasma (a) and plasma from a patient treated with antipyrine (b). The plasma antipyrine concentration was 18 μg/ml. Peaks A and B are phenacetin (internal standard) and antipyrine respectively.

For conditions see text.
<table>
<thead>
<tr>
<th>Plasma antipyrine concentration</th>
<th>Mean recovery %</th>
<th>SD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>101.5</td>
<td>1.7</td>
</tr>
<tr>
<td>40</td>
<td>104.2</td>
<td>1.9</td>
</tr>
<tr>
<td>30</td>
<td>100.5</td>
<td>2.5</td>
</tr>
<tr>
<td>20</td>
<td>102.3</td>
<td>4.0</td>
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<td>10</td>
<td>99.3</td>
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<tr>
<td>5</td>
<td>101.4</td>
<td>9.3</td>
</tr>
<tr>
<td>Mean</td>
<td>101.5</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Table 6. Results of replicate gas-liquid chromatographic analyses of human plasma containing added antipyrine.
antipyrine was run with the plasma samples and the drug concentration in an unknown sample U was calculated from the following formula:

\[ U = \frac{25Y}{X} \mu g/ml \]

where \( Y \) is the peak height ratio of antipyrine to phenacetin of unknown sample and \( X \) the corresponding ratio for the aqueous standard.

(iv) **Comparison with the spectrophotometric method**

Sixty-seven plasma samples obtained from 12 hospital patients at varying times after oral administration of 18 or 25 mg/kg of antipyrine were analysed by both the gas-liquid chromatographic assay and the spectrophotometric method of Brodie et al. (1949) using the precipitation procedure. The results obtained by both methods were comparable, but the spectrophotometric method generally gave a higher value (Fig. 17). The plasma antipyrine half-life was calculated in each of the 12 patients using both methods. A shorter mean half-life was obtained by gas-liquid chromatography (10.6 ± 3.9 h) than by spectrophotometry (11.4 ± 3.1 h) but the difference was not statistically significant (\( p > 0.2 \)). The mean standard deviation of the spectrophotometric method for 25 plasma samples containing 10 to 50 μg/ml of antipyrene was 7.7%. 
Figure 17. Comparison of gas-liquid chromatographic and spectrophotometric estimation of antipyrine in plasma.

67 plasma samples from 12 patients were assayed by both methods.
ASSAY OF PLASMA LIGNOCAINIE ALONE

Plasma lignocaine was assayed by the modification according to Prescott and Nimmo (1971) of Keeneghan's method (1968). Glass columns 3 or 4 ft long \( \frac{1}{4} \) in o.d. packed with 3% HI EFF 8BP on 80/100 mesh Gas Chrom Q were used. The nitrogen carrier gas flow was 60 ml/min and the oven and flame ionisation detector temperatures were 210 °C and 300 °C respectively. The standard deviation of the method was approximately 6.1% for plasma lignocaine concentration 0.05 to 10 µg/ml. The recovery from plasma was complete (98.7 ± SD of 6.6%, n = 40).
The combined assay of lignocaine, EGX, GX is sensitive, specific and has good reproducibility. The internal standard used (APT) was not ideal since it is structurally different from the compounds of interest and eluted much more rapidly than EGX and GX. In addition it did not undergo acetylation. Although it was not possible to find more suitable internal standards for these two compounds, calibration curves were linear down to 0.05 µg/ml and APT was therefore considered satisfactory.

The retention times were constant if the oven temperature was allowed to stabilise at 200°C for at least 3 min at the end of each temperature programmed run. Potential sources of error include loss of sample during the evaporation stage and incomplete derivative formation.

The main disadvantage was the time taken for the initial establishment of optimal conditions for operation of the nitrogen detector, but once these were determined there was very little day to day variation in response. Although the assay was reasonably reproducible and specific, the estimation of 4-hydroxyxylidine in biological samples can only be considered an approximation since the compound is very unstable. Other potential sources of error include loss of 4-hydroxyxylidine during the extraction and evaporation stages and incomplete derivative formation.

Since this work was started, several investigators have described /
described methods for the GLC assay of lignocaine metabolites. Di Fazio and Brown (1971) first described a method for the assay of EGX and GX in plasma by direct chromatography of chloroform extracts on 10% UCW 98 on Chromosorb W. The ranges of linearity claimed by these authors were 0.4 µg/ml to 40 µg/ml for GX and 0.08 µg/ml to 40 µg/ml for EGX. Apart from stating that GX could not be extracted quantitatively from plasma or water, few details were given. It is most unlikely that EGX and GX could be chromatographed directly on a "non-polar" liquid phase such as UCW98 without serious absorption losses. Keenaghan and Boyes (1972) extracted lignocaine and its metabolites into dichloromethane and after reaction with heptafluorobutyric anhydride, chromatographed the extracts without the use of internal standard. Again, in this study no details of the method such as reproducibility, specificity and recovery were given. Strong and Atkinson (1972) and Strong, Parker and Atkinson (1973) described a method for the assay of lignocaine, EGX and GX based on mass fragmentography after prior gas-liquid chromatography of plasma extracts using a mixed liquid phase of 3% 6:1 SE 30 : OV 17. This method is complex, demands specialised knowledge and requires very expensive instruments. Again, few details of the methods were given. The mass fragmentograms of EGX and GX showed gross tailing. In spite of the use of expensive and sophisticated instrumentation the sensitivity of the method was very poor as GX could not be measured at all at concentrations below 1 µg/ml. In addition the standard curve for EGX was non linear below 0.4 µg/ml.
All these recent methods have serious limitations and are unsuitable for accurate quantitative clinical study of lignocaine metabolism.

**Antipyrine assay**

The estimation of antipyrine in plasma by gas-liquid chromatography was mentioned recently by Shoeman, Kauffman, Azarnoff and Boulos (1972), but few details were given and an internal standard was not used. The simple assay described here is quicker than the standard spectrophotometric method and reproducibility is better. The drug is estimated directly without conversion to a derivative and the chromatographic step is completed within 3 min.

The spectrophotometric method gave a higher antipyrine plasma concentration and a slightly longer half-life than the gas-liquid chromatographic assay. The explanation is not clear. However, there is a small but variable increase in absorption when sodium nitrite is added to blank plasma, and metabolites such as 4-hydroxyantipyrine and 3-hydroxymethylantipyrine (Yoshimura, Shimeno and Tsukamoto, 1968) might cause additional interference.
SUMMARY

1. A sensitive and specific method has been developed for the simultaneous GLC assay of lignocaine, EGX and GX in human plasma and urine.

Lignocaine, EGX and GX are extracted from alkaline plasma or urine into dichloromethane and acetylated with acetic anhydride in the presence of anhydrous pyridine. Gas chromatography was carried out on a 3% HI EFF 8BP column in a Hewlett-Packard Model 402 GLC equipped with a nitrogen sensitive flame ionisation detector. APT was used as internal standard. The limits of detection in plasma were 0.01 µg/ml for lignocaine and EGX and 0.05 µg/ml for GX. The standard deviations for plasma samples containing 1 µg/ml of each drug were 2.3%, 4.6% and 6.5% respectively.

With the use of the nitrogen-sensitive flame ionisation detector, the sensitivity of the assay was increased, the solvent front signal was reduced, interference was minimised and it was possible to temperature programme using a single column without baseline drift.

2. A method has also been developed for the estimation of 4-hydroxyxylidine in urine. 4-Hydroxyxylidine is very unstable. The conjugated metabolite is hydrolysed by incubation with β glucuronidase and sulphatase (glusulase) and extracted at pH 7.2 with dichloromethane. N-butyryl-p-aminophenol is used as internal standard. The compounds were acetylated during evaporation and chromatography was carried out on a 4 ft x 6 o.d. glass column packed with 1% Carbowax 20 M/
20 M on Gas Chrom Q 50/100 mesh. The standard deviation for urine samples containing 50 µg/ml was 2.0%. The mean recovery from urine was $127.4\% \pm 11.2\%$ (SD).

3. A simple gas-liquid chromatographic method for the estimation of antipyrine in plasma is described. The drug is extracted from alkaline plasma into chloroform and after concentration is chromatographed directly using a mixed liquid phase of 0.5% SE 30 and 0.5% Carbowax 20 M on Gas Chrom Q. The standard deviation of the method for plasma containing 10 to 50 µg/ml of antipyrine was 2.8%. The GLC assay gave lower plasma antipyrine concentrations than the standard spectrophotometric method.
METHODOLOGY - PROOF OF IDENTITY OF LIGNOCaine

METABOLITES IN URINE
INTRODUCTION

The identity of lignocaine and its metabolites was established by simple indirect evidence. After an overnight fast, urine from a healthy male volunteer was collected. The subject then ingested 400 mg lignocaine hydrochloride (as two 200 mg tablets) with 40 ml of water. Urine was collected for 8 h and aliquots were used for the identification of lignocaine and its metabolites.

CHAPTER 1 THE IDENTIFICATION OF LIGNOCAIN, BGX AND GX

1. Gas-liquid chromatographic methods

0.5 ml 5N NaOH was added to 2 ml of control urine, urine passed after taking lignocaine and an aqueous solution containing lignocaine EGX and GX (10 μg/ml each). After mixing, 5 ml of dichloromethane containing APT was added. The tubes were shaken for 20 min and after centrifuging the aqueous or urine layer was discarded. The whole procedure was repeated with the addition of 25 μl of acetic anhydride and 5 μl of pyridine to the dichloromethane extracts. After incubation, the dichloromethane, acetic anhydride and pyridine were evaporated as already described. The residues were dissolved in 25 μl ethanol and 1 to 4 μl aliquots injected onto different columns in the GLC. The retention times of the peaks obtained from urine extracts which were absent from the control urine were measured and compared to those of authentic lignocaine, EGX and GX before and after acetylation.

2. Solvent extraction
2. Solvent extraction

The extraction of lignocaine and its metabolites from urine into dichloromethane, ethyl acetate and n-hexane was studied.

3. Effect of pH on extraction of drugs

2 ml aliquots of urine sample, aqueous standard containing 10 µg/ml each of lignocaine, EGX and GX or urine blank acidified with 0.5 ml of N HCl were extracted into 5 ml dichloromethane. The procedure was repeated but the samples were made alkaline with 0.5 ml 5N NaOH. The organic extracts were acetylated and chromatographed using the same conditions as stated in Section II for the assay of lignocaine, EGX and GX. APT was used as internal standard.

Chromatographic conditions

(a) Column 3% XE 60 on Gas Chrom Q 80/100 mesh. Carrier gas flow rate (Nitrogen) 25 ml/min. Oven temperature 185°C. Detector - flame ionisation. Chart speed 0.25 in/min.

(b) Column 5% SE 52 on Chromosorb G 80/100 mesh. Helium carrier flow rate 50 ml/min. Hydrogen 30 ml/min. Oven temperature 180°C. Detector - Nitrogen sensitive flame ionisation. Chart speed 0.25 in/min.

(c) (i) Column 3% HI EFF 8BP on Gas Chrom Q. The same conditions as in (b) except that oven temperature was 200°C.

(ii) For acetylated derivatives on 3% HI EFF 8BP column. The same conditions were used as described in Section II for combined lignocaine, EGX and GX assay.

(d) For acetylated derivatives on 5% SE 52 column. The same conditions as in (b) were used.
4. Thin layer chromatographic (TLC) identification. Lignocaine, EGX, GX and "cyclic metabolites" M₁ and a related compound M₂.

\[ M_1 = N-1\text{-ethyl 2 methyl-N-3(2,6 dimethylphenyl)-4-}
\text{imidazolidinone metabolite} \]

\[ M_2 = N-1\text{-ethyl-N-3(2,6 dimethylphenyl)-4-imidazolidinone} \]

Materials

Glass plates 3 mm thick measuring 20 cm x 20 cm were coated with 0.3 mm thick silica gel C (slurry prepared by mixing 30 g with 60 ml of water). The plates were activated by heating to 110°C for 20 min. Three solvent systems were used:

(i) Ether : Ethanol 8:2
(ii) Ether alone
(iii) Ethyl acetate : methanol : water : acetic acid in a proportion of 6 : 3 : 0.9 : 0.1

Procedures

(a) One dimensional thin-layer chromatographic studies

To 2 ml of urine sample and urine blank was added 0.5 ml 5N NaOH. The sample was extracted with 5 ml of dichloromethane and the procedure was repeated for nine 2 ml aliquots of the urine sample and control urine. The dichloromethane extracts were pooled and evaporated to dryness. The residue was dissolved in 30 μl of absolute ethanol. A standard ethanol solution containing 200 μg/ml each of lignocaine, EGX, GX, "cyclic metabolites M₁ and M₂" was prepared. 2.5 μl /

* E. Merck, A.G. Darmstadt, Germany
2.5 μl of the standard solution and 10 μl each of the extracts of the urine sample and blank were spotted side by side on the TLC plate. Separate solutions of each authentic compound in ethanol (200 μg/ml) were also spotted on the plate. All spots were placed along the same horizontal line about 1 in from the lower edge of the plate.

Two identical plates were prepared. One was developed in the ether : ethanol solvent and the other in ether alone. When the solvent fronts were about 2 cm from the upper edge of the silica edge, the plates were removed, dried in a stream of warm air and sprayed. The spraying agent was made up by mixing 5 μl of platinic chloride/potassium iodide solution with 50 ml 0.5% ninhydrin in isopropylalcohol. (platinic chloride/potassium iodide solution was made by dissolving 0.25 g platinic chloride and 5 g potassium iodide in 100 ml distilled water). The plates were dried in an oven at 100°C for 20 min. The Rf values of the spots were measured.

(b) Two dimensional thin-layer chromatographic studies

2 μl of sample urine and blank urine extracts and 1 μl of the mixture of reference compounds in ethanol were placed at the left lower corner on three separate chromatographic plates. The plates were placed vertically in the same tank containing ether : ethanol (8 : 2). When the solvent front was about 2 cm from the top the plates were removed and dried in a stream of warm air. They were then placed in a second tank containing ethyl acetate : methanol : water : acetic acid (6 : 3 : 0.9 : 0.1) solvent system. The second solvent moved at right angles to the first solvent system. After about /
about 90 min, the plates were removed, dried and sprayed with the ninhydrin/platinic chloride reagent. The Rf values of the spots on the two plates were measured.

RESULTS

(a) GC identification of lignocaine, EGX and GX

Gas-liquid chromatograms of urine extracts, reference compounds and blank urine on 3% XE 60 and 5% SE 52 columns are shown in Figs. 18 (a) and (b). The retention times of lignocaine, EGX and GX are shown in Table 7.

Chromatograms of the acetyl derivatives on 3% HI EFF 8BP columns are shown in Fig. 19 which also shows that the three compounds were not extracted from acidified urine or aqueous solution. The retention times of the acetyl derivatives are shown in Table 8.

(b) Solvent extraction

Authentic compounds and the compounds in urine were not extracted into n-hexane at alkaline pH.

Thin layer chromatography

The Rf values of the spots obtained from urine extracts confirmed the presence of EGX and GX in the urine after the ingestion of lignocaine hydrochloride on all plates. Fig. 20 shows the plate developed in the ether solvent system. The Rf values obtained with the two-dimensional TLC are shown in Tables 9 (a) and (b) (Fig. 21). A spot from the urine extract had the same Rf values as those of M_1 (77 : 8 and 80 : 85). In addition to lignocaine, EGX and GX there were 3 other spots which were present in the sample urine extract, but not in the blank urine. It is possible that these represent other unknown metabolites of lignocaine.
Figure 18a. For conditions see text.
GAS-LIQUID CHROMATOGRAMS OF LIGNOCaine AND ITS METABOLITES E.G.X. AND G.X. EXTRACTS FROM ALKALINE URINE. A.P.T. WAS USED AS THE INTERNAL STANDARDS.
COLUMN 5% S.E. 52 ON CHROMOSORB G 80/100 MESH.

Figure 18b. For conditions see text.
<table>
<thead>
<tr>
<th>Column</th>
<th>Temperature °C</th>
<th>Lignocaine</th>
<th>EGX</th>
<th>CX</th>
</tr>
</thead>
<tbody>
<tr>
<td>3% HI EFF 85P</td>
<td>200</td>
<td>2.9</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>5% SE 52</td>
<td>180</td>
<td>3.5</td>
<td>2.7</td>
<td>2</td>
</tr>
<tr>
<td>3% XE 60</td>
<td>180</td>
<td>5.4</td>
<td>5.9</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Table 7. Retention times of lignocaine, EGX and CX on columns of differing polarity.
Figure 19. For conditions see text.
<table>
<thead>
<tr>
<th>Column</th>
<th>Temperature °C</th>
<th>Lignocaine</th>
<th>EGX</th>
<th>GX</th>
</tr>
</thead>
<tbody>
<tr>
<td>3% HI EFF 8BP</td>
<td>Programmed 200 to 245 °C/min after 2 min delay</td>
<td>3</td>
<td>11.5</td>
<td>17.1</td>
</tr>
<tr>
<td>5% SE 52</td>
<td>180</td>
<td>3.4</td>
<td>6.9</td>
<td>6.3</td>
</tr>
<tr>
<td>3% XE 60</td>
<td>200</td>
<td>3.8</td>
<td>6.2</td>
<td>11.0</td>
</tr>
</tbody>
</table>

Table 8. Retention times of lignocaine and acetyl derivatives of EGX and GX.
Figure 20. Thin layer chromatogram of extracts of blank urine (B), and urine (U) obtained from a healthy volunteer after ingestion of 400 mg lignocaine HCl. Mix represent chromatograms of a mixture containing authentic lignocaine, EGX, GX, M2 and M1.

The chromatographic plate was developed in ether.

For conditions see text.
**Authentic reference compound**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean Rf of 3 experiments</th>
<th>Colour of spot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solvent 1</td>
<td>Solvent 2</td>
</tr>
<tr>
<td>GX</td>
<td>0.09</td>
<td>0.30</td>
</tr>
<tr>
<td>EGX</td>
<td>0.22</td>
<td>0.25</td>
</tr>
<tr>
<td>M₂</td>
<td>0.68</td>
<td>0.79</td>
</tr>
<tr>
<td>M₄</td>
<td>0.77</td>
<td>0.85</td>
</tr>
<tr>
<td>Lignocaine</td>
<td>0.80</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Table 9 (a).

**Urine Extracts**

<table>
<thead>
<tr>
<th>Spots in numbers</th>
<th>Mean Rf of 3 experiments</th>
<th>Colour of spot</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solvent 1</td>
<td>Solvent 2</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.07</td>
<td>0.28</td>
<td>Brick-red (faint)</td>
</tr>
<tr>
<td>2</td>
<td>0.10</td>
<td>0.23</td>
<td>Yellow-orange</td>
</tr>
<tr>
<td>3</td>
<td>0.12</td>
<td>0.41</td>
<td>Brick-red</td>
</tr>
<tr>
<td>4</td>
<td>0.18</td>
<td>0.22</td>
<td>Purple-red</td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
<td>0.27</td>
<td>Red</td>
</tr>
<tr>
<td>6</td>
<td>0.80</td>
<td>0.28</td>
<td>Brown</td>
</tr>
<tr>
<td>7</td>
<td>0.80</td>
<td>0.85</td>
<td>Faint red</td>
</tr>
</tbody>
</table>

Table 9 (b).

Tables 9 (a) and (b). Rf values on 2-dimensional thin-layer chromatograms.

Solvent 1 = Ether:ethanol 8:2

Solvent 2 = Ethylacetate:methanol:water:acetic acid 6:3:0.9:0.1
Figure 21. Two-dimensional thin layer chromatogram of urine extract from a healthy volunteer after ingestion of 400 mg lignocaine HCl. The plate was developed first in ether : ethanol (8 : 2) tank and then in a second tank containing ethyl acetate : methanol : water : acetic acid (6.3 : 0.9 : 0.1) solvent system. For details see text.

Lig = lignocaine. EGX = ethylglycylxylidide.
GX = glycylxylidide.
M_1 = N-1-ethyl 2 methyl - N - 3 (2,6 dimethylphenyl)-4- imidazolidinone.

? = unknown compounds.
Lignocaine was well separated from all other spots in the ether system. However, in the ether : ethanol system the drug was not well separated from M₁. A faint spot which had the same Rf value as M₂ was seen on the plate developed in ether. However, from the one-dimensional TLC studies it was not possible to confirm the presence of M₁ and M₂.
CHAPTER 2

IDENTIFICATION OF 4-HYDROXYXYLIDINE

No free 4-hydroxyxylidine could be extracted from urine at pH 7.2 into dichloromethane. Acid and gluclase hydrolysis of urine was carried out prior to extraction according to the methods described earlier.

Preliminary identification

After acid hydrolysis, the pH of the urine sample, the urine blank and a reference solution containing 20 μg/ml of 4-hydroxyxylidine was adjusted to 7.2. The 4-hydroxyxylidine in the aqueous reference solution and the urine was extracted into dichloromethane, and after evaporation, the residues were redissolved in 20 μl of chloroform. 2 μl aliquots were injected into the GLC. The retention times of peaks from the urine sample extract and standard solutions were compared.

Columns:

(i) 3% HI EFF SBP on Gas Chrom Q 100/120 mesh.
Oven temperature: 200°C.
Nitrogen carrier flow: 30 ml/min.
Chart speed: 0.25 in/min.

(ii) 5% SE 52 on Chromosorb G 80/100 mesh.
Oven temperature: 150°C.
Nitrogen carrier flow: 25 ml/min.
Chart speed: 0.25 in/min.

(iii) 10% OV 17 on Gas Chrom Q 80/100 mesh.
Oven temperature: 210°C.
Nitrogen carrier gas flow: 30 ml/min.
Chart speed: 0.25 in/min.

Derivative formation

Trimethyl silyl derivatives and acetyl derivatives of extracts from urine sample, control urine and reference aqueous solution containing 20 μg/ml 4-hydroxyxylidine were prepared as described earlier.

The TMSI derivative was chromatographed on the 3% HI EFF 8BP column. Conditions:
- Oven temperature: 170°C.
- Nitrogen carrier flow: 25 ml/min.
- Chart speed: 0.25 in/min.

The acetyl derivatives were chromatographed on the 3% HI EFF 8BP column and on a 1% Carbowax 20 M column. Conditions:

(i) 3% HI EFF 8BP on Gas Chrom Q 100/120 mesh.
- Oven temperature: 210°C.
- Helium carrier flow: 50 ml/min.

(ii) 1% Carbowax 20 M on Gas Chrom Q 80/100 mesh.
- Oven temperature 205°C.
- Nitrogen carrier flow: 25 ml/min.

Results

The retention times of 4-hydroxyxylidine and its TMSI and acetyl derivatives in the extracts of urine and aqueous solution of the authentic compound were identical. These are shown in Table 10. Fig. 22 shows chromatograms of TMSI derivatives of 4-hydroxyxylidine in urine and aqueous extracts.
<table>
<thead>
<tr>
<th>Column</th>
<th>°C</th>
<th>Absolute retention time at °C</th>
<th>Relative retention time (4 amino - 3 methyl 1 phenol = 1.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3% HI EFF 8BP</td>
<td>200</td>
<td>2.0</td>
<td>1.5</td>
</tr>
<tr>
<td>5% SE 52</td>
<td>150</td>
<td>2.5</td>
<td>1.7</td>
</tr>
<tr>
<td>10% OV 17</td>
<td>210</td>
<td>1.8</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Table 10 (a). Retention times of 4-hydroxyxylidine on columns of differing polarity.

<table>
<thead>
<tr>
<th>Derivatives</th>
<th>Column</th>
<th>Absolute retention time</th>
<th>Absolute retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TMSI</td>
<td>Acetyl</td>
<td></td>
</tr>
<tr>
<td>Column</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3% HI EFF 8BP</td>
<td>3.6 at 170°C</td>
<td>6.5 at 210°C</td>
<td></td>
</tr>
<tr>
<td>1% Carbowax 20 M</td>
<td>-</td>
<td>6.3 at 205°C</td>
<td></td>
</tr>
</tbody>
</table>

Table 10 (b). Retention times of silyl and acetyl derivatives of 4-hydroxyxylidine.
Figure 22. Gas liquid chromatogram of aqueous extract containing 4-hydroxyxylidine and an extract of urine obtained from a healthy volunteer after ingestion of 400 mg lignocaine HCl. The extracts were chromatographed as TMSI derivatives.

For conditions see text.
CHAPTER 3

OTHER METABOLITES OF LIGNOCaine

2.0 ml of urine was hydrolysed with 1 ml of 3 N HCl for 1 h at 100°C. After cooling, the pH of the urine was adjusted to 9.0 with 5 N NaOH and 2 M phosphate buffer, pH 10.7. The urine was extracted twice with 4 ml of dichloromethane. After shaking, the combined dichloromethane extracts were evaporated and the residue dissolved in 20 µl absolute ethanol. 2.0 ml of blank urine was similarly hydrolysed and extracted. 2 µl of the ethanol solution was chromatographed on a 3% HI EFF 8BP column under the following conditions:

Oven temperature: 195°C. programmed at 5°C/min to 250°C after an initial delay of 6 min.

2 µl of reference ethanol solution containing 100 µg/ml each of 2,6 xylidine, 4-hydroxyxylidine, lignocaine, 4-hydroxy lignocaine, EGX and GX were also injected under the same conditions and the retention times of the peaks measured.

Derivatives

2 µl acetic anhydride and 5 µl pyridine were added to the urine extract used in the above study. After evaporation of the acetic anhydride and pyridine, the residue was redissolved in 20 µl absolute ethanol. 2 µl aliquots were chromatographed on 3% HI EFF 8BP and 3% XE 60 columns. The oven temperature was held at 180°C for 2 min and then programmed at 5°C per min to 250°C. The reference solutions of lignocaine, acetyl derivatives of 2, 6 xylidine, 4-hydroxyxylidine, 4-hydroxy lignocaine, EGX and GX were also chromatographed.
chromatographed under the same conditions. Preliminary identification was made on the basis of the peak retention times. Further identification was not attempted.

Results

Fig. 23 shows chromatograms obtained from extracts of acid hydrolysed urine sample and urine blank before and after acetylation. The presence of 2,6 xylidine and 4-hydroxy lignocaine in urine was confirmed. Many other chromatographic peaks were present in sample urine but absent from the control urine extracts. These were judged to be probable unknown metabolites of lignocaine.

CYCLIC 'METABOLITES' OF BRECK AND TRAGER

Peaks with the same retention times as the two 'cyclic metabolites' were never seen when alkaline urine was extracted with dichloromethane as above. However, a small peak with the same retention time as M₄ (2.2 min) was seen after exhaustive extraction of large volumes of urine with ether.
GAS LIQUID CHROMATOGRAMS OF LIGNOCAINE AND ITS METABOLITES IN ACID HYDROLYSED URINE EXTRACTED AT pH 9.

A 24 HIEFF B8P glass column was used.

Figure 23a. Gas-liquid chromatogram of extracts of acid hydrolysed urine from a healthy volunteer after ingestion of 400 mg lignocaine HCl. The urine was extracted at pH 9. The peaks represent lignocaine (L), 4-hydroxyxylidine (4-OH ylidine) EGX and GX. The peaks absent from the control urine probably represent unidentified lignocaine metabolites. The numbers represent the amplifier attenuation.

For conditions see text.
Figure 23b. Gas-liquid chromatograms of authentic lignocaine, EGX and GX and of extracts of acid hydrolysed urine. The urine was extracted at pH 9. The lignocaine metabolites were chromatographed as acetyl derivatives. For conditions see text.

? probably represent unidentified metabolites of lignocaine.
DISCUSSION

These studies provide evidence that in man, metabolites of lignocaine include ethylglycyl xylidide, glycylyxylidide, 2,6-xylidine, 4-hydroxyxylidide, 4-hydroxy lignocaine and M₃. 3-hydroxy lignocaine could also have been present, but could not be separated from 4-hydroxy lignocaine under the conditions employed.

Since this work was started three reports have appeared concerning the metabolism of lignocaine in man. Breck and Trager (1971) identified lignocaine, EGX, xylidine and a 'cyclic metabolite'. N'-ethyl-2-methyl N₃(2,6-dimethylphenyl)-4-imidazolidinone in human urine after oral lignocaine administration. They were unable to detect hydroxy metabolites. According to these workers, cyclic metabolites gave the largest peak on chromatograms of an ether extract of alkaline urine. Details of the extraction procedure and gas chromatographic conditions were not provided. In the present study this 'cyclic metabolite' was not observed in dichloromethane extracts of alkaline urine, but could just be detected after the exhaustive extraction of a large volume of urine with ether.

Keenaghan and Boyes (1972) identified and quantitated several urinary metabolites of lignocaine in rats, guinea pigs, dogs and man. After oral administration of lignocaine hydrochloride, EGX, GX, 3-hydroxy EGX, 3-hydroxy lignocaine, 2,6-xylidine, 4-hydroxyxylidine and lignocaine were detected in the urine of all the species studied. Boyes (personal communication, 1973) could not detect the 'cyclic metabolite' of Breck and Trager and suggested that the cyclic metabolite /
metabolite is an artefact formed from EGX under the conditions of extraction used by Breck and Trager. Strong and Atkinson (1972) and Strong, Parker and Atkinson (1973) have detected EGX and GX in plasma and urine of patients receiving large intravenous infusions of lignocaine. Thomas and Meffin (1972) have also identified EGX and small quantities of 3-hydroxy and 4-hydroxy lignocaine in man after oral administration of lignocaine.

The results of thin-layer chromatography of alkaline urine extracts and the gas-liquid chromatography of acid-hydrolysed urine extracted at pH 9.0 suggest that metabolites of lignocaine other than those mentioned are formed in man. It was not possible to identify these 'metabolites'. Some peaks may be N-oxides of EGX and GX since it has been recently shown that N-oxidation is a major route of amine metabolism (Beckett, Mitchard and Shihab, 1971; Beckett and Al-Sarraj, 1972; Beckett, Vaughan and Essien, 1972; Mitchard, Kendall and Chan, 1972). Oxidation of the methyl groups on the aromatic ring is another possible route of metabolism.
SUMMARY

1. Lignocaine, EGX and GX were identified in dichloromethane extracts of alkaline urine following oral administration of lignocaine.

2. After acid hydrolysis, 4-hydroxyxylidine, 2,6-xylidine and 4-hydroxy lignocaine were detected when urine was extracted at pH 9.0. Several unidentified GLC peaks and TLC spots were probably other unknown lignocaine metabolites.

3. The 'cyclic metabolite' (N'-ethyl-2-methyl 4-(2,6-dimethyl-phenyl)-4-imidazolidinone) could only be detected in multiple ether extracts of large volumes of alkaline urine. It is probable that the 'cyclic metabolite is an artefact produced during extraction.
METABOLIC AND PHARMACOKINETIC STUDIES OF LIGNOCaine IN HEALTHY VOLUNTEERS
In preliminary studies in man ethylglyclylxyldide (EGX), glyclylxyldide (GX) and 4-hydroxyxylidine were found to be the most important metabolites of lignocaine from a quantitative point of view.

Lignocaine metabolism was therefore studied in healthy volunteers to determine (1) the rate of biotransformation of lignocaine, (2) the relative rates of formation and excretion of lignocaine metabolites, (3) the total amounts of lignocaine and its metabolites excreted in the urine in 48 hours and (4) the effect of route of administration on lignocaine metabolism and its excretion.
A

METABOLIC STUDIES

(a) Oral administration

After an overnight fast, 4 healthy male volunteers aged between 23 and 35 years (mean 29 years) weighing 64.9 to 74.5 kg (mean 70.9 kg) ingested two tablets each containing 200 mg of lignocaine hydrochloride together with 40 ml of water. None of the subjects had taken any drug during the preceding four weeks. They all remained sitting during the first 3 h of the study. Coffee and lunch were allowed after 2 and 4 h respectively. Urine was collected hourly for the first 10 h, then from 10 to 12, 12 to 14, 14 to 24, 24 to 36 and 36 to 48 h. No attempt was made to control urine pH by the administration of ammonium chloride or sodium bicarbonate. The urine pH and volume were measured and aliquots stored at -20°C until the time of analysis.

Venous blood was sampled at frequent intervals through an indwelling polythene catheter with a three-way tap placed in an anterior cubital vein. The plasma was immediately separated and stored at -20°C.

(b) Intramuscular administration

After an interval of 10 to 14 days the same volunteers received an injection of 200 mg of lignocaine hydrochloride in 2 ml of sterile water into the deltoid muscle, venous blood was withdrawn from the opposite arm vein and the study was continued under the same conditions as described above for oral lignocaine.
(c) Urinary excretion of lignocaine and its metabolites

Additional studies were carried out in one healthy male volunteer given an intramuscular injection of 200 mg of lignocaine hydrochloride and 7 other volunteers (6 males and 1 female) who ingested 400 mg of lignocaine hydrochloride as tablets. The experimental conditions were the same as described above except that only total 24 and 48 h urine collections were made.

(d) Analytical methods

The concentrations of lignocaine, EGX, GX and GX in plasma and urine, and of 4-hydroxyxylidine in urine were determined as described in Section II.

(e) Concentrations of lignocaine, EGX and GX between plasma and whole blood

Venous blood was taken from a healthy volunteer 1, 2 and 4 h after 400 mg of oral lignocaine. At 4 h each sample was divided and lignocaine and its metabolites in plasma and in whole blood were estimated without delay.
Pharmacokinetic Analysis

(a) Plasma and urine half-life of lignocaine and its metabolites

The plasma half-life of lignocaine and its metabolites was determined from the following formula:

\[ t^{\frac{1}{2}} = \frac{\log 2}{b} \]

where \( t^{\frac{1}{2}} \) is the plasma half-life and \( b \) the slope of the semi-logarithmic plot of plasma concentration against time. Only data points on the linear terminal portions of the plasma concentration-time curve were used. The half-life of urinary excretion of lignocaine and its metabolites was calculated similarly using the plot of log-urinary excretion rate against time (mid points of collection periods). The slope was calculated by the method of least squares.

The overall elimination of drugs following absorption and distribution usually obeys first order kinetics (Gibaldi, 1971; Goldstein, Aronow and Kalman, 1969). In such circumstances the rate of decline of plasma drug concentration is proportional to the total amount of drug in the body. If \( C_t \) is plasma concentration at time \( t \), \( C_0 \) the plasma concentration at time 0, and \( k_e \) the overall elimination rate constant then

\[ C_t = C_0 e^{-k_e t} \quad \text{(Gibaldi, 1971)} \quad (1) \]

\[ \ln C_t = \ln C_0 - k_e t \quad (2) \]

Converting to \( \log_{10} \) (2) becomes

\[ \log_{10} C_t = \log_{10} C_0 - \frac{k_e t}{2.3} \quad (3) \]
A plot of \( \log_{10} C_t \) versus \( t \) gives a straight line with a slope \( b = -\frac{ke}{2.3} \) which intercepts the \( y \)-axis at \( C_0 \).

If \( b \) is substituted in equation (3) then

\[
\log_{10} \left( \frac{C_t}{C_0} \right) = -bt \quad (4)
\]

\( t_\frac{1}{2} \) is the time taken for the drug concentration in the body to fall by 50%.

Therefore substituting \( \frac{1}{2} \) for \( \frac{C_t}{C_0} \) and \( t_\frac{1}{2} \) for \( t \), equation (4) becomes:

\[
\log_{10} \left( \frac{1}{2} \right) = -bt_\frac{1}{2} \quad (5)
\]

Therefore

\[
\log_{10} 1 - \log_{10} 2 = -bt_\frac{1}{2} \quad (6)
\]

\[
t_\frac{1}{2} = \frac{-\log_{10} 2}{b} \]

(b) Overall elimination rate constant (\( ke \))

\( ke \) is obtained from the following equation:

\[
ke = \frac{\log_e 2}{t_\frac{1}{2}} = \frac{0.693}{t_\frac{1}{2}} \quad (\text{Goldstein et al., 1969})
\]

(c) Plasma lignocaine clearance

The plasma clearance of lignocaine is the volume of plasma 'cleared' of drug per minute and can be calculated as the product of \( ke \) and \( Vd \) where \( Vd \) is the apparent volume of distribution. The apparent volume of distribution is defined as the volume in which the total amount of drug in the body would be uniformly distributed to give the observed plasma concentration.

\( Vd/ \)
\[ Vd = \frac{\text{Total amount of drug in the body}}{\text{Concentration of drug in the plasma}} \]

In this study it has been assumed that the Vd of lignocaine is 1.7 L/kg as determined by Thomson, Rowland and Melmon (1971).

(d) **Area under the plasma concentration - time curve**

Three assumptions have been made:

(i) that the elimination of lignocaine is a first order process.

(ii) that the area under the plasma concentration - time curve (plotted on arithmetic scale) was proportional to the amount of drug reaching systemic circulation (Gibaldi, 1971).

(iii) that there was complete absorption of lignocaine after intramuscular injection.

The areas under the concentration time curve (sq cm) from 0 to 6 h after administration were measured with an Albright's Planimeter. Since the oral dose was double the intramuscular dose, the percentage of orally administered lignocaine reaching the systemic circulation from 0 to 6 h is:

\[ \frac{\text{Area under the oral curve} \times 100}{\text{Area under the i.m. curve} \times 2} \]

(e) **Urinary recovery of lignocaine and its metabolites**

The urinary recovery of lignocaine and its metabolites was determined on a molar basis in relation to the administered dose of lignocaine. 400 mg of lignocaine HCl is equivalent to 346.10 mg of lignocaine base, 304.60 mg of EGX base, 263.20 mg of GX base and 202.37 mg of 4-hydroxyxylidine.
(f) **Statistical methods**

(i) Unless stated otherwise mean values are given with the standard error of the mean.

(ii) Student's t test was used for comparison of sample means. The two-tail t test was used.
CHAPTER 3

RESULTS

A PLASMA CONCENTRATIONS

(a) Lignocaine

(i) Oral administration

Oral lignocaine was absorbed rapidly and the mean individual peak concentration of $0.82 \pm 0.14 \mu g/ml$ was reached in 30 min in two subjects and 45 min in the other two. The drug disappeared from plasma with a half-life of $1.4 \pm 0.01$ h (Table 11 (a) : Fig. 24). Lignocaine could not be measured accurately in the plasma after 8 h. (concentrations < 0.01 $\mu g/ml$).

(ii) Intramuscular injection

The mean individual peak concentration was $1.25 \pm 0.20 \mu g/ml$ and this was reached in 15 min in one volunteer and at 30 min in the other three. The drug was therefore rapidly absorbed. Taking the difference in dose into account plasma concentrations were significantly higher at all times after intramuscular than after oral administration ($p < 0.05$ at all concentrations). The drug disappeared with a mean half-life of $1.6 \pm 0.04$ h (Table 11 (a) : Fig. 25). There was no statistically significant difference in the mean plasma lignocaine $t_\frac{1}{2}$ between the two routes (Table 11 (a)).

(iii) Areas under the concentration-time curve

The areas under the concentration time curves were $39.1 \pm 6.58$ sq cm and $68 \pm 11.6$ sq cm after oral and intramuscular lignocaine /
Lignocaine

<table>
<thead>
<tr>
<th>Route</th>
<th>Concentration (µg/ml)</th>
<th>Time of Peak (min)</th>
<th>Half-life (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>± 0.82 - 0.14</td>
<td>37.5 - 4.33</td>
<td>± 1.4 - 0.01</td>
</tr>
<tr>
<td>i.m.</td>
<td>± 1.25 - 0.20</td>
<td>26.3 - 3.8</td>
<td>± 1.6 - 0.04</td>
</tr>
<tr>
<td>p value</td>
<td>&lt; 0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 11 (a). NS = Not statistically significant.

EGX

<table>
<thead>
<tr>
<th>Route</th>
<th>Concentration (µg/ml)</th>
<th>Time of Peak (min)</th>
<th>Apparent Half-life (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>± 0.66 - 0.11</td>
<td>37.5 - 4.33</td>
<td>± 2.3 - 0.10</td>
</tr>
<tr>
<td>i.m.</td>
<td>± 0.18 - 0.02</td>
<td>112.5 + 7.5</td>
<td>± 3.6 - 0.3</td>
</tr>
<tr>
<td>p value</td>
<td>&lt; 0.01</td>
<td>&lt; 0.001</td>
<td>&lt; 0.02</td>
</tr>
</tbody>
</table>

Table 11 (b).

GX

<table>
<thead>
<tr>
<th>Route</th>
<th>Concentration (µg/ml)</th>
<th>Time of Peak (h)</th>
<th>Apparent Half-life (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>± 0.27 - 0.05</td>
<td>3.00 - 0.58</td>
<td>15</td>
</tr>
<tr>
<td>i.m.</td>
<td>± 0.13 - 0.01</td>
<td>5.50 - 0.50</td>
<td>-</td>
</tr>
<tr>
<td>p value</td>
<td>&lt; 0.05</td>
<td>&lt; 0.02</td>
<td></td>
</tr>
</tbody>
</table>

Table 11 (c).

* Calculated from individual values.
** Calculated from the mean curve.

Tables 11 (a), (b) and (c). Mean peak plasma concentrations of lignocaine, EGX and GX following oral (400 mg) and intramuscular (200 mg) lignocaine HCl in 4 healthy volunteers.
Figure 24. Mean plasma concentrations of lignocaine EGX and GX after oral doses of 4.00 mg of lignocaine HCl in 4 healthy volunteers.
Figure 25. Mean plasma concentrations of lignocaine EGX and GX after intramuscular doses of 200 mg of lignocaine HCl in 4 healthy volunteers.
lignocaine respectively. The difference was not statistically significant \( (p > 0.05 < 0.1) \) (Table 12). However, if the difference in dose is taken into account the area under the curve after intramuscular lignocaine was significantly higher than that after oral lignocaine \( (p < 0.01) \) \( (136 \pm 23.21 \text{ and } 39.1 \pm 6.58 \text{ sq cm after i.m. and oral lignocaine respectively}) \). If it is assumed that intramuscular absorption was complete only about 29% of the oral dose reached the systemic circulation intact during the first 6 h.

(iv) plasma clearances and elimination rate constants

These are summarised in Table 12. The mean clearances were 8.61 and 7.49 ml/kg/min after intramuscular and oral lignocaine respectively and the differences were not statistically significant. The mean elimination rate constants were 0.52 h\(^{-1}\) and 0.46 h\(^{-1}\) after intramuscular and oral lignocaine respectively and again these values were not statistically significantly different.

(b) ethylglycyxlidide (EGX)

(i) oral administration

EGX was detected in plasma following oral lignocaine in 15 min and in each individual the initial rise in its concentration paralleled that of lignocaine. The individual peak EGX concentration occurred at 30 min in two subjects and at 45 min in the other two. The mean of individual
<table>
<thead>
<tr>
<th>Route of administration and dose</th>
<th>Plasma clearance (ml/min/kg)</th>
<th>Elimination Rate constant (h⁻¹)</th>
<th>Area under concentration-Time curve (sq cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral 400 mg</td>
<td>8.61 ± 0.37</td>
<td>0.52 ± 0.02</td>
<td>68.0 ± 11.6</td>
</tr>
<tr>
<td>i.m. 200 mg</td>
<td>7.49 ± 0.70</td>
<td>0.46 ± 0.02</td>
<td>39.1 ± 6.58</td>
</tr>
<tr>
<td>p value</td>
<td>NS</td>
<td>NS</td>
<td>&gt; 0.05 &lt; 0.1</td>
</tr>
</tbody>
</table>

The volume of distribution of lignocaine in healthy volunteers was assumed to be 1.7 L/kg (Thomson, Rowland and Helmon, 1971).

Table 12. Oral and intramuscular lignocaine – Comparison of pharmacokinetic data after administration of lignocaine HCl in 4 healthy volunteers.
individual peak concentration was $0.66 \pm 0.11 \mu g/ml$. The mean apparent plasma half-life of EGX was $2.3 \pm 0.10$ (Table 11 (b)). The mean plasma concentrations are shown graphically in Fig. 24.

(ii) Intramuscular injection

Following intramuscular injection of lignocaine, EGX was detected at 30 min (Fig. 25) and the individual peak plasma concentrations were reached at 1.5 h in one subject and at 2 h in the other three. The mean of the individual peak plasma concentration was $0.18 \mu g/ml$ and this was significantly lower than the corresponding value after oral lignocaine ($p < 0.01$) (Table 11 (b)).

Taking the difference in dose into account plasma concentrations of EGX were significantly lower after intramuscular lignocaine in the first two hours ($p < 0.025$) but did not differ significantly from those obtained after oral administration during the period 2 to 6 h. The mean plasma half-life ($3.6 \pm 0.3$ h) was significantly longer than that obtained after the oral route of administration ($p < 0.02$).

(c) Glycylxylidide (GX)

(i) Oral administration

Plasma concentration data for GX are given in Table 11 (c) and shown in Fig. 24. GX was not detected in plasma until 45 min after oral lignocaine. It reached maximum concentration /
concentration at 2 h in two subjects and at 4 h in the other two. The plasma concentrations were low and the mean of the individual peak concentrations was $0.27 \pm 0.05 \mu g/ml$. The metabolite was eliminated very slowly. The plasma half-life in two subjects could not be measured as the plasma concentrations did not fall during the period of observation. In the other two the plasma half-life values were 11 and 16 h respectively.

A gas-liquid chromatogram of plasma extracts from a volunteer given oral lignocaine is shown in Fig. 26. GX was not present at 30 min and the concentrations at 5 and 8 h were similar. Lignocaine concentration had fallen from 0.89 at 30 min to 0.05 $\mu g/ml$ at 8 h.

**(ii) Intramuscular injection**

GX was not detected in plasma until 2 h after intramuscular lignocaine (Fig. 25). The plasma concentrations were consistently low and peak levels were reached at 4 h in one individual and at 6 h in the other three. The mean individual peak plasma concentration was $0.13 \pm 0.01 \mu g/ml$ and this was significantly lower than that obtained after oral lignocaine ($p < 0.05$). Taking the dose into account plasma mean GX concentrations did not differ significantly from those obtained after oral administration. It was not possible to estimate the plasma half-life of GX as blood samples were taken for only 8 h.

(d) /
Figure 26. Gas liquid chromatogram of plasma extracts from a healthy volunteer (a) ½ h (b) 5 h and (c) 8 h after ingestion of 400 mg lignocaine HCl. APT was used as internal standard. EGX and GX were chromatographed as acetyl derivatives.
(d) Distribution of lignocaine and its metabolites between blood cells and plasma

More lignocaine was extracted from plasma than from an equal volume of whole blood obtained from the same subject. Thus after an oral dose the plasma whole blood ratio measured at 1 h was 1.3 : 1 rising to 1.8 : 1 at 4 h. The ratio increased as plasma lignocaine concentrations decreased with time suggesting a delay in equilibration between plasma and red blood cells (Table 13).

In contrast, the amounts of EGX extracted from plasma and whole blood were about the same during the first two hours suggesting more rapid equilibration between red cells and plasma. The ratio increased from 1.03 : 1 to 1.21 : 1 from 1 to 4 h (Table 13). As the plasma concentration fell, more EGX was extracted from plasma than from whole blood.

The amounts of GX extracted from whole blood and plasma were essentially the same during the period of study (Table 13). The haematocrit in this subject was 49%. 

<table>
<thead>
<tr>
<th>Time after ingestion (h)</th>
<th>Concentrations (μg/ml)</th>
<th>Lignocaine a</th>
<th>Lignocaine b</th>
<th>EGX a</th>
<th>EGX b</th>
<th>GX a</th>
<th>GX b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>0.90</td>
<td>0.60</td>
<td>0.63</td>
<td>0.61</td>
<td>0.22</td>
<td>0.20</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.39</td>
<td>0.24</td>
<td>0.54</td>
<td>0.51</td>
<td>0.28</td>
<td>0.30</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.18</td>
<td>0.10</td>
<td>0.40</td>
<td>0.33</td>
<td>0.28</td>
<td>0.28</td>
</tr>
</tbody>
</table>

a = concentration in plasma  
b = concentration in whole blood

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Plasma : Whole blood ratio</th>
<th>Lignocaine</th>
<th>EGX</th>
<th>GX</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>1.3 : 1</td>
<td>1.03 : 1</td>
<td>1.1 : 1</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>1.5 : 1</td>
<td>1.06 : 1</td>
<td>0.93 : 1</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>1.8 : 1</td>
<td>1.21 : 1</td>
<td>1.0 : 1</td>
</tr>
</tbody>
</table>

Table 13. Concentration of lignocaine, EGX and GX in plasma and whole blood obtained from a healthy volunteer after 400 mg of oral lignocaine HCl.
(a) Lignocaine EGX and GX

(i) Excretion rate

The mean rates of excretion of the three compounds during the first 12 h after oral and i.m. administration are shown in Figs. 27 and 28. Lignocaine was excreted very rapidly following i.m. injection and the mean estimated urinary excretion half-life was 1.5 h. EGX was excreted with a mean half-life of 2.5 h but it was not possible to estimate the mean excretion half-life of GX since the rate actually increased during the period of study (Fig. 28). However, after oral lignocaine the mean excretion half-life of lignocaine, EGX and GX were 1.3, 2.4 and 11 h respectively. Apart from GX the excretion half-life of the compounds were essentially the same (Fig. 27).

There was considerable fluctuation in the rate of excretion of the three compounds particularly after oral lignocaine. This variation may have been due to changes in urine pH since the rate of excretion of the three compounds increased sharply whenever the urine pH fell (Fig. 29).

The excretion half-life of lignocaine was the same as its plasma half-life after both routes of administration, but after i.m. injection the excretion half-life of EGX was shorter than its plasma half-life.

(ii) Cumulative excretion

The /
Figure 27. Mean urinary excretion rate (in mg ± S.E./h) of lignocaine, G6X and GX after oral doses of 400 mg lignocaine HCl in 4 healthy volunteers.
Figure 28. Mean urinary excretion rate (in mg ± S.E./h) of lignocaine, EGX and GX after intramuscular injection of 200 mg lignocaine HCl in 4 healthy volunteers.
The rate of excretion of lignocaine, EGX and GX (mg/h) under conditions of fluctuating urinary pH.

The subject ingested 400 mg lignocaine HCl.
The cumulative excretion of lignocaine, EGX and GX following oral and intramuscular lignocaine is shown in Figs. 30 and 31.

Lignocaine excretion was virtually complete by 12 h and very small amounts were detected in the 12 to 24 h urine sample. After oral administration, less than 1% (0.53 ± 0.03%) of the dose was excreted unchanged in 48 h even though the mean urine pH was 6.18. In contrast, after i.m. administration 2.94 ± 0.9%(p < 0.05) of the dose was excreted unchanged in 48 h (Table 14).

About 4% of the dose was excreted as EGX in 48 h after oral and intramuscular lignocaine and EGX could not be detected in the urine after 36 h.

Only small amounts of GX were excreted in the urine initially, but the amount excreted gradually increased with time. It was still present in the urine in easily measurable concentration 48 h after oral and i.m. lignocaine.

The total urinary recovery of the dose of lignocaine as GX was 6.54 ± 1.4% and 11.2 ± 1.9% following oral and intramuscular administration respectively. The difference was not statistically significant (p < 0.1 > 0.05) (Table 14). There was considerable individual variation in the amount of GX excreted in 48 h and recovery ranged from 6 to 16% after intramuscular injection.

Fig. /
Table 14. 48 h urinary recovery of lignocaine and its metabolite after oral and intramuscular lignocaine HCl (400 mg and 200 mg respectively) in 4 healthy subjects.
Figure 30. Mean cumulative excretion (per cent) of lignocaine, EGX and GX after oral administration of 400 mg lignocaine HCl to 4 healthy volunteers.
Figure 31. Mean cumulative excretion (per cent) of lignocaine, EGX and GX after intramuscular injection of 200 mg lignocaine HCl to 4 healthy volunteers.

-♦-♦-♦ = lignocaine, o---o---o = EGX, x---x---x = GX
Fig. 32 shows gas-liquid chromatogram of extracts of urine obtained from a healthy volunteer at 4 to 5, 10 to 12 and 36 to 48 h after oral lignocaine. All three compounds were easily measurable at 4 to 5 and 10 to 12 h, but only GX was present in easily measurable concentration from 36 to 48 h.

(b) Urinary excretion of 4-hydroxyxylidine

(i) Rate of excretion

4-hydroxyxylidine is apparently formed rapidly since large quantities were present in urine obtained during the first hour after lignocaine administration (Fig. 33). After oral lignocaine the rate of excretion was highest during the second hour and remained relatively constant until after the fourth hour when it decreased with a mean half-life of 4 h. There was considerable variation in the rate of excretion. For example, the excretion rate during the second hour ranged from 7.63 to 19.00 mg/h after oral administration.

After intramuscular lignocaine the rate of excretion of 4-hydroxyxylidine reached a peak of 7.76 ± 2.09 mg/ml at the second hour. It fluctuated between 2 and 5 h after lignocaine administration and was subsequently excreted with a mean half-life of 3.2 h.

(ii) Cumulative urinary excretion

The maximum excretion of 4-hydroxyxylidine occurred during the /
Figure 32.

GAS CHROMATOGRAM OF EXTRACTS OF URINE FROM A HEALTHY VOLUNTEER (a) 0 to 5 hours, (b) 10 to 12 hours, (c) 26 to 48 hours after ingestion of 400 mg LIDOCAINE HCL. ACET-P-TOLUIDIDE (APT) WAS USED AS INTERNAL STANDARD. ETHYLGLYXYLIDE (EGX) AND GEYLEXYLIDE (GX) WERE CHROMATOGRAPHED AS THE ACETYL DERIVATIVES. LIG = LIDOCAINE.
Figure 33. Mean rate of excretion (mg/h) of 4-hydroxyxylidine after (a) intramuscular (b) and oral ingestion of 200 and 400 mg of lignocaine HCl respectively in 4 healthy volunteers.
the first 12 h. Thus 86.1% and 86.6% of the total amount measured was excreted in 12 h after intramuscular and oral doses of lignocaine respectively. However small amounts of 4-hydroxyxyllidine were still being excreted after 24 h (Fig. 34) but urine concentrations of the compound below 3 μg/ml could not be determined accurately because of interfering peaks.

A larger proportion of the dose of lignocaine was recovered as 4-hydroxyxyllidine in 24 h following intramuscular than oral administration. The values obtained were 52.7 ± 7.4% (range 37.3 to 71.7%) and 32.0 ± 3.6% (range 26.7 to 42.7%) respectively (p < 0.05). Fig. 35 shows gas-liquid chromatograms of acetylated 4-hydroxyxyllidine in urine extracts of one of the subjects after oral lignocaine.

Large amounts of 4-hydroxyxyllidine were excreted in the urine during the second hour (77.7 μg/ml) and during the fourth hour (65 μg/ml) after lignocaine administration.

(c) Total urinary recovery of lignocaine and its metabolites

(i) Healthy volunteers

The total urinary excretion data is summarised in Table 14. Only 42.5 ± 4.5% of the oral dose of lignocaine was recovered in the urine in 48 h as lignocaine, EGX, GX and 4-hydroxyxyllidine. However a significantly higher proportion (70.4 ± 8.4%) was recovered after intramuscular lignocaine. The difference between these values was statistically significant (p = < 0.05). It should be noted that the excretion /
Figure 34. Mean cumulative excretion (per cent) of 4-hydroxy-xylidine in 4 healthy volunteers after oral and intramuscular administration of lignocaine HCl.
Figure 35. Gas liquid chromatogram of extracts of urine from a healthy volunteer (A) 2 h and (b) 4 h after ingestion of lignocaine HCl. 4-hydroxyxylidine (4-OH xyl) and N-butyryl-p-aminophenol (NBA) were chromatographed as acetyl derivatives. The concentration of 4-hydroxylidine in the second hour and fourth hour urine samples were 77.7 µg/ml and 65 µg/ml respectively.
excretion of GX and 4-hydroxyxylidine were not complete by the end of the period of observation.

Further studies with oral lignocaine

The total 48 h urinary excretion of lignocaine and its metabolites in another group of 7 healthy volunteers taking 400 mg of lignocaine orally is shown in Table 15. Only 46.2 ± 3.3% of the total dose was recovered as lignocaine, EGX, GX and 4-hydroxyxylidine. The recovery of the 4 compounds was very similar to previous results in the 4 healthy volunteers (Tables 14 and 15). Again there was considerable individual variation in the 48 h urinary recovery of the lignocaine and its metabolites. The variation in the excretion of lignocaine, EGX and GX may in part be due to differences in urine pH. There were statistically significant negative correlations between the recovery of lignocaine (p < 0.05), EGX (p < 0.01), GX (p < 0.001) and the mean individual 24 h urine pH (Fig. 35). The 24 h total recovery of these compounds was high in subjects whose urinary pH was low. There was no significant correlation between the recovery of lignocaine, EGX and GX and the 24 h urine volume.
### Table 15. 4.8 h urinary recovery of lignocaine and its metabolites after oral lignocaine HCl (400 mg) in 7 healthy volunteers.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Urine pH</th>
<th>Percentage of dose recovered as:</th>
<th>Total recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lignocaine</td>
<td>EGX</td>
</tr>
<tr>
<td>1</td>
<td>5.8</td>
<td>1.68</td>
<td>6.1</td>
</tr>
<tr>
<td>2</td>
<td>6.0</td>
<td>1.21</td>
<td>4.2</td>
</tr>
<tr>
<td>3</td>
<td>5.8</td>
<td>0.60</td>
<td>6.0</td>
</tr>
<tr>
<td>4</td>
<td>6.2</td>
<td>0.49</td>
<td>5.4</td>
</tr>
<tr>
<td>5</td>
<td>6.1</td>
<td>0.71</td>
<td>2.9</td>
</tr>
<tr>
<td>6</td>
<td>6.8</td>
<td>0.70</td>
<td>2.9</td>
</tr>
<tr>
<td>7</td>
<td>6.6</td>
<td>0.33</td>
<td>2.0</td>
</tr>
<tr>
<td>Mean</td>
<td>6.18</td>
<td>0.82</td>
<td>4.2</td>
</tr>
<tr>
<td>± SE</td>
<td>0.14</td>
<td>0.18</td>
<td>0.6</td>
</tr>
</tbody>
</table>
The relation between pH and lignocaine, EGX and GX recovered. n = 12 subjects.

Figure 36. The relation between urine pH and the recovery (percent) of lignocaine, EGX and GX in 12 healthy volunteers. Seven received 400 mg lignocaine HCl orally (a) and 5 received 200 mg lignocaine HCl intramuscularly (c).
SUMMARY OF RESULTS

1. Lignocaine was rapidly absorbed after oral and intramuscular administration and it was extensively metabolised. Only between 0.3 and 5% of the dose was excreted unchanged 48 h after administration.

About 30% of an oral dose of 400 mg of lignocaine reached the systemic circulation intact during the first 6 h. The plasma half-life of the drug was about 1.5 h.

2. EGX is apparently formed rapidly since it was detected in plasma within 15 min of ingestion of lignocaine. The plasma half-life of EGX was between 2.3 and 3.5 h.

About 4% of the dose of lignocaine (intramuscular or oral) was excreted in the urine as EGX in 48 h and renal excretion was pH dependent. The mean excretion half-life was 2.5 h after intramuscular administration.

3. GX appeared in plasma more slowly reaching a maximum concentration of between 0.11 and 0.34 μg/ml in 2 to 6 h. GX was eliminated very slowly with a plasma half-life of about 15 h.

The urinary excretion of GX was pH dependent and accounted for 3.6 to 16% of the administered dose of lignocaine.

4. Lignocaine and EGX could not be measured in the urine after 24 and 36 h respectively. In contrast GX could still be easily measured at 48 h.

5. /
5. Conjugated 4-hydroxyxylidine appeared in the urine rapidly. Between 26.7 and 72% of the dose of lignocaine was excreted in the urine in 24 h as 4-hydroxyxylidine. The half-life of urinary excretion was 3.2 to 4 h.

6. Lignocaine, EGX, GX and 4-hydroxyxylidine accounted for about 42 and 70% of the dose of lignocaine after oral and intramuscular administration respectively.

7. The proportion of the dose recovered as lignocaine, GX and 4-hydroxyxylidine was higher after intramuscular than after oral lignocaine.
A Comparisons with previous studies

(a) Plasma lignocaine concentrations

The present pharmacokinetic data are in good agreement with previously reported studies. These are summarised in Table 16. For example, estimates of plasma lignocaine half-life have ranged from 87 to 108 min and the values obtained in the present study were 96 and 84 min following intramuscular and oral lignocaine respectively.

After an oral dose of 500 mg of lignocaine hydrochloride, peak plasma lignocaine concentrations of 0.6 to 1.1 µg/ml were obtained between 45 and 60 min (Boyes, Scott, Jebson, Godman and Julian, 1971). These values compare with corresponding concentrations of between 0.54 and 1.00 µg/ml at 30 and 45 min observed in the present studies. Eisenger and Hellier (1969) however, recorded a peak plasma lignocaine concentration of 2.4 µg/ml in one subject at 30 min after an oral dose of 500 mg lignocaine. On the other hand, Parkinson, Margolin and Dickinson (1970) claimed to have achieved plasma concentrations from 5 to 16 µg/ml after 250 and 500 mg of lignocaine. These workers used the non-specific methyl orange method which presumably also measured metabolites and possibly other drugs taken at the same time. Their findings must be discounted.

There is no published data concerning the plasma concentrations of EGX and 6X in healthy volunteers receiving lignocaine.

(b) Urinary excretion of lignocaine and its metabolites

Published data on the urinary excretion of lignocaine and its
<table>
<thead>
<tr>
<th>Authors</th>
<th>Route and dose</th>
<th>ke - 1 h (± SD %)</th>
<th>Clearance (ml/min/kg) (± SD %)</th>
<th>t1/2 min (± SD %)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rowland, Thomson, Guichard and Melmon (1971)</td>
<td>i.v. bolus (100 mg)</td>
<td>0.0076 ± 12.5</td>
<td>9.94 ± 20</td>
<td>92.3 ± 20</td>
<td>5</td>
</tr>
<tr>
<td>Thomson, Rowland and Melmon (1971)</td>
<td>i.v. (50 mg)</td>
<td>-</td>
<td>9.2 ± 8</td>
<td>108 ± 6.5</td>
<td>10</td>
</tr>
<tr>
<td>Boyes, Scott, Jebson, Godman and Julian (1971)</td>
<td>i.v. infusion (250 mg/h)</td>
<td>0.008</td>
<td>87 ± 5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Adjepo-Yamoah (1973)</td>
<td>i.m. (200 mg)</td>
<td>0.0076 ± 8</td>
<td>7.49 ± 9.3</td>
<td>96 ± 5.0</td>
<td>4</td>
</tr>
<tr>
<td>Adjepo-Yamoah (1973)</td>
<td>oral (400 mg)</td>
<td>0.0086 ± 18.6</td>
<td>8.61 ± 7.4</td>
<td>84 ± 14.9</td>
<td>4</td>
</tr>
</tbody>
</table>

The Vd of lignocaine was assumed to be 1.70 L/kg (Thomson et al., 1971).

Table 16. Comparison of plasma lignocaine kinetics.
its metabolites in healthy volunteers is summarised in Table 17. There is reasonable agreement in the recovery of EGX. However, there is an eleven-fold difference in the recovery of lignocaine, presumably due to effects of dose, route of administration and urine pH. The recoveries of GX and 4-hydroxyxylidine also vary considerably.

Keenaghan and Boyes (1972) are the only investigators to attempt a comprehensive quantitation of total 24 h urinary excretion of lignocaine and its metabolites. Following oral lignocaine (250 mg) Keenaghan and Boyes claimed to have recovered about twice as much 4-hydroxyxylidine and four times as much lignocaine in 24 h as in the present study. On the other hand the recovery of GX was about half of that observed in the present study. Keenaghan and Boyes observed small quantities of the dose of lignocaine excreted as 3-hydroxy-lignocaine (1.1%), 3-hydroxy EGX (0.3%) and 2,6, xylidine (1.0%). The observed discrepancies between the results of Keenaghan and Boyes may be due to many factors.

These authors studied only two subjects. Clearly studies carried out in only 2 volunteers are unlikely to be representative of the general population because of the great individual variation in the pattern of urinary recovery of lignocaine and its metabolites. No details of the urine pH were given and the precision of the methods was not stated. In addition, no internal standard was used in the assay of 4-hydroxyxylidine.

The rate of metabolism may be dose-dependent. Keenaghan and Boyes administered 250 mg whilst the present author gave 400 mg lignocaine hydrochloride orally.

Unconjugated /
<table>
<thead>
<tr>
<th>Route</th>
<th>Dose (mg)</th>
<th>N</th>
<th>Metabolites</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Route</td>
<td>Dose (mg)</td>
<td>N</td>
<td>Metabolites</td>
</tr>
<tr>
<td>Beckett, Boyes and Appleton (1966) (12 h recovery)</td>
<td>i.v. 50&quot;</td>
<td>3</td>
<td>6.1</td>
<td>4.2</td>
</tr>
<tr>
<td>Keenaghan and Boyes (1972)</td>
<td>Oral 250</td>
<td>2</td>
<td>2.8</td>
<td>3.7</td>
</tr>
<tr>
<td>Thomas and Meffin (1972)</td>
<td>Oral 100</td>
<td>3</td>
<td>0.86</td>
<td>2.8</td>
</tr>
<tr>
<td>Present studies</td>
<td>Oral 400&quot;</td>
<td>7</td>
<td>0.82</td>
<td>4.21</td>
</tr>
<tr>
<td>&quot;</td>
<td>Oral 400&quot;</td>
<td>4</td>
<td>0.5</td>
<td>4.00</td>
</tr>
<tr>
<td>&quot;</td>
<td>i.m. 200&quot;</td>
<td>4</td>
<td>2.49</td>
<td>3.93</td>
</tr>
</tbody>
</table>

* as base, urine acidified with NH₄Cl. **" 48 h urine recovery.

Table 17. 24 h urine recovery of lignocaine and its metabolites in man expressed as a percentage of dose.
Unconjugated 4-hydroxyxylidine is very unstable and this presumably explains why it was not detected by Hollunger (1960b) or by Breck and Trager (1971). The conditions of hydrolysis of unconjugated 4-hydroxyxylidine and extraction of free 4-hydroxyxylidine employed by Keenaghan and Boyes may have resulted in a greater recovery. This seems unlikely since in the present study enzyme hydrolysis yielded larger amounts of 4-hydroxyxylidine than the acid hydrolysis used by Keenaghan and Boyes.

The methods employed by the present author were unsuitable for the quantitation of 2,6-xylidine, 3 or 4-hydroxy lignocaine and 3 or 4-hydroxy EGX.
SITES OF LIGNOCAINE METABOLISM

(a) The liver

Lignocaine is probably metabolised by the liver (Boyes, Scott, Jebson, Godman and Julian, 1971). Harrison and Alderman (1971) claimed that lignocaine was metabolised to the extent of 70 ± 16% by the liver in 17 patients undergoing cardiac catheterisation. This assumption was based on the hepatic extraction of lignocaine. These workers assumed (incorrectly) that hepatic arterial-venous differences in blood lignocaine concentrations were due to hepatic metabolism. The possibility of hepatic uptake and storage of the drug was not considered. A serious criticism of this work is that "steady state" lignocaine concentrations were assumed to have been reached within 2 h of starting a constant intravenous lignocaine infusion. In fact a constant infusion must be maintained for at least 6 h in healthy volunteers to achieve 95% of the steady state plasma concentration (Boyes, Scott, Jebson, Godman and Julian, 1971).

Many lipid soluble drugs are metabolised by hepatic microsomal enzymes (Parke, 1968; Gillette, 1971; Williams, 1971; Mannering, 1972; Davies and Thorgeirsson, 1971). According to Hollunger (1960 a, b, c) lignocaine is metabolised by rabbit hepatic microsomal enzymes and the same system is presumably responsible for the biotransformation of the drug in man. Hepatic microsomal reactions of possible relevance to lignocaine metabolism include aromatic hydroxylation, aliphatic hydroxylation, N-dealkylation, N-oxidation and conjugation.

(b) Other possible sites of lignocaine metabolism

Other /
Other possible sites of lignocaine metabolism include the gut lumen (Bacterial flora), the gut wall and the kidneys (Åkerman, Åström, Ross and Tele, 1966; Åström, 1971). Gut bacteria may metabolise certain drugs such as cyclamate (Williams, 1971). However, lignocaine was not appreciably metabolised by human gut bacteria (Adjepon-Yamoah, Nimmo, Parkinson - unpublished observations). Bacterial \( \beta \)-glucuronidase may also hydrolyse conjugated drugs and metabolites entering the gut through biliary excretion giving rise to an entero-hepatic circulation of the compounds. Stilboestrol may be cited as an example (Williams, 1971).

After oral and intravenous administration of radioactive (tritiated) lignocaine to rats, peak intestinal radioactivity was observed at 2 and 4 h respectively (Keenaghan and Boyes, 1972). These authors postulated an entero-hepatic circulation of lignocaine metabolites and such a mechanism could play some role in the elimination of hydroxylated lignocaine and its metabolites.

According to Dollery, Davies and Conolly (1971) 90% of oral dose of isoprenaline may be conjugated in the gut wall during absorption. It is possible that oral lignocaine could be extensively metabolised by some of the mechanisms outlined above.
As judged by the appearance of lignocaine metabolites in the plasma and urine, a probable sequence of lignocaine metabolism can be proposed (Fig. 37). It seems likely that lignocaine is first fairly quickly de-ethylated to EGX since both compounds reached peak plasma concentrations at the same time in each individual. Part of the EGX formed then probably undergoes rapid amide hydrolysis to form 2,6-xylidine (Hollunger, 1960b) which in turn is hydroxylated and conjugated. Thus maximum excretion of 4-hydroxyxylidine occurred within 2 to 4 h of lignocaine administration. Conjugated 4-hydroxyxylidine was quantitatively the most important end product of lignocaine biotransformation. GX appeared in plasma slowly and peak concentrations were observed at a time when lignocaine concentrations were falling rapidly. It is therefore reasonable to assume that EGX is slowly de-ethylated to form GX which is probably not extensively metabolised since it has a long apparent biological half-life and its excretion continued long after EGX has become undetectable in urine.

It is possible that 4-hydroxyxylidine could be formed partly from lignocaine, EGX or GX after hydroxylation of the aromatic ring.
Figure 37. A scheme of the metabolism of lignocaine in man.

The compounds which are underlined have been identified in the present study. The arrows indicate the proposed sequence of formation of lignocaine metabolites.
Quantitative and qualitative differences in lignocaine metabolites were observed in the volunteers given the drug orally and intramuscularly suggesting route-dependent metabolism. Thus the total urinary recovery of lignocaine and its metabolites was higher and the proportion of the dose recovered as lignocaine, GX and 4-hydroxyxylyidine was greater after intramuscular than after oral administration. There are several possible explanations. Absorption from the gastrointestinal tract could have been incomplete. However, the speed of absorption as judged by the rapid rise in plasma concentration (Fig. 24) makes this unlikely. In addition, lignocaine is completely absorbed from the gut in dogs (Boyes and Keenaghan, 1971).

It has been suggested that biliary excretion of a drug is facilitated if the molecular weight exceeds 300 and if there are polar groups (Plaa, 1972). If conjugated hydroxy metabolites of lignocaine are formed with molecular weights greater than 300 biliary excretion may be expected. It is possible that this route of excretion may be greater after oral than after parenteral lignocaine since metabolism in the gut may occur. Route-dependent metabolism of isoprenaline and propanolol occurs (Dollery, Davies and Conolly, 1971). Drugs absorbed from the gastrointestinal tract reach the systemic circulation after first passing through the portal circulation. The hepatic extraction ratio of lignocaine is very high (about 60 - 70%) and in such circumstances metabolism /
metabolism is likely to be different after oral and parenteral administration. Oral lignocaine is apparently metabolised extremely rapidly. In the present work, plasma lignocaine concentrations were very low after oral administration, and it was calculated that about 30% of the dose entered the systemic circulation during the first 6 h. This value compares with 34.8% calculated by Boyes and Keenaghan (1971).

The metabolism of drugs such as dicoumarol, probenecid, phenylbutazone and diphenylhydantoin is dose-dependent (Dayton and Perel, 1971) presumably due to saturation or self inhibition of drug metabolising enzymes. The oral dose of lignocaine in the present study was twice that of the intramuscular dose. In addition lignocaine and its metabolites may also compete for the same enzymes. For example, Hollunger found that lignocaine competitively inhibited the amide hydrolysis of EGX (Hollunger, 1960c) and there could also have been competition between lignocaine, EGX and GX for N-dealkylation.

The higher urinary recovery of lignocaine after intramuscular injection is probably related directly to the higher plasma concentration. Similarly, the greater recovery of GX after intramuscular lignocaine may be explained by the slower elimination of its precursors, i.e. lignocaine and EGX. However, this does not account for the similar recovery of EGX after both routes. The differences are unlikely to be due to changes in urine pH. It is not known why the urinary excretion of 4-hydroxyxylidine was route dependent.
E. CONCLUDING REMARKS

EGX and GX are excreted more slowly than the parent drug. This may be due to larger volumes of distribution and or decreased plasma clearance and slow release from the tissues. The greater plasma : whole blood ratio of lignocaine compared with EGX and GX is consistent with larger Vd of these metabolites. EGX and GX have about 60% of the antiarrhythmic activity of lignocaine and similar central nervous system toxicity (Section I). Cumulation of these active metabolites may therefore be very relevant to therapeutic and toxic effects during prolonged infusion of lignocaine. Drug metabolism is often stated to result in the formation of more polar derivatives which have little or no pharmacological activity and are rapidly excreted. These generalisations are only partly true of GX and EGX.
THE METABOLISM OF LIGNOCaine AND ANTIpyRINE FOLLOWING
ANAESTHESIA IN SURGICAL PATIENTS
CHAPTER 1

LIGNOCaine METABOLISM IN LAPAROSCOpy PATIENTS

INTRODUCTION

Lignocaine is often administered to patients before or during surgery to control ventricular arrhythmias or to produce local analgesia yet nothing is known about the effect of anaesthesia and surgery on the disposition of the drug.

In a study in which lignocaine was administered orally for the prophylaxis of ventricular arrhythmias in 6 patients undergoing laparoscopy, Prescott, Benton and Scott (quoted by Prescott, 1971) noted that plasma lignocaine concentrations were higher 4 h after the administration than in unanaesthetised volunteers. It was suggested that halothane modified liver function and reduced the rate of lignocaine metabolism. These workers, however, studied the patients for only 4 h and it is possible that the high plasma concentrations of lignocaine were due to continuing absorption from the gut. The metabolism of lignocaine in laparoscopy patients was therefore studied in greater detail. Since halogenated hydrocarbon anaesthetic agents such as halothane have been alleged to be hepatotoxic (Kirkman, Dundee, Clarke, Mitchell and Neil, 1965; Brodie et al 1971; Vorne and Arvela, 1971) control studies of lignocaine disposition were carried out in healthy unanaesthetised volunteers and in patients undergoing laparoscopy with and without halothane anaesthesia.
METHODS

Healthy volunteers

After an overnight fast, 7 healthy ambulant unanaesthetised volunteers (4 males and 3 females) aged between 22 and 37 years, ingested two tablets each containing 200 mg lignocaine hydrochloride together with 40 ml of water. Coffee was taken 2 h later and lunch was allowed after 4 h. Venous blood was sampled at frequent intervals through a polythene annula in an anterior cubital vein connected to a three-way tap. The patency of the vein and cannula was maintained by flushing with 2 ml of saline containing 10 units of heparin after each sample was withdrawn.

Patients

Twelve healthy patients aged between 22 and 39 years (mean 30 years) scheduled for laparoscopy and tubal diathermy for sterilisation volunteered for the study. Except for 5 patients who had been on oral contraceptives none of the patients gave a history of regular drug intake and none had any past history or clinical evidence of hepatic or renal, cardio-vascular or gastrointestinal disease.

The patients received oral lignocaine as described above for healthy volunteers 0.5 to 2 h (mean 1.2 h) before induction of anaesthesia. Venous blood was sampled at intervals for 12 h.

Anaesthesia and laparoscopy

Premedication consisted solely of 0.6 mg atropine sulphate given 1 h preoperatively. No narcotic was given to any of the patients preoperatively or postoperatively. Induction was carried out with thiopentone 400 mg intravenously. In 6 patients gallamine 60 mg was given and anaesthesia was maintained with halothane 2% and a mixture /
mixture of nitrous oxide (3 litres/min) and oxygen 1 litre/min) in a semiclosed system incorporating a carbon dioxide absorber. Respiration was spontaneous throughout. The other 6 patients received gallamine 80 mg, halothane was omitted and respiration was controlled using intermittent positive pressure.

Laparoscopy was performed after insufflation of 2 to 3 litres of carbon dioxide through a Veres needle into the peritoneal cavity. The resulting abdominal pressure was in the range of 15 - 20 cm H₂O. The laparoscope was introduced through a sub-umbilical incision 1.5 cm long and sterilization was carried out by diathermy of the fallopian tubes through a separate incision (Fig. 38).

The duration of carbon dioxide insufflation of the peritoneal cavity lasted 4 to 5 min and the duration of anaesthesia varied from 8 to 15 min. There were no operative complications. All patients were ambulant 3 h after laparoscopy and were discharged home the following day.

**Plasma lignocaine**

Plasma was stored at -20°C until the time of analysis.

Lignocaine was estimated as described in Section II.

**Statistical analyses** were carried out as in Section IV.
Figure 38. The procedure of laparoscopy. The laparoscope is introduced in the first incision (A) and the diathermy instrument is inserted into the second incision (B).
RESULTS

There were no consistent differences between the mean plasma lignocaine concentrations in the two groups of anaesthetised patients and their data have therefore been pooled for comparison with the control subjects (Table 18).

Lignocaine absorption was much slower and the drug disappeared from the plasma more slowly in the patients than in the healthy volunteers (Figs. 39 and 40). Thus the mean peak plasma lignocaine concentration occurred at 45 min in the healthy volunteers as compared with 3 h in the patients. However, the means of the individual peak plasma lignocaine concentrations were similar in volunteers and anaesthetised patients (Table 19). The estimated mean plasma lignocaine half-life was 1.3 h in control subjects and 2.8 in the patients.

After 9 h the half-life was prolonged to 4.8 h in the patients undergoing laparoscopy.

There was no correlation between the individual plasma lignocaine half-life values and the duration of anaesthesia. However, there was a negative correlation between the calculated individual apparent plasma lignocaine half-life and the time of start of anaesthesia after lignocaine ingestion (Fig. 41). The correlation coefficient was $-0.54$ ($p > 0.05 < 0.10$).

Although mean plasma lignocaine concentrations were much higher in healthy volunteers up to 2 h, the concentrations were much higher in the patients at all times after 2 h (Table 19 and Figs. 39 and 40). For example, at 6 h the mean plasma lignocaine concentration in laparoscopy patients was more than five times higher than that in healthy volunteers (Table 19 and Figs. 39 and 40).
<table>
<thead>
<tr>
<th>Time after ingestion (h)</th>
<th>0.25</th>
<th>0.5</th>
<th>0.75</th>
<th>1</th>
<th>1.25</th>
<th>1.5</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>With halothane</td>
<td>0.06</td>
<td>0.12</td>
<td>0.19</td>
<td>0.18</td>
<td>0.22</td>
<td>0.23</td>
<td>0.28</td>
<td>0.39</td>
<td>0.21</td>
<td>0.10</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>±0.03</td>
<td>±0.03</td>
<td>±0.01</td>
<td>±0.24</td>
<td>±0.07</td>
<td>±0.06</td>
<td>±0.06</td>
<td>±0.13</td>
<td>±0.07</td>
<td>±0.03</td>
<td>±0.02</td>
<td>±0.01</td>
<td></td>
</tr>
<tr>
<td>Without halothane</td>
<td>0.05</td>
<td>0.16</td>
<td>0.08</td>
<td>0.22</td>
<td>0.24</td>
<td>0.24</td>
<td>0.28</td>
<td>0.34</td>
<td>0.30</td>
<td>0.21</td>
<td>0.08</td>
<td>0.06</td>
</tr>
<tr>
<td>±0.05</td>
<td>±0.04</td>
<td>±0.09</td>
<td>±0.08</td>
<td>±0.09</td>
<td>±0.09</td>
<td>±0.07</td>
<td>±0.05</td>
<td>±0.06</td>
<td>±0.02</td>
<td>±0.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Number of observations are given in parentheses

Table 18. Mean plasma lignocaine concentrations (µg/ml ± SE) in laparoscopy patients anaesthetised with and without halothane.
<table>
<thead>
<tr>
<th></th>
<th>Mean of individual peak concentrations</th>
<th>Mean concentrations at h</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy volunteers</td>
<td>0.67 ± 0.04</td>
<td></td>
<td>0.51</td>
<td>0.52</td>
<td>0.31</td>
<td>0.18</td>
<td>0.04</td>
</tr>
<tr>
<td>Laparoscopy patients</td>
<td>0.55 ± 0.07</td>
<td></td>
<td>0.14</td>
<td>0.20</td>
<td>0.29</td>
<td>0.47</td>
<td>0.21</td>
</tr>
<tr>
<td>P value</td>
<td>N.S.</td>
<td>&lt;0.001</td>
<td>N.S.</td>
<td>&lt;0.001</td>
<td>N.S.</td>
<td>&lt;0.02</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

N.S. = Not significant.

Table 19. Plasma lignocaine concentrations (µg/ml ± SE) in 12 laparoscopy patients and 7 healthy volunteers.
Mean plasma lignocaine concentrations after ingestion of 400mg lignocaine hydrochloride in 12 laparoscopy patients and 7 healthy volunteers.

Figure 39. Mean plasma lignocaine concentrations after ingestion of 400 mg lignocaine HCl in 12 laparoscopy patients and 7 unanaesthetised healthy volunteers.
Rate of fall in plasma concentrations of lignocaine following an oral dose in 12 laparoscopy patients and 7 healthy volunteers.

Figure 40. Rate of fall of plasma concentration of lignocaine following oral dose of 400 mg in 12 laparoscopy patients and 7 healthy volunteers.
Figure 4.1. The relation between apparent plasma lignocaine half-life and the time of starting anaesthesia after ingestion of 400 mg lignocaine HCl in 12 laparoscopy patients.
SUMMARY

Lignocaine absorption is slowed and its elimination impaired in laparoscopy patients anaesthetised with or without halothane.
CHAPTER 2  EFFECT OF ATROPINE ON THE ABSORPTION OF ORALLY ADMINISTERED LIGNOCaine IN HEALTHY VOLUNTEERS AND IN ANAESTHETISED PATIENTS

INTRODUCTION

In the previous Chapter it was found that lignocaine absorption was delayed in laparoscopy patients and that the drug disappeared from the plasma abnormally slowly. A possible explanation for the delayed disappearance of the drug from plasma is continuing absorption from the gut. Absorption could be slowed if gastric secretion and motility were decreased thereby decreasing the rate of tablet disintegration and dissolution. A delay of gastric emptying could also slow down lignocaine absorption. Drugs are poorly absorbed from the stomach but are usually absorbed much more rapidly from the intestine. This applies even to ethanol and acidic drugs such as aspirin, warfarin and barbiturates (Magnussen, 1968; Siurala, Mustala and Jussila, 1969; Kekki, Pyörälä, Mustala, Salmi, Jussila and Siurala, 1971; Kojima, Smith and Doliusio, 1971). Heading, Nimmo, Prescott and Tothill (1973) have shown that the rate of gastric emptying may have marked effects on the rate of absorption on orally administered drugs. The anticholinergic drug propantheline delays gastric emptying and slows paracetamol absorption in man, while metoclopramide, a drug which stimulates gastric emptying, increases the rate of paracetamol absorption (Nimmo, Heading, Tothill and Prescott, 1973). Propantheline also delays the absorption of riboflavin (Levy, Gibaldi and Procknal, 1972).

Since /
Since all the previously described laparoscopy patients had been premedicated with atropine only, further studies were carried out in healthy volunteers and in laparoscopy patients to determine the effect of atropine on the absorption of lignocaine and its disappearance from the plasma.

METHODS

Healthy volunteers

Four healthy ambulant unanaesthetised male volunteers aged between 27 and 35 years (mean 31 years) received 400 mg lignocaine hydrochloride orally. The procedure for lignocaine administration and blood sampling was as described in the previous study. One week later the administration of lignocaine hydrochloride was repeated in the same subjects, but 0.6 mg atropine sulphate was injected intramuscularly into the gluteal region at the same time as the lignocaine was taken.

Patients

Twelve patients aged between 22 and 48 years (mean 31 years) with no previous history of hepatic, renal, gastrointestinal or cardiovascular disease, scheduled for laparoscopy were studied. All the patients were in good health at the time of the study and none gave a history of regular intake of drugs. Premedication consisted of atropine only. The patients were divided into two equal groups.

Six patients ingested 400 mg lignocaine hydrochloride as described above one hour before premedication with 0.6 mg atropine sulphate given intramuscularly into the gluteal region. Induction of anaesthesia /
Anaesthesia was started approximately two hours (mean 2.2 range 1.8 - 2.8 h) after the lignocaine was taken.

The other 6 patients also received lignocaine hydrochloride orally but the intramuscular atropine (0.6 mg) was administered at the same time. Anaesthesia was induced approximately 1 h later (mean 1.2 h, range 0.5 - 2 h).

Anaesthesia and laparoscopy

Anaesthesia was induced with intravenous thiopentone (400 mg) and gallamine (60 mg). It was maintained with 2% halothane and a mixture of nitrous oxide and oxygen (3 : 1) in a semiclosed system containing a carbon dioxide absorber. Respiration was spontaneous throughout and the duration of anaesthesia varied from 9 to 15 min (mean 10 min). Laparoscopy was performed without complications.

Plasma lignocaine

Plasma was stored at -20°C and assayed for lignocaine as described previously.

RESULTS

Healthy unanaesthetised volunteers

Atropine significantly delayed the time taken to reach maximum plasma lignocaine concentrations. Thus the mean of the individual times taken to attain peak concentrations was significantly increased from 34 ± 3.8 to 75 ± 10 min. Atropine did not have any significant effect on the mean individual peak plasma lignocaine concentrations. However, when atropine was given, the mean overall plasma concentrations were lower during the first hour but higher after 2 h /
The mean plasma lignocaine half-life before and after atropine was unchanged (1.4 h).

Laparoscopy patients

The mean plasma lignocaine concentrations during the first two hours were significantly lower in the patients receiving lignocaine and atropine simultaneously than in those given lignocaine 1 h before the atropine. This relationship was reversed after 3 h, however, and the concentrations were then significantly higher in the former group (Fig. 43: Table 21). From Fig. 43 it can be seen that in patients given atropine and lignocaine at the same time there was a temporary fall in the plasma lignocaine concentrations soon after induction of anaesthesia whereas no such effect was observed when atropine was given after peak plasma lignocaine concentrations had been reached. Induction of anaesthesia and laparoscopy apparently caused a further marked inhibition of lignocaine absorption.

The time taken to reach peak plasma lignocaine concentrations was much longer in the patients given atropine and lignocaine simultaneously than in the patients receiving atropine 1 h after lignocaine. The mean values were 3.3 ± 0.8 and 1.0 ± 0.2 h respectively (p = < .02).

As in healthy unanaesthetised volunteers, there was no significant difference in the mean of individual peak plasma lignocaine concentrations in the two groups of patients. Because of the delay in absorption when the two drugs were given together plasma lignocaine half-life comparison could not be made.

SUMMARY Atropine delayed lignocaine absorption in healthy volunteers and /
and anaesthetised laparoscopy patients. It had no marked effect on the elimination of the drug from plasma in the healthy volunteers. Laparoscopy and anaesthesia further inhibited the absorption of lignocaine in the patients.
<table>
<thead>
<tr>
<th></th>
<th>Mean time to peak concentration (min ± SE)</th>
<th>Mean peak concentration (µg/ml ± SE)</th>
<th>Mean plasma lignocaine concentration (µg/ml) ± SE</th>
<th>0.5 h</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without atropine</td>
<td>3.4 ± 3.8</td>
<td>0.78 ± 0.04</td>
<td>0.75 ± 0.05 0.62 ± 0.05 0.31 ± 0.02 0.19 ± 0.01</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With atropine</td>
<td>7.5 ± 0.3</td>
<td>0.66 ± 0.03</td>
<td>0.38 ± 0.09 0.49 ± 0.04 0.41 ± 0.04 0.32 ± 0.04</td>
<td>0.17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>&lt; 0.02</td>
<td>NS</td>
<td>&lt; 0.01 NS NS NS &lt; 0.02 &lt; 0.005</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 0.6 mg atropine sulphate i.m.

** Calculated from data from each individual

NS = Not significant

Table 20. Plasma lignocaine concentrations in four healthy unanaesthetised volunteers following oral administration of 400 mg of lignocaine hydrochloride with and without atropine.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean time to peak concentration (h ± SE)</th>
<th>Mean peak concentration (μg/ml ± SE)</th>
<th>Mean plasma lignocaine concentration (μg/ml ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignocaine 1 h before atropine</td>
<td>1.0 ± 0.2</td>
<td>0.76 ± 0.17</td>
<td>0.47 ± 0.15 0.68 ± 0.14 0.35 ± 0.06 0.23 ± 0.03 0.16 ± 0.02</td>
</tr>
<tr>
<td>Atropine and lignocaine simultaneously</td>
<td>3.3 ± 0.8</td>
<td>0.73 ± 0.17</td>
<td>0.06 ± 0.0 0.25 ± 0.13 0.18 ± 0.03 0.35 ± 0.13 0.57 ± 0.17</td>
</tr>
<tr>
<td>p value</td>
<td>&lt; 0.02</td>
<td>NS</td>
<td>&lt; 0.025 &lt; 0.05 &lt; 0.05 NS &lt; 0.05</td>
</tr>
</tbody>
</table>

* six patients received atropine one hour after the lignocaine and six received both drugs simultaneously. Anaesthesia was induced one hour after premedication with atropine.

** Calculated from data from each individual.

NS = Not significant.

Table 21. Plasma lignocaine concentrations following an oral dose of 400 mg of lignocaine hydrochloride in 12 laparoscopy patients premedicated with atropine.
Figure 42. Mean plasma lignocaine concentrations in 4 healthy volunteers following oral administration of 400 mg lignocaine HCl with and without 0.6 mg atropine given intramuscularly.
Arrows indicate induction of anaesthesia.

Lignocaine 1 hr. before Atropine

Lignocaine and Atropine at same time

Figure 43. Mean plasma lignocaine concentrations in laparoscopy patients.

Six patients were given 0.6 mg atropine intramuscularly 1 h after 400 mg oral lignocaine HCl and 6 patients were given both drugs simultaneously.
CHAPTER 3 THE EFFECT OF ANAESTHESIA ON THE DISPOSITION OF LIGNOCaine GIVEN INTRAMUSCULARLY

INTRODUCTION

In the previous studies it was shown that lignocaine absorption and elimination were abnormal in laparoscopy patients. To overcome the problems associated with gastrointestinal absorption, the effect of laparoscopy and anaesthesia on lignocaine metabolism was studied following i.m. administration. Studies were carried out in healthy volunteers and laparoscopy patients.

Methods

Healthy volunteers

After an overnight fast 5 healthy male volunteers aged between 27 and 36 years (mean 32 years) received 200 mg lignocaine hydrochloride (in 2 ml sterile water) by injection into the deltoid muscle of one arm. The subjects were kept sitting for 3 h. Venous blood was withdrawn from the opposite arm through an indwelling catheter. Coffee and lunch were allowed at 2 and 4 h respectively.

Patients

Ten patients were studied. Eight were scheduled for laparoscopy and 2 were scheduled for dilatation and curettage (D & C). Their ages ranged from 25 to 40 years (mean 34). Lignocaine was given intramuscularly into the deltoid muscle as described above for the unanaesthetised volunteers at ½ h before induction of anaesthesia. Atropine /
Atropine (0.6 mg) was given intramuscularly into the gluteal region at the same time as the lignocaine.

Induction, anaesthesia and laparoscopy were carried out as described previously for the patients who received halothane. There were no complications.

Plasma lignocaine concentrations were measured as described previously. The plasma half-life was calculated using individual values obtained after 2 h from the time of injection.

**RESULTS**

At 30 min and after 4 h the mean plasma concentrations in the patients were significantly higher than in the unanaesthetised volunteers (Table 22 : Fig. 44). The mean plasma concentrations were comparable from 1 to 4 h.

As in the previous studies, lignocaine disappeared from the plasma more slowly in the patients than in the healthy volunteers (Fig. 44 : Tables 23 (a) and (b)). The mean plasma lignocaine half-life from 2 to 9 h was 2.80 ± 0.3 h and 1.50 ± 0.2 h in the patients and unanaesthetised volunteers respectively. This difference was statistically significant (p < 0.01). There was no obvious difference between the D & C patients and the laparoscopy patients.

**SUMMARY**

After intramuscular administration lignocaine disappeared from the plasma more slowly in laparoscopy patients than in healthy volunteers.
Figure 4. Mean plasma lignocaine concentrations in 5 healthy unanaesthetised patients and 10 laparoscopy patients after 200 mg intramuscular injection of lignocaine HCl.
<table>
<thead>
<tr>
<th></th>
<th>Plasma lignocaine concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 h</td>
</tr>
<tr>
<td><strong>Healthy volunteers</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>± 0.06</td>
</tr>
<tr>
<td><strong>Anaesthetised patients</strong></td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td>± 0.09</td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td>&lt; 0.005</td>
</tr>
</tbody>
</table>

Table 22. Mean plasma lignocaine concentrations (μg/ml ± SE) in 10 anaesthetised patients and 5 healthy volunteers after intramuscular injection of 200 mg lignocaine HCl.
Table 23 (a). Plasma lignocaine half-life values (2 to 9 h) in laparoscopy and D & C patients after intramuscular injection of 200 mg lignocaine HCl.

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Plasma lignocaine $t_1/2$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
</tr>
<tr>
<td>4</td>
<td>2.8</td>
</tr>
<tr>
<td>5</td>
<td>3.0</td>
</tr>
<tr>
<td>6</td>
<td>3.5</td>
</tr>
<tr>
<td>7</td>
<td>3.0</td>
</tr>
<tr>
<td>8</td>
<td>3.8</td>
</tr>
<tr>
<td>9</td>
<td>3.0</td>
</tr>
<tr>
<td>10</td>
<td>3.5</td>
</tr>
<tr>
<td>Mean</td>
<td>2.8</td>
</tr>
<tr>
<td>± SEM</td>
<td>± 0.3</td>
</tr>
</tbody>
</table>

*D & C patients.*

Table 23 (b). Plasma lignocaine half-life values (2 to 9 h) in healthy volunteers after intramuscular injection of 200 mg lignocaine HCl.

<table>
<thead>
<tr>
<th>Subject Number</th>
<th>Plasma lignocaine $t_1/2$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>1.3</td>
</tr>
<tr>
<td>4</td>
<td>1.8</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
</tr>
<tr>
<td>Mean</td>
<td>1.5</td>
</tr>
<tr>
<td>± SEM</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table 23 (b). Plasma lignocaine half-life values (2 to 9 h) in healthy volunteers after intramuscular injection of 200 mg lignocaine HCl.
CHAPTER 4
EFFECTS OF ANAESTHESIA AND SURGERY
ON THE METABOLISM OF ANTIPYRINE

INTRODUCTION

The procedures of laparoscopy, dilatation and curettage (D & C) normally last for only about 15 min. In the previous chapters the patients (except those having D & C) were ambulant 3 h after the procedure. However, the elimination of lignocaine in the previous studies was abnormally slow for as long as 10 h after anaesthesia.

Very little is known of effects of anaesthesia on drug metabolism and impairment could result in exaggerated and prolonged pharmacological effects. It is important therefore to determine to what extent the observed abnormalities in lignocaine metabolism apply to other commonly used drugs which are metabolised in the liver.

The plasma antipyrine half-life is widely used to assess hepatic microsomal drug metabolising enzyme activity (O'Malley, Crooks, Duke and Stevenson, 1971; Davies and Thorgeirsson, 1971). Antipyrine is rapidly absorbed from the gastrointestinal tract and distributed throughout total body water (Soberman, Brodie, Levy, Axelrod, Hollander and Steele, 1949). In healthy subjects it has a plasma half-life of about 12 h (O'Malley et al., 1971) and is extensively metabolised in man since only about 6% is excreted unchanged in the urine. In addition, the lack of obvious subjective pharmacological effects make it particularly suitable for clinical studies. The plasma antipyrine half-life was therefore measured in patients undergoing /
undergoing laparoscopy and other gynaecological surgical procedures. The effect of the duration of anaesthesia on the antipyrine half-life was also studied.

Short anaesthesia

**PATIENTS AND METHODS**

Nine patients were studied and each patient served as her own control. Their ages ranged from 26 to 52 years (mean 33.2 years) and apart from 3 patients on oral contraceptives none of the patients gave a history of regular intake of drugs. All were in good health and there was no history or clinical evidence of renal, cardiac, gastrointestinal and hepatic disease.

After an overnight fast of 10 h, each patient ingested 18 mg/kg of antipyrine dissolved in 50 ml of orange juice at about 6 to 7 h pre-operatively. Venous blood was sampled through an indwelling catheter in an arm vein at 0, 2, 3 and 5 h and immediately before induction of anaesthesia. Blood was sampled again post-operatively for up to 25 h after antipyrine administration.

Premedication consisted of intramuscular atropine (0.6 mg) given 1 h pre-operatively. No narcotic analgesics were given.

Induction and maintenance of anaesthesia was as described for laparoscopy patients anaesthetised with halothane.

The type of surgical operation and the duration of anaesthesia for each patient are shown in Table 21. Six patients had laparoscopy, 2 had D & C (one with cone biopsy) and a third had termination of pregnancy by suction. The mean duration of anaesthesia was 11.2 min (range 8 to 15 min).
<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Age</th>
<th>Drug history</th>
<th>Operation</th>
<th>Duration of anaesthesia (min)</th>
<th>Antipyrine half-life (h)</th>
<th>Before Anaesthesia</th>
<th>After Anaesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28</td>
<td>-</td>
<td>Lap TD</td>
<td>9</td>
<td>9.5</td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>2 years oral contraceptive</td>
<td>Lap TD</td>
<td>10</td>
<td>14.2</td>
<td>14.8</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>-</td>
<td>Cone biopsy and D &amp; C</td>
<td>15</td>
<td>8.5</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>39</td>
<td>4 years oral contraceptive</td>
<td>Lap TD</td>
<td>10</td>
<td>19.0</td>
<td>18.8</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>-</td>
<td>Lap TD</td>
<td>8</td>
<td>12.5</td>
<td>13.2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>52</td>
<td>-</td>
<td>D &amp; C</td>
<td>11</td>
<td>9.5</td>
<td>10.3</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>27</td>
<td>-</td>
<td>Lap TD</td>
<td>10</td>
<td>11.4</td>
<td>14.5</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>33</td>
<td>-</td>
<td>Suction Termination</td>
<td>16</td>
<td>13.4</td>
<td>9.8</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>34</td>
<td>1 year oral contraceptive</td>
<td>Lap TD</td>
<td>12</td>
<td>16.7</td>
<td>23.3</td>
<td></td>
</tr>
<tr>
<td>Mean ± SE</td>
<td></td>
<td></td>
<td></td>
<td>11.2 ± 1.7</td>
<td>12.7 ± 1.2</td>
<td>13.9 ± 1.5</td>
<td></td>
</tr>
</tbody>
</table>

Table 24. Clinical details and antipyrine half-life in 9 patients before and after anaesthesia of short duration. Lap TD = Laparoscopy and tubal diathermy

D & C = Dilatation and curettage
There were no complications and apart from the patients who had cone biopsy and termination of pregnancy, all were ambulant about 3 h post-operatively. The laparoscopy patients were discharged home the following day and the other patients on the third post-operative day.

Long anaesthesia

Five patients were studied. Surgery consisted of hysterectomy in 3 patients with menorrhagia and dysmenorrhoea and anterior pelvic floor repair in two who presented with stress incontinence. Clinical details and the duration of anaesthesia are shown in Table 25.

Antipyrine was administered in a dose of 25 mg/kg dissolved in 50 ml of orange juice at 10.0 p.m. on the night before surgery. All patients had fasted for 3 h before antipyrine administration. At 7.0 a.m. on the following day (about 6 h before surgery) venous blood was sampled hourly and immediately before induction of anaesthesia. Blood was sampled again post-operatively and at 2, 5, 8 and 17 h after surgery.

All patients were premedicated 1 h before induction with 0.6 mg atropine sulphate and 5 mg diamorphine i.m. Anaesthesia was induced by intravenous injection of 4.00 mg of thiopentone and 120 mg of gallamine and was maintained with 1.5% halothane and nitrous oxide : oxygen (3 : 1) in a semidosed system containing a carbon dioxide absorber. The pulse and blood pressure were monitored before, during and after the procedures and the diastolic pressure did not fall below 70.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Drug history</th>
<th>Operation</th>
<th>Duration of anaesthesia (min)</th>
<th>Antipyline half-life (h) Before surgery</th>
<th>Antipyline half-life (h) After surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38</td>
<td>Oral contraceptive</td>
<td>Hysterectomy</td>
<td>70</td>
<td>13.6</td>
<td>10.5</td>
</tr>
<tr>
<td>2</td>
<td>44</td>
<td>Mucaine</td>
<td>Hysterectomy</td>
<td>55</td>
<td>15.8</td>
<td>11.6</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>None</td>
<td>Hysterectomy</td>
<td>110</td>
<td>10.0</td>
<td>14.6</td>
</tr>
<tr>
<td>4</td>
<td>44</td>
<td>Oral contraceptive</td>
<td>Anterior pelvic floor repair</td>
<td>60</td>
<td>15.8</td>
<td>13.7</td>
</tr>
<tr>
<td>5</td>
<td>48</td>
<td>None</td>
<td>Anterior pelvic floor repair</td>
<td>60</td>
<td>10.8</td>
<td>8.1</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>41.4 ± 2.6</td>
<td></td>
<td></td>
<td>71 ± 10</td>
<td>13.2 ± 1.2</td>
<td>11.7 ± 1.2</td>
</tr>
</tbody>
</table>

Table 25. Clinical details and antipyline half-life before and after anaesthesia of long duration.
70 mm Hg in any of the patients. All the patients were discharged home by the eighth post-operative day without complications.

Liver function tests

Normal values for serum bilirubin, alkaline phosphatase, aspartate and alanine amino transaminase and plasma proteins were obtained in all the patients on the day before surgery, immediately after the procedure and 5 days after surgery.

Plasma antipyrine

Plasma was stored at -20°C until time of analysis. Antipyrine concentration was measured by the precipitation method of Brodie et al., 1949. In each patient the antipyrine half-life during the pre-operative period was compared with the corresponding value after the procedure.

RESULTS

Short anaesthesia

The plasma antipyrine half-life values before and after anaesthesia for each patient are shown in Table 24. There was no consistent change in the antipyrine half-life after anaesthesia. In patients Nos. 1, 3, 5 and 7 there was a small increase in the antipyrine half-life after surgery but in patient No. 8 there was a small decrease. In patient No. 9 the half-life increased from 16.7 to 23.3 h. These changes are small (except in patient No. 9) and of no therapeutic significance and may reflect analytical errors. The mean antipyrine half-life before the start of anaesthesia was $12.7 \pm 1.17$ h and /
and did not differ significantly from the mean antipyrine half-life of 13.9 ± 1.54 h in the post-operative period.

**Long anaesthesia**

Plasma antipyrine half-life values before and after surgery are shown in Table 25. Again there was no consistent change in the antipyrine half-life after surgery. In 4 patients there was a small decrease in the antipyrine half-life, but in patient No. 3 it increased from 10.0 to 14.6 h. These changes could not be clearly related to the duration and type of surgery or previous drug therapy. Following surgery the mean antipyrine half-life decreased from 13.2 ± 1.22 h to 11.7 ± 1.7 h, but the difference was not statistically significant.

**SUMMARY**

Lignocaine absorption and elimination is impaired in patients anaesthetised with thiopentone, nitrous oxide, halothane and gallamine while undergoing laparoscopy or D & C, but there was no consistent effect of these factors on antipyrine elimination in patients undergoing various gynaecological procedures.
CHAPTER 5

DISCUSSION

A LIGNOCaine Metabolism

The plasma concentration of lignocaine at any given time depends on the relative rates of absorption, distribution and elimination. The present findings indicate that the absorption of lignocaine from the gastrointestinal tract is delayed and suggest that its metabolism is depressed in patients undergoing laparoscopy and minor gynaecological procedures.

(a) Absorption

The delay in absorption is probably due to the combined effects of atropine premedication, anaesthesia and laparoscopy. In unanaesthetised volunteers, atropine alone delayed the gastrointestinal absorption of lignocaine but had no effect on the rate of elimination as judged by the plasma half-life. The plasma lignocaine half-life was significantly prolonged in the laparoscopy patients who received the drug intramuscularly and this suggests that lignocaine metabolism was depressed since it is unlikely that continuing absorption could account for the higher plasma concentrations.

It has been said that weakly basic lipid soluble drugs are absorbed rapidly from the relatively alkaline medium of the small intestine while little or no absorption occurs from the acid gastric contents (Brodie, 1964). Lignocaine is a weak base with a pKa 7.85 (Eriksson, 1966) and therefore probably absorbed largely from the small intestine. Delayed gastric emptying will slow down the transfer of the drug from the stomach to the sites of maximum absorption.
absorption in the small intestine. Atropine has anticholinergic activity and probably delayed lignocaine absorption by inhibition of gastric emptying. Other anticholinergic agents have been shown to slow down the absorption of drugs in man by this mechanism (Levy, Gibaldi and Procknal, 1972: Nimmo, Heading, Tothill and Prescott, 1973).

In addition, the rate of absorption of orally administered drugs may depend on the dissolution rate of dosage form in aqueous solutions (Katchen, 1971: Sjogren, 1971). A reduction in gastrointestinal motility and secretion produced by atropine could have slowed down lignocaine absorption partially by this mechanism.

The combination of anaesthesia and laparoscopy had marked additional inhibitory effect on the absorption of lignocaine. The mechanisms involved are unknown, but thiopentone, the inhalational anaesthetics, or laparoscopy, could have had further inhibitory effects on gastric emptying and gastointestinal motility.

Additional possible contributory factors include the mechanical effects of peritoneal insufflation, reduction in splanchnic blood flow and increased sympathetic activity resulting from painful stimuli in lightly anaesthetised patients (Marshall, Jebson, Davie and Scott, 1972).

(b) Distribution

The plasma half-life of a drug depends both on the plasma clearance and the volume of distribution (Vd). The volume of distribution in turn may depend on factors such as intracellular and extracellular pH gradients, plasma protein and tissue binding, regional /
regional blood flow and cardiac output (Waddell and Butler, 1957).

It is not known whether the Vd of lignocaine was changed by laparoscopy or anaesthesia. However, Marshall, Jebson, Davie and Scott (1972) have shown that in laparoscopy patients there is respiratory acidosis accompanied by an increase in pulse rate, arterial pressure and central venous pressure, but without significant changes in cardiac output. In another study Kelman, Swapp, Smith, Benzie and Gordon (1972) showed that increases in intra-abdominal pressure paralleled increases in femoral venous pressure. This suggests regional hypovolaemia could have occurred in the abdomen possibly reducing hepatic portal blood flow. Lignocaine is a weakly basic drug and respiratory acidosis could cause an increase in the plasma lignocaine concentrations due to a shift from intracellular to extracellular water. Waddell and Butler (1957) observed an opposite effect with phenobarbital, an acidic drug with a pKa 7.2 and Hayes (1971) stated that the myocardial uptake of lignocaine may be reduced by severe acidosis. Acidosis would tend to increase the renal clearance of lignocaine, but this would have an insignificant effect on the half-life since even with maximum urine acidity only about 6% of a dose of lignocaine is excreted unchanged in the urine (Beckett, Boyes and Appleton, 1966). It is also possible that the plasma protein and tissue binding of lignocaine could have been modified by acidosis or competitive displacement by the other drugs received by the patients. In such circumstances the half-life would be reduced rather than increased.

It /
It may be concluded that any haemodynamic changes which altered the Vd of lignocaine would have occurred only during the short period of anaesthesia. The plasma lignocaine concentrations in all patients remained abnormally high long after surgery and changes in the volume of distribution are unlikely to account for the prolonged plasma lignocaine half-life.

(c) Elimination

Since lignocaine is eliminated largely by metabolism in the liver, possible explanations for the prolonged lignocaine half-life are decreased hepatic blood flow or impaired hepatic microsomal enzyme metabolism of the drug.

Hepatic blood flow can be a major determinant of the clearance of rapidly metabolised drugs. According to Gillette (1971) the half-life depends on the rate of hepatic blood flow (BFR) in litres per minute according to the following equation:

\[
\frac{t_2^1}{0.693 \text{ Vd}} = \frac{Cv}{BFR \left(1 - \frac{1}{Ca}\right)}
\]

where \(t_2^1\) = biological half-life in minutes

- \(Vd\) = volume of distribution in litres
- \(Cv\) = the ratio of drug concentration in hepatic venous blood to the mean drug concentration in hepatic arterial blood and portal blood.
- \(1 - \frac{Cv}{Ca}\) = hepatic extraction ratio.

**Extraction ratio for lignocaine**
Extraction ratio for lignocaine

The Vd = 1.70 ± 0.21 litres/kg (Thomson, Rowland and Melmon, 1971) and the Vd for a 70 kg weight man = 119 litres. The plasma half-life of lignocaine is approximately 90 min for a healthy volunteer and the hepatic blood flow in man is about 1.5 litres/min. The hepatic extraction ratio is therefore

\[
\frac{0.693 \times 119}{1.5 \times 90} = 0.61
\]

This compares with the experimental value of 0.70 determined by Harrison et al. (1970).

Extraction ratio for antipyrine

The Vd for a 70 kg man is about 50 litres and the mean plasma half-life for normal subjects = 12.1 ± 0.4 h (O'Malley et al., 1971) The hepatic extraction ratio is therefore:

\[
\frac{0.693 \times 50}{1.5 \times 726} = 0.03
\]

Whitsett, Dayton and McNay (1970) observed a positive correlation between hepatic blood flow and the hepatic removal of oxyphenbutazone in dogs and prolongation of the half-life of glutethamide following overdosage in patients who are hypotensive may be due to decreased liver perfusion (Mahler, 1970). However, a reduction in hepatic blood flow can only produce an appreciable change in the plasma half-life of drugs which have a high hepatic extraction ratio and a relatively /
relatively large volume of distribution (Gillette, 1971). Thus the prolongation of lignocaine half-life could have been due to a reduction in hepatic blood flow by anaesthesia or the procedure.

Available evidence suggests that liver blood flow can be reduced by anaesthesia. Shackman, Graber and Melrose (1953) observed a 30% decrease in hepatic blood flow, a 34% decrease in hepatic and splanchnic oxygen consumption and a decreased clearance of bromsulphthalein (BSP) from the peripheral blood. The decrease in hepatic blood flow occurred regardless of changes in systemic blood pressure and it was suggested that liver and splanchnic vascular resistance was increased. During laparoscopy intra-abdominal pressure following peritoneal insufflation rose to 15 to 20 cm of water and this probably caused a decrease in hepatic portal vein flow since the portal pressure is normally only 20 to 30 cm of water (Taylor, 1954) and the intrasinusoidal pressure is only 2 to 4 cm of water (Dodson, 1971). Small decreases in intra-abdominal pressure increases hepatic blood flow (Brauer, 1963: Taylor, 1954).

Diminished hepatic blood flow could decrease lignocaine metabolism through a reduction in the amount of the drug reaching the liver and by causing relative hypoxia since about 80% of liver blood flow is from the portal system.

Hypercarbia is another possible factor in hepatic dysfunction. Morris and Feldman (1963) found that the post-operative retention of BSP was increased by hypercarbia during halothane anaesthesia. Julian and Scott (1972) found that the arterial P CO₂ increased /
increased from a mean of 43.2 to 60.8 mm Hg during laparoscopy and it is possible that hypercarbia contributed to impairment of hepatic metabolism in these patients.

In "in vitro" rat liver preparations, halothane has a biphasic action on hepatic oxidative metabolism of drugs. Thus the metabolism of hexobarbitone, aminopyrine, pentobarbitone and amylobarbitone was inhibited whilst that of aniline was enhanced by halothane (Brown, 1971). Anaesthetic gases also depress rat liver microsomal glucuronyl transferase activity (Brown, 1972). However, Rahn, Dayton and Frederickson (1969) have shown that halothane anaesthesia does not alter thiopentone metabolism in man. Other investigators have shown that halothane is no more hepatotoxic than other anaesthetic gases in patients (Stephen, Lawrence, Favian, Bourgeois-Gavardin, Dent and Grosskrentz, 1958): Kirwan, Dundee, Clarke, Mitchell and Neil, 1965). These observations and the fact that plasma lignocaine concentrations were similar in patients anaesthetised with halothane and those without halothane (Chapter 1 of this Section) suggest that hepatotoxicity due to halothane alone is unlikely to account for the slow disappearance of lignocaine from the plasma.

All the patients studied received thiopentone for induction of anaesthesia. There is no clear evidence that this drug is hepatotoxic although Dundee (1955) suggested that in doses exceeding 750 mg the drug was hepatotoxic on the basis of urinary excretion of urobilinogen for 3 days after anaesthesia. Acute administration of some hepatic /
hepatic microsomal enzyme inducing agents can cause an initial inhibition of drug metabolism (Kato, Chiesara and Vassenelli, 1964). Thiopentone could perhaps interfere with lignocaine metabolism through this mechanism.

Thiopentone, halothane and lignocaine are all metabolised by hepatic enzymes (Goodman and Gilman, 1970 : Brown and Vandam, 1971 : Hollunger, 1960 a and b). It has been suggested that hepatic microsomal enzymes can be saturated by high drug concentrations (Dayton and Perel, 1971 : Weiner, Shapiro, Axelrod, Cooper and Brodie, 1950). It is difficult to say whether saturation occurred in the present studies, but there could have been competition when several drugs were given simultaneously.

The slow fall in plasma lignocaine concentrations was not due to delayed absorption as the disappearance of the drug was also slow after intramuscular injection. In conclusion, it is likely that several factors contributed to the reduced rate of disappearance of lignocaine from the plasma in laparoscopy patients.
ANTIPYRINE METABOLISM

There was no consistent change in the plasma antipyrine half-life in patients undergoing different gynaecological operations although in some it was slightly shortened while in others it was prolonged after anaesthesia. If reduction in hepatic blood flow during anaesthesia and surgery contributed significantly to the slow elimination of lignocaine, such a mechanism would be expected to have little effect on antipyrine half-life since its extraction ratio is only about 3% and its volume of distribution is small.

These studies suggest that changes in the rate of metabolism of some drugs can occur during anaesthesia and surgery. Not all drugs are equally affected however. Karlin and Kutt (1970) described diphenylhydantoin intoxication in an epileptic patient after halothane anaesthesia. For reasons which are obscure, these workers attributed this effect to impaired diphenylhydantoin metabolism due to halothane hepatotoxicity. Perhaps some cases of drug interaction during anaesthesia may be due to impaired hepatic microsomal enzyme activity or reduced hepatic blood flow.
SUMMARY

1. The rate of metabolism of lignocaine and antipyrine was studied in patients undergoing laparoscopy and other surgical procedures.

2. The absorption of lignocaine following oral administration was delayed and the drug disappeared from plasma much more slowly than in healthy unanaesthetised volunteers.

3. After intramuscular injection lignocaine disappeared more slowly from plasma of anaesthetised patients than from healthy volunteers.

4. The delayed gastro-intestinal absorption of lignocaine may be due to inhibition of gastric motility by atropine and to the effects of anaesthesia and laparoscopy.

5. The combined effects of reduced hepatic blood flow and inhibition of hepatic microsomal enzyme activity (which lasted many hours) may have contributed to the reduced clearance of lignocaine from the plasma of anaesthetised patients.

6. The plasma antipyrine half-life was not significantly altered by anaesthesia and surgery.

7. Since antipyrine has a low hepatic extraction ratio, small changes of hepatic blood flow would not be expected to influence the plasma half-life of the drug.
LIGNOCaine AND ANTIFPyRINE METABOLISM IN PATIENTS WITH CHRONIC LIVER DISEASE
CHAPTER 1

INTRODUCTION

Drug metabolism may be abnormal in patients with severe acute liver damage but the results of studies on the effects of chronic liver disease have been conflicting (Prescott and Stevenson, 1973). Severe central nervous system toxicity was observed in a cirrhotic patient given relatively large doses of lignocaine (600 to 800 mg in 4 h) (Selden and Sasahara, 1967). Thomson, Rowland and Melmon (1971) reported a reduction in plasma lignocaine clearance and a three-fold increase in plasma lignocaine half-life in patients with severe cirrhosis. In the latter studies the volume of distribution of lignocaine was increased but this was not statistically significant. Since some patients who receive lignocaine may have impaired liver function a study was undertaken to determine the effect of chronic liver disease on lignocaine metabolism.

The antipyrine plasma half-life was also determined as an independent measure of hepatic drug metabolising activity.
CHAPTER 2

PATIENTS AND METHODS

Patients

Four patients suffering from different liver diseases were studied. The diagnosis was based on history, clinical and biochemical evidence. In patients 2, 3 and 4 this was confirmed by liver biopsy. The clinical details are given in Table 26. All patients were hospitalized and all drug therapy was stopped at least 24 h before the study except in the case of patient No. 2 in whom spironolactone was stopped only on the day of the study. None of the patients was in hepatic failure or precoma, none had impaired renal function and all gave informed consent.

Lignocaine administration

400 mg of lignocaine hydrochloride was administered orally after an overnight fast under the same conditions as described for healthy volunteers. Urine collections were made hourly during the first 10 h when possible and collections were continued for 48 - 72 h.

Antipyrine administration

At least 4 days later patients Nos. 2, 3 and 4 were given 18 mg/kg of antipyrine dissolved in 50 ml of orange juice. Venous blood was collected at 0, 4, 8, 12, 16 and 24 h.

Analytical procedures

The concentrations of lignocaine and its metabolites were determined by the methods described in Section II. Plasma antipyrine was estimated by the GLC method described in Section II.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>Therapy</th>
<th>Alkaline phosphatase (mg/100 ml)</th>
<th>Protein (g/100 ml)</th>
<th>Units/litre</th>
<th>Units/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66</td>
<td>71</td>
<td>M</td>
<td>Alcoholic cirrhosis with hepatomegaly</td>
<td>Vitamin K₁</td>
<td>1.8</td>
<td>4.5</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>67</td>
<td>M</td>
<td>Alcoholic cirrhosis and hepatoma Portal hypertension</td>
<td>Bendrofluazide Spironolactone Phenobarbitone Diazepam Vitamin K₁ Neomycin</td>
<td>3.1</td>
<td>2.4</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>F</td>
<td>Chronic active hepatitis</td>
<td>Prednisolone Potassium chloride Vitamin K₁ Spironolactone Frusemide</td>
<td>11.8</td>
<td>2.1</td>
<td>170</td>
</tr>
<tr>
<td>4</td>
<td>43</td>
<td>54</td>
<td>F</td>
<td>Primary biliary cirrhosis hepatospleno- megaly</td>
<td>Cholestyramine Nitrarepam Chlorpheniramine</td>
<td>25.2</td>
<td>-</td>
</tr>
</tbody>
</table>

Normal values

| SGPT = Alaline transaminase. |
| SGOT = Serum aspartate transaminase. |

Table 26. Clinical and biochemical details of patients with chronic liver disease.
CHAPTER 3

RESULTS

A LIGNOCaine METABOLISM

(a) Plasma concentrations

The individual plasma concentrations of lignocaine, EGX and GX are shown in Figs. 45 to 48. Mean values obtained from four healthy volunteers given oral lignocaine are shown in Fig. 49 for comparison.

(i) Lignocaine

Except in patient No. 4, plasma lignocaine concentrations were higher than those observed in the healthy volunteers. For example, in patient No. 2 (with cirrhosis and hepatoma) the peak plasma lignocaine concentration was 1.7 μg/ml compared to 0.82 ± 0.14 in healthy volunteers (Figs. 46 and 49). In patient No. 3 (with chronic active hepatitis) the peak plasma concentration was 1.3 μg/ml and eight hours after the lignocaine was given the plasma concentration was grossly elevated at 1.1 μg/ml (Fig. 47). In healthy volunteers the drug was usually unmeasurable in plasma eight hours after oral administration. The peak plasma lignocaine concentrations were reached at 1 to 3 h whereas in healthy volunteers peak concentrations were reached between 1/2 and 3/4 h.

The areas under the plasma lignocaine concentration time-curve from 0 to 6 h were considerably increased in patients Nos. 2 and 3. The values were 93.7 and 109.5 sq cm respectively. The areas under the curve were within normal limits (39.1 ± 6.6 sq cm) for patients Nos. 1 and 4. (Table 27).

In /
Table 27. The areas under the concentration time-curve 0 - 6 h in 4 patients with liver disease after 400 mg of lignocaine orally.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Area under curve (sq cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40.4</td>
</tr>
<tr>
<td>2</td>
<td>93.7</td>
</tr>
<tr>
<td>3</td>
<td>109.5</td>
</tr>
<tr>
<td>4</td>
<td>27.2</td>
</tr>
<tr>
<td>Healthy volunteers (n = 4)</td>
<td>39.1 ± 6.58</td>
</tr>
</tbody>
</table>
In all four patients the lignocaine plasma half-life was prolonged. The individual values ranged from 2.3 to 19 h (Table 28). The mean value for 4 healthy volunteers is $1.4 \pm 0.01$ h. In patient No. 3 (with chronic active hepatitis) the elimination of lignocaine was grossly abnormal and there was virtually no decline in plasma concentrations over the period of observation (Fig. 47).

(ii) EGX

The initial and maximum plasma concentrations of EGX were abnormally low in all patients except for patient No. 1 (Figs. 45 to 49). The mean individual peak concentration of EGX was $0.36 \pm 0.05 \mu g/ml$ whereas the corresponding value for normal subjects was $0.66 \pm 0.11 \mu g/ml$. The plasma concentration of EGX declined abnormally slowly and the half-life of EGX ranged from 3 to 4.5 h (Table 28). It was not possible to measure the plasma EGX half-life in the patient with grossly prolonged lignocaine half-life (patient No. 3) since the concentration of EGX was still rising at 6 h.

(iii) GX

GX was not measurable at any time in the plasma of patients Nos. 3 and 4. The concentrations could not have exceeded $0.05 \mu g/ml$ during the period of observation. In patient No. 1 the concentrations were virtually normal but in patient No. 2 GX concentrations did not exceed $0.1 \mu g/ml$ (Figs. 45 to 48). GX half-life could not be measured as the plasma concentrations in patients Nos. 1 and 2 did not fall.

(b) /
<table>
<thead>
<tr>
<th>Patient</th>
<th>Lignocaine</th>
<th>EGX</th>
<th>Antipyrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.3</td>
<td>3.0</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>2.8</td>
<td>4.5</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>19.0</td>
<td>-</td>
<td>137</td>
</tr>
<tr>
<td>4</td>
<td>2.6</td>
<td>3.2</td>
<td>12.3</td>
</tr>
<tr>
<td>Healthy Volunteers</td>
<td>1.4 ± S.E. 0.01</td>
<td>2.3 ± S.E. 0.1</td>
<td>12 ± S.D. 2.5</td>
</tr>
</tbody>
</table>

* O'Malley and Stevenson (1973) for 65 controls ± S.D.

Table 28  Apparent plasma half-life (h) of lignocaine, EGX and antipyrine in 4 patients with chronic liver disease.
Figure 45. Plasma concentrations of lignocaine (Lig), EGX and GX in a patient with alcoholic cirrhosis (No. 1) after ingestion of 400 mg lignocaine HCl.
Figure 46. Plasma concentrations of lignocaine (Lig), EGX and GX in a patient with alcoholic cirrhosis and hepatoma (No. 2) after ingestion of 400 mg lignocaine HCl.
Figure 47. Plasma concentrations of lignocaine and EGX in a patient with chronic active hepatitis (No. 3) after ingestion of 400 mg lignocaine HCl.

GX was not measurable.
Plasma concentrations of lignocaine (Lig) and EGX in a patient with chronic biliary cirrhosis (No. 4) after ingestion of 400 mg lignocaine HCl. EGX was not measurable.
Figure 49. Mean plasma concentrations of lignocaine, EGX and GX in 4 healthy volunteers after ingestion of 400 mg lignocaine HCl.
(b) Urinary excretion of lignocaine, EGX, GX and 4-hydroxyxylidine

The 48 h urinary recoveries of lignocaine and its metabolites for each patient are summarised in Table 29.

(i) Lignocaine

In all the patients the recovery of unchanged lignocaine was much higher than in healthy volunteers. The mean recovery in the patients was $6.9 \pm 1.7\%$ whereas the corresponding recovery in 7 healthy volunteers was $0.8 \pm 0.18\%$ and the difference was statistically significant ($p < 0.005$). The differences could not be explained on the basis of urine pH. Indeed in patients 2, 3 and 4 the mean urine pH was rather high 6.1, 7.1 and 6.8 yet the recovery was 10.5, 8.0 and 2.0%, respectively.

Lignocaine was still detectable in the urine at 60 h whereas it cannot normally be detected after 24 h.

(ii) EGX

Relatively large amounts of EGX were recovered in the urine in 48 h in patients Nos. 2 and 3 (8.0 and 9.7%) whereas the percentage recovery of the compound was within normal limits ($4.2 \pm 0.6\%$) in patients Nos. 1 and 4. The mean urinary recovery of EGX in the patients and normal volunteers however did not significantly differ (Table 29).

(iii) GX

All four patients excreted significantly less GX in the urine ($mean 1.2 \pm 0.39\%$) than the healthy volunteers ($6.2 \pm 1.18\%$) $p < 0.02$.
(iv) 4-hydroxyxylidine

The mean urinary recovery of lignocaine as 4-hydroxyxylidine was 15.7 ± 3.3% and this compared with 33.8 ± 2.7% in healthy volunteers (p < 0.005). Patient No. 3 only excreted 8.8% of the dose of lignocaine as 4-hydroxyxylidine (Table 29).

Total urinary excretion of lignocaine and its metabolites

The excretion of lignocaine and its metabolites was apparently delayed since after 48 h only 27 to 32% of the dose of lignocaine was accounted for as the unchanged drug, EGX, GX and 4-hydroxyxylidine. The corresponding recovery in healthy volunteers was 46.2 ± 3.3% (Table 29).

In normal volunteers the mean ratio of total lignocaine and its metabolites to lignocaine in the urine was 55.4 but the mean ratio in the patients was only 6.5.

B  PLASMA ANTIPYRINE HALF-LIFE (Table 28)

Antipyrine plasma half-life was grossly abnormal in patient No. 3 with chronic active hepatitis (137 h) and was also prolonged in patient No. 2 with hepatoma (24 h). The antipyrine half-life was normal in patient No. 4 with primary biliary cirrhosis (12.3 h).
Table 29. Percentage of dose of lignocaine excreted into 48 h urine as lignocaine, EGX, GX and 4-hydroxyxyridine following oral lignocaine in 4 patients with chronic liver disease.
These studies demonstrate that lignocaine and antipyrine metabolism can be grossly impaired in patients with severe chronic liver disease. The most striking abnormalities were observed in the patient with chronic active hepatitis, and the other with hepatoma and cirrhosis. In these two patients there was also a striking impairment in antipyrine elimination. Indeed the grossly prolonged plasma lignocaine half-life of 19 h and that of antipyrine of 137 h found in the patient with chronic active hepatitis are the longest ever described. In the patient with chronic biliary cirrhosis poor absorption of lignocaine may have occurred in addition to impaired metabolism of the drug since malabsorption is a recognised feature of this condition (Sherlock, 1971). In this patient however, antipyrine metabolism as judged by its plasma half-life was normal. The drug is mostly metabolised by 4-hydroxylation (Stevenson, 1973 - personal communication) and it may be that the enzymes involved in drug metabolism are damaged to different degrees by the type of liver disease, its severity and duration.

It is possible that the lower total urinary recovery of lignocaine and its metabolites may be due to altered biotransformation of the drug to different metabolites not measured in the present study. An additional explanation is that the rate of metabolism of the drug is much slower than normal as the drug was still detectable in the urine at 60 h after a single oral dose in two patients.

Several factors may explain the abnormally slow metabolism of /
of lignocaine in all 4 and of antipyrine in 2 out of 3 patients. Some of these include a decrease in functional liver cell mass by tumour or fibrosis and disturbances of microsomal enzyme activity by hypoxia or retention of bile acids (Prescott and Stevenson, 1973: McLuen and Fouts, 1961: Gillette, 1971: Sherlock, 1971: Groszmann, Kotelanski, Cohn and Khatri, 1972: Cohn, Khatri, Groszmann and Kotelanski, 1972). Porto-systemic and porto-pulmonary shunting which occurs in cirrhosis (Sherlock, 1971) may be another contributory factor since absorbed lignocaine or antipyrine may bypass the liver.

The role of the drugs taken by the patients on lignocaine and antipyrine metabolism is difficult to determine. Paradoxically, the two patients (Nos. 2 and 3) with the greatest abnormalities in lignocaine and antipyrine metabolism were on spironolactone which is a microsomal drug metabolising enzyme stimulant (Gillette, 1971). In addition patient No. 2 was on phenobarbitone, No. 3 on prednisolone and No. 4 on chlorpheniramine which are also microsomal inducing agents (Gillette, 1971: Levi, Sherlock and Walker, 1968: Breckenridge, Burke, Davies and Orme, 1973) and could have overcome the reduced lignocaine and antipyrine metabolism by the liver.

The number of patients in this study is small and the results must be interpreted with caution. The paradoxical normal antipyrine half-life in the presence of disturbed lignocaine metabolism illustrates the danger inherent in the assessment of microsomal drug metabolising activity by measurement of antipyrine half-life alone.

The role of changes in the volume of distribution cannot be assessed but gross abnormalities of lignocaine and antipyrine elimination could not be explained solely on this basis.
SUMMARY

1. In 4 patients with different types of chronic liver disease, plasma lignocaine half-life was much longer than in healthy volunteers. In a severely ill patient with chronic active hepatitis the t½ of the drug was as long as 19 h.

2. Concentrations of the drug were much higher in three of these patients over the 8 h period.

3. Plasma EGX concentrations were generally lower than those obtained in healthy volunteers.

4. Plasma GX was measurable only in 2 of the patients and again the concentrations were low compared to those obtained in healthy volunteers.

5. Combined urinary recovery of lignocaine and its metabolites in 4-8 h urine was low in the patients.

6. Compared to healthy volunteers significantly more lignocaine was recovered in urine (2 to 10.5%) in 4-8 h and in 2 patients the drug was still detectable in the urine 60 h after ingestion.

7. In a patient with chronic active hepatitis and another with alcoholic cirrhosis and hepatoma relatively large amounts of EGX were recovered in the urine (9.7 and 8% normal 4.2 ± 0.63) which suggests that further metabolism of EGX was diminished.

8. The amounts of 4-hydroxyxylidine excreted in 4-8 h was generally low ranging from 8.6 to 23.6% (normal 33 ± 2.52).

9. Antipyrine t½ was grossly prolonged to 136.8 h in a patient with chronic active hepatitis and to 24 h in another who had hepatoma and cirrhosis but was normal in a patient with primary biliary cirrhosis.
LIGNOCaine AND ANTIPYRINE METABOLISM IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION AND CARDIAC FAILURE
CHAPTER 1

INTRODUCTION

Lignocaine has been widely used as an antiarrhythmic drug in patients with myocardial infarction in Coronary Care Units (CCU) Harrison and Alderman, 1971; Hayes, 1971; Jewitt, Kishon and Thomas, 1968). The drug is usually given by continuous intravenous infusion for 24 to 48 h and has even been given for as long as 5 days (Jewitt et al., 1968) yet very little is known of the metabolism of lignocaine in these patients. After prolonged intravenous infusion the elimination of the drug from plasma even in the absence of cardiac failure is abnormally slow (Prescott and Nimmo, 1971). Thomson et al. (1971) observed a reduced apparent volume of distribution and plasma clearance of lignocaine in patients with cardiac failure with unspecified aetiology. In the present studies the apparent plasma half-life of the active lignocaine metabolites EGX and GX have been shown to be long in healthy volunteers, i.e. about 2.3 h for EGX and 11 to 15 h for GX.

This study was therefore carried out to investigate

(a) the plasma concentrations of lignocaine during constant infusion in patients with acute myocardial infarction, with cardiac failure and cardiogenic shock.

(b) the metabolism of lignocaine during and after constant intravenous infusion.

(c) the effects of cardiac failure and shock on the metabolism of lignocaine.

(d) the possibility of cumulation of EGX and GX during prolonged administration of lignocaine.

Hepatic microsomal drug metabolising activity was assessed independently by serial measurements of the plasma antipyrine half-life in some of the patients whilst in the Coronary Care Unit, and at least 10 days later.
CHAPTER 2

METHODS

Patients

Nine male patients who were admitted to the C.C.U. The Royal Infirmary of Edinburgh, with acute myocardial infarction or cardiac failure were studied. All had ventricular arrhythmias which required lignocaine therapy. Clinical details of the patients are shown in Tables 30 and 31. Patients 1 to 8 had ventricular arrhythmias complicating acute myocardial infarction. Patients 1 to 5 had no clinical evidence of cardiac failure on admission. Patients 6 and 7 had transient mild to moderate degree of cardiac failure, (dyspnoea, raised jugular venous pressure, crepitations at the lung bases and no marked radiological evidence of pulmonary oedema) and patient 8 developed severe cardiogenic shock with unrecordable blood pressure 25 h after admission. He died 24 h later. The ninth patient was admitted with acute cardiac failure due to uncontrolled ventricular tachycardia, but had no evidence of myocardial infarction.

Liver function was assessed by serial measurements of serum bilirubin, alkaline phosphatase and serum alanine transaminase (SGPT) activity. The serum aspartate transaminase activity (SGOT) was also measured but since serum activity increases after myocardial infarction it could not be used to assess hepatic function. The SGPT increased in patients 2 to 8 (Table 31).

Renal function was assessed by serial measurement of creatinine clearance, blood urea nitrogen, urine output and plasma electrolytes.

* Coronary Care Unit
<table>
<thead>
<tr>
<th>Patient</th>
<th>Weight (kg)</th>
<th>Blood Pressure (mmHg)</th>
<th>Diagnosis</th>
<th>Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>63</td>
<td>70.0</td>
<td>Myocardial infarction</td>
<td>Ampicillin, Trifluorperazine, Atropine, 60</td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>79.0</td>
<td>Myocardial infarction</td>
<td>Frusemide, Diazepam, *Bendrofluozide, 40</td>
</tr>
<tr>
<td>3</td>
<td>56</td>
<td>76.4</td>
<td>Myocardial infarction</td>
<td>*Procainamide, Diazepam, 50</td>
</tr>
<tr>
<td>4</td>
<td>41</td>
<td>82.3</td>
<td>Myocardial infarction</td>
<td>*Digoxin, Procainamide, Diazepam, 60</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>95.5</td>
<td>Myocardial infarction</td>
<td>Frusemide, Diazepam, 40</td>
</tr>
<tr>
<td>6</td>
<td>68</td>
<td>82.7</td>
<td>Myocardial infarction</td>
<td>*Quinidine, Digoxin, Amylobarbitone, Diazepam, *Solbutamol, 50</td>
</tr>
<tr>
<td>7</td>
<td>62</td>
<td>66.8</td>
<td>Myocardial infarction</td>
<td>Ampicillin, Frusemide, Atropine, 60</td>
</tr>
<tr>
<td>8</td>
<td>42</td>
<td>65.0</td>
<td>Myocardial infarction</td>
<td>Digoxin, Frusemide, Dextran, Ampicillin, Practolol, unrecordable</td>
</tr>
<tr>
<td>9</td>
<td>76</td>
<td>65.5</td>
<td>Cardiac failure only</td>
<td>Metoclopramide, Digoxin, **Thyroxine, Frusemide, *Procainamide, 70</td>
</tr>
</tbody>
</table>

* Therapy during convalescence.  ** Preadmission therapy.

Table 30. Clinical details of 9 patients with acute myocardial infarction and or cardiac failure.
<table>
<thead>
<tr>
<th>Patient number</th>
<th>Lowest creatinine clearance ml/min</th>
<th>Blood urea Nitrogen mg/100 ml</th>
<th>SGOT unit/L</th>
<th>**HBD unit/L</th>
<th>*SCP K unit/L</th>
<th>Alkaline phosphatase K.A.units/100 ml</th>
<th>SGPT Units/L</th>
<th>Bilirubin mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>94</td>
<td>49</td>
<td>232</td>
<td>696</td>
<td>880</td>
<td>11</td>
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<td>93</td>
<td>407</td>
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<td>35</td>
<td>76</td>
<td>916</td>
<td>1710</td>
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<td>56</td>
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<td>6</td>
<td>56</td>
<td>70</td>
<td>178</td>
<td>624</td>
<td>364</td>
<td>20</td>
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<td>7</td>
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<td>43</td>
<td>384</td>
<td>1280</td>
<td>2000</td>
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<td>73</td>
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<tr>
<td>8</td>
<td>43</td>
<td>68</td>
<td>372</td>
<td>992</td>
<td>1040</td>
<td>12</td>
<td>73</td>
<td>0.2</td>
</tr>
<tr>
<td>9</td>
<td>86</td>
<td>88</td>
<td>18</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Normal range: 100 - 120 mg/100 ml, 15 - 40 mg/dl, 10 - 40 mg/dl, 100 - 240 U/L, 10 - 60 U/L, 2 - 13 K.A.units/100 ml, 10 - 35 Units/L, 0.3 - 1 mg/dl

* Serum creatine phosphokinase.
** Serum hydroxybutyric dehydrogenase.

Table 31. Biochemical details of 9 patients with acute myocardial infarction and/or cardiac failure.
electrolytes. The results were difficult to interpret because most of the patients received diuretic therapy. Apart from patient 8 who developed oliguria 25 h after starting lignocaine therapy, none of the patients developed severe renal impairment (Tables 31 and 40).

The patients stayed in the C.C.U. from 49 to 72 h and were then transferred to the general medical wards. Whilst in the C.C.U. the electrocardiogram was monitored continuously in each patient and the blood pressure, pulse rate and clinical state were recorded at regular intervals.

With the exception of patient 9, who had been on thyroxine 0.15 mg/day for 30 years, none of the patients had been taking drugs regularly before admission. In addition to lignocaine, all patients received warfarin, nitrazepam, cyclizine and morphine whilst in the C.C.U. Additional drugs given are shown in Table 30.

Lignocaine administration

After an initial i.v. bolus of 100 mg of lignocaine, an infusion was started as soon as possible using a Braun constant infusion pump (the Perfuser) through a long polythene catheter tubing connected to an arm vein via a three-way tap. The lignocaine was infused at a rate of 1.4 mg/min in patients 1 to 8. Patient 9 received 1.3 mg/min of lignocaine HCl. The duration of lignocaine infusion varied from 40 to 46 h. Additional lignocaine (200 mg) was given over a 10 min period to patient 6, 24 h after the infusion was started.

Blood sampling /
Blood sampling

During the infusion venous blood was sampled at 8 hourly intervals from the opposite arm. In the patients with cardiac failure blood samples were also taken at 1, 2 and 4 h during the infusion. Blood was withdrawn at frequent intervals after stopping the lignocaine infusion.

Antipyrine administration

Patients 6, 7 and 9 ingested 18 mg/kg of antipyrine dissolved in 50 ml orange juice on the morning after the day of admission, as described by O'Malley et al., (1971). Venous blood was withdrawn at 0, 4, 8, 12, 16 and 24 h. The experiment was repeated 12 days later when the patients were convalescent.

The apparent volume of distribution of antipyrine

The straight portion of the semilogarithmic plot of antipyrine concentration versus time was back extrapolated to time 0 h.

The Vd was obtained from the following

\[
\text{Total administered dose} \div \text{Concentration at time 0 h.}
\]

(Breckenridge et al., 1973).

Plasma clearance of antipyrine

This was obtained as follows

\[
\text{Clearance} = \frac{0.693 \times \text{Vd}}{t_\frac{1}{2}}
\]

where \(t_\frac{1}{2}\) is the plasma antipyrine half-life.
Urine collection

Serial urine collections were made for up to 7 days after starting the lignocaine therapy. The pH and volume were measured and aliquots were stored at -20°C until the time of analysis.

Analytical procedures

The concentrations of lignocaine, EGX, GX, 4-hydroxyxylidine and antipyrine in plasma and urine were measured by the GLC methods described in Section II.
A

RESULTS

LIGNOCAINE METABOLISM

(a) Plasma concentrations of lignocaine, EGX and GX during infusion

The plasma concentrations of lignocaine, EGX and GX during infusion are shown in Tables 32 to 34 and Figs. 50 to 54.

(i) Lignocaine

Patients without cardiac failure (1 to 5)

The mean plasma lignocaine concentration rose from 1.36 ± 0.05 µg/ml at 8 h to 2.76 ± 0.26 µg/ml at 40 h after starting the infusions (Fig. 50). The individual values are given in Table 32.

Patients with mild to moderate cardiac failure (6, 7 and 9)

After an initial fall the mean plasma lignocaine concentration rose to 3.2 µg/ml at 8 h, and then rose progressively to 4.1 µg/ml at 46 h after starting the lignocaine infusion. The plasma concentrations were higher than those in the patients without cardiac failure (Table 32, Figs. 50 to 52).

Patient with severe cardiac failure and cardiogenic shock (8)

There was considerable fluctuation in the plasma concentrations. At 7 h the plasma concentration was the same as the mean value obtained for patients 6, 7 and 9 (3.3 µg/ml). At 25 h the patient developed severe cardiogenic shock and the plasma lignocaine concentration subsequently rose steeply to 6.5 µg/ml at 40 h (Fig. 53).

The plasma lignocaine concentrations in the patients with myocardial infarction are compared in Fig. 54.

No /
## Table 32.

Plasma concentrations of lignocaine (μg/ml) during a constant i.v. infusion of 1.4 mg/min of the drug in patients with myocardial infarction or cardiac failure.

<table>
<thead>
<tr>
<th>Patients without cardiac failure</th>
<th>Patient number</th>
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<td>2</td>
</tr>
<tr>
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<td>Mean ± SE</td>
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</tr>
<tr>
<td></td>
<td>±0.05</td>
<td>±0.15</td>
</tr>
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<td>Patients with mild to moderate cardiac failure</td>
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<td><strong>9</strong></td>
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<td>1.83</td>
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<tr>
<td>Mean</td>
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</tr>
<tr>
<td>Patient with cardiac failure and shock</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>3.29</td>
<td>3.57</td>
<td>2.82</td>
</tr>
</tbody>
</table>

* Patient 9 received 1.3 mg/min of lignocaine infusion. He had no myocardial infarction.
** Patient 6 received additional lignocaine HCl (200 mg) i.v. at 24 h.
No so-called 'steady state' plasma lignocaine concentrations were attained in these patients and the plasma concentrations rose during constant infusion of the drug for 40 to 46 h. There was no significant correlation between the individual plasma lignocaine concentrations at 4.0 h and age \( (r = 0.19) \), SCFPK \( (r = -0.49) \) or SGPT \( (r = -0.43) \) activity. There was a negative correlation between the individual lignocaine concentrations at 4.0 h and the lowest diastolic pressure \( (r = -0.60, p < 0.05) \) and body weight \( (r = -0.69, p < 0.05) \). Higher plasma lignocaine concentrations were attained earlier in the patients with cardiac failure.

(ii) EGX

The plasma concentrations of EGX during the infusion are shown in Table 33.

Patients without cardiac failure (1 to 5)

The mean EGX concentration after 8 h of lignocaine infusion was \( 0.41 \pm 0.07 \mu g/ml \) and it remained relatively steady until 4.0 h of infusion when it rose to \( 0.47 \pm 0.05 \mu g/ml \). (Fig. 50).

Patients with mild to moderate cardiac failure (6, 7 and 9)

After an initial fall the mean plasma EGX concentration rose gradually from \( 0.16 \mu g/ml \) at 2 h to \( 0.80 \mu g/ml \) at 4.0 h after starting the infusion. Compared to the patients without cardiac failure the mean plasma EGX concentrations between 24 and 46 h after starting infusion were higher (Figs. 51 and 52).

Patient with severe cardiac failure and shock (8)

The EGX plasma concentration was relatively high at \( 0.58 \mu g/ml \) after 7 h of lignocaine infusion. The concentration of this metabolite /
<table>
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<th>3</th>
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<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>0.30 0.47 0.56 0.67 0.76 0.83 0.92 1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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</tr>
<tr>
<td>6</td>
<td>0.30 0.47 0.56 0.67 0.76 0.83 0.92 1.00</td>
<td>-</td>
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</tr>
<tr>
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<tr>
<td>8</td>
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<td>-</td>
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</tr>
</tbody>
</table>

**Patients**

- 1: **without cardiac failure**
- 2: **with mild to moderate cardiac failure**
- 3: **with cardiac failure and shock**
- 4: **Patient 9 had no myocardial infarction. He was given lignocaine at 1.3 mg/min.**

**Patients**

- 5: **Mean ± SE**
- 6: **Mean**
- 7: **Mean ± SE**
- 8: **Mean ± SE**
- 9: **Mean ± SE**

**Patients**

- 1: **without cardiac failure**
- 2: **with mild to moderate cardiac failure**
- 3: **with cardiac failure and shock**
- 4: **Patient 9 had no myocardial infarction. He was given lignocaine at 1.3 mg/min.**

**Patients**

- 5: **Mean ± SE**
- 6: **Mean**
- 7: **Mean ± SE**
- 8: **Mean ± SE**
- 9: **Mean ± SE**
metabolite fell to 0.31 μg/ml at the time when the blood pressure was unrecordable, but rose again to 0.58 μg/ml at the end of the infusion (after 40 h). (Fig. 53).

(iii) GX

The plasma GX concentrations during the infusion are shown in Table 34.

Patients without cardiac failure (1 to 5)

The mean GX concentration was 0.49 ± 0.11 μg/ml 8 h after starting the infusion and there was only a very slow cumulation of the metabolite in the plasma (Fig. 50). The mean plasma concentrations of GX were usually higher than the mean EGX concentrations. (Tables 33 and 34).

Patients with mild to moderate cardiac failure (6, 7, and 9)

Compared to the patients without cardiac failure the GX concentrations were generally low throughout the period of study. Thus the mean GX concentration at 8 h was only 0.26 μg/ml and rose gradually to 0.34 μg/ml at 40 h and 0.47 μg/ml at 46 h of lignocaine infusion respectively. (Table 34). Unlike patients 1 to 5, the plasma GX concentrations in these patients were lower than those of EGX. (Figs. 51 and 52).

Patient with cardiac failure and shock (8)

In this patient the plasma GX concentrations were always lower than those of EGX and rose from 0.20 μg/ml after 7 h of infusion to 0.50 μg/ml at the end of the infusion although there was a transient fall at 32 h. (Fig. 53).

(b) /
<table>
<thead>
<tr>
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<th></th>
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<td>46</td>
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<td>±0.11 ±0.05 ±0.05 ±0.04 ±0.05 ±0.06</td>
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<tr>
<td>9</td>
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<td>0.12</td>
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<td>0.28</td>
<td>0.34</td>
<td>0.47</td>
<td></td>
</tr>
</tbody>
</table>

* Patient 9 received 1.3 mg/min lignocaine

Table 34. Plasma concentrations of GX (µg/ml) during a constant i.v. infusion of 1.4 mg/min lignocaine HCl in patients with myocardial infarction and cardiac failure.
Figure 50. Mean plasma concentrations of lignocaine, EGX and GX during constant i.v. infusion of 1.4 mg/min lignocaine HCl in 5 patients with acute myocardial infarction and no cardiac failure. (Patients 1 - 5).
Figure 51. Mean plasma concentrations of lignocaine, EGX and GX during constant i.v. infusion of 1.0 mg/min lignocaine HCl in 2 patients admitted with acute myocardial infarction complicated by mild cardiac failure. (Patients 6 and 7).
Figure 52. Plasma concentrations of lignocaine, EGX and GX during a constant i.v. infusion of 1.3 mg/min lignocaine HCl in a patient (No. 9) with acute cardiac failure, but no evidence of myocardial infarction.
Figure 53. Plasma concentrations of lignocaine, EGX and GX during constant i.v. infusion of 1.4 mg/min lignocaine HCl in a patient (No. 8) with acute myocardial infarction who developed cardiogenic shock 25 h after starting the infusion.
Plasma lignocaine concentrations in 8 patients with acute myocardial infarction during a constant i.v. infusion of 1.4 mg/min lignocaine HCl. Five patients had no clinical evidence of cardiac failure. Two had clinical signs of mild cardiac failure on admission and one patient developed severe cardiogenic shock 25 h after starting lignocaine infusion.
(b) **Plasma concentrations of lignocaine and its metabolites after stopping intravenous lignocaine infusion**

The plasma concentrations of lignocaine, EGX and GX after stopping the infusion are shown in Tables 35 to 37 and Figs. 55 to 58.

(i) **Lignocaine**

In all the 9 patients the rate of decline of plasma lignocaine concentrations was slow and did not appear to be a single exponential process. (Figs. 55 to 58). The plasma half-life values given are therefore only approximations as the log – concentration time curve was curvilinear except for patients 8 and 9. (Table 38 and Figs. 55 to 57).

**Patients without cardiac failure (1 to 5)**

There was an initial rapid but irregular decline in the plasma concentrations during the first 2 h. There was a second phase of decline with an apparent individual mean plasma half-life of \(2.8 \pm 0.23\) h from 2 to 6 h after stopping the lignocaine infusion, but after 6 h the half-life increased to \(4.4 \pm 0.30\) h (Table 38).

24 h after stopping the infusion the mean plasma lignocaine concentration had fallen to only \(0.04 \pm 0.01\) \(\mu\)g/ml. (Fig. 55, Table 35).

**Patients with mild cardiac failure (6, 7 and 9)**

The mean plasma lignocaine declined rapidly from \(4\) \(\mu\)g/ml to 2.3 \(\mu\)g/ml at 2 h after stopping the lignocaine infusion. The mean rate of disappearance of lignocaine from the plasma decreased progressively and the mean terminal apparent half-life in patients 6, 7 and 9 from 6 to 24 h was \(12.0\) h. (Table 38). The plasma lignocaine half-life /
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<td>Patients with cardiac failure and shock</td>
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</tr>
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<td>5.36</td>
<td>5.87</td>
<td>Died at</td>
<td>9 h</td>
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</tbody>
</table>

* Patient 9 had no myocardial infarction.

Table 35. Plasma lignocaine concentrations (µg/ml) after discontinuing constant infusion of lignocaine in patients with myocardial infarction and cardiac failure.
half-life in patient 9 was abnormally prolonged (15.7 h) (Fig. 57).

The mean plasma lignocaine concentration 24 h after stopping the lignocaine infusion was 0.25 µg/ml.

**Patient with cardiac failure and shock (8)**

Lignocaine concentrations were only measured for 8 h after stopping the infusion in this patient. The concentrations did not decline and remained very high in the range of 5.0 to 6.46 µg/ml. This patient died 9 h after the lignocaine infusion was stopped. (Fig. 58, Table 35).

(ii) **EGX**

(Table 36)

**Patients without cardiac failure (1 to 5)**

The mean plasma EGX concentration fell from 0.40 ± 0.04 µg/ml with an apparent initial half-life of 3 h to 0.19 ± 0.02 µg/ml at 4 h. The plasma concentrations subsequently declined with a mean half-life of 5.2 ± 0.39 h to 0.02 µg/ml 24 h after stopping the lignocaine infusion (Fig. 55, Table 36). Thus the plasma half-life of EGX is slightly longer than that of lignocaine in these patients.

**Patients with mild to moderate cardiac failure (6, 7 and 9)**

The mean plasma EGX concentrations remained high between 0.75 and 0.56 µg/ml during the first 4 h. The mean plasma concentration then declined with an apparent half-life of 10.3 h (Table 38). In patient 9 the plasma EGX concentration actually increased to 1.2 µg/ml 6 h after stopping the infusion. The concentration then fell to 0.27 µg/ml 24 h after the infusion was stopped. (Figs. 56 and 57, Tables 36 and 38).

The /
### Table 36: Plasma concentrations of ECGX (μg/ml) after discontinuing a constant infusion of 1.4 mg/min lignocaine HCl in patients with myocardial failure.

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<th>Time after stopping the infusion (h)</th>
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<th>1/2</th>
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<td>0.01</td>
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<td>3</td>
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<tr>
<td>Mean ± SE</td>
<td>0.40 ± 0.04</td>
<td>0.37 ± 0.05</td>
<td>0.35 ± 0.04</td>
<td>0.32 ± 0.04</td>
<td>0.28 ± 0.02</td>
<td>0.19 ± 0.02</td>
<td>0.16 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.07 ± 0.005</td>
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<td><strong>Patients with mild to moderate cardiac failure</strong></td>
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<td>0.72</td>
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<td>0.79</td>
<td>0.57</td>
<td>0.41</td>
<td>0.31</td>
<td>0.28</td>
<td>0.20</td>
<td>0.06</td>
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<td>1.04</td>
<td>1.04</td>
<td>1.13</td>
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<td>0.84</td>
<td>0.80</td>
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<td>0.74</td>
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<td>0.54</td>
<td>0.41</td>
<td>0.36</td>
<td>0.12</td>
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<td><strong>Patient with cardiac failure and shock</strong></td>
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</tr>
<tr>
<td>8</td>
<td>0.56</td>
<td>0.48</td>
<td>0.43</td>
<td>0.32</td>
<td>0.34</td>
<td>0.32</td>
<td>0.38</td>
<td>Died at 9 h</td>
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</table>

Table 36. Plasma concentrations of ECGX (μg/ml) after discontinuing a constant infusion of 1.4 mg/min lignocaine HCl in patients with myocardial failure.
The mean EGX concentrations after stopping the infusion in these patients were higher than the corresponding values obtained from the patients without failure. Statistical analysis was not done because of the small numbers.

**Patient with cardiac failure and shock (8)**

In this patient there was little change in the plasma concentrations of EGX. The concentration fell from 0.56 µg/ml to 0.32 µg/ml at 2 h and then actually rose to 0.38 at 8 h (Fig. 58, Table 36).

(iii) **GX**

The plasma GX concentrations after stopping the lignocaine infusion are shown in Table 37.

**Patients without cardiac failure (1 to 5)**

There was an initial rapid fall in the mean concentrations from 0.54 ± 0.04 µg/ml to 0.46 ± 0.03 µg/ml during the first half hour and then remained relatively steady until 4 h. (Fig. 55). The mean concentrations then decreased with a mean apparent half-life of 16.1 ± 2.12 hours to 0.16 ± 0.02 µg/ml at the 24th hour after stopping the lignocaine infusion. (Table 38)

**Patients with mild to moderate cardiac failure (6, 7 and 9)**

The mean plasma GX concentrations in these patients remained fairly constant between 0.38 and 0.44 µg/ml 0 - 24 h after stopping the infusion. It was not possible to estimate the plasma half-life of this metabolite in patient 6 as the plasma concentrations fluctuated between 0.20 and 0.08 µg/ml from 2 to 24 h after stopping the lignocaine infusion. In patient 9 without myocardial infarction, the /
<table>
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<tr>
<th>Patient number</th>
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<th>1/2</th>
<th>1</th>
<th>2</th>
<th>4</th>
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<td>0.41</td>
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<td>0.35</td>
<td>0.34</td>
<td>0.35</td>
<td>0.30</td>
<td>0.29</td>
<td>0.25</td>
<td>0.24</td>
<td>0.30</td>
<td>0.18</td>
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<tr>
<td>2</td>
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<td>0.43</td>
<td>0.43</td>
<td>0.42</td>
<td>0.38</td>
<td>0.39</td>
<td>0.36</td>
<td>0.39</td>
<td>0.22</td>
<td>0.13</td>
</tr>
<tr>
<td>3</td>
<td>0.52</td>
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<td>0.48</td>
<td>0.52</td>
<td>0.50</td>
<td>0.43</td>
<td>0.38</td>
<td>0.38</td>
<td>0.33</td>
<td>0.20</td>
<td>0.24</td>
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<tr>
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<td>0.58</td>
<td>0.57</td>
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<td>0.50</td>
<td>0.36</td>
<td>0.37</td>
<td>0.32</td>
<td>0.19</td>
<td>0.19</td>
<td>0.24</td>
</tr>
<tr>
<td>5</td>
<td>0.48</td>
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<td>0.43</td>
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<td>0.26</td>
<td>0.19</td>
<td>0.15</td>
<td>0.11</td>
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<td>0.11</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>0.54</td>
<td>0.49</td>
<td>0.46</td>
<td>0.44</td>
<td>0.40</td>
<td>0.34</td>
<td>0.29</td>
<td>0.25</td>
<td>0.16</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>Patients with mild to moderate cardiac failure</strong></td>
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<tr>
<td>6</td>
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<td>0.27</td>
<td>0.22</td>
<td>0.20</td>
<td>0.13</td>
<td>0.08</td>
<td>0.11</td>
<td>0.11</td>
<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>7</td>
<td>0.36</td>
<td>0.37</td>
<td>0.32</td>
<td>0.31</td>
<td>0.32</td>
<td>0.30</td>
<td>0.26</td>
<td>0.25</td>
<td>0.18</td>
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<td>9</td>
<td>0.67</td>
<td>0.67</td>
<td>0.67</td>
<td>0.75</td>
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<td>0.75</td>
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<td>0.44</td>
<td>0.40</td>
<td>0.42</td>
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<td>0.47</td>
<td>0.39</td>
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<td>0.39</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.50</td>
<td>0.51</td>
<td>0.48</td>
<td>0.32</td>
<td>0.38</td>
<td>0.35</td>
<td>0.48</td>
<td>Died</td>
<td>at 9 h</td>
<td>0.48</td>
<td>0.48</td>
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</table>

Table 37. Plasma concentrations of GX (µg/ml) after discontinuing a constant infusion of 1.4 mg/min lignocaine HCl in patients with myocardial infarction and cardiac failure.
### Table 38.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Lignocaine $t_{1/2}$ (h)</th>
<th>EGX $t_{1/2}$ (h)</th>
<th>GX $t_{1/2}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 - 6</td>
<td>6 - 24</td>
<td>4 - 24</td>
</tr>
<tr>
<td>1 - 5</td>
<td>2.8</td>
<td>4.4</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>±0.23</td>
<td>±0.30</td>
<td>±0.39</td>
</tr>
<tr>
<td>6</td>
<td>2.8</td>
<td>9.0</td>
<td>10.0</td>
</tr>
<tr>
<td>7</td>
<td>2.2</td>
<td>11.4</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td>*26.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>15.7</td>
<td>12.5</td>
</tr>
<tr>
<td>Mean</td>
<td>-</td>
<td>12.0</td>
<td>10.3</td>
</tr>
</tbody>
</table>

* measured from 12 and 24 h samples only.

** measured from 6 to 24 h samples.

Apparent plasma half-life ($t_{1/2}$) of lignocaine, EGX and GX in patients with myocardial and/or cardiac failure after discontinuing a constant infusion of 1.4 mg/min lignocaine HCl.
Figure 55. Fall in the mean plasma concentrations of lignocaine, EGX and GX after discontinuing constant i.v. lignocaine infusions in patients (1 - 5) with myocardial infarction, but without cardiac failure. The patients received the drug for periods of 40 to 46 h.
Figure 56. Fall in the mean plasma concentrations of lignocaine, EiX and GX after discontinuing lignocaine infusion in 2 patients (6 and 7) with acute myocardial infarction complicated by mild acute cardiac failure. The lignocaine was given for 46 h.
Figure 57. Fall in plasma concentrations of lignocaine, EGX and GX after discontinuing lignocaine infusion in a patient (No. 9) who had ventricular arrhythmias and cardiac failure, but no evidence of myocardial infarction. He received 1.3 mg/min lignocaine for 45 h. The concentrations of EGX and GX were high several hours after the infusion was stopped.
Figure 58. Plasma concentrations of lignocaine, EGX and GX after stopping lignocaine infusion in a patient who developed cardiogenic shock 25 h after starting the infusion. There was virtually no decline in the concentrations of lignocaine and its metabolites. The patient died 9 h after stopping the lignocaine infusion.
the plasma concentrations of GX actually rose from 0.67 at the end of the infusion to 1.05 µg/ml at 12 h and then declined with a plasma half-life of 38.4 h to 0.86 µg/ml at 24 h after stopping the infusion (Fig. 57). The plasma GX concentrations in this patient were higher than those obtained in the other 8 patients after stopping the infusion. The plasma half-life in patient 6 was 26.2 h between 12 and 24 h after the lignocaine infusion was stopped. Fig. 56 shows the combined results in patients 6 and 7. The mean plasma GX concentrations were lower than those obtained in patients 1 to 5 and 9 (Table 37).

Patient with severe cardiac failure and shock (8)
There was little change in the plasma GX concentrations during the period of observation and it varied between 0.51 and 0.32 µg/ml. (Fig. 58, Table 37).

(c) Urinary excretion
The total urinary recovery of lignocaine and its metabolites from 0 to 40 h and 0 to 160 h after starting the lignocaine infusion is shown in Tables 39 a and b. The cumulative excretion of lignocaine, EGX, GX and 4-hydroxyxylidine is shown in Figs. 59 to 66.

(i) Lignocaine

In patients 1 to 5 only 2.7 ± 0.39% of the administered dose of lignocaine was recovered unchanged in the urine in the first 40 h. (Table 39 a). The corresponding recoveries in patients 6, 7 and 9 were 6.5%, 3.9% and 5.0% respectively.

In patient 8, 4.0% of the dose of lignocaine was recovered unchanged /
### Table 39a.

Total excretion of lignocaine and its metabolites in urine in patients with myocardial infarction 0 - 40 h after starting lignocaine infusion.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Mean urine volume (litres)</th>
<th>Percentage of dose excreted as</th>
<th>Total recovered %</th>
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<tr>
<td></td>
<td>pH 0 - 40 h</td>
<td>Lignocaine</td>
<td>EGX</td>
</tr>
<tr>
<td>1 - 5</td>
<td>5.5 ± 0.03</td>
<td>2.7</td>
<td>2.0</td>
</tr>
<tr>
<td>6, 7 &amp; 9**</td>
<td>5.6 ± 0.60</td>
<td>5.4</td>
<td>5.1</td>
</tr>
<tr>
<td>8*</td>
<td>5.1 ± 0.04</td>
<td>4.0</td>
<td>0.9</td>
</tr>
</tbody>
</table>

* Collections were made from 0 - 36 and 0 - 45 h.
** Patient 9 had cardiac failure only.

### Table 39b.

Total excretion of lignocaine and its metabolites in patients with myocardial infarction 0 - 160 h after starting lignocaine infusion.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Mean urine volume (litres)</th>
<th>Percentage of dose excreted as</th>
<th>Total recovered %</th>
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<td>pH 0 - 160 h</td>
<td>Lignocaine</td>
<td>EGX</td>
</tr>
<tr>
<td>1 - 5</td>
<td>5.6 ± 0.03</td>
<td>3.3</td>
<td>2.5</td>
</tr>
<tr>
<td>6, 7 &amp; 9**</td>
<td>5.8 ± 1.36</td>
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<td>6.2</td>
</tr>
<tr>
<td>8*</td>
<td>5.1 ± 0.04</td>
<td>4.1</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* Patient 8 died 9 h after stopping lignocaine infusion.
** Patient 9 had cardiac failure only.
*** Collections were made from 0 - 154 and 0 - 169 h.
unchanged at 0 - 4.0 h. The urine in all the patients was acidic (pH ranged between 5.1 and 5.9). The excretion of lignocaine often continued many hours after stopping the infusion, particularly in patients 6, 7 and 9 (Figs. 59 to 62).

(ii) **EGX**

Only a small fraction of the administered dose of lignocaine was excreted from 0 to 160 h as EGX in patients 1 to 5 and in patient 8. In the latter patient only 1% of the dose of lignocaine had been excreted as EGX by the time he died (Fig. 61).

However, urine was collected for only 45 h. The proportion of the dose of lignocaine excreted as EGX from 0 - 160 h was higher in the other three patients with cardiac failure (6, 7 and 9) (Tables 39 a and b) than in those without. The percentage recovered as EGX in patients 6, 7 and 9 5 days after stopping the infusion ranged from 4.5 to 8.2% (Figs. 59 - 62).

(iii) **GX**

Very small amounts of GX were excreted in all patients. Even in the absence of cardiac failure and with acidic urine only 2.9 ± 0.31% of the dose of lignocaine had been recovered in the urine as GX 5 days after stopping the lignocaine infusion (Tables 40 a and b).

The recovery of GX in the urine was even less in patients with mild cardiac failure or shock (Tables 39 a and b).

The excretion of the compound was slow and was easily measured in the urine several days after stopping the infusion (Figs. 59 - 62).

(iv) /
Figure 59. Mean cumulative excretion (%) of lignocaine, EGX and GX during and after a constant i.v. infusion of 1.4 mg/min lignocaine HCl in patients (1 - 5) with acute myocardial infarction and without cardiac failure. The lignocaine was infused for 40 to 46 h. (The points at the stated times were extrapolated from cumulative excretion curves of each patient).
Figure 60. Cumulative excretion (%) of lignocaine, EGX and GX during and after a constant i.v. infusion of 1.4 mg/min lignocaine HCl in patient 6 with myocardial infarction complicated by cardiac failure. The drug was infused for 46 h.
Figure 61. Cumulative excretion (%) of lignocaine, EGX, and GX during and after a constant i.v. infusion of 1.4 mg/min lignocaine HCl in patient 8 with acute myocardial infarction who developed cardiogenic shock 25 h after starting the infusion. This patient received the drug for 40 h and died 9 h after the infusion was stopped.
Figure 62. Cumulative excretion (%) of lignocaine, EGX and GX during and after a constant infusion of 1.3 mg/min lignocaine HCl in a patient (No. 9) with acute cardiac failure and ventricular arrhythmia. He had no evidence of myocardial infarction.
(iv) 4-hydroxyxylidine

4-hydroxyxylidine was quantitatively the most important metabolite and the recovery of this compound was higher in the patients without cardiac failure (Tables 39 a and b). Thus 62.4 ± 3.05% of the administered dose of lignocaine was recovered in the first 40 h as 4-hydroxyxylidine in patients 1 to 5, whereas the corresponding mean recovery in patients 6, 7 and 9 was 23%. During the same period only 15.2% of the administered dose of lignocaine was recovered as 4-hydroxyxylidine in patient 8.

The excretion of this compound continued several days after stopping the infusion particularly in patients 6, 7 and 9 (Figs. 63 to 66).

(v) Total urinary recovery of lignocaine and its metabolites

The total urinary recovery of the administered dose of lignocaine as unchanged drug, EGX, GX and 4-hydroxyxylidine is shown in Tables 39 a and b.

In patients without cardiac failure or shock 77.2 ± 2.4 of the dose of lignocaine was recovered in the urine up to 160 h whereas the total recovered within the same period was much less in those with cardiac failure (mean 48.7%). However, the values in patient 9 were underestimated as one urine sample was lost the first day after stopping the infusion.

In patient 8 only 20.8% of the dose of lignocaine was recovered, but death occurred 9 h after stopping the lignocaine infusion.
Figure 63. Mean cumulative excretion (%) of 4-hydroxyxylidine during and after a constant i.v. infusion of 1.4 mg/min lignocaine HCl in patients (1 - 5) with myocardial infarction and no cardiac failure. The duration of infusion was 4.0 to 4.6 h. (The points were extrapolated from individual cumulative excretion curves).
Figure 6L. Cumulative excretion (%) of 4-hydroxyxydine during and after a constant i.v. infusion of 1.4 mg/min lignocaine HCl in patient 7 with myocardial infarction complicated by mild cardiac failure. The duration of the infusion was 46 h.
Cumulative excretion (%) of 4-hydroxyxylidine during and after a constant i.v. infusion of 1.4 mg/min lignocaine HCl in patient 8 with acute myocardial infarction and cardiogenic shock. The patient died 9 h after stopping the infusion.
Figure 66. Cumulative excretion (%) of 4-hydroxyxylidine during and after a constant i.v. infusion of 1.3 mg/min lignocaine HCl in patient 9 with acute cardiac failure but no evidence of myocardial infarction.
ANTIPYRINE METABOLISM

The plasma antipyrine half-life, its apparent volume of distribution and its plasma clearance are shown in Tables 40 a, b and c. In all three patients the plasma antipyrine half-life was prolonged and the mean value was 23.2 h. When the study was repeated 10 to 12 days later during convalescence there was a substantial reduction in the antipyrine half-life in patients 6 and 7 and a smaller reduction in patient 9. The mean antipyrine half-life during convalescence was 15.0 h (Table 40 a).

The mean apparent volume of distribution of antipyrine was 28.8 litres when the patients were acutely ill, but this increased to 35.0 litres when they were clinically better (Table 40 b).

In all three patients there was a considerable reduction in the plasma antipyrine clearance when they were acutely ill. Thus the mean plasma antipyrine clearance increased from 14.3 to 27.9 ml/min when the patients recovered from the acute illness (Table 40 c). The change in antipyrine clearance was however smaller in patient 9 and larger in patient 7. Thus there was impairment of antipyrine metabolism in the patients with acute mild to moderate cardiac failure. However, these pharmacokinetic calculations have been based on the assumptions that (1) in each patient the urinary clearance of antipyrine was negligible and was not greatly changed during the two studies and (2) that plasma protein binding of the drug did not change. It was also assumed that absorption was complete and that induction of hepatic enzyme drug metabolising activity did not occur.
### Table 4.0a

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### Table 4.0b

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### Table 4.0c

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<td>6</td>
<td>17.5</td>
<td>25.6</td>
</tr>
<tr>
<td>7</td>
<td>13.8</td>
<td>39.8</td>
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<tr>
<td>9</td>
<td>11.7</td>
<td>18.4</td>
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<tr>
<td>Mean</td>
<td>14.3</td>
<td>27.9</td>
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Antipyrine plasma half-life, apparent volume of distribution and plasma clearance in 3 patients with cardiac failure in the C.C.U. and during convalescence.

* Antipyrine (18 mg/kg) was given on the day after admission to the C.C.U. and again 10 - 12 days later during convalescence.
CHAPTER 4

DISCUSSION

A LIGNOCAIN METABOLISM

The results obtained in these studies cannot be compared directly with those obtained in healthy volunteers given parenteral lignocaine. The physiological state of the patients was changing during the study, they had received several other drugs in addition to lignocaine and the lignocaine was given by a different route and over a prolonged period.

(a) During infusion

Even when lignocaine was infused at a constant rate of 1.4 mg/min the plasma concentrations of the drug were within the alleged therapeutic levels of 1.2 to 6.0 μg/ml (Harrison and Alderman, 1971). In the presence of cardiac failure or shock, plasma lignocaine concentrations can reach potentially toxic levels. Prescott and Nimmo (1971) also observed a steep rise in plasma lignocaine concentrations in a patient with cardiogenic shock given only 0.7 mg/min of constant lignocaine infusion.

A true 'steady state' plasma lignocaine concentration was not achieved in these patients and the concentrations rose during the infusion. There was presumably a reduction in the apparent volume of distribution (Vd) and or plasma clearance of the drug.

(i) Changes in the Vd of lignocaine

Prescott and Nimmo (1971) calculated that both the apparent volume of distribution and plasma clearance of lignocaine were reduced in patients with acute myocardial infarction even in the absence /
absence of cardiac failure. However, these calculations were based on the assumption that steady state plasma lignocaine concentrations had been reached.

Hayes (1971) also observed that the plasma lignocaine concentrations rose towards the end of 24 h of a constant lignocaine infusion but no satisfactory explanation was given. Hayes claimed that a 'steady state' plasma lignocaine concentration was reached within 8 to 12 h.

The Vd of lignocaine in the present studies could not be measured and it may not have been constant because of the changing haemodynamic and physiological state in the patients. The blood pressure in all these patients, except No. 9, fell gradually during the first 24 to 48 h. In addition there may have been altered redistribution of the drug caused by changes in regional blood flow and peripheral vasoconstriction.

Patient 8 was in severe cardiac failure with cardiogenic shock and additional factors could have been metabolic acidosis with a fall in blood pH. This would have the effect of decreasing tissue uptake of lignocaine.

The role of other drugs given to the patients in altering the distribution of lignocaine cannot be assessed. Some of the patients received diuretic therapy. In addition, as the clinical state of the patients improved, there was marked diuresis. A possible rise in plasma lignocaine concentration as a result of haemoconcentration may have been a contributory factor in the rise in plasma lignocaine concentrations during the infusion.
(ii) Changes in the metabolic clearance of lignocaine during infusion

Even when the urine was acid less than 5% (range 1.8 - 4.5%) of the administered dose of lignocaine was excreted unchanged in the patients without cardiac failure. The drug was apparently extensively metabolised in these patients and about 70% of the administered dose could be accounted for as EGX, GX and 4-hydroxyxylidine 40 h after starting administration of the drug. In these patients therefore it may be inferred that major reduction in lignocaine clearance did not occur 0 to 40 h after starting the lignocaine therapy. These findings give no information on the rate of lignocaine metabolism but an initial reduction in the plasma clearance of the drug during the first few hours of infarction cannot be excluded.

In the patients with myocardial infarction and mild to moderate cardiac failure (6 and 7), only about 34% of the administered dose of lignocaine was excreted as unchanged drug, EGX, GX and 4-hydroxyxylidine after 40 h of starting lignocaine therapy. The plasma clearance of lignocaine was probably greatly depressed since its concentrations rose to high levels at 8 h after starting the infusion, remained high and, in addition, the concentrations of EGX and GX were low particularly during the first 32 h after starting the infusion.

In patient 8 the 40 h urinary recovery of lignocaine and its metabolites was even lower but this could have been due to both a greatly reduced lignocaine metabolic clearance and pre-renal failure which /
which must have been present. The drop in plasma lignocaine concentration observed at the time when this patient was severely hypotensive may be partly due to haemodilution as a result of intravenous fluids which this patient received at that time. The plasma lignocaine rose steeply soon after the onset of shock and the metabolism of the drug must have been markedly depressed. It has been shown by Thomson et al (1971) that the clearance of lignocaine is not affected by severe renal disease.

In patient 9 the proportion of the dose of administered lignocaine excreted as EGX, GX and 4-hydroxyxylidine after 40 h was much less (30%) than the corresponding values for patients 1 to 5 (about 70%), but there was a progressive rise in the plasma concentration of EGX and GX with infusion time. These findings could be explained by a low initial metabolic clearance of lignocaine since on admission the patient was acutely ill with reduced cardiac output due to ventricular tachycardia. When the arrhythmia was controlled the haemodynamic state improved, hepatic blood flow probably increased with improvement of hepatic function and hence the rate of lignocaine metabolism was accelerated.

The cause of reduced metabolic clearance of lignocaine in the patients with cardiac failure or shock is not known. Possible factors include impaired hepatic function due to reduced hepatic blood flow (Sherlock, 1971), raised venous pressure and hypoxia. Central liver cell necrosis occurs in shock (Eilenberg and Osserman, 1951) and in myocardial infarction (Clarke, 1950). In patient 8 central /
central lobular necrosis was reported soon after death. Hepatic function was difficult to assess in these patients, but patients 2 to 8 had transient rise in SGPT and the alkaline phosphatase values obtained were near the upper limit of normal. In patient 6 it was higher than normal.

(iii) Additional factors

The effect of other drugs on the metabolism of lignocaine cannot be readily assessed. All the patients received morphine which may inhibit drug metabolism (Gillette, 1971: Chaplin, Sladek and Mannering, 1968). Patient 9 had been on long term thyroxine therapy (0.15 mg/day). Since thyroxine may inhibit drug metabolism (Conney and Garren, 1961) it cannot be excluded as a possible cause of inhibition of lignocaine metabolism in this patient.

Competition between lignocaine and its metabolites EGX and GX for the same microsomal enzymes may have occurred, but it is difficult to understand why such a competition should have occurred mainly in the patients with cardiac failure or shock. In these patients the conversion of EGX to GX may have been inhibited since the plasma concentrations of GX remained lower than that of EGX. The relationship was reversed in patients without cardiac failure. The higher urinary recovery of EGX in those with failure compared to those without is consistent with this hypothesis.

Another factor which may have contributed to the progressive rise in the plasma lignocaine concentrations during the infusion may have been the saturation of the lignocaine metabolizing enzymes as the concentration /
concentration of the drug in the liver increased. There is evidence that saturation of drug metabolising enzymes occurs with some drugs (Dayton and Perel, 1971).

(b) **After stopping the lignocaine infusion**

After discontinuing the lignocaine infusion the drug disappeared abnormally slowly in all the patients. The decline in plasma levels did not follow first order kinetics and the plasma lignocaine half-life increased progressively.

The apparent plasma lignocaine half-life values from 2 to 6 h after discontinuing the infusion in the patients without cardiac failure agree with those obtained by Prescott and Nimmo (1971).

The increase in the lignocaine half-life is consistent with slow release of the drug from tissues which are poorly perfused with blood. The transfer of the drug from tissues to plasma could become a rate limiting step in the elimination of lignocaine from the body. A reduced metabolic clearance of lignocaine cannot be ruled out even in the absence of cardiac failure.

The extremely long apparent plasma half-life of lignocaine EGX and GX in the patients with cardiac failure and the virtual failure of elimination of these compounds from the plasma of the shocked patient can be explained by a markedly reduced hepatic metabolism of these compounds. Such gross abnormalities cannot be conceivably due to changes in the volume of distribution alone. The very low urinary recovery of GX and 4-hydroxyxylidine in these patients is consistent with this view.

(c) /
(c) **Urinary excretion**

The results are difficult to interpret because of variation in urine flow rate due to diuretic therapy and cardiac failure. The excretion data obtained in patients with cardiac failure or shock probably gives a true index of impaired drug metabolism since renal clearance of lignocaine is not significantly altered by urine flow (Eriksson and Granberg, 1965).

The relatively higher recovery of EGX in these patients suggests selective impairment of drug metabolism. The general pattern of total excretion of lignocaine and its metabolites compare with those obtained in the patients with chronic liver disease. In both groups of patients there was increased recovery of the dose of administered lignocaine as unchanged drug and EGX, but a decreased recovery of the dose as GX and 4-hydroxyxylidine. This similarity further supports the hypothesis that lignocaine metabolism by the liver was impaired in patients with acute and mild to moderate cardiac failure and or shock.
ANTIPYRINE METABOLISM

Initially the plasma antipyrine half-life was abnormally prolonged in all three patients. The antipyrine half-life values obtained during convalescence were all shorter and within the normal range of 12.1 ± 0.4 h (O'Malley et al., 1971) in two patients.

The prolonged plasma antipyrine half-life during the acute illness cannot be solely explained on the basis of the reduced apparent volume of distribution (Vd) of the drug observed since the Vd was virtually unchanged in one patient (6) and the plasma clearance of the drug was reduced in all three.

When the patients were acutely ill a reduction of hepatic blood flow may have occurred as a result of reduced cardiac output. Since the extraction ratio of antipyrine is low, i.e. 0.03 and its Vd is low, any reduction in liver blood flow could not have contributed significantly to the abnormally long plasma antipyrine half-life in these patients.

The shorter plasma antipyrine half-life and the increased clearance of the drug during convalescence are not likely to be due to induction of hepatic microsomal drug metabolising enzyme since the patients did not receive drugs which are known microsomal inducers when they were in the wards.

During convalescence the plasma antipyrine half-life in patient 9 was relatively long. A possible explanation may be due to the fact that he was older (76 years) since the antipyrine half-life increases with age. (O'Malley et al., 1971). In addition, antipyrine metabolism in this patient may have been inhibited by chronic thyroxine therapy. It is interesting to note that although this patient/
patient had the longest plasma lignocaine half-life (15.7 h) his plasma antipyrine half-life was the shortest (20 h) during the acute illness.

Since the renal elimination of antipyrine is negligible, (Breckenridge et al., 1973) the most likely explanation for the changes in the plasma antipyrine half-life, its Vd and its clearance is that the hepatic metabolism of the drug was markedly impaired during the acute illness.

CLINICAL IMPLICATIONS

Although EGX and GX are pharmacologically active metabolites of lignocaine, the plasma concentrations of each were generally below 1 μg/ml except in patient 9. The antiarrhythmic activity of each of these metabolites is only about 60% of that of lignocaine (Astrom, 1971; Smith and Duce, 1971; Boyes, 1972, personal communication) and these metabolites are unlikely to have contributed significantly to the antiarrhythmic effects of lignocaine in these patients. However, the concentrations of EGX and GX achieved in the myocardium is unknown and the combined concentrations of these active metabolites may be significant.

Patient 9 developed nausea towards the end of the infusion when the total concentration of these bases was rather high (5.6 μg/ml). It is tempting to postulate that this was due to the combined actions of lignocaine, EGX and GX on the brain. However, this patient was also on digoxin therapy (0.75 mg daily). EGX and GX have been shown to cause emesis and convulsions in animals. Strong and Atkinson (1972)
(1972) claimed to have measured an EGX plasma concentration of 2.6 \( \mu g/\text{ml} \) in a patient treated with lignocaine and without any evidence stated that this was largely the cause of central nervous system toxicity. No details of the dose and duration of lignocaine administered, the clinical state of the patient or other administered drugs were given. Strong, Parker and Atkinson, (1973) attributed central nervous system toxicity in another patient to EGX and GX, but again they did not provide sufficient details in support of this claim. Higher plasma concentrations might have been obtained in the present studies if larger doses of lignocaine had been given.

An important observation in this study is that very high plasma concentrations of lignocaine may be attained in the presence of cardiac failure or shock. Furthermore, hepatic metabolism of other drugs will be grossly abnormal under these conditions. Ristola and Pyörälä (1972) were unable to demonstrate prolongation of the plasma warfarin half-life in a group of patients with acute myocardial infarction and cardiac failure. However, in some patients with severe cardiac failure the plasma warfarin half-life was prolonged initially.

It is clear that regular monitoring of plasma lignocaine concentrations is essential during chronic infusions in patients with cardiac failure.
SUMMARY

1. During a constant intravenous infusion of 1.4 mg/min lignocaine HCl for 4.0 to 4.6 h 'steady-state' plasma levels of the drug were not attained in 8 patients with myocardial infarction. The plasma concentrations increased during the infusion.

2. In 5 patients with no cardiac failure the mean plasma lignocaine concentration rose from 1.36 \( \pm \) 0.05 \( \mu g/ml \) at 8 h to 2.76 \( \pm \) 0.26 \( \mu g/ml \) at 4.0 h after starting the infusion. These concentrations are within the alleged 'therapeutic range' for antiarrhythmic activity of the drug.

In 4 patients with cardiac failure with or without myocardial infarction, the plasma concentrations were much higher and exceeded the potentially toxic concentration of 6 \( \mu g/ml \) in 1 patient with cardiogenic shock.

3. There was a slow but steady accumulation of the active metabolites ethylglycylxylidide (EGX) and glycylxylidide (GX) during the lignocaine infusion. In 8 patients the plasma concentrations of each of these metabolites were low and did not exceed 1 \( \mu g/ml \).

In 1 patient with acute cardiac failure only the plasma EGX concentration rose to 1 \( \mu g/ml \) after 4.0 h of lignocaine infusion. Plasma GX concentrations were generally higher than those of EGX in patients without cardiac failure or shock, but the relationship was reversed in patients with cardiac failure.
4. When the infusion was discontinued, plasma lignocaine concentrations declined at a decreasing rate except in the patient with severe cardiac failure and shock, where the plasma lignocaine concentrations did not fall. The mean apparent plasma half-life of the drug between 6 and 24 h after discontinuing the infusion was $4.4 \pm 0.3$ h in the patients without cardiac failure. In 2 patients with myocardial infarction complicated by mild to moderate degree of cardiac failure the corresponding values were 9.0 and 11.4 h. In the patient with cardiac failure only this was 15.7 h.

5. The plasma concentrations of EGX also declined at a decreasing rate in all patients except the patient with severe cardiac failure and shock. The mean apparent plasma half-life of EGX between 4 and 24 h after stopping the lignocaine infusion was $5.2 \pm 0.39$ h in the 5 patients without cardiac failure and 10.3 h in the 3 patients with mild to moderate cardiac failure. In the patient with severe cardiac failure and shock there was no fall in the plasma EGX concentrations.

6. In the patients without cardiac failure GX disappeared from the plasma with a mean apparent half-life of $16.1 \pm 2.12$ h, but the half-life was grossly prolonged to about 40 h in the patients with mild to moderate cardiac failure. In the patient with severe /
severe cardiac failure and shock there was little change in the plasma GX concentrations during the 8 h period after the lignocaine infusion was discontinued.

7. In the patients without cardiac failure 77.2 ± 2.4% of the total dose of the lignocaine was accounted for as unchanged drug, EGX, GX and 4-hydroxyxylidine in the urine in 7 days. In the patients with mild to moderate cardiac failure the corresponding mean recovery was 48.7%. In the patient with cardiogenic shock only 20.8% was recovered.

8. In the patients with cardiac failure the proportion of the dose recovered as unchanged lignocaine and EGX in the urine was higher than in the patients without cardiac failure. Conversely, more 4-hydroxyxylidine was recovered in the patients with mild to moderate cardiac failure. Less than 4% of the dose of lignocaine appeared in the urine as GX and even less was recovered in the patients with mild to moderate cardiac failure.

9. The urinary excretion of lignocaine and its metabolites continued for several days after the infusion was stopped.

10. The antipyrine plasma half-life was prolonged in the 3 patients with cardiac failure. During convalescence the antipyrine plasma half-life was decreased in all 3 patients. The antipyrine plasma clearance was low in these patients, but this /
this was nearly doubled during convalescence.

11. These findings are consistent with impairment of lignocaine and antipyrine metabolism in patients with myocardial infarction, particularly in the presence of cardiac failure or shock. The metabolism of other drugs may also be abnormal in these patients.
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