THE EFFECT OF ALCOHOL, ISONIAZID, RIFAMPICIN, PARACETAMOL AND HEXANE ON HEPATIC GLUCONEOGENESIS AND BROMOSULPHTHALEIN CLEARANCE

by

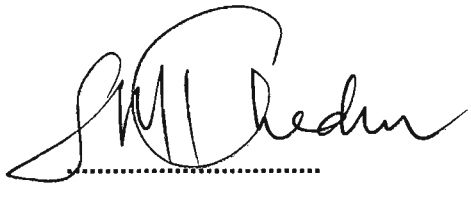
SHAUN MAHABEER KHEDUN

Submitted in partial fulfilment of the requirements for
the degree of
MASTER OF SCIENCE IN MEDICINE
in the
Department of Experimental and Clinical Pharmacology,
University of Natal
1988

Durban, 1988

DECLARATION

I, **SHAUN MAHABEER KHEDUN**, DECLARE THAT THE WHOLE DISSERTATION, UNLESS SPECIFICALLY INDICATED TO THE CONTRARY IN THE TEXT, IS MY OWN ORIGINAL WORK AND HAS NOT BEEN SUBMITTED FOR A DEGREE AT ANY OTHER UNIVERSITY.



.....

S M KHEDUN

The work reported in this dissertation was carried out in the DEPARTMENT OF EXPERIMENTAL AND CLINICAL PHARMACOLOGY, UNIVERSITY OF NATAL, DURBAN, SOUTH AFRICA under the supervision of Professor W P Leary.

To my parents, the late Mr and Mrs R Khedun

ACKNOWLEDGEMENTS

I express my appreciation and thanks to Professor W P P Leary and Dr B Maharaj of the Department of Experimental and Clinical Pharmacology, University of Natal, for their goodwill, guidance, assistance and discussion they so readily and generously afforded me in this project.

Special appreciation and gratitude is extended to Mr Ken van der Byl for his expertise and help with regard to the statistical analysis used in this project.

I would also like to acknowledge Mr Lockett and Dr Botha of the Department of Experimental and Clinical Pharmacology for their encouragement.

A special word of thanks and appreciation to Mrs Odette French not only for typing this dissertation, but also for all the patience she showed in the re-typing of my working drafts.

I wish to express my gratitude to:
The University of Natal Faculty of Medicine Research Fund for awarding me a research grant for this study.

Ciba Geigy and Lennons for the supply of rifampicin and isoniazid respectively.

The Anatomical Pathology Department for examination of tissue samples.
The Medical Illustration Unit for producing the illustrations.
The technical staff of the Department of Pharmacology for their interest and useful suggestions.

ABSTRACT

The first workers to use the isolated perfused rat liver for the study of gluconeogenesis were Corey and Britton (1941). Subsequently, other investigators found the modified method of Miller et al (1951) to be more suitable. This technique, with modifications introduced by Mortimore (1961) and Hems et al (1966) was used in the present study.

The isolated liver is perfused through the portal vein with saline, supplemented by bovine serum albumin and washed human erythrocytes, under a pressure of about 20cm of water, maintained by a reservoir of adjustable height. The perfusate which passes through the liver enters the inferior vena cava and passes, via a cannula, to a collecting vessel from which it is pumped to the top of a multiple bulb oxygenator and then returned to the liver for re-perfusion. This technique has proved to be a satisfactory means of assessing changes in the metabolic status of hepatic cells in response to starvation and exposure to halothane.

The study described here was performed to determine whether the isolated liver perfusion technique can be used to measure the effects on liver perfusion of therapeutic and supra-therapeutic doses of various drugs, some of which have been reported to affect liver metabolism adversely in the intact animal.

Liver function was assessed by studying gluconeogenesis and bromosulphthalein clearance. Alcohol and hexane were administered in toxic doses, rifampicin and isoniazid in high doses and paracetamol in therapeutic doses.

Inbred male Wistar rats were used for these studies. Hexane was injected subcutaneously, while the other drugs were given per os on 7 consecutive days each week for a period of 90 days; with the exception of the control group in the hexane study, all the control groups were untreated.

Pyruvate, a precursor for gluconeogenesis (synthesis of glucose from non-carbohydrate sources) is an excellent substrate for the formation of oxaloacetate, which is probably an obligatory intermediate in the pathway to glucose synthesis. It has been used over a number of years by different investigators who have studied gluconeogenesis using the isolated liver perfusion technique. It was used for the same purpose in the present study.

Methylene blue, a redox dye, capable of oxidising NADH to NAD^+, was used to determine whether an altered NADH : NAD^+ ratio would have any effect on the output of glucose in the ethanol, paracetamol and hexane studies. Fructose, a non-NAD^+ dependent precursor of glucose, was also used for this purpose in the ethanol study.

All the drugs studied were found to inhibit gluconeogenesis. This was shown by a decrease in glucose levels and an increase in lactate : pyruvate ratios in the perfusion medium of experimental livers. The decreased glucose production by the experimental livers, which occurred pari passu with an increased pyruvate utilization, indicates that in these animals pyruvate was used for the production of other compounds such as lactate. In contrast, glucose production and pyruvate utilization were increased in the control group indicating that pyruvate was used mainly for the production of glucose.

In the ethanol group, impaired gluconeogenesis was probably due to a change in the NADH : NAD^+ ratio; when methylene blue was introduced into the perfusion medium of this group the output of glucose was high.

Impaired gluconeogenesis in the paracetamol and hexane-treated groups was probably related to the non-availability of oxaloacetate or impairment of the activity of key enzymes involved in gluconeogenesis; when methylene blue was added to the perfusion medium of these animals the glucose output remained low.

Except for the rifampicin study, bromosulphthalein clearance was impaired in all the experimental groups. Histological examination of liver tissue obtained from the hexane-treated animals demonstrated severe fatty change.

In conclusion, these studies have demonstrated that the isolated liver perfusion technique is a suitable method of evaluating the effect of therapeutic and supra-therapeutic doses of some drugs which affect hepatic function.

Ethanol, isoniazid, rifampicin, paracetamol (in therapeutic doses) and hexane were found to alter liver function as evidenced by impaired gluconeogenesis and bromosulphthalein clearance. In addition, histological evidence of liver damage was noted in rats treated with hexane.

TABLE OF CONTENTS

LIST OF TABLES

RIFAMPICIN

PARACETAMOL

HEXANE.

LIST OF FIGURES

HEXANE

CHAPTER 1

INTRODUCTION

INTRODUCTION

The liver is the major site of gluconeogenesis with the kidney becoming an important site during starvation and acidosis (Exton and Park, 1967; Exton, 1972). Attempts to study gluconeogenesis in rat liver slices met with major difficulties. The rate of gluconeogenesis obtained with substrates such as succinate, malate, fumarate, glutamate and aspartate were unexpectedly low or even nil. Pyruvate and lactate formed glucose but less rapidly than expected (Hems, et al 1966). These observations show that liver slices have limited scope in the study of gluconeogenesis and it was largely as a result of this finding that the technique of the isolated perfused rat liver was developed.

Initially, workers perfused livers of larger animals, mainly dogs, because the surgical and chemical analytical techniques available were on "macro" scale. The first to use the perfused rat liver were Corey and Britton (1941), Trowell (1942) and Miller, Bly, Watson and Bale (1951). The method of Miller et al (1951) proved to be the most acceptable to subsequent workers and this technique, with modifications introduced by Mortimore (1961) and Hems et al (1966), was the starting point of this investigation. Miller et al perfused the isolated liver through the portal vein with heparinized rat blood under a pressure of about 20 cm of water, maintained by a reservoir of adjustable height. Mortimore (1961) perfused the liver in situ, isolating the circulation from the donor animal. Schimassek (1963) replaced blood as perfusate with a saline medium containing bovine albumin and bovine erythrocytes. The perfusion medium used in this study was that of Hems et al (1966) which consisted of physiological saline (Krebs and Hensleit 1932), bovine serum albumin and washed human red cells.

Many experiments were carrried out in which modifications of the perfusion medium were tested, using the flow of medium through the liver and the rate of glucose formation from lactate as criteria of efficacy. Omission of albumin from the medium caused gross swelling of the liver and exudation of fluid from the surface. Varying the concentration of albumin between 1.5% and 6% made no difference. De-ionized haemaccel, a plasma expander, used instead of albumin, gave a good perfusion flow but some haemolysis occurred and the rate of glucose formation from lactate was reduced. Replacement of albumin (2.6%) by dextran (40 or 80) led to clumping of red blood cells and a cessation of flow (Gronwall, 1957). Omission of red cells made no difference, provided that the livers weighed at least 5 g and that the flow was rapid, not less than 30 ml/min.

This technique, with various modifications, has been used over a number of years by different investigators in the study of gluconeogenesis (Hems et al, 1966; Ross et al, 1966), lactate production (Woods and Krebs, 1971) and glycogen, carbohydrate, fatty acid and xylitol metabolism (Sokal et al, 1959; Ross et al, 1967; Krebs and Hems, 1970; Woods and Krebs, 1973). It has also been used to investigate the effects of alcohol on gluconeogenesis (Forsander et al, 1965; Krebs et al, 1969; Krebs et al, 1969) and albumin synthesis (Kirsch et al, 1973). In addition, this technique was used to test bromosulphthalein clearance after the administration of nafenopin (Gartner et al, 1982), halothane (Biebuyck et al, 1970), norbolethone (Bassan et al, 1971) chlorpromazine (Kendler et al, 1971) and albumin (Gumucio et al, 1984). The technique is based upon the cannulation of the portal vein and the inferior vena cava just below the right atrium. The liver is isolated by a tie on the abdominal aorta. The liver is perfused through a cannula in the portal vein. After passing through the liver, the perfusate is collected by a cannula tied into the vena cava just below the right atrium.

The technique was incorporated in the design of the present study for two reasons:

i) The isolated rat liver provides the possibility of studying many aspects of liver metabolism with much greater accuracy than is practical in the whole organism.

ii) The perfused organ has proved to be a better experimental model than liver slices and homogenates because much of the normal metabolic capacity as well as the control mechanisms are lost on disturbing the structure of the organ.

The object of this thesis has been to examine whether the isolated liver perfusion technique can be used to measure the effects on liver function of therapeutic and supra-therapeutic doses of drugs, some of which have been reported to affect liver metabolism adversely in the intact animal.

The drugs used were alcohol, isoniazid, rifampicin, paracetamol and hexane. Alcohol was chosen because its toxic effects on the liver are well documented; metabolic events such as hypoglycaemia and histological abnormalities including fatty change, alcoholic hepatitis and cirrhosis can complicate alcoholism (MacSween, 1983). Isoniazid and rifampicin are first line drugs in the treatment of tuberculosis; jaundice may complicate therapy with either of these drugs (Davies and Glowinski, 1961; Maddrey, 1981; Capelle et al, 1972; Kenwright and Levi, 1974). Paracetamol, a commonly used non-narcotic analgesic, is a major component of many formulations available for the relief of colds, fever, headache and cough (Ameer and Greenblatt, 1977). Liver damage with overdose has been well documented (Record et al, 1981; Davis, 1986; Monteagudo and Folb, 1987). Chronic hepatic inflammation and fibrosis due to low doses of the drug have been reported

(Bonkowsky, 1978) but no literature regarding chronic dosing with therapeutic doses of this drug is available. Solvent abuse or glue-sniffing has become an increasing problem in the young and a wide variety of substances have been involved (Glaser et al, 1962; Watson, 1979). Benzine and glue, which contain hexane in high concentrations, are commonly inhaled by individuals intent upon producing a state of euphoria (Edward, 1982). The effects of hexane on the liver have not previously been studied, whereas its toxic effects on the nervous system are well known (Spencer et al, 1980; Lallo et al, 1981). Liver function was assessed by studying gluconeogenesis and bromosulphthalein clearance.

CHAPTER 2

MATERIALS AND METHODS

MATERIALS AND METHODS

Experimental Animals

85 Wistar rats were used during the investigation of the effects of toxic doses of alcohol and hexane, high doses of isoniazid and rifampicin, and therapeutic doses of paracetamol on hepatic function in vitro.

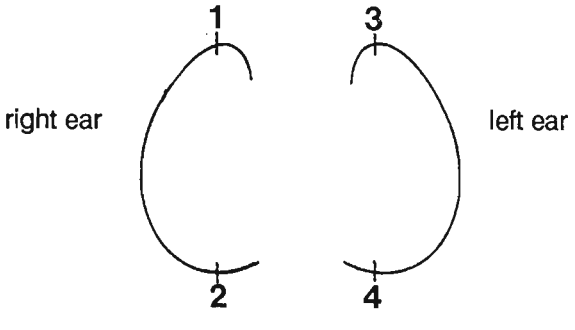
All were inbred male rats obtained from the animal colony, University of Natal Medical School.

Caging

All the animals were kept in polypropylene boxes with metallic grills in the animal colony. The rats were housed three to a cage.

Numbering of Rats

The rats were individually earmarked for identification, by nicking the ears according to the diagram below.



Higher numbers were indicated by more than one nick.

Bedding

Wood shavings (pine and meranti wood) were placed in each box to a depth of about 4 cm to serve as bedding. Bedding was changed on a weekly basis.

Routine Diet

Pelleted rat food was provided in food hoppers and water were given by bottle. Both food and water was supplied ad libitum and changed thrice weekly. The composition of the standard laboratory rat food is shown in Table 1.

Environmental conditions

The rats were maintained under conventional temperature and humidity conditions.

TABLE 1

Constituents of United Oil Epol Rat Cubes

Protein	18.71%	Vitamin	A		21124.00 I.U./kg of feed
Fibre	3.71%	Vitamin	D_3		1677.00 I.U.
Fat	4.31%		E		37.3 mg/kg feed
Sugar	2.14%		B_2	Riboflavine	5.7 mg/kg
Calcium	1.06%			Pantothenic acid	14.8 mg/kg
NaCl	0.63%			Niacin	53.5 mg/kg
Na Phosphates	0.76%			Choline HCL	1117.8 mg/kg
Available Phosphates	0.61%		B_1	Thiamine	5.8 mg/kg
70% from animal phosphates				Folic acid (Folicla)	2.1 mg/kg
Energy	1285.2 kJ/kg			K3	4.2 mg/kg
Amino Acids			B_6	Pyrodoxine	8.4 mg/kg
Arginine	1.16%		B_{12}		Trace
Lysine	0.93%		Biotin		Trace
Methionine	0.38%		Amantioxidine		178.2 mg/kg
Cystine	0.44%	Minerals			
Tryptophan	0.16%	Calcium			10.63 g/kg feed
Histidine	0.33%	Phosphorous	7.56 g/kg		
Leucine	1.44%	Magnesium	1.42 g/kg		
Isoleucine	0.73%	Na			2.5 mg/g
Phenylalanine	0.74%	K			4.43 mg/g
Tyrosine	0.55%	Mn			17.6 mg/kg
Threonine	0.90%	$MnS0_4$			104.8 mg/kg
Valine	0.95%	$ZnS0_4$			429.6 mg/kg (172 mg Zn/kg)
Glycine	0.77%	$CuS0_40$			828.2 mg/kg
		Cobalt SO_4	10.5 mg/kg		
	9.48%	$FeSO_4$			471.4 mg/kg
Available	8.66%	Ash			4.5%

The drugs used in the study, their dosage and route of administration are shown in Table 2.

TABLE 2

DRUG	DOSE/DAY	DURATION	ROUTE OF ADMINISTRATION
Alcohol	25% soln	90 days	per os
Isoniazid	2.5mg	90 days	per os
Rifampicin	4.0mg	90 days	per os
Paracetamol	0.005g	90 days	per os
Hexane	0.2ml	90 days	subcutaneously

The doses used were toxic (alcohol and hexane), high (isoniazid and rifampicin) and therapeutic (paracetamol).

Statistical Analysis of Results

Statistical analyses of results were carried out using the Mann Whitney Rank Sum test with a p value of <0.05 accepted as the limit of significance for both gluconeogenesis and bromosulphthalein clearance.

Perfusion Medium

Constituents

The medium used was that described by Hems, Ross, Berry and Krebs (1966). The perfusion medium contained :-

a) physiological saline

b) bovine serum albumin

c) washed human red blood cells.

The physiological saline was prepared to the method of Krebs and Hensleit (1932). Composition of the physiological saline is set out in appendix A.

Purification of Bovine Serum Albumin

5g of bovine serum albumin was dissolved in 100 ml of saline (Krebs and Hensleit, 1932) by stirring. This solution was dialysed against 250ml of saline for 48 hours, and then repeated. Since the bovine serum albumin was acidic, pH was adjusted to 7.4 by the addition of N NaOH.

Preparation of Red Blood Cells

Human red blood cells stored at 4°C and no longer suitable for transfusion were obtained from the blood bank at King Edward VIII Hospital. 100ml of the whole blood was centrifuged at 1500 rpm for 20 minutes. The buffy layer and plasma were removed by suction and red cells washed 4 times with physiological saline gassed with carbogen (95% O_2 : 5% CO_2). The washing fluid was removed by centrifugation for 10 min at 1500rpm and washed cells made up to 100 ml with saline.

Preparation of Perfusion Medium

The total volume of perfusion medium was 150 ml which consisted of 100 ml of purified bovine serum albumin (3.3% w/v) and 50 ml of red blood cell suspension.

Methods Employed for Biochemical Analysis

Pyruvate was measured by the U.V. method of Czok R and Lamprecht W (1974) which included sample deproteinization by hypochloric acid.
Lactate by the U.V. method of Gutmann I and Wahlefeld A W (1974) which included sample deproteinization by 0.6 N hypochloric acid.
Glucose by the Beckman enzymatic glucose reagent method (Hexokinase endpoint).
Bromosulphthalein clearance spectrophotometrically by the method of Henry et al (1959) which included sample deproteinization by acetone.

Operative Technique

Rats were anaesthetised by intraperitoneal injection with a fresh solution of sodium pentobarbital (60 mg/kg) and then 0.1 ml of heparin was administered via the saphenous vein. Cannulae were inserted as shown in (Fig. 1). The bile duct was first cannulated after which cannulation of the portal vein was achieved. Finally a cannula which consisted of a portex tubing cut to a sharp-point to facilitate piercing of the right atrium, was placed in the inferior vena cava proximal to the liver. All cannulae were secured by ties, using silk 3/0.

Fig. 1 Diagram of the operative procedure for in situ perfusion of the rat liver.
The portal vein and bile duct are cannulated, the portal vein being tied distally. The cannula in the inferior vena cava is inserted through the wall of the right atrium and tied in place above the diaphragm. The inferior vena cava is tied below the liver. The arrows demonstrate the direction of flow.

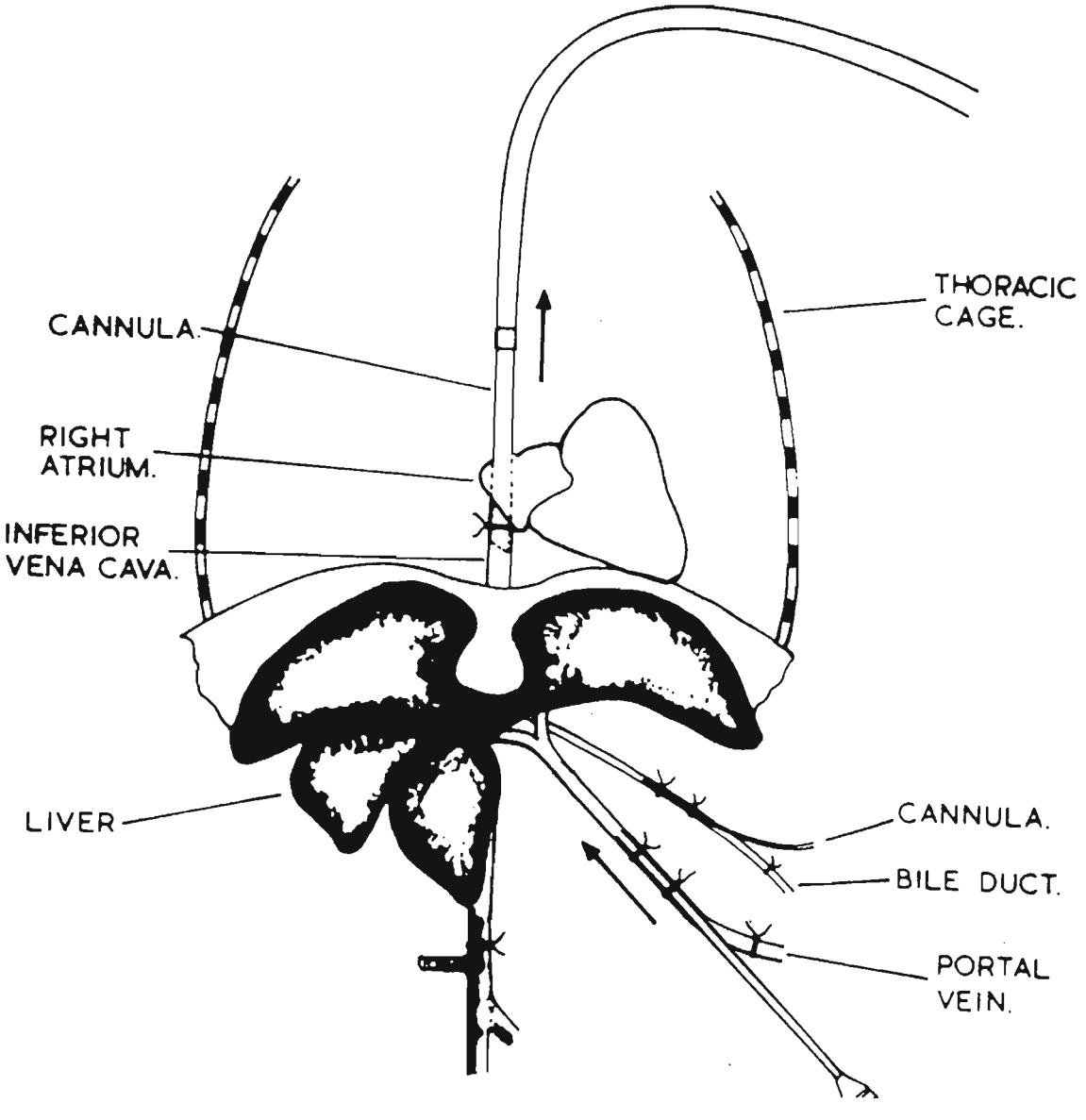
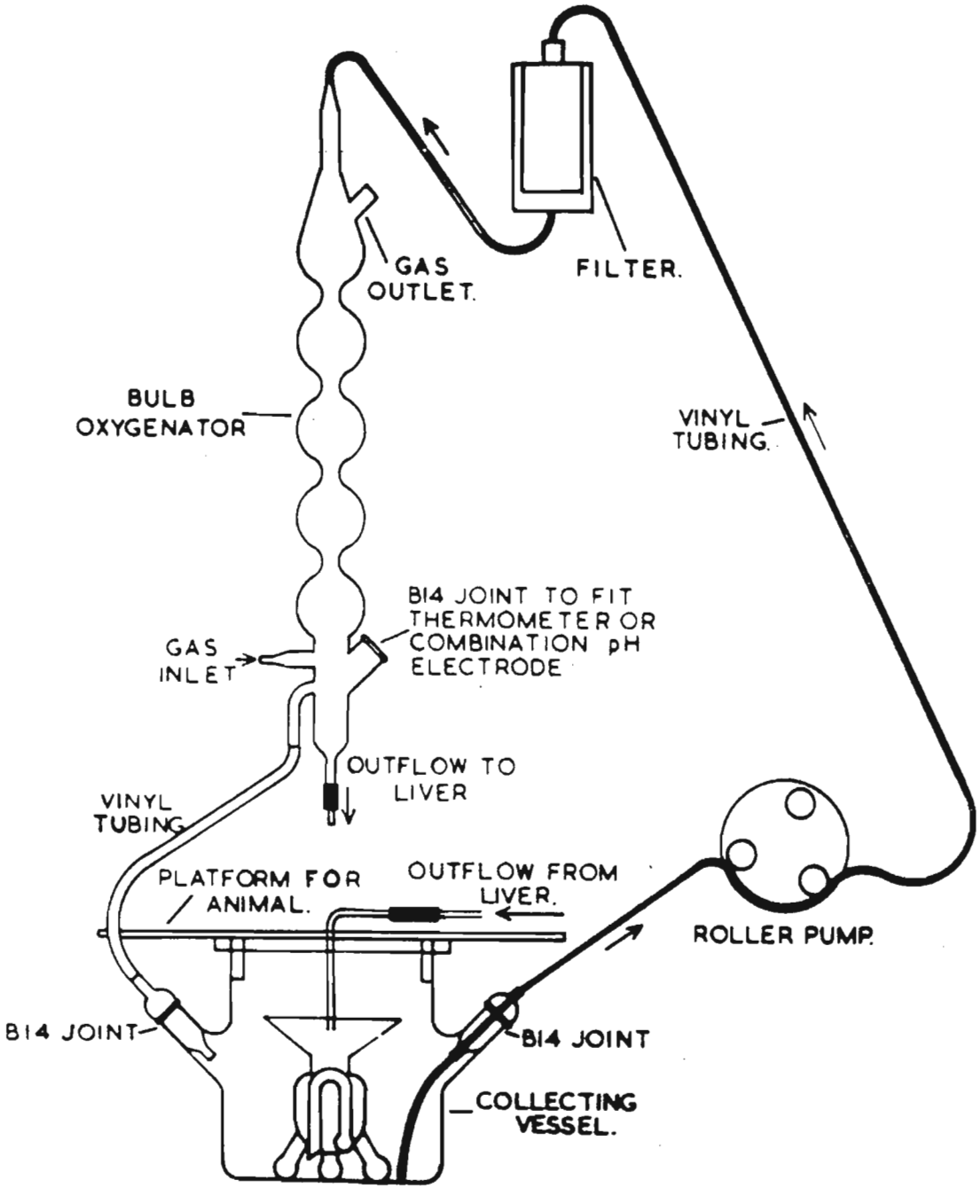


Fig. 2 Diagram of the liver perfusion apparatus showing the direction of circulation of the medium. The medium in the collecting vessel is pumped through the nylon mesh filter to the top of the oxygenator. The medium then passes to the reservoir and enters the portal vein; the excess medium returns to the collecting vessel. The venous outflow is directed into the syphon.



Glucose Formation

Fructose (500mg) a non-NAD^+ dependent precursor of glucose was added at the start of the perfusion which continued for 75 minutes. The capacity of the alcohol injured liver to synthesise glucose from fructose was measured by taking samples at 15 minute intervals for the measurement of glucose. 1ml 10% solution of methylene blue, a redox dye capable of oxidising NADH to NAD^+, was added at start of perfusion which continued for 75 minutes.

Glucose synthesis, by the isolated liver, damaged by drug administration, was measured by taking five samples at 15 minute intervals for the measurement of glucose levels.

Microscopy

The liver tissue from the hexane study was fixed for 24 hours in 10% buffered formalin solution, embedded in paraffin wax and 5um thick sections cut. All the sections were stained with haemotoxylin and eosin; sections were cut at least two levels in each block of tissue.

CHAPTER 3

ALCOHOL

ALCOHOL

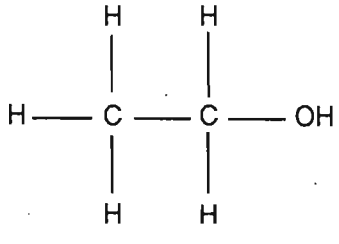
Introduction

While there are many alcohols in use, this study deals exclusively with ethanol. It is consumed in a vast variety of forms ranging from ales (3-6% alcohol by weight) to distilled spirits such as gin and whisky (35% or more alcohol by weight). It is also used in the parenteral nutrition of patients who are unable to take food or liquid by mouth.

Physical and Chemical Properties

Ethanol is a clear, colourless, mobile, volatile, readily inflammable liquid with a characteristic spiritous odour and burning taste. Ethanol can be produced in nature by fermentation of carbohydrates or synthetically. It has a boiling point of about 78°C with a specific gravity of 0.806. It is miscible with water, acetone, chloroform, ether, glycerol and almost all other organic solvents. The molecular formula of ethanol is C_2H_5OH and the molecular weight is 46,07g.

Structure of Ethanol



Absorption

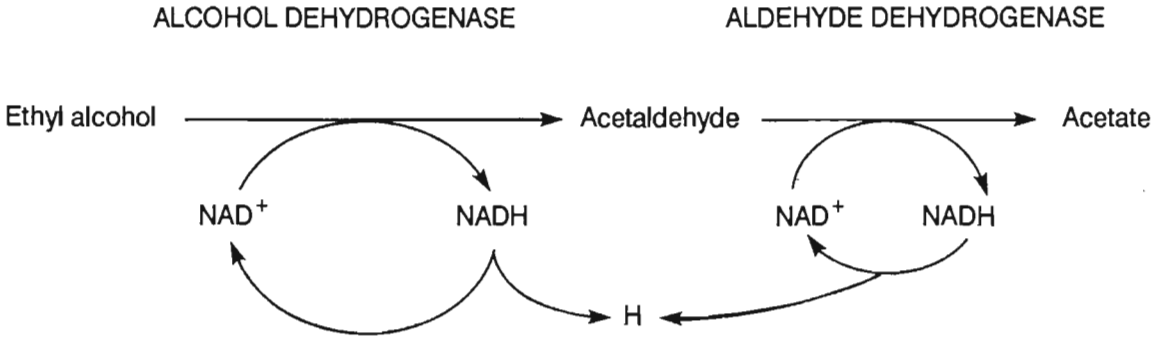
Over half of alcohol absorption, after oral ingestion, occurs in the stomach where the process is rapid, but it is even more rapid in the small intestine. Absorption is complete when the alcohol content of the gastrointestinal tract reaches equilibrium with the remainder of the body water, both intracellularly and extracellularly.

Metabolism

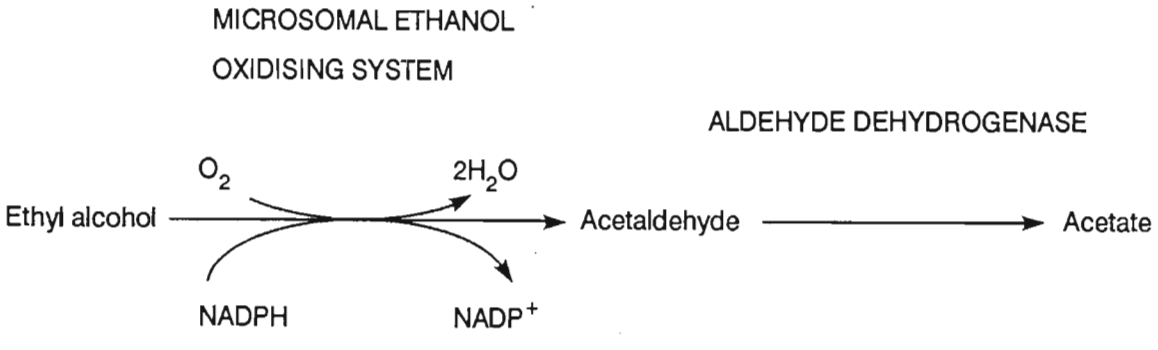
The toxic effects of ethanol on the liver are, in part, determined by the fact that it is preferentially metabolised in the hepatocytes, and only about 2-10% of the absorbed ethanol is eliminated via the lungs and kidneys; skeletal muscle cannot utilize ethanol. In the liver cell about 90% of the absorbed ethanol is metabolised in the cytosol by the action of alcohol dehydrogenase (ADH) and is converted to acetalydehyde with the release of (H^+) ions. The major and minor pathways of ethanol metabolism are illustrated schematically in (Fig.3).

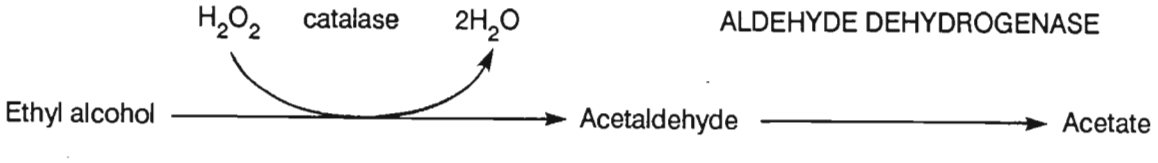
Fig. 3 Schematic representation of pathways of ethanol metabolism

MAJOR PATHWAY



MINOR PATHWAYS





Nicotinamide adenine dinucleotide (NAD^+) acts as an H^+ ion receptor and is converted to NADH, consequently changing the NADH : NAD^+ ratio and the redox state within the cell. This change in the NADH : NAD^+ ratio exerts a number of metabolic effects which include:

a. The accumulation of free NADH with a resultant increase in NADH : NAD^+ ratio;
b. An increase in lactate : pyruvate ratio and development of hyperlactaemia (but usually the acidosis is not severe);
c. Inhibition of the tricarboxylic acid cycle through the reduction reactions which require NAD^+;
d. Possible inhibition of hepatic gluconeogenesis which results in hypoglycaemia.

The rate-limiting step in ethanol metabolism appears to be the availability of NAD^+ and this, in turn, will depend on the rate of re-oxidation of NADH, a mitochondrial function.

Experimental Design

Since isolated liver perfusion is an established method of studying gluconeogenesis in vitro (Hems et al, 1966), it appeared likely that this technique would be suitable for determining the effects of chronic administration of drugs on liver function. In order to confirm this hypothesis, the effect of a known hepatotoxin, ethanol, on gluconeogenesis and bromosulphthalein clearance was studied using this method. Ethanol was chosen for investigation because it produces hypoglycaemia by inhibiting gluconeogenesis (Field et al, 1963; Forsander et al, 1965; Frienkel et al, 1965; Kaden et al, 1969; Krebs et al, 1969; Lochner et al, 1967; and Madison, 1969).

Materials and Methods

Four groups of 6 rats, each weighing 200-210g, were used in this study. Fluids to drink were given ad libitum; the control group were given tap water and the three experimental groups a solution of 25% alcohol in water for a period of 90 days. The livers of control and of one experimental group of rats were perfused for 75 minutes with a medium containing pyruvate (500umoles), a gluconeogenic precursor, and subsequently these livers were perfused with a medium containing bromosulphthalein (1.25 mg/100g rat weight) for an additional 20 minutes; those of the 2nd experimental group of rats were perfused for 75 minutes with a medium containing pyruvate (500umoles) and a 1ml 10% solution of methylene blue, a redox dye capable of oxidising NADH to NAD^+, and those of the 3rd experimental group of rats with fructose (500mg), a non-NAD^+ dependent precursor of glucose, for 75 minutes. The animals were randomly chosen for perfusion.

Serial specimens were obtained at 15 minute intervals for the analysis of glucose, lactate and pyruvate and at 5 minute intervals for bromosulphthalein clearance from perfusate and biliary excretion of the dye.

Results

After drinking ethanol for 12 weeks, the rats appeared healthy and not visibly different from control animals. The condition of the livers remained good during two hours of perfusion at 37°C as judged by gross appearance and linear rates of glucose production. The time course of glucose and lactate production by the livers of control and experimental animals, pyruvate concentrations, lactate:pyruvate ratios and rates of glucose and lactate production are shown in Tables 3a and 3b respectively and illustrated graphically (Figs. 4-7).

Gluconeogenesis

When the livers were perfused with a medium containing pyruvate (500umoles) for 75 minutes, measurable amounts of glucose and lactate were formed. Glucose formation was linear, the average glucose concentration derived from the perfusate after 75 minutes perfusion was 6.33 mmoles/l, and formation at an average rate of 0.12 mmoles/min per g wet weight of liver in the control group, while in the experimental group the glucose concentration was 1.97 mmoles/l, and formation at an average rate of 0.02 mmoles/min per g wet weight of liver.

Lactate formation was linear, the mean lactate concentration derived from the perfusate at the end of perfusion was 2.63 mmoles/l and formation at a mean rate of 0.02 mmoles/min per g wet weight of liver in the control group while in the ethanol-treated group the concentration was 3.10 mmoles/l and formation at a mean rate of 0.03 mmoles/min per g wet weight of liver.
305 umoles of pyruvate was utilised in the control group such that the lactate:pyruvate ratio was at a value of about 13 whereas 385 umoles was used in the ethanol group such that the lactate:pyruvate ratio was at a value of about 26. The rate of glucose formation, glucose and pyruvate concentrations were decreased and lactate:pyruvate ratios increased in the experimental group compared to controls ($p<0.05$ for all comparisons).

Glucose Formation

1ml 10% methylene blue (a redox agent capable of oxidising NADH to NAD^+) was added to the perfusion medium containing pyruvate at the beginning of the perfusion. Methylene blue has been reported to reverse alcohol inhibition of gluconeogenesis. Methylene blue overcame the ethanol inhibition of pyruvate conversion to glucose and the glucose concentration was higher with methylene blue (11.33mmoles/l) than that of pyruvate alone (1.97 mmoles/l). A significant increase in glucose production was noted after the addition of methylene blue ($p<0.05$; Fig 8).
Fructose (500mg), a non-NAD^+ dependent precursor of glucose, was added at the start of the perfusion. Ethanol did not inhibit fructose utilization or its conversion to glucose. After 75 minutes of perfusion the glucose concentration derived from perfusate containing fructose was 7.43 mmoles/l and 1.97 mmoles/l with pyruvate alone. Since fructose has no requirement for NAD^+, inhibition would not be expected. A significant increase in glucose production was observed after the addition of fructose ($p<0.05$; Fig. 9).

Bromosulphthlein Clearance

Bromosulphthalein was added, at a dosage of 1.25mg/100g donor rat weight, to the perfusate reservoir 80 minutes after beginning the perfusion. The time course of bromosulphthalein clearance from perfusate and biliary excretion of the dye are shown in Table 3c. The bromosulphthalein percentage retention was 45.6% after 12 weeks of ethanol feeding compared to 9.4% in control animals. The clearance of bromosulphthalein from perfusate ($p<0.005$; Fig. 10) and biliary excretion of dye ($p<0.05$ for all comparisons except 5 minute biliary excretion level; Fig. 11) were impaired in the experimental animals.

Table 3a

TIME-COURSE OF THE PRODUCTION OF LACTATE, AND GLUCOSE BY PERFUSED LIVERS OF CONTROL RATS (n=6)

UNDER AEROBIC CONDITIONS (500 umoles of pyruvate added to perfusion medium)

The results are expressed as MEAN ± SEM. The initial volume of the medium was 150 ml; it decreased every 15 minutes by 10ml. (removed for analysis). The livers had a mean wet weight of 6.48g.

TIME (minutes)	15		30		45		60		75	
CONC. OF METABOLITE (mmoles/l)										
LACTATE	2.09	±0.11	2.28	±0.13	2.44	±0.13	2.68	±0.11	2.63	±0.16
PYRUVATE	0.190	±0.01	0.208	±0.04	0.202	±0.01	0.187	±0.01	0.190	±0.008
GLUCOSE	3.05	±0.17	3.69	±0.22	4.73	±0.57	5.42	±0.49	6.33	±0.52
LACTATE:PYRUVATE ratios	11.41	±1.1	10.98	±0.65	12.19	±0.79	14.72	±1.30	13.82	±0.85
METABOLIC RATES (mmoles / min per g wet weight of liver)										
GLUCOSE FORMATION			0.09		0.16		0.11		0.14	
LACTATE FORMATION			0.03		0.02		0.04		−0.007	

Table 3b

TIME-COURSE OF THE PRODUCTION OF LACTATE, AND GLUCOSE BY PERFUSED LIVERS OF EXPERIMENTAL RATS (n=6)

UNDER AEROBIC CONDITIONS (500 umoles of pyruvate added to perfusion medium)

The results are expressed as MEAN ± SEM. The initial volume of the medium was 150ml; it decreased every 15 minutes by 10 ml. (removed for analysis). The livers had a mean wet weight of 6.23g.

TIME (minutes)	15		30		45		60		75	
CONC. OF METABOLITE (mmoles/l)										
LACTATE	2.78	±0.24	3.13	±0.43	2.92	±0.26	2.97	±0.27	3.10	±0.29
PYRUVATE	0.108	±0.004	0.112	±0.004	0.111	±0.004	0.107	±0.004	0.127	±0.001
GLUCOSE	1.28	±0.04	1.5	±0.13	1.75	±0.26	2.13	±0.20	1.97	±0.13
LACTATE:PYRUVATE ratios	25.83	±2.4	27.45	±3.1	26.72	±3.1	27.77	±2.9	24.72	±1.1
METABOLIC RATES (mmoles / min per g wet weight of liver)										
GLUCOSE FORMATION		0.03		0.04		0.06		−0.02		
LACTATE FORMATION		0.06		−0.03		0.008		0.02		

Table 3c

TIME-COURSE OF BROMOSULPHTHALEIN CLEARANCE BY PERFUSED LIVERS OF CONTROL AND EXPERIMENTAL RATS

The results are expressed as MEAN ± SEM. BSP was added, at a dosage of 1.25 mg / 100 g body weight, to the perfusion medium.

TIME (minutes)	0		5		10		15		20	
CONTROLS (6)	0.85	±0.008	0.61	±0.008	0.579	±0.01	0.18	±0.008	0.08	±0.004
EXPERIMENTALS (6)	1.03	±0.08	0.51	±0.03	0.490	±0.08	0.46	±0.08	0.47	±0.05

TIME-COURSE OF BILIARY EXCRETION OF BROMOSULPHTHALIEN

TIME	0		5		10		15		20	
CONTROLS (n=6)	0.07	±0.03	0.30	±0.12	0.55	±0.23	0.61	±0.25	0.79	±0.33
EXPERIMENTALS (N=6)	0.27	±0.11	0.30	±0.12	0.28	±0.11	0.33	±0.14	0.33	±0.13

Fig. 4 Glucose concentrations derived from perfusate of isolated perfused livers of control and alcohol-treated rats

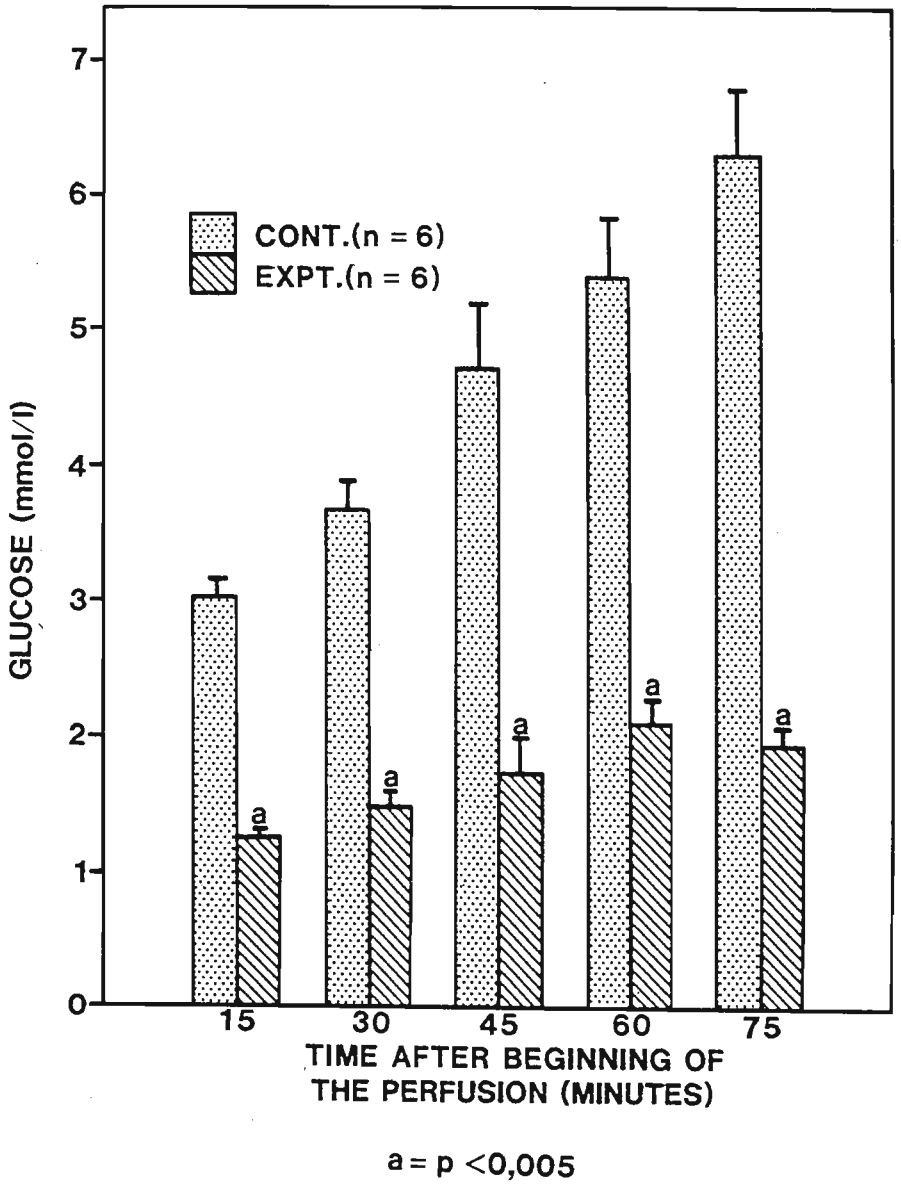


Fig. 5 Lactate concentrations derived from perfusate of isolated perfused livers of control and alcohol-treated rats

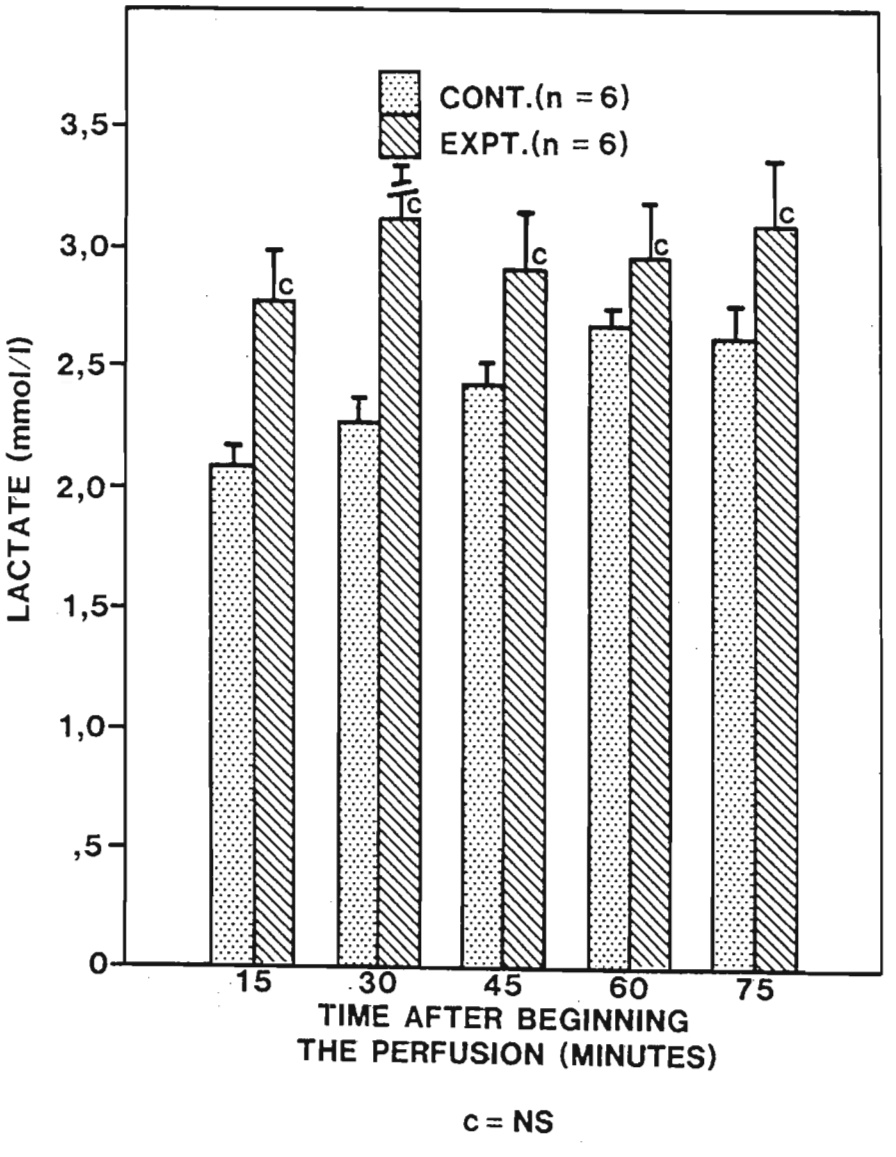


Fig. 6 Pyruvate concentrations derived from perfusate of isolated perfused livers of control and alcohol-treated rats

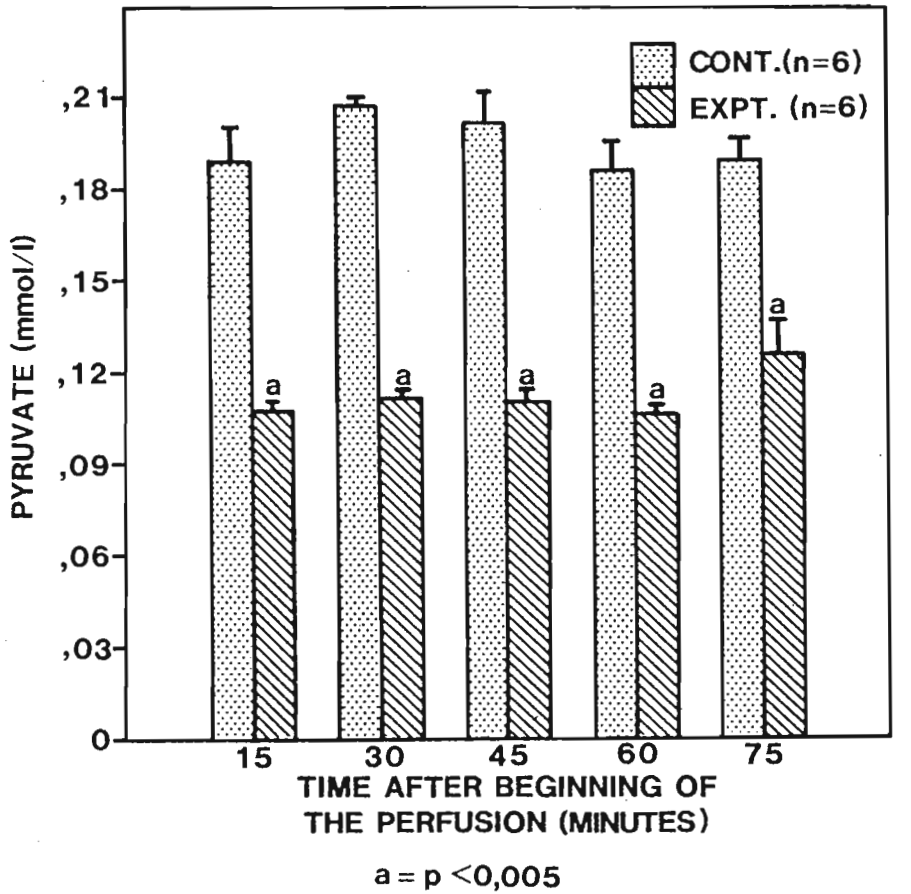
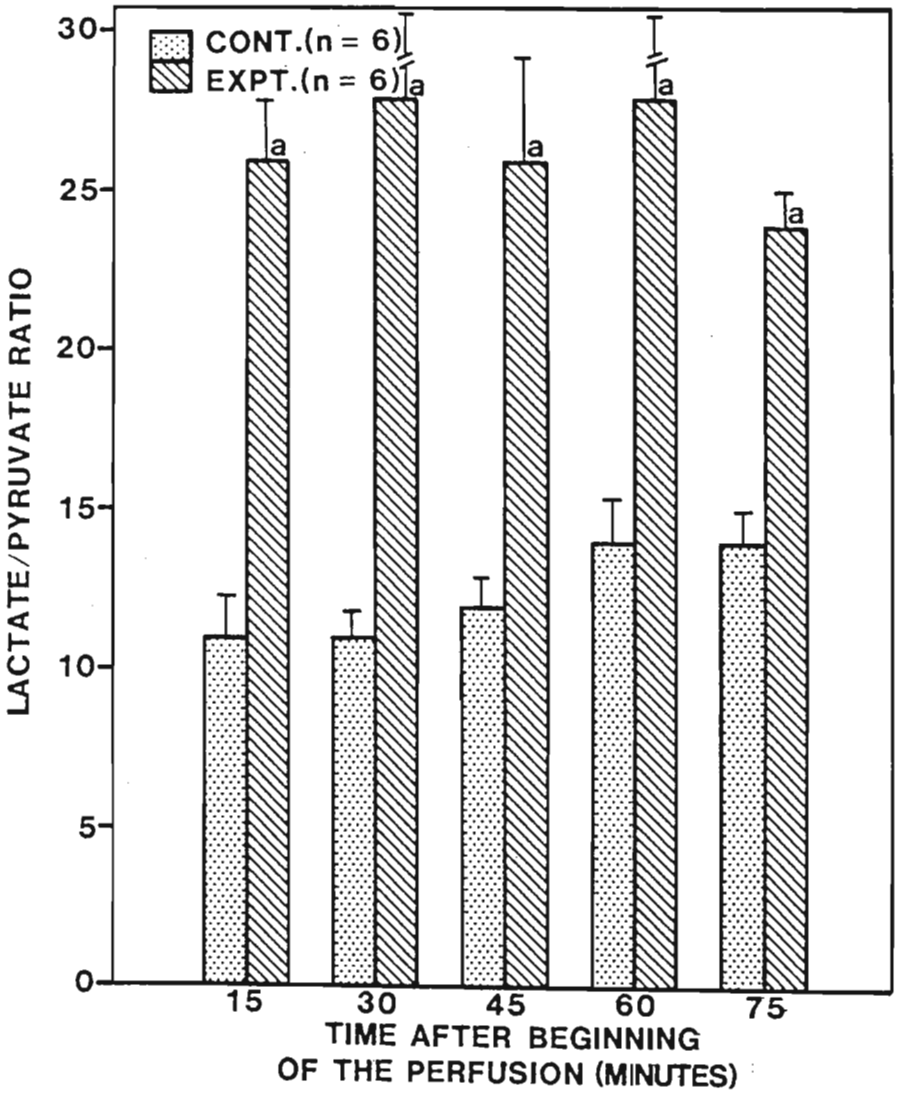


Fig. 7 Lactate:Pyruvate ratios derived from perfusate of isolated perfused livers of control and alcohol-treated rats



a = p < 0,005

Fig. 8 The effect of methylene blue, a redox agent, on glucose concentrations derived from perfusate of isolated perfused livers of control (experimental group 1) and alcohol-treated rats (experimental group 2)

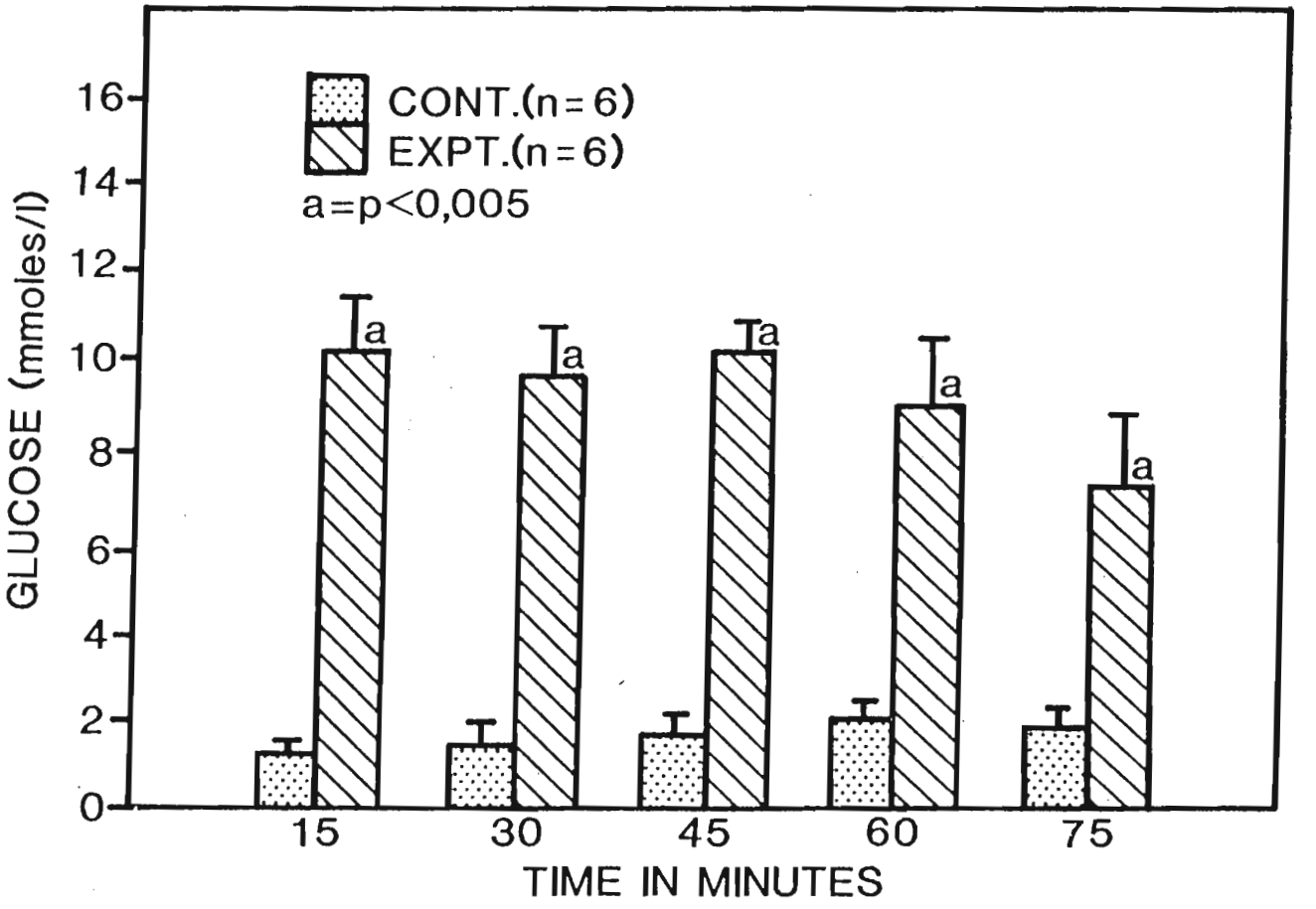


Fig. 9 The effect of fructose, a non-NAD$^+$ dependent precursor, on glucose concentrations derived from perfusate of isolated perfused livers of control (experimental group 1) and alcohol-treated rats (experimental group 3)

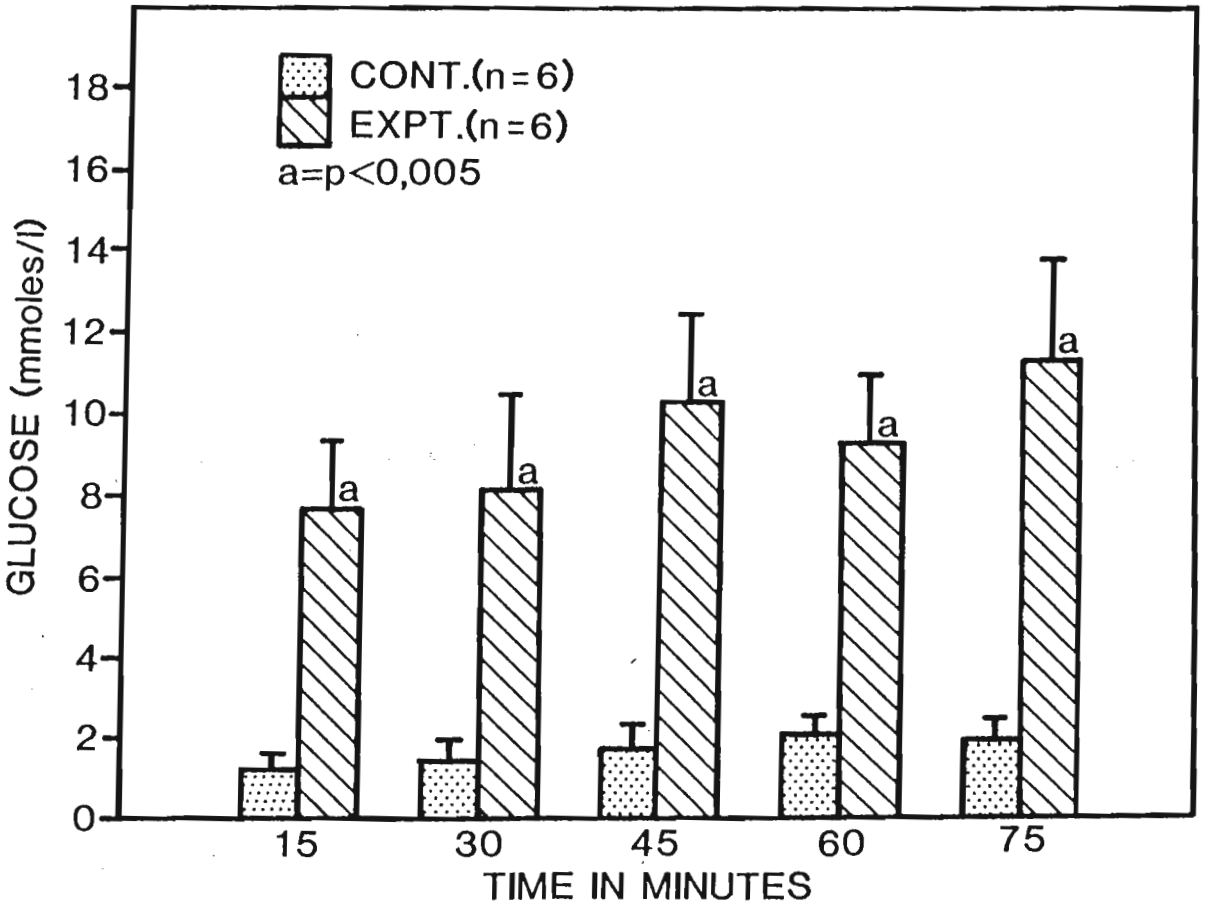


Fig. 10 Bromosulphthalein clearance derived from perfusate of isolated perfused livers of control and alcohol-treated rats

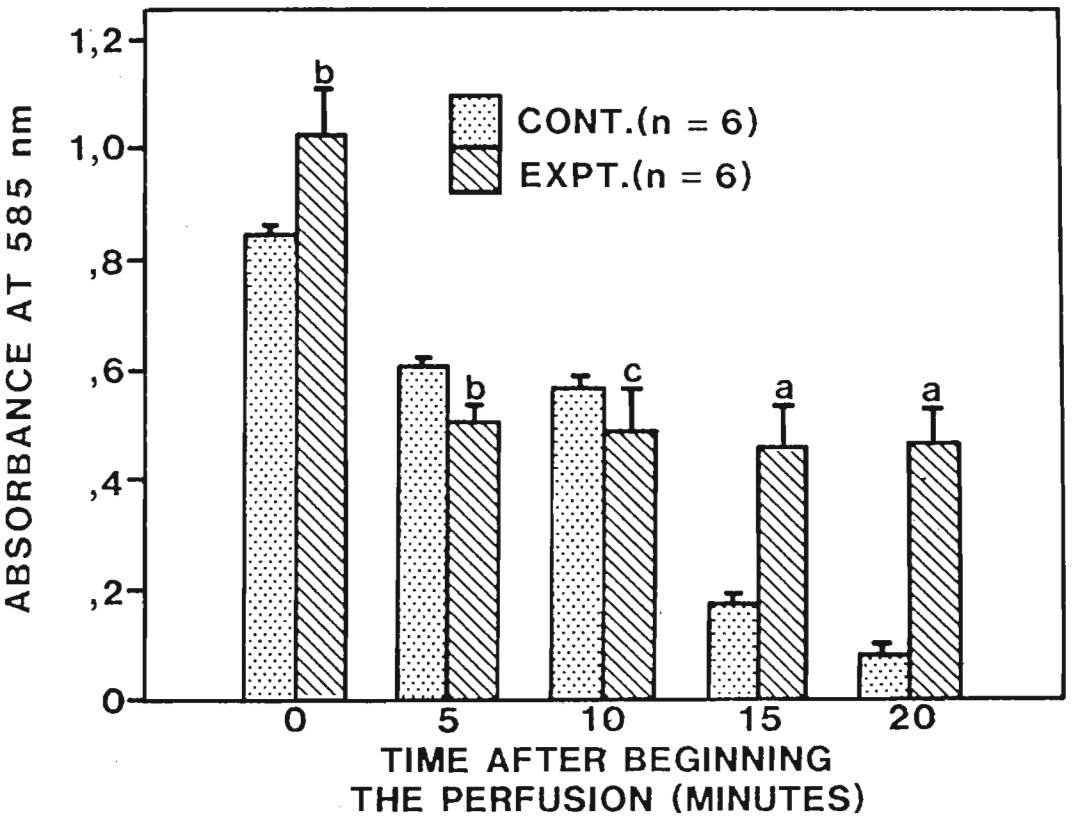
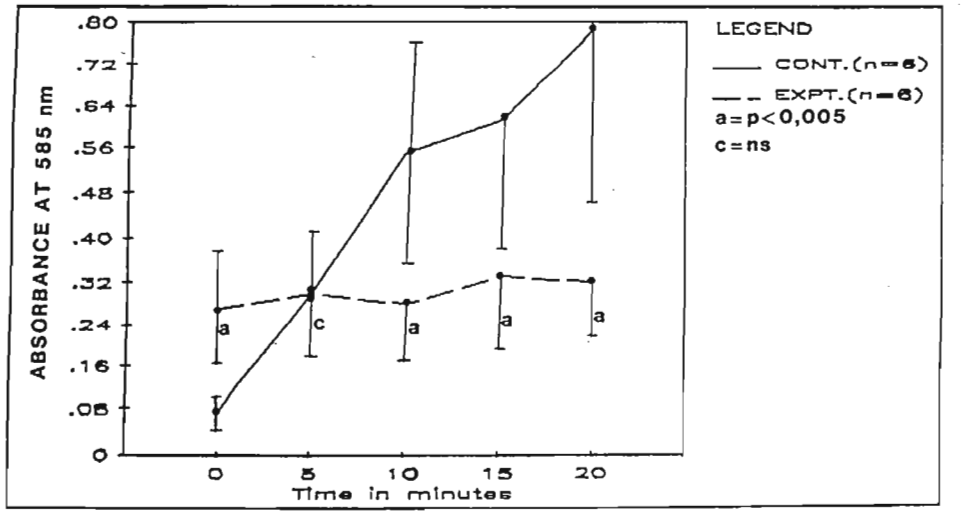


Fig. 11 Biliary excretion of bromosulphthalein derived from the bile of control and alcohol- treated rats



DISCUSSION

When pyruvate, a substrate for gluconeogenesis, was added to the perfusion medium, pyruvate utilization and glucose production were increased in the controls, indicating that the added pyruvate was utilized for the production of glucose. In contrast, glucose production by the alcohol-treated livers decreased, indicating that gluconeogenesis was impaired. This finding is in agreement with that of other workers (Freinkel et al, 1965; Kreisberg et al, 1971; Wilson et al, 1981).

The addition of methylene blue to the perfusion medium containing pyruvate resulted in a significant increase in glucose production by the experimental animals. Since methlyene blue is a redox agent capable of oxidising NADH to NAD^+, these findings support the view that an increased free NADH : free NAD^+ ratio generated during ethanol metabolism is the primary mechanism by which gluconeogenesis is inhibited (Lochner et al, 1967; Madison 1968). Other workers have reported that alcohol suppressed glucose production in fasting dogs with portocaval shunts, and that methylene blue reversed this effect (Madison et al, 1967). These observations are consistent with the hypothesis that alcohol inhibits gluconeogenesis by lowering hepatic cytoplasmic redox potential, as a consequence of its metabolism. Such alterations in NADH:NAD^+ have been demonstrated by direct measurements (Smith and Newman, 1959). and indirectly by lactate : pyruvate ratios (Forsander et al, 1965). Ethanol oxidation results in an increase in cytosolic and mitochondrial reduced nicotinamide-adenine-dinucleotide to oxidised-nicotinamide -adenine-dinucleotide (NADH : NAD^+) ratios. The cytosolic alcohol dehydrogenase reaction, the major ethanol-oxidising mechanism, results in acetaldehyde production. The acetaldehyde is further oxidised to acetate. These two reactions produce 2 mol NADH per mol of ethanol oxidised (Fig. 3).

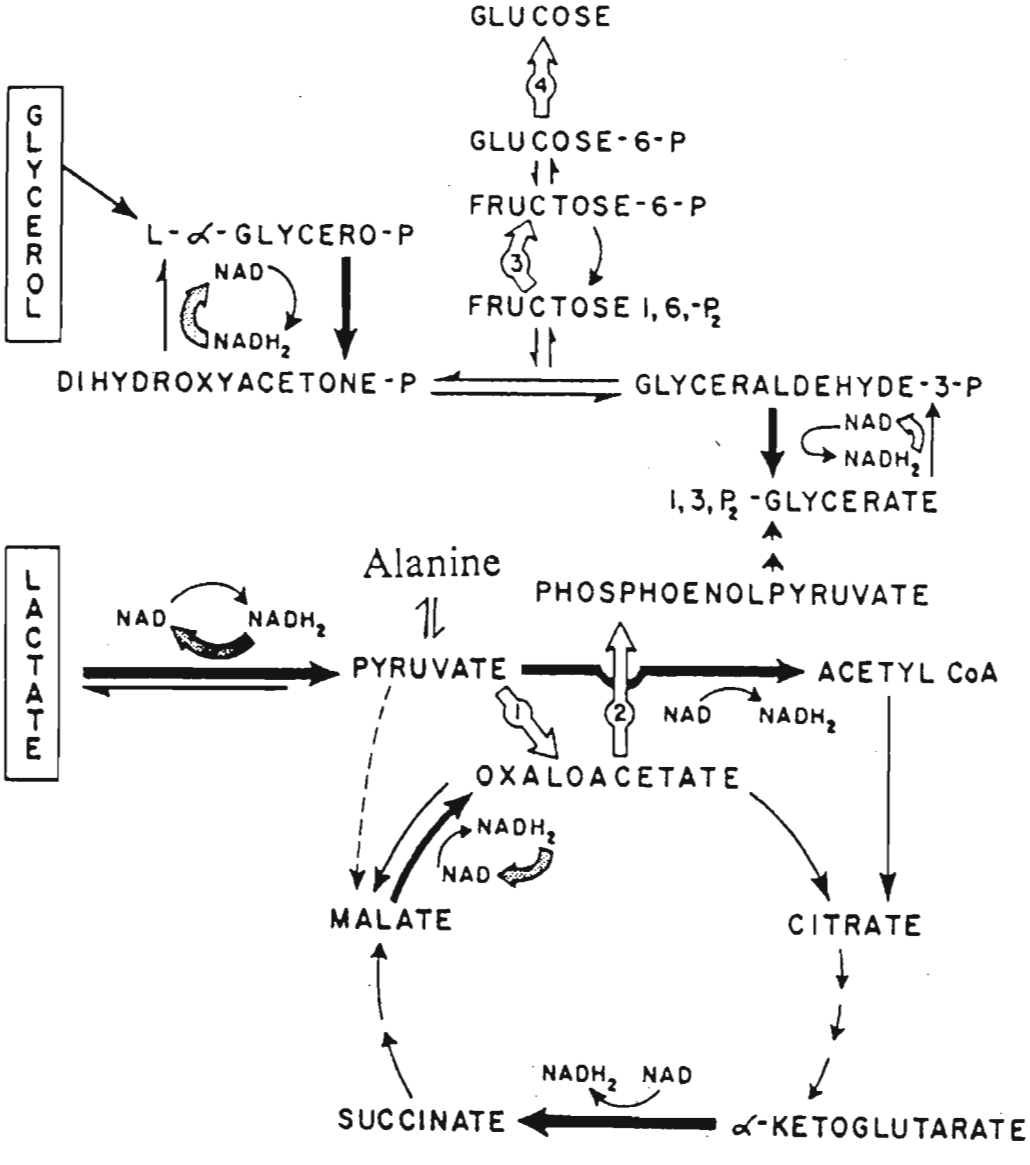
During ethanol metabolism the rate of NAD^+ reduction exceeds mitochondrial NADH oxidation, thus increasing the cytosolic NADH : NAD^+ ratio. The shift in redox potential would be expected to decrease the availability of substrates (pyruvate, alanine, glycerol) for gluconeogenesis.

When fructose was added to the perfusion medium instead of pyruvate, glucose production by the alcohol-treated livers was significantly increased. Since fructose is a non-NAD^+ dependent precursor of glucose, glucose production in response to fructose would not be inhibited by an alteration of NADH : NAD^+ ratio. Other studies have also shown that ethanol does not inhibit glucose production from fructose (Kaden et al, 1969; Field et al, 1963; Madison, 1968; Tygstrup et al, 1965). Our study also confirms that starvation is not a pre-requisite for ethanol inhibition of gluconeogenesis (Kreisberg et al, 1971).

In the presence of decreased glucose production, the increased lactate : pyruvate ratio in the experimental animal would suggest that the majority of the pyruvate was utilised to form lactate. However lactate levels were not increased in the experimental group. It is likely, therefore, that pyruvate entered another biochemical pathway. One possibility is that pyruvate was converted to L-alanine in the presence of an increased NADH : NAD^+ ratio. This contention is supported by the observation that methylene blue produced a rapid reversal of the inhibition of glucose production by alcohol in this study. Other possibilities include the conversion of pyruvate to acetyle Co-A or malate (Fig 12).

The study of Krebs et al (1969) and Kaden et al (1969), also on the effect of alcohol on gluconeogenesis using the isolated livers of fed and fasted rats, previously reported increased lactate concentrations, increased lactate:pyruvate ratios and decreased pyruvate concentrations but the pyruvate conversion to glucose was not inhibited by ethanol when these livers were perfused with a medium containing pyruvate and ethanol, which is contrary to our findings.

Fig. 12 Pathways of hepatic gluconeogenesis. The specific enzymes upon which gluconeogenesis is dependent are numbered within the large arrows. The NAD^+-dependent points in the pathway of gluconeogenesis are shown by the bold solid arrows. The direction of reactions in the presence of an elevated $NADH:NAD^+$ ratio is depicted by the stippled curved arrows.



In another situation when the livers of fed and fasted rats were perfused with a medium with or without ethanol, the following changes were observed. With livers from fed rats glucose was released from the liver into the perfusion medium. This release was slightly greater when ethanol was present. With livers from starved rats, no release of glucose was observed, and when ethanol was added a marked uptake of glucose from the medium was found. The lactate:pyruvate ratios in the perfusion medium increased from 10 to 87 with livers from fed rats and from 20 to 171 with livers from starved rats when the livers were perfused with ethanol in the medium. A simultaneous release of lactate and pyruvate into the medium occurred. The discrepancy in results of gluconeogenesis in the present experiments was most probably due to the chronic alcohol treatment of the rats.

The clearance of bromosulphthalein from plasma is a useful means of evaluating liver function. In the present investigation a marked retention in perfusate and decreased biliary excretion of bromosulphthalein was observed in alcohol-treated rats. During the twenty minute perfusion, most of the dye was cleared from the perfusate with increased biliary excretion of the dye in the control animals whereas in the experimental animals, after an initial high clearance from perfusate, the concentration of the dye remained constant with decreased biliary excretion of the dye till the end of the experiment.

The precise mechanism of increased bromosulphthalein retention in this setting is not clear. Previous studies in rats have shown that acute ethanol administration induces both a loss of glutathione from liver and an inhibition of glutathione synthesis (Speisky et al, 1985; Lauterberg et al, 1984; Morton and Mitchell, 1985). Another study showed that patients with alcoholic liver disease had decreased hepatic glutathione compared to patients with liver disease unrelated to alcohol (Shaw et al, 1983). Since the rate limiting step in the elimination of bromosulphthalein from plasma into bile of man and experimental animals is probably the conjugation of the dye with hepatic glutathione prior to its excretion into the bile, it is possible that reduced glutathione levels were responsible for the decreased bromosulphthalein clearance from perfusate observed.

CHAPTER 4

ISONIAZID

ISONIAZID

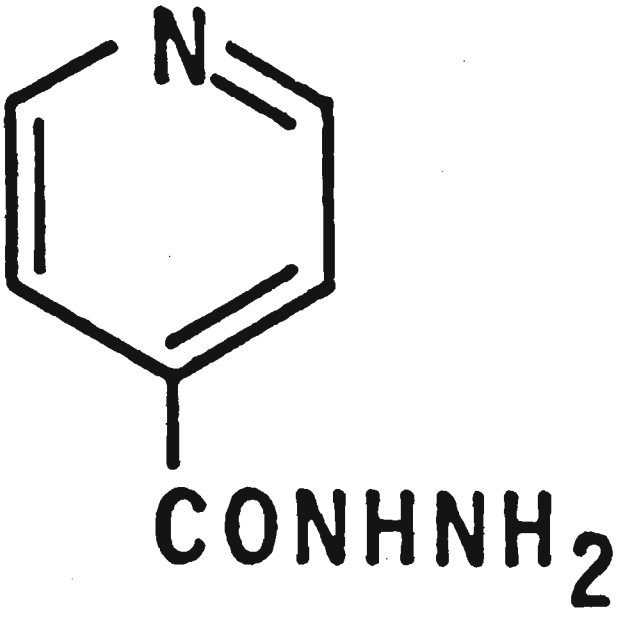
Introduction

Isoniazid is the hydrazide of isonicotinic acid, and has been the most widely used drug in the treatment of tuberculosis with excellent therapeutic efficacy and good patient compliance (Gillman et al, 1980; Maddrey and Boitnett, 1973; Zimmerman, 1978).

Physical and Chemical Properties

It occurs as a colourless, odourless, white crystalline powder with a taste slightly sweet at first and then bitter. Melting point 170°C to 174°C. Solubility ranges from 1 in 8 of water, 1 in 45 to 50 of alcohol, and 1 in 1000 of chloroform; very slightly soluble in ether. The molecular formula is $C_6H_7N_3O$ with molecular weight of 137,1g.

Structure of Isoniazid



Kinetic Data

Isoniazid is readily absorbed from the gastrointestinal tract when administered either orally or parenterally. Peak concentrations appear in the blood 1 to 2 hours after dosing by mouth. The drug is not considered to be bound appreciably to plasma proteins and diffuses into all body tissues and fluids including cerebrospinal fluid.

Metabolism

From 75% to 95% of a dose of isoniazid is excreted in the urine in 24 hours, mostly as metabolites. The main excretory products are the result of enzymatic acetylation, (acetylisoniazid), and enzymatic hydrolysis, (isonicotinic acid). Small quantities of an isonicotinic acid conjugate, probably isonicotinyl glycine, one or more isonicotinyl hydrazones and traces of N-methyl isoniazid are also detectable in urine. The drug is metabolised principally by acetylation with a bi-modal distribution. T=1hr in fast acetylators, 3hr in slow acetylators. The difference between fast and slow acetylators depends upon the amount of hepatic N-acetyl transferase present. It is known that the capacity for rapid acetylation is inherited as an autosomal dominant character while slow acetylation is thought to be transmitted by a recessive gene. The ratio of fast : slow acetylators is 40 : 60 in Europe, 85 : 15 in Japan and 100 : 0 in Eskimos. These differences have clinical consequences. Increased drug toxicity including erythematosus-like syndrome may occur among slow acetylators; slow acetylators require low doses of isoniazid. Fast acetylators are more at risk from toxic effects referable to the metabolites of isoniazid. However it is important to emphasize that there is no conclusive evidence of a difference in therapeutic efficacy or in the incidence of toxicity related to the rate of acetylation of isoniazid.

Experimental Design

Because of its potent bactericidal action combined with a low incidence of serious adverse effects, isoniazid has been accepted as a first line agent in the treatment and prophylaxis of tuberculosis. The recommended daily dosage of this drug in adults is 5mg/kg with a maximum of 300mg; administration of therapeutic doses (and those exceeding the recommended dose) of this compound has been complicated by liver damage (Maddrey, 1981; Martin and Arthaud, 1970).

The study of gluconeogenesis and bromosulphthalein clearance using the isolated liver perfusion model has been found to be a good method of evaluating the effect of toxic doses of drugs, eg ethanol on liver function. In this investigation, this model was used to determine the effects of isoniazid on liver function when the substance was administered to a group of rats for a 3 month period in a dose equivalent to two and a half times the recommended adult dose.

Materials and Methods

10 male Wistar rats (175 - 190 g) of the University of Natal inbred strain were kept in stainless steel cages with plastic bottoms. Each animal was individually earmarked for identification and randomly assigned to either a control or an experimental group, each consisting of 5 rats. All the animals were fed on the same diet in the form of commercial rat cubes (Epol). Water was given ad libitum. The animals in the experimental group were given 2.5mg isoniazid per os daily for 90 days. The control group was untreated. The livers of experimental and control animals were perfused for 75 minutes with a medium containing pyruvate (500umoles), a gluconeogenic precursor, and subsequently these livers were perfused with a medium containing bromosulphthalein (1.25mg/100g rat weight) for an additional 20 minutes. The animals were randomly chosen for perfusion. Serial specimens were obtained for analysis.

Results

Livers perfused for 2 hours showed no visible swelling or evidence of local or general ischaemia. The time course of glucose and lactate production by the livers of control and experimental animals, pyruvate concentrations and lactate : pyruvate ratios are shown in Tables 4a and 4b respectively and illustrated graphically (Figs. 13-16).

Gluconeogenesis

Pyruvate (500umoles) was added 10 minutes after the start of the perfusion; 290 umoles of pyruvate was removed from the perfusion medium in the control group compared to 330 umoles in the experimental group. The average lactate concentration was 2.99 mmoles/l at the end of the perfusion and formation was at an average rate of 0.04 mmoles/min per g wet weight of liver in the control group such that the lactate: pyruvate ratio was about 13 whereas the concentration was 3.01 mmoles/l and formation was at an average rate of 0.1 mmoles/min per g wet weight of liver such that the lactate:pyruvate ratio was about 18 in the experimental group. Glucose formation was linear in both groups at a mean rate of formation of 0.09 mmoles/min g wet weight of liver in the control group compared to 0.02 mmoles/min per g wet weight of liver in the isoniazid-treated rats. The mean glucose concentrations at the end of perfusion was 5.42 mmoles/l in the control

group compared to 2.07 mmoles/l in the experimental group. The rate of glucose formation and glucose concentration were decreased and lactate:pyruvate ratios were increased in the experimental group compared to controls ($p<0.05$ for all comparisons except 30 and 60 minute lactate:pyruvate ratios).

Bromosulphthalein

Bromosulphthalein (1.25 mg/100g weight of rat) was added 80 minutes after the start of the perfusion which continued for an additional twenty minutes. The time course of bromosulphthalein clearance from perfusate and biliary excretion of dye are shown in Table 4c. About 8.2% bromosulphthalein retention was observed in the control group while 51.6% was observed in the experimental group. The clearance of bromosulphthalein from perfusate ($p<0.05$; Fig. 17) and biliary excretion of bromosulphthalein ($p<0.05$; Fig. 18) was impaired in the isoniazid-treated group.

Table 4a

TIME-COURSE OF THE PRODUCTION OF LACTATE, AND GLUCOSE BY PERFUSED LIVERS OF CONTROL RATS (n=5)

UNDER AEROBIC CONDITIONS (500 umoles of pyruvate added to perfusion medium)

The results are expressed as MEAN ± SEM. The initial volume of the medium was 150 ml; it decreased every 15 minutes by 10 ml. (removed for analysis). The livers had a mean wet weight of 6.81g.

TIME (minutes)	15		30		45		60		75	
CONC. OF METABOLITE (mmoles/l)										
LACTATE	2.14	±0.06	2.56	±0.20	2.41	±0.16	2.94	±0.28	2.99	±0.19
PYRUVATE	0.267	±0.01	0.215	±0.02	0.189	±0.01	0.186	±0.01	0.198	±0.01
GLUCOSE	2.69	±0.04	3.35	±0.13	4.32	±0.31	4.92	±0.56	5.42	±0.76
LACTATE : PYRUVATE ratios	8.20	±0.20	13.00	±2.40	13.40	±1.70	16.20	±1.40	15.60	±1.00
METABOLIC RATES (mmoles/min per g wet weight of liver)										
GLUCOSE FORMATION			0.09		0.14		0.09		0.07	
LACTATE FORMATION			0.06		−0.02		0.08		0.007	

Table 4b

TIME-COURSE OF THE PRODUCTION OF LACTATE, AND GLUCOSE BY PERFUSED LIVERS OF EXPERIMENTAL RATS (n=5)

UNDER AEROBIC CONDITIONS (500 umoles of pyruvate added to perfusion medium)

The results are expressed as MEAN ± SEM. The initial volume of the medium was 150 ml; it decreased every 15 minutes by 10 ml. (removed for analysis). The livers had a mean wet weight of 6.52 g.

TIME (minutes)	15		30		45		60		75	
CONC. OF METABOLITE (mmoles/l)										
LACTATE	2.93	±0.26	2.76	±0.12	2.67	±0.16	2.96	±0.26	3.01	±0.14
PYRUVATE	0.201	±0.004	0.191	±0.02	0.179	±0.01	0.159	±0.01	0.131	±0.01
GLUCOSE	1.41	±0.09	1.54	±0.08	1.93	±0.27	2.09	±0.16	2.07	±0.30
LACTATE:PYRUVATE ratios	14.63	±1.30	15.95	±3.30	17.76	±2.40	19.61	±2.7	24.39	±3.20
METABOLIC RATES (mmoles/min per wet weight of liver)										
GLUCOSE FORMATION		0.02		0.06		0.02		0.003		
LACTATE FORMATION		−0.03		−0.01		0.04		0.007		

Table 4c

TIME-COURSE OF BROMOSULPHTHALEIN CLEARANCE BY PERFUSED LIVERS OF CONTROLS AND EXPERIMENTAL ANIMALS

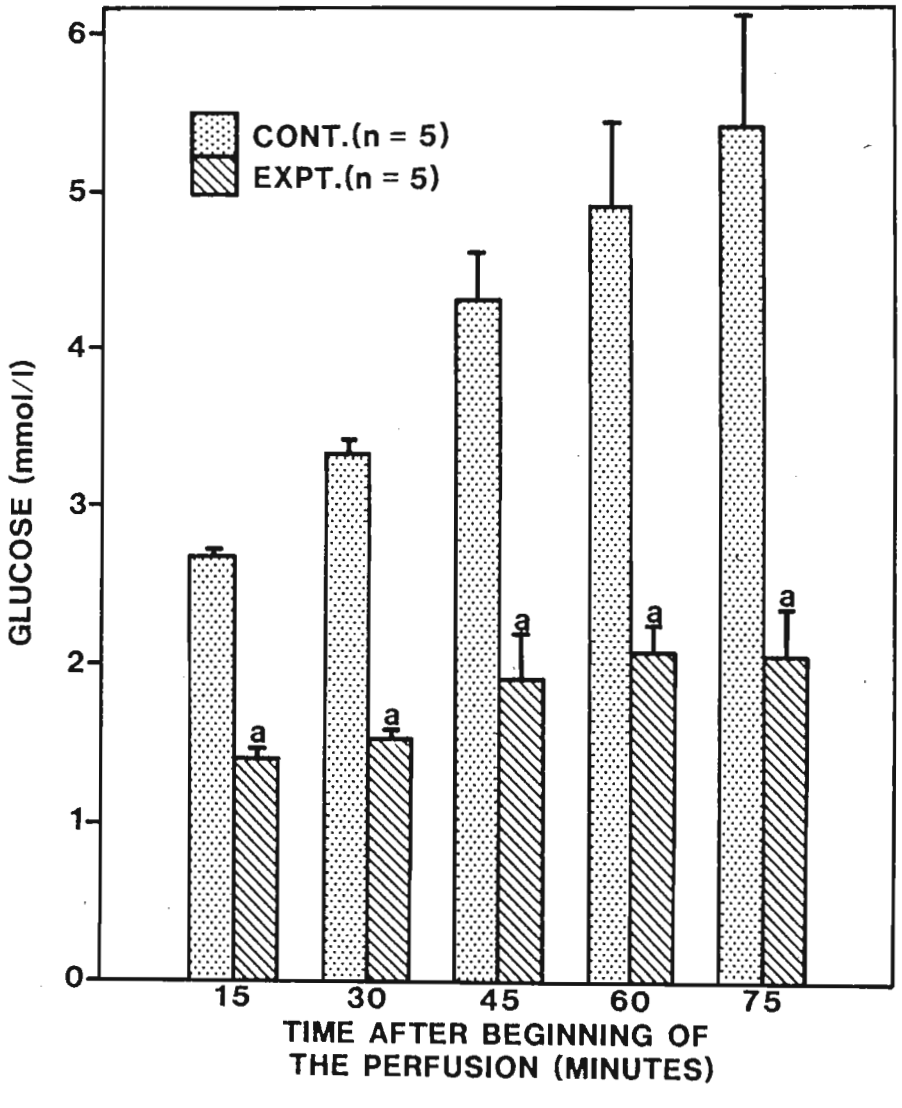
The results are expressed as MEAN ± SEM. BSP was added, at a dosage of 1.25 mg / 100 g weight of rat, to the perfusion medium.

TIME (minutes)	0		5		10		15		20	
CONTROLS (5)	0.85	±0.01	0.60	±0.01	0.531	±0.01	0.20	±0.004	0.07	±0.008
EXPERIMENTALS (5)	0.62	±0.01	0.38	±0.04	0.39	±0.008	0.34	±0.01	0.32	±0.008

TIME-COURSE OF BILIARY EXCRETION OF BROMOSULPHTHALEIN

TIME (minutes)	0		5		10		15		20	
CONTROLS (5)	0.09	±0.04	0.27	±0.12	0.49	±0.22	0.62	±0.28	0.79	±0.35
EXPERIMENTALS (5)	0.20	±0.09	0.33	±0.15	0.31	±0.14	0.33	±0.15	0.38	±0.17

Fig. 13 Glucose concentrations derived from the perfusate of isolated perfused livers of control and isoniazid-treated rats



a = p <0,005

Fig. 14 Lactate concentrations derived from perfusate of isolated perfused livers of control and isoniazid-treated rats

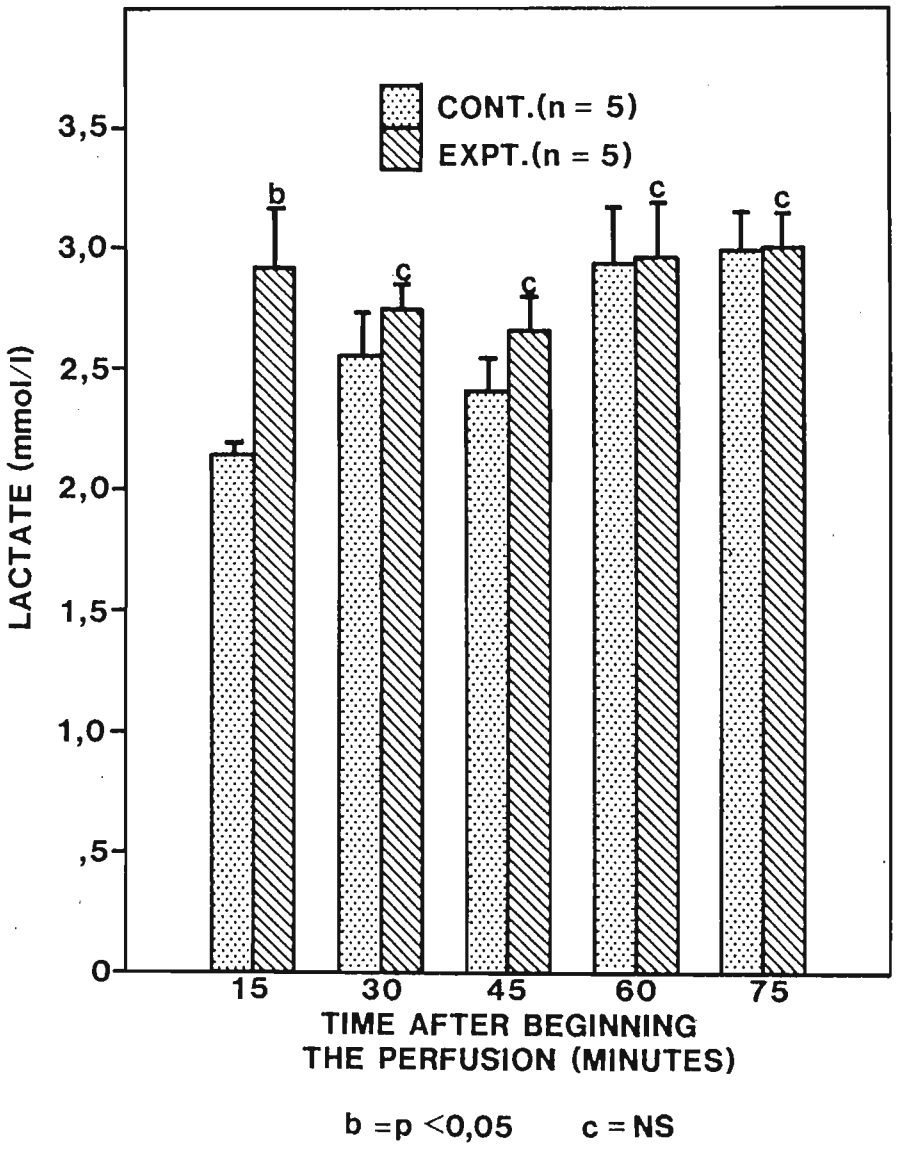
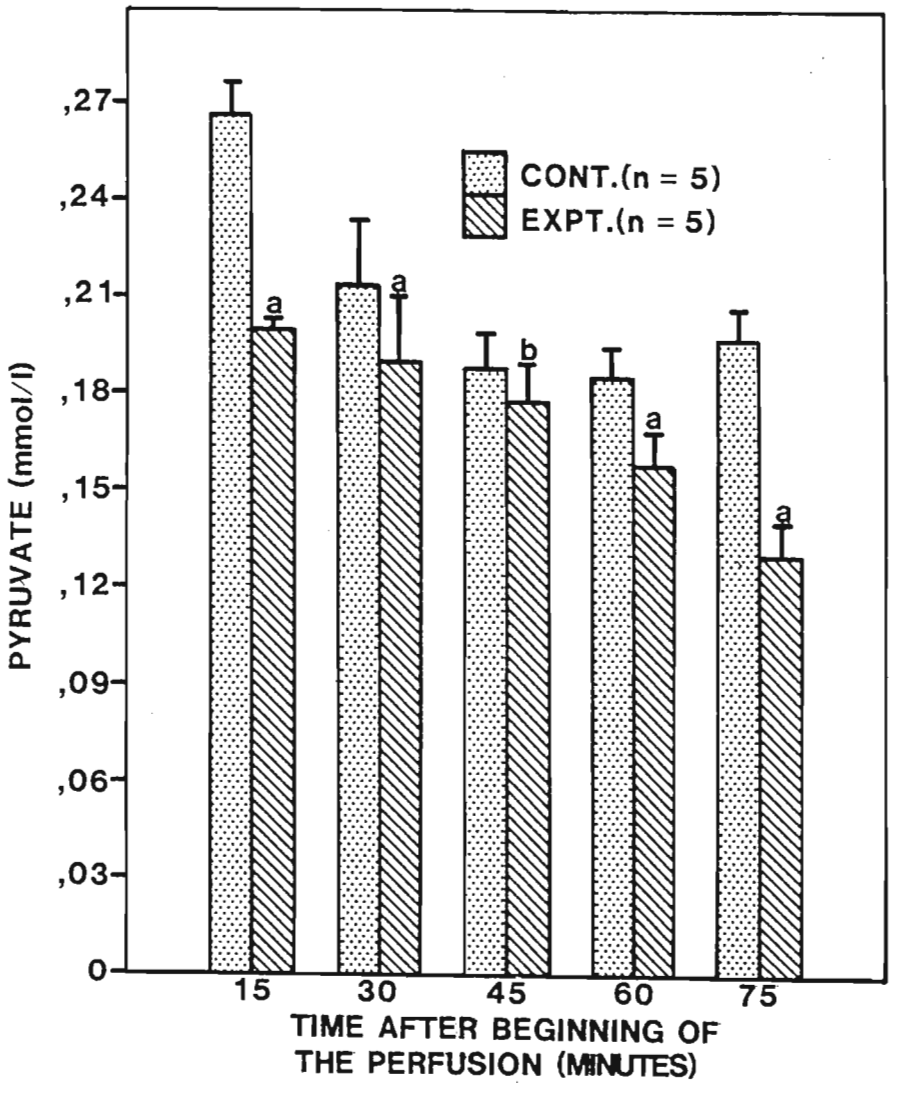


Fig. 15 Pyruvate concentrations derived from perfusate of isolated perfused livers of control and isoniazid-treated rats



a = p < 0,005 b = p < 0,05

Fig. 16 Lactate:Pyruvate ratios derived from perfusate of isolated perfused livers of control and isoniazid-treated rats

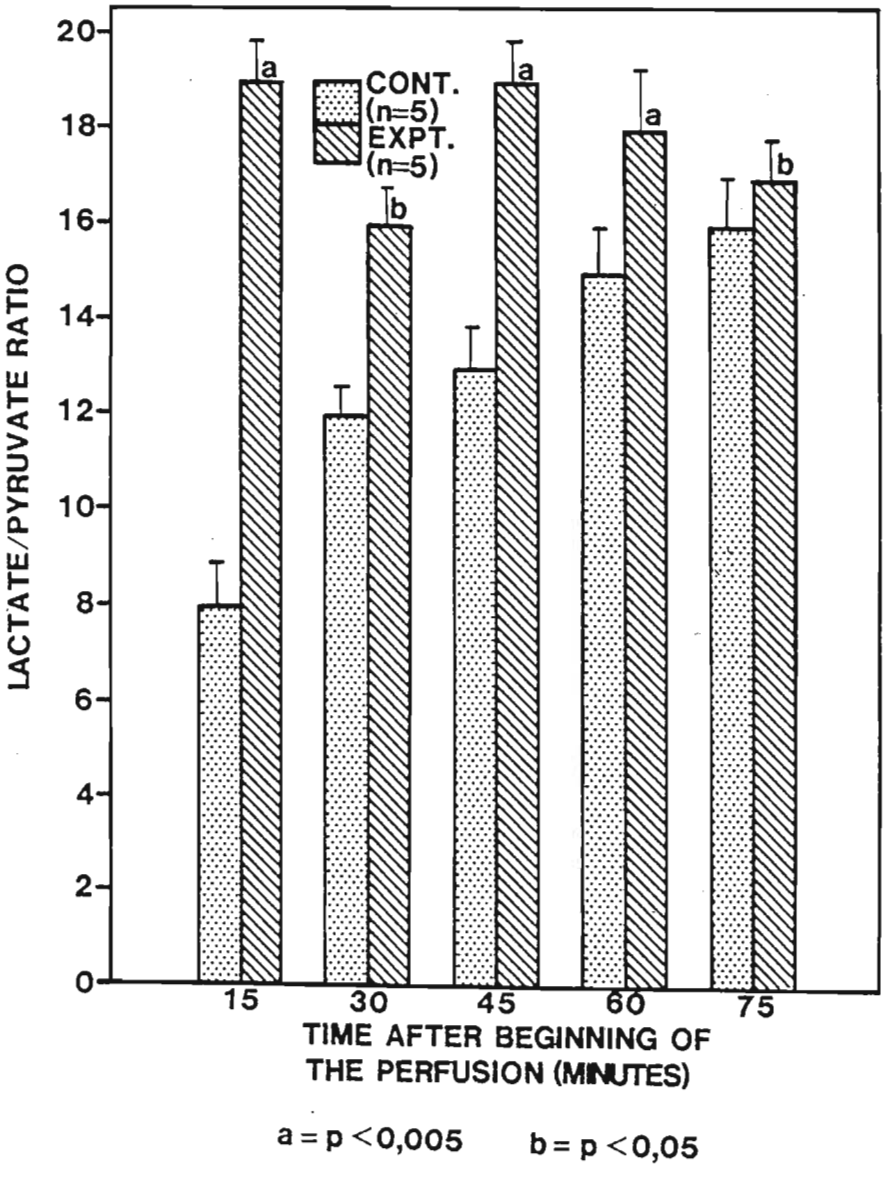


Fig. 17 Bromosulphthalein clearance derived from perfusate of isolated perfused livers of control and isoniazid-treated rats

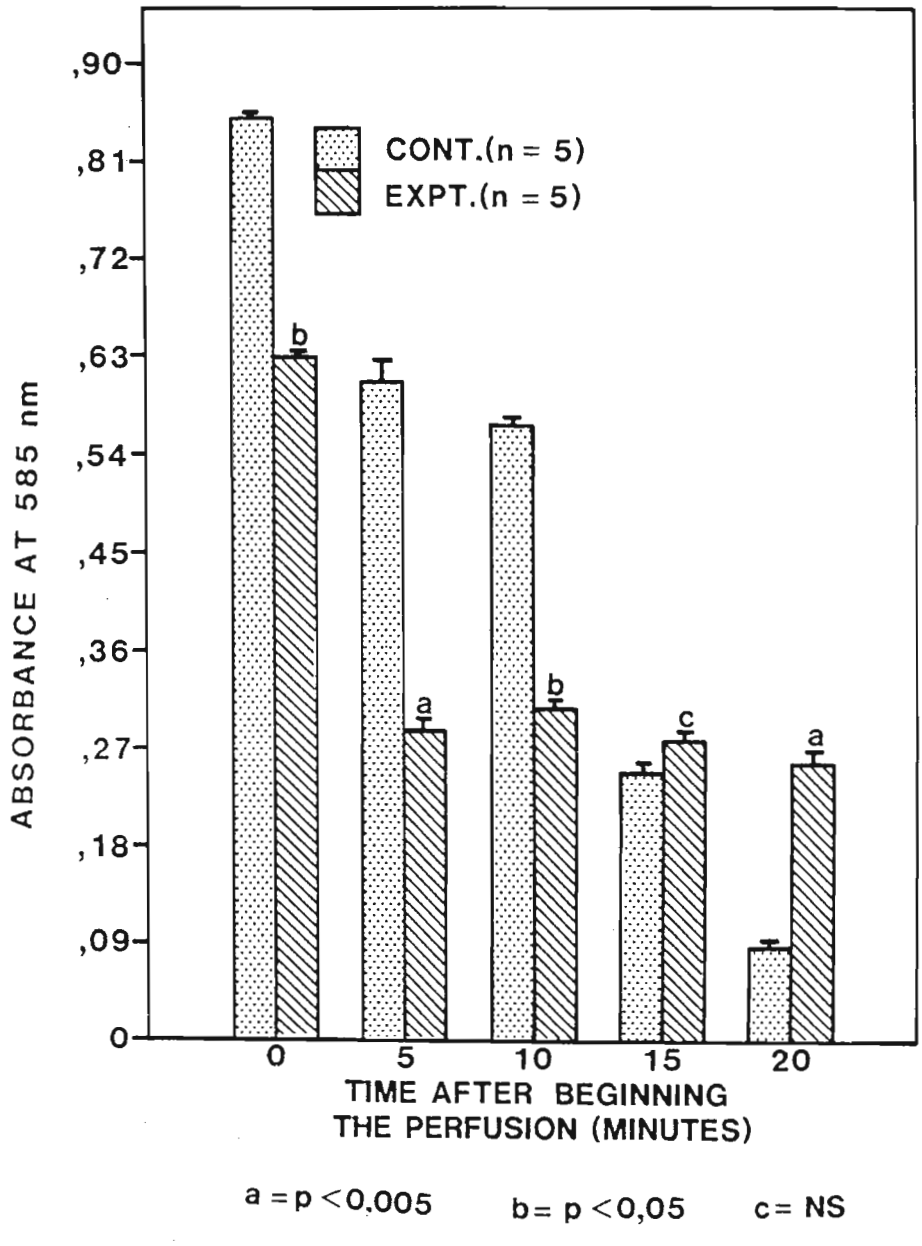
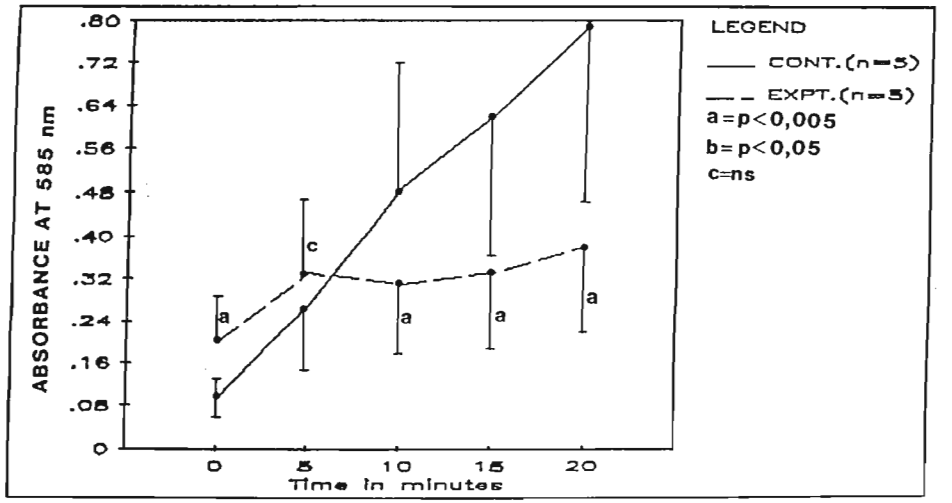


Fig. 18 Biliary excretion of bromosulphthalein derived from bile of control and isoniazid-treated rats.



Discussion

Isoniazid has been an important agent in the treatment of tuberculosis since the early 1950's. Despite a few case reports of associated liver injury, it was initially considered relatively safe and was not generally regarded as an important hepatotoxin. In the early 1970's, however, an outbreak of isoniazid - associated hepatitis triggered a reassessment of its hepatotoxic potential, and the clinical, epidemiologic, and biochemical investigations of the ensuing decade leave little doubt that isoniazid can cause usually mild, but occasionally fatal, hepatic injury, although the possible mechanism or mechanisms involved remain in some doubt. Mild hepatotoxicity is a seemingly benign response occuring in approximately 10% of all the subjects receiving single drug isoniazid chemoprophylaxis (Bailey et al, 1974; Mitchell et al, 1975; Litt et al, 1976; Mitchell et al, 1975; Mitchell et al, 1976). It may be entirely asymptomatic, and is most often characterised by a mild increase in serum transaminase activity during the first few months of treatment (Mitchell et al, 1975). In virtually all cases, the response appears to be transient and self limited and liver function tests revert to the normal despite continued administration of the drug.

In this study, glucose production from the livers of isoniazid treated rats was decreased, indicating that gluconeogensis was impaired. The increased lactate : pyruvate ratio in the experimental livers would suggest that an alteration in redox state of cytosol may be the mechanism underlying the impaired gluconeogenesis. In contrast, the rate and amount of pyruvate utilization and of glucose production by the control livers were increased, indicating that the pyruvate that was added to the perfusion medium was utilized for the production of glucose.

In the presence of decreased glucose production, increased lactate : pyruvate ratios (15 and 75 minutes ratios only) in the isoniazid-treated animals would suggest that the majority of pyruvate was utilised to form lactate. However lactate levels were not increased in these experiments. It is likely therefore that pyruvate entered another biochemical pathway. One possibility is that pyruvate was converted to L-alanine. Other possibilities include the conversion of pyruvate to acetyl Co-A or malate (Fig 12).

The study of Rubin et al (1952), also using supra-therapeutic doses of isoniazid (17.5 mg/kg) in dogs, reported central nervous system dysfunction, including convulsions and jaundice (with marked fatty degeneration of liver) resulting when this dose was given to two animals, the symptoms appearing after two to four weeks. Benson et al (1952) also reported slight liver pathology in dogs which had received isoniazid 5 and 10 mg/kg. In another study involving 32 patients receiving 16 - 24 mg/kg, one developed convulsive seizures with subsequent encephalopathy and 14 developed neuritis. The incidence of hepatic dysfunction was much less definite. Changes in

standard liver function tests appeared in approximately one half of the 17 patients studied; but were found on repeated testing in only 4 patients. While these changes were real, they were transient in every case. Thus, a daily dosage of 17.5 mg/kg in dog and 20mg/kg in man apparently has a high degree of neural toxicity, but significant hepatic toxicity was not observed at this dosage in patients even though it had been observed in dogs.

In the present investigation, a marked retention in perfusate and decreased biliary excretion of bromosulphthalein was observed in isoniazid - treated rats. During the 20 minute perfusion, most of the dye was cleared from perfusate in the control animals with increased biliary excretion of the dye; in experimental animals, after an initial high clearance, from perfusate, the concentration of the dye remained constant with decreased biliary excretion of the dye till the end of the experiment. The decrease in bromosulphthalein clearance from the perfusate and the biliary excretion of the dye in isoniazid-treated rats was presumed to be as a result of depressed activities of some enzymes and altered permeability of cell membranes.

This study demonstrates that isoniazid inhibits gluconeogenesis and bromosulphthalein clearance from perfusate and biliary excretion of the dye in rats when given in a dose two and a half times the recommended adult human dose. Whether or not prolonged supra-therapeutic doses of isoniazid will prove superior to conventional doses in tuberculous patients is uncertain at present but will be the subject of further study. The data available from the present and previous studies, suggests that it is inadvisable to prescribe doses of isoniazid beyond conventional dose as hepatic disorders, including hepatitis, may well occur.

CHAPTER 5

RIFAMPICIN

RIFAMPICIN

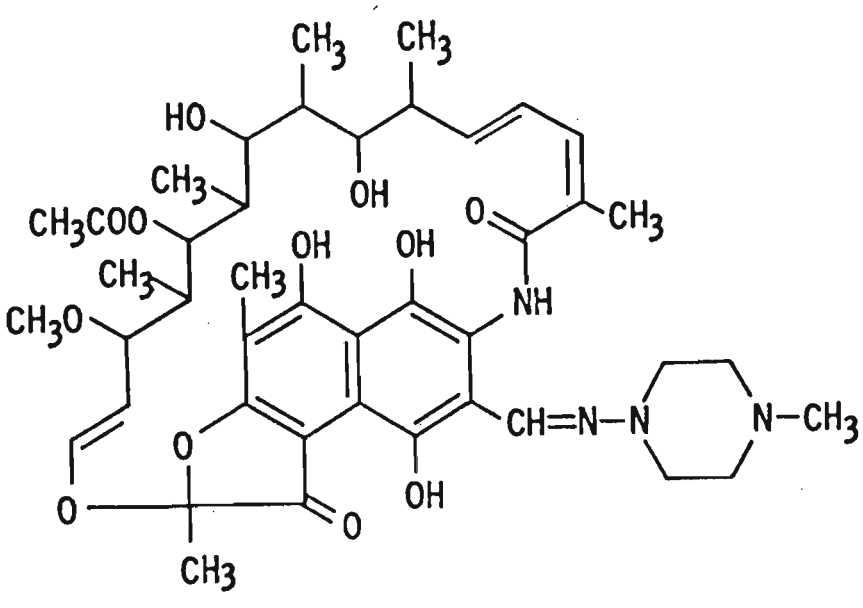
Introduction

Numerous reports have been published showing rifampicin to be a potent antituberculous drug, comparable with isoniazid in its activity in both animals and humans (Cannetti et al, 1968; Pines, 1971). Rifampicin is an antibiotic derived from the family of rifamycins; it is produced by Streptomyces mediterranei and mainly used for its antituberculous action (Vall-Spinonsa et al, 1970). Rifampicin is also a drug of choice for chemoprophylaxis of meningococcal disease and shows promise in the treatment of certain non-mycobacterial disease and as an adjunct in the treatment of leprosy. The fear of drug resistance has prevented its wider use, despite its excellent activity against staphylococci, some Gram-negative bacilli and legionellae. Drug resistance can emerge quite rapidly if rifampicin is used alone. There has been a steady increase in the use of rifampicin to treat Staphylococcus epidermidis infections which are commonly extremely sensitive. In such cases, however, it is appropriate to combine rifampicin with another drug such as flucloxacillin, to reduce the risk of drug resistance. Rifampicin is also used in the treatment of legionnaire's disease.

Physical and Chemical Properties

Rifampicin is a tasteless, brick red to reddish-brown crystalline powder. It is a zwitterion and is soluble in organic solvents and in water at acidic pH. The molecular formula is $C_{43}H_{58}N_4O_{12}$ with molecular weight of 823g.

Structure of Rifampicin



Kinetic Data

Rifampicin is well-absorbed. The oral administration of rifampicin produces peak concentrations in plasma in 2 to 4 hours. Rifampicin is distributed throughout the body and is present in effective concentrations in many organs and body fluids including the cerebrospinal fluid. This is exemplified by the fact that the drug may impart orange red colour to urine, faeces, saliva, sputum, tears and sweat.

Metabolism

It is partly metabolised in the liver by de-acetylation followed by glucuronidation to inactive metabolites and partly excreted into bile unchanged with consequent re-circulation. The rest is eliminated unchanged in the urine. $T^1/_2$ = 3 hours. Biliary excretion increases progressively during the first 2 weeks of treatment and $T^1/_2$ shortens.

Experimental Design

Rifampicin has achieved first line status in the treatment of tuberculosis. The administration of therapeutic doses of this compound has been complicated by the development of hepatic damage in the form of hepatitis (Scheuer et al, 1974). The recommended daily dosage of this drug in adults is 10mg/kg, with a maximum of 600mg. An investigation using the isolated rat liver perfusion technique was conducted to determine whether the administration of rifampicin, in doses exceeding those that are recommended, would affect gluconeogenesis and bromosulphthalein clearance by the liver.

Materials and Methods

12 male Wistar rats (190 - 200 g) of the University of Natal inbred strain were kept in stainless steel cages with plastic bottoms. Each was individually ear marked for identification and randomly assigned to either a control or an experimental group, each with 6 rats. All the animals were fed on the same diet in the form of commercial rat cubes (Epol). Water was given ad libitum. The animals in the experimental group were given 4 mg rifampicin per os daily for 90 days; the control group was untreated. The livers of control and experimental rats were perfused for 75 minutes with a medium containing pyruvate (500umoles), a gluconeogenic precursor.

Immediately after perfusion with the medium containing pyruvate, the livers of both the groups were perfused with a medium containing bromosulphthalein (1.25mg/100g rat weight) for an additional 20 minutes. The animals were randomly chosen for perfusion. Serial specimens were obtained for analyses.

Results

Evidence of satisfactory functioning of liver in this study was indicated by the absence of patchy colouring of the liver, swelling and formation of exudate on the surface of the liver after two hours of perfusion. The time course of glucose and lactate production by livers of control and experimental animals, pyruvate concentrations and lactate : pyruvate ratios are shown in Tables 5a and 5b respectively and illustrated graphically (Figs. 19-22).

Gluconeogenesis

When the livers of the control rats were perfused with a medium containing pyruvate (500 umoles), there was an increase in glucose and lactate contents of the medium. Mean glucose concentration derived from the perfusate after 90 minutes of perfusion was 4.55 mmoles/l and glucose formation was at an average rate of 0.07 mmoles/min per g wet weight of liver while lactate concentration was 2.63 mmoles/l and formation at an average rate of 0.002 mmoles/min per g wet weight of liver. The pyruvate concentration in the medium dropped by 300 umoles such that the lactate:pyruvate ratio was at a value of about 12.

Similarly when the livers of rifampicin-treated rats were perfused with medium containing pyruvate (500 umoles) there was a rapid increase in lactate and a steady but slow increase in glucose contents of the medium. Lactate concentration was 3.03 mmoles/l at the end of the perfusion with formation at an average rate of 0.02 mmoles/min per g wet weight of liver. The pyruvate content of the medium decreased by 385 umoles such that the lactate:pyruvate ratio was approximately 24.

The glucose concentration was 1.78 mmoles/l and the formation was at an average rate of 0.006 mmoles/min per g wet weight of liver. The rate of glucose formation and glucose concentration were decreased and lactate:pyruvate ratios increased in the experimental group compared to controls ($p<0.05$ for all comparisons).

Bromosulphthalein Clearance

Bromosulphthalein was added, at a dosage of 1.25 mg/100g body weight of rat, 80 minutes after the beginning of the perfusion which continued for an additional 20 minutes. The bromosulphthalein clearance from perfusate in the control and experimental group was similar. No biliary excretion of dye could be detected in the scanty bile collected.

Table 5a

TIME-COURSE OF THE PRODUCTION OF LACTATE, AND GLUCOSE BY PERFUSED LIVERS OF CONTROL RATS (n=6) UNDER AEROBIC CONDITIONS (500 umoles of pyruvate added to perfusion medium)

The results are expressed as MEAN ± SEM. The initial volume of the medium was 150 ml; it decreased every 15 min by 10 ml. (removed for analysis). The livers had a mean wet weight of 6.28 g.

TIME (minutes)	15		30		45		60		75	
CONC. OF METABOLITE (mmoles/l)										
LACTATE	2.60	±0.33	2.65	±0.09	2.85	±0.08	2.43	±0.18	2.63	±0.06
PYRUVATE	0.200	±0.01	0.212	±0.01	0.211	±0.01	0.210	±0.004	0.196	±0.004
GLUCOSE	2.84	±0.10	3.05	±0.21	3.68	±0.23	3.57	±0.32	4.77	±0.65
LACTATE:PYRUVATE ratios	13.30	±1.9	12.67	±0.78	13.92	±0.98	11.53	±0.72	13.43	±0.28
METABOLIC RATES (mmoles / min per g wet weight of liver)										
GLUCOSE FORMATION			0.03		0.10		0.01		0.19	
LACTATE FORMATION			0.008		0.03		−0.06		0.03	

Table 5b

TIME-COURSE OF THE PRODUCTION OF LACTATE, AND GLUCOSE BY PERFUSED LIVERS OF EXPERIMENTAL RATS (n=6)

UNDER AEROBIC CONDITIONS (500 umoles of pyruvate added to perfusion medium)

The results are expressed as MEAN ± SEM. The initial volume of the medium was 150ml; it decreased every 15 minutes by 10ml. (removed for analysis). The livers had a mean wet weight of 6.18 g.

TIME (minutes)	15		30		45		60		75	
CONC. OF METABOLITE (mmoles/l)										
LACTATE	2.58	±0.16	2.55	±0.24	2.73	±0.23	3.07	±0.07	3.03	±0.35
PYRUVATE	0.110	±0.004	0.115	±0.004	0.108	±0.004	0.118	±0.008	0.135	±0.008
GLUCOSE	1.99	±0.22	1.83	±0.19	1.82	±0.17	1.86	±0.16	1.78	±0.19
LACTATE:PYRUVATE ratios	23.61	±1.9	22.26	±2.2	25.16	±1.9	26.73	±2.1	23.20	±3.7
METABOLIC RATES (mmoles / min per g wet weight of liver)										
GLUCOSE FORMATION		−0.02		−0.001		0.006		−0.01		
LACTATE FORMATION		−0.004		0.03		0.06		−0.006		

Table 5c

TIME-COURSE OF BROMOSULPHTHALEIN CLEARANCE BY PERFUSED LIVERS OF CONTROLS AND EXPERIMENTAL ANIMALS

The results are expressed as MEAN ± SEM. BSP was added, at a dosage of 1.25 mg / 100g body weight, to the perfusion medium.

TIME (minutes)	0		5		10		15		20	
CONTROLS (6)	0.84	±0.008	0.58	±0.02	0.467	±0.03	0.25	±0.01	0.05	±0.01
EXPERIMENTALS (6)	0.80	±0.02	0.62	±0.04	0.48	±0.03	0.26	±0.10	0.05	±0.02

Fig. 19 Glucose concentrations derived from perfusate of isolated perfused livers of control and rifampicin-treated rats

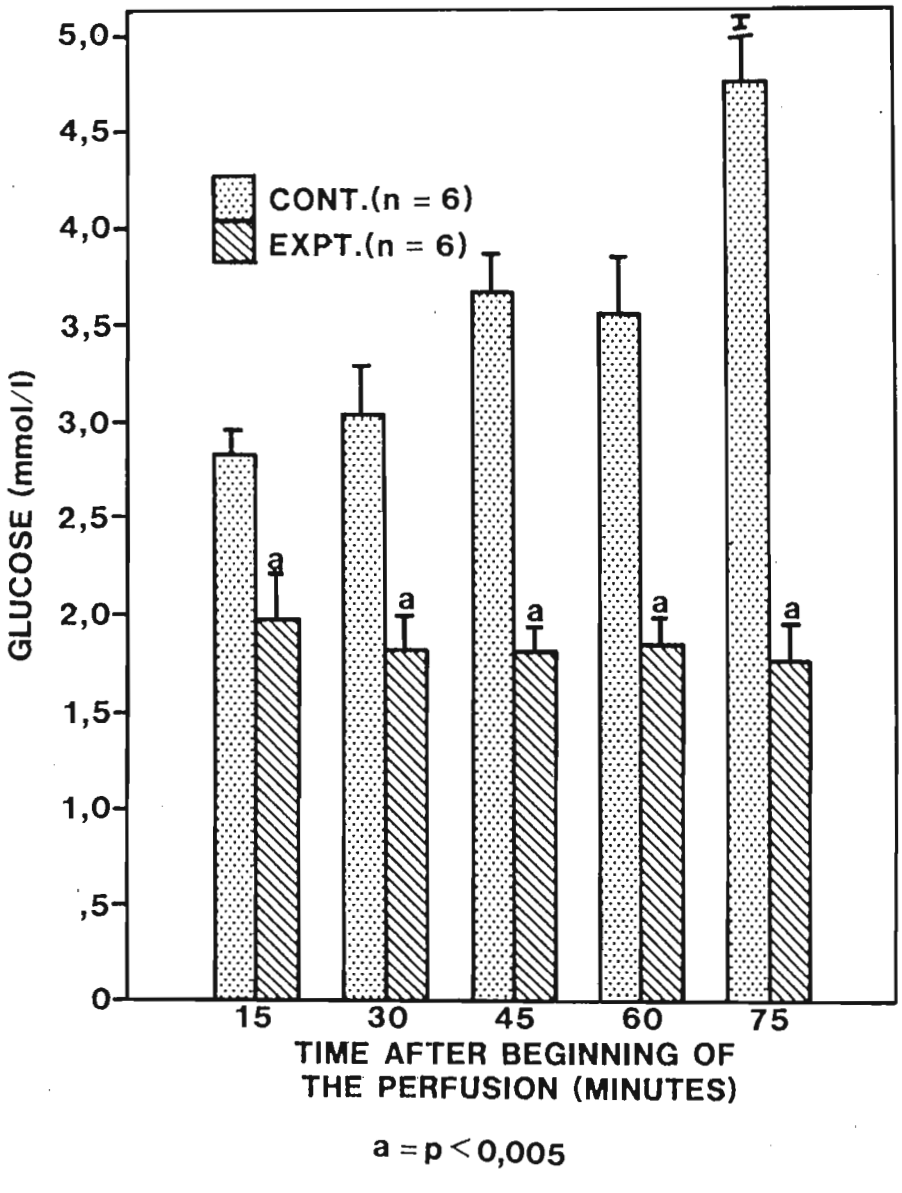
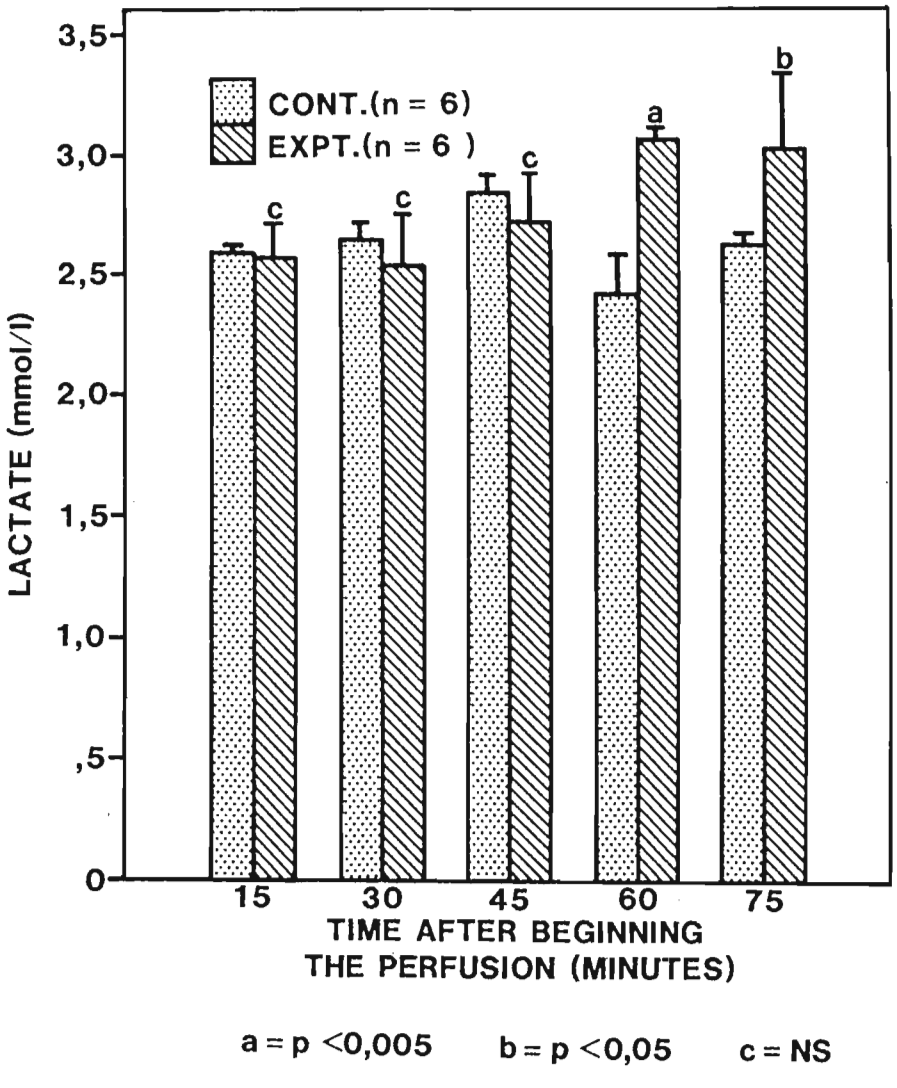


Fig. 20 Lactate concentrations derived from perfusate of isolated perfused livers of control and rifampicin-treated rats



a = p <0,005 b = p <0,05 c = NS

Fig. 21 Pyruvate concentrations derived from perfusate of isolated perfused livers of control and rifampicin-treated rats

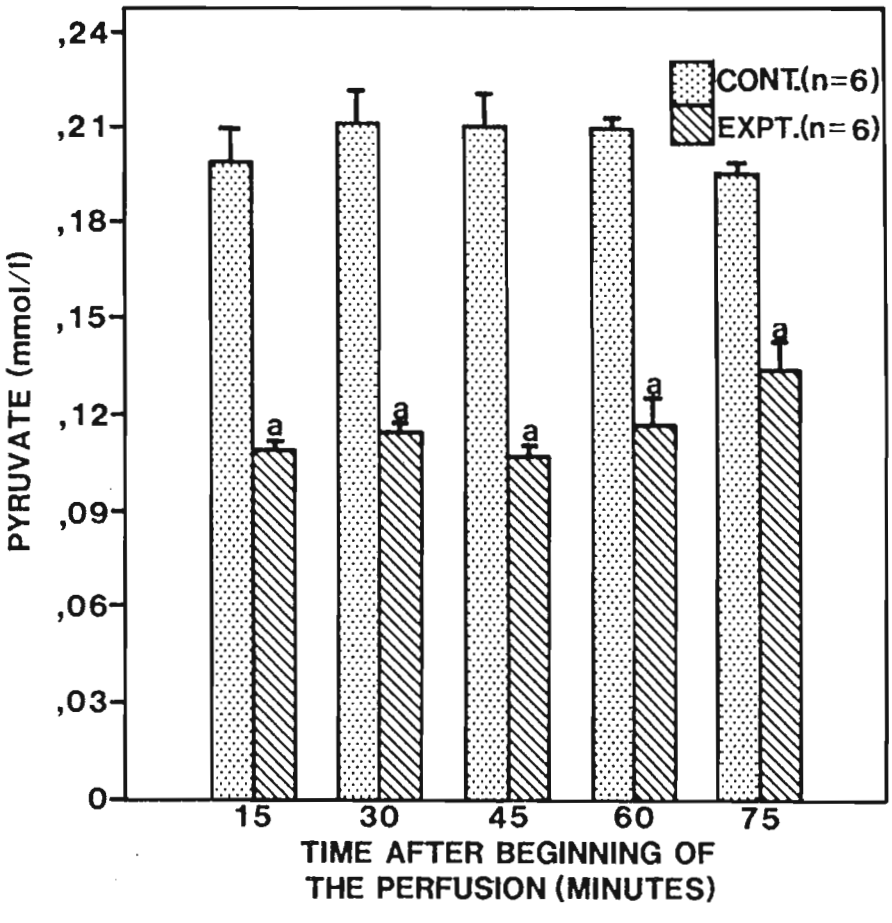
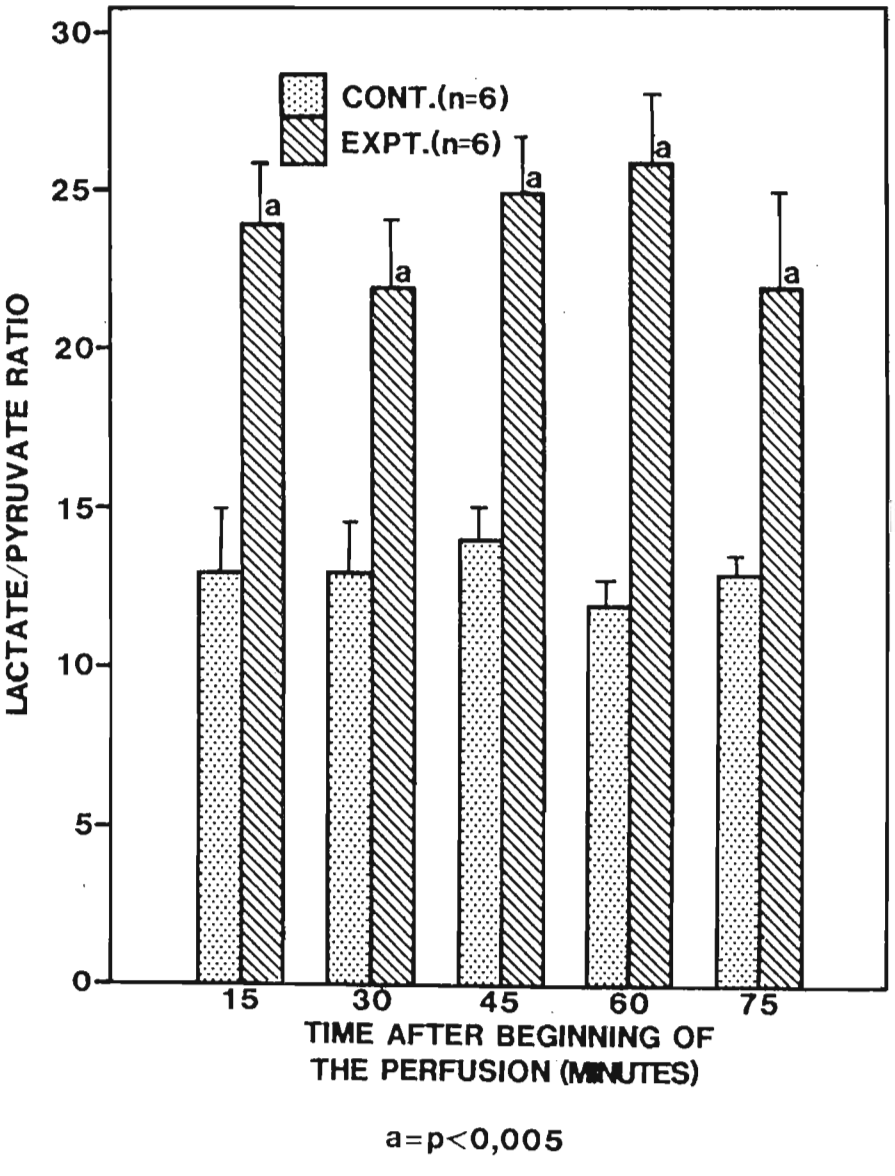


Fig. 22 Lactate:Pyruvate ratios derived from perfusate of Isolated perfused livers of control and rifampicin-treated rats



a=p<0,005

Discussion

Rifampicin has achieved first line status in the treatment of tuberculosis because of its low toxicity and high therapeutic efficacy. It is metabolised in the liver mainly by de-acetylation followed by glucuronidation.

In the present study, glucose production and pyruvate utilization were increased in control animals compared to experimental animals indicating that the pyruvate added to the perfusion medium was utilized mainly for the production of glucose. In contrast the glucose production by rifampicin-treated animals was decreased compared with control animals indicating that gluconeogenesis was impaired. In the presence of decreased glucose production, the increased lactate : pyruvate ratios in the experimental animals would suggest that the majority of the pyruvate was utilized to form lactate. However lactate levels were not increased in the experimental animals. It is likely therefore that pyruvate entered another biochemical pathway. One possibility is that pyruvate was converted to L-alanine.

Other possibilities include the conversion of pyruvate to acetyl Co-A or malate.
There was no difference between the 2 groups for bromosulphthalein clearance from perfusate; no biliary excretion of bromosulphthalein could be detected in the scanty bile collected from livers of rifampicin-treated rats.

Liver function and serum concentration of rifampicin, a highly cholephilic antibiotic, have been studied after the ingestion of a single dose of rifampicin in 12 patients and during treatment with 600 mg of rifampicin per day for 17 days in 8 patients. Rifampicin caused a considerable decrease in the bromosulphthalein plasma disappearance rate and a slight increase in bilirubin (Capelle et al, 1972). This apparent competition between bromosulphthalein and bilirubin which involved mainly uptake, but also excretion by the liver cells was always rapidly reversible when treatment was discontinued. These workers conclude that on the basis of these findings, it is impossible to interpret a bromosulphthalein test during treatment with rifampicin; the bromosulphthalein test in a patient treated with rifampicin can only be interpreted 24h after rifampicin has been stopped. Since the present study was conducted while the rats were receiving rifampicin, it is not possible to draw any conclusions from the results of bromosulphthalein clearance.

The liver damage that follows rifampicin administration is believed to be idiosyncratic. However, in the present investigation, rifampicin administration in a dose equivalent to 2 times the recommended adult dose for a 3 month period resulted in impaired gluconeogenesis in all the experimental rats.

The information obtained from the study is inadequate. However, since therapeutic doses of rifampicin may be associated with hepatobiliary dysfunction, supra therapeutic doses of the drug are not recommended.

CHAPTER 6

PARACETAMOL

PARACETAMOL

Introduction

Paracetamol, a medication available over the counter without a prescription, is a major component of many formulations available for the relief of headaches, fever, coughs and cold.

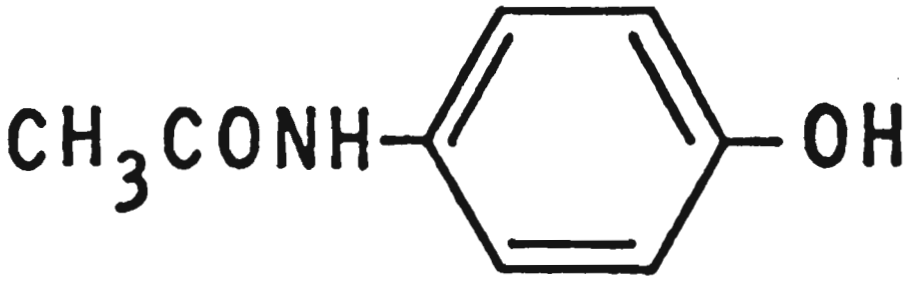
Physical and Chemical Properties

It occurs as a crystalline powder or white odourless crystals with a bitter taste. Melting point ranges from 168°C to 172°C. The solubility of the substance varies with different solvents.

Kinetic Data

Following oral ingestion, paracetamol is rapidly absorbed from the gastrointestinal tract and peak plasma concentrations are reached in 30-60 minutes. The drug is uniformly distributed throughout most body fluid and is only fractionally bound to plasma proteins (25%). It is primarily metabolised by conjugation with glucuronide or sulphate, and the plasma half life ranges between 1-3 hours. The molecular formula is $C_8 H_9 NO_2$ with molecular weight of 151,2g.

Structure of Paracetamol

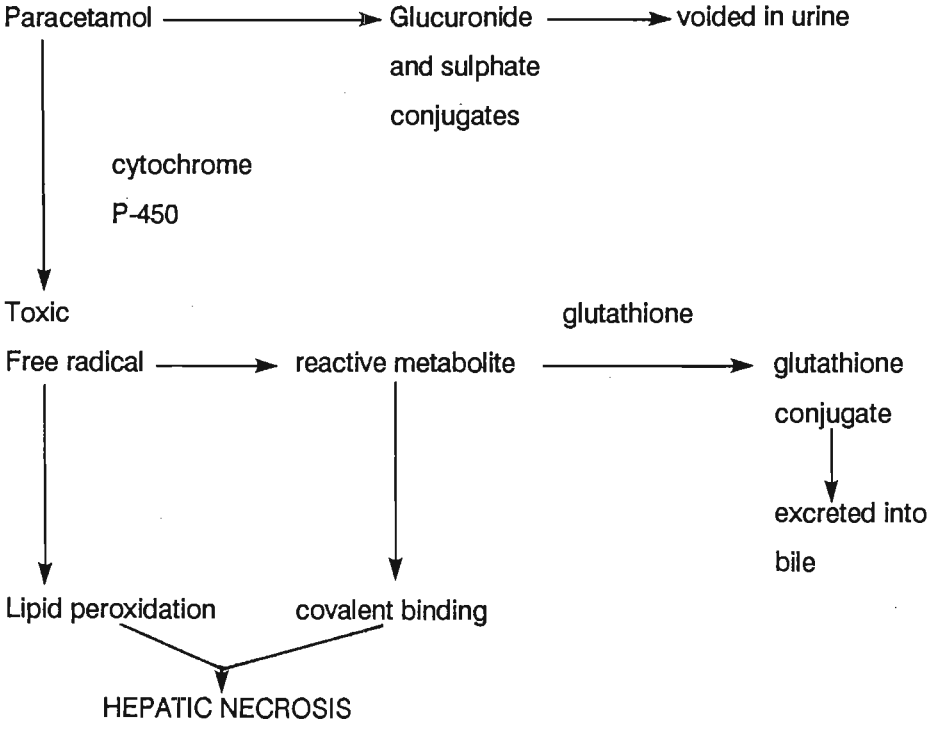


Metabolism

A number of drugs including paracetamol, a widely used non-narcotic analgesic, produce hepatotoxicity by generating chemically reactive metabolites which react indiscriminately with vital cellular macro molecules. In therapeutic doses paracetamol is metabolised, the major part being converted to glucuronide and sulphate conjugates and very little (5-10%) of the drug is excreted unchanged in the urine. (Fig. 23). A very small proportion (in the liver) is converted by hepatic microsomal enzymes to a highly unstable and potentially hepatotoxic metabolite, N-acetylimidoquinone, which is rapidly inactivated by conjugation with hepatic glutathione. Hepatic glutathione plays a vital role in protecting against peroxidative damage, detoxifying the reactive paracetamol metabolite by acting as a ligand to form a paracetamol glutathione conjugate or as a reducing agent to convert the reactive metabolite back to parent compound. The resulting paracetamol - glutathione conjugate is then excreted into the bile.

However, after an overdose, the sulphation and glutathione pathways become saturated. The proportion excreted as conjugates decreases with the increasing dose of the drug. The depletion of hepato-cellular glutathione occurs because the rate of formation of the metabolite of drug exceeds the rate at which the liver can synthesise glutathione for conjugation. The reactive metabolite accumulates within the liver and arylates essential cell structures.

Fig 23. Possible mechanisms of hepatotoxicity from paracetamol.



It has also been demonstrated that paracetamol can undergo metabolism to a highly reactive and electrophilic free radical. Free radicals are highly unstable and chemically reactive compounds because they contain an unpaired electron. Donation of this electron to oxygen leads to formation of highly reactive superoxide, and when a free radical comes into proximity with unsaturated lipids, as in cell membranes, it initiates a self-perpetuating peroxidative decomposition, with disruption of membrane structure and function.

It is now recognised that paracetamol hepatotoxicity occurs not only in persons who ingest massive amounts of the drug but also in some individuals who ingest quantities in sub-therapeutic doses.

Experimental Design

Paracetamol is a widely used analgesic. Overdose with this compound results in hepatotoxicity which may be fatal. In vivo studies of glucose metabolism and bromosulphthalein clearance have shown that impaired gluconeogenesis and bromosulphthalein clearance often occurs after liver damage due to paracetamol overdose. Earlier studies have shown that in chronic alcoholics paracetamol hepatotoxicity occurs with therapeutic or near therapeutic doses of paracetamol (Seeff et al, 1986; Mclain et al, 1980; Johnson et al, 1981). A recent report showed that in two patients, with a high daily alcohol consumption, liver damage exhibiting clinical and morphological features of paracetamol hepatotoxicity occurred; although the patients took only therapeutic doses of paracetamol (Floren et al, 1987). In order to test the hypothesis that the isolated liver perfusion technique could be a valuable means of assessing the effect of therapeutic doses of drugs on liver function, this investigation was conducted to determine the effects of therapeutic doses of paracetamol on gluconeogenesis and bromosulphthalein clearance using this technique.

Materials and Methods

24 male Wistar rats (170 - 190g) of the University of Natal inbred strain were kept in stainless steel cages with plastic bottoms. Each was individually earmarked for identification and randomly assigned to 3 groups, one control and 2 experimental, each with 8 rats. All the animals were fed on the same diet in the form of commercial rat cubes (Epol). Water was given ad libitum. The animals in the experimental groups were given 0.005g paracetamol per os daily for 90 days.

The control group was untreated. The livers of the control (Group A) and one experimental group (Group B) were perfused for 75 minutes with a medium containing pyruvate (500umoles), a gluconeogenic precursor, and subsequently the livers of control rats were perfused with a medium containing bromosulphthalein (1.25mg/100g rat weight) for an additional 20 minutes. The second experimental group (Group C) which was reduced to 6 because of the death of 2 rats during the course of the study, was perfused with a medium containing pyruvate (500umoles) and 1ml 10% solution of methylene blue and subsequently these livers were perfused with a medium containing bromosulphthalein (1.25mg/100g rat weight) for an additional 20 minutes. The animals were randomly chosen for perfusion. Serial specimens were obtained for analyses.

Results

The success of the isolated liver perfusion technique was judged mainly by the completeness with which the blood was expelled from the liver as indicated by the quick fading of the organ's colour. The time-course of glucose and lactate production by the livers of control and experimental animals, pyruvate concentrations and lactate:pyruvate ratios are shown in Tables 6a and 6b respectively and illustrated graphically (Figs. 24-27).

Gluconeogenesis

Results of control and experimental perfusion with pyruvate (500 umoles) shows that livers of control rats shed appreciable amounts of glucose and lactate during 75 minutes of perfusion. The mean glucose concentration was 3.69 mmoles at the end of perfusion and the average rate of glucose formation was 0.07 mmoles/min per g wet weight of liver while the lactate concentration was 3.07 mmoles/l after 75 minutes of perfusion and the mean rate of lactate formation was 0.04 mmoles/min per g wet weight of liver. About 300 umoles of pyruvate were utilized such that the lactate:pyruvate ratio was at a value of about 12.

In the experimental rats the mean glucose concentration obtained from perfusate was 1.95 mmoles/l at the end of perfusion and the glucose formation was at an average rate of 0.03 mmoles / min g wet weight of liver while the mean lactate concentration derived from the perfusate at the end of the perfusion was 4.34 mmoles/l and the rate of lactate formation was 0.06 mmoles/min per g wet weight of liver. The experimental livers utilised about 350 umoles of pyruvate such that the lactate:pyruvate ratio was about 30.

The rate of glucose formation and glucose concentration was decreased, lactate levels and lactate:pyruvate ratios were increased in the experimental groups compared to control ($p<0.05$ for all comparisons except 15 minute lactate levels).

Glucose Formation

1ml 10% methylene blue (a redox agent capable of oxidising NADH to NAD^+) was added at the start of perfusion which continued for 75 minutes. In two experiments, one with pyruvate plus methylene blue (0.78mmoles/l) and the other with pyruvate alone (1.95mmoles/l), glucose concentration was low in both experiments. Methylene blue did not overcome the paracetamol inhibition of pyruvate conversion to glucose. Methylene blue had no effect on glucose output, in fact even lower than that of pyruvate alone ($p <$ 0,05; Fig. 28).

Bromosulphthalein Clearance

Bromosulphthalein was added at a dosage of 1.25 mg/100g body weight of rat to the perfusion medium 80 minutes after the beginning of the perfusion. The time course of bromosulphthalein clearance from perfusate and the biliary excretion of the dye are shown in Table 6c. The percentage of bromosulphthalein retention in the perfusate of control animals was 8.3% while that of the paracetamol-treated group was 41.6%. Bromosulphthalein clearance from perfusate ($p<0.005$; Fig. 29) and biliary excretion of the dye ($p<0.05$; Fig. 30) were impaired in the experimental livers compared to controls.

Table 6a

TIME-COURSE OF THE PRODUCTION OF LACTATE, AND GLUCOSE BY PERFUSED LIVERS OF CONTROL RATS (n=8)

UNDER AEROBIC CONDITIONS (500 umoles of pyruvate added to perfusion medium)

The results are expressed as MEAN ± SEM. The initial volume of the medium was 150 ml; it decreased every 15 minutes by 10 ml. (removed for analysis). The livers had a mean wet weight of 6.08g.

TIME (minutes)	15		30		45		60		75	
CONC. OF METABOLITE (mmoles/l)										
LACTATE	1.84	±0.07	2.23	±0.22	2.38	±0.11	2.42	±0.12	3.07	±0.14
PYRUVATE	0.235	±0.07	0.182	±0.01	0.191	±0.04	0.190	±0.04	0.205	±0.04
GLUCOSE	2.14	±0.1	2.45	±0.09	2.78	±0.25	3.66	±0.36	3.69	±0.28
LACTATE:PYRUVATE ratios	7.90	±0.38	13.57	±2.2	13.19	±1.6	13.36	±1.4	15.51	±1.5
METABOLIC RATES (mmoles / min per g wet weight of liver)										
GLUCOSE FORMATION			0.05		0.05		0.14		0.004	
LACTATE FORMATION			0.06		0.02		0.006		0.10	

Table 6b

TIME-COURSE OF THE PRODUCTION OF LACTATE, AND GLUCOSE BY PERFUSED LIVERS OF EXPERIMENTAL RATS (n=8)

UNDER AEROBIC CONDITIONS (500 umoles of pyruvate added to perfusion medium)

The results are expressed as MEAN ± SEM. The initial volume of the medium was 150 ml; it decreased every 15 min by 10 ml. (removed for analysis). The livers had a mean wet weight of 6.01g.

TIME (minutes)	15		30		45		60		75	
CONC. OF METABOLITE (mmoles/l)										
LACTATE	2.71	±0.47	3.26	±0.36	3.91	±0.46	4.08	±0.31	4.34	±0.51
PYRUVATE	0.115	±0.003	0.120	±0.01	0.134	±0.01	0.129	±0.01	0.123	±0.01
GLUCOSE	0.93	±0.14	0.99	±0.18	1.20	±0.17	1.19	±0.14	1.95	±0.25
LACTATE:PYRUVATE ratios	21.01	±4.5	28.12	±3.5	31.03	±4.7	33.81	±3.9	36.22	±4.7
METABOLIC RATES (mmoles / min per g wet weight of liver)										
GLUCOSE FORMATION			0.01		0.03		−0.001		0.1	
LACTATE FORMATION			0.09		0.11		0.03		0.04	

Table 6c

TIME-COURSE OF BROMOSULPHTHALEIN CLEARANCE BY PERFUSED LIVERS OF CONTROL AND EXPERIMENTAL ANIMALS

The results are expessed as MEAN ± SEM. BSP was added, at a dosage of 1.25 mg / 100g body weight, to the perfusion medium.

TIME (minutes)	0		5		10		15		20	
CONTROLS (8)	0.84	±0.007	0.59	±0.01	0.51	±0.002	0.22	±0.007	0.07	±0.003
EXPERIMENTALS (6)	0.72	±0.06	0.31	±0.02	0.29	±0.02	0.31	±0.03	0.30	±0.02

TIME-COURSE OF BILIARY EXCRETION OF BROMOSULPHTHALEIN

TIME (minutes)	0		5		10		15		20	
CONTROLS (n=8)	0.14	±0.05	0.27	±0.09	0.38	±0.13	0.46	±0.16	0.71	±0.25
EXPERIMENTS (n=6)	0.19	±0.08	0.18	±0.07	0.26	±0.11	0.31	±0.13	0.38	±0.16

Fig. 24 Glucose concentrations derived from perfusate of isolated perfused livers of control and paracetamol-treated rats

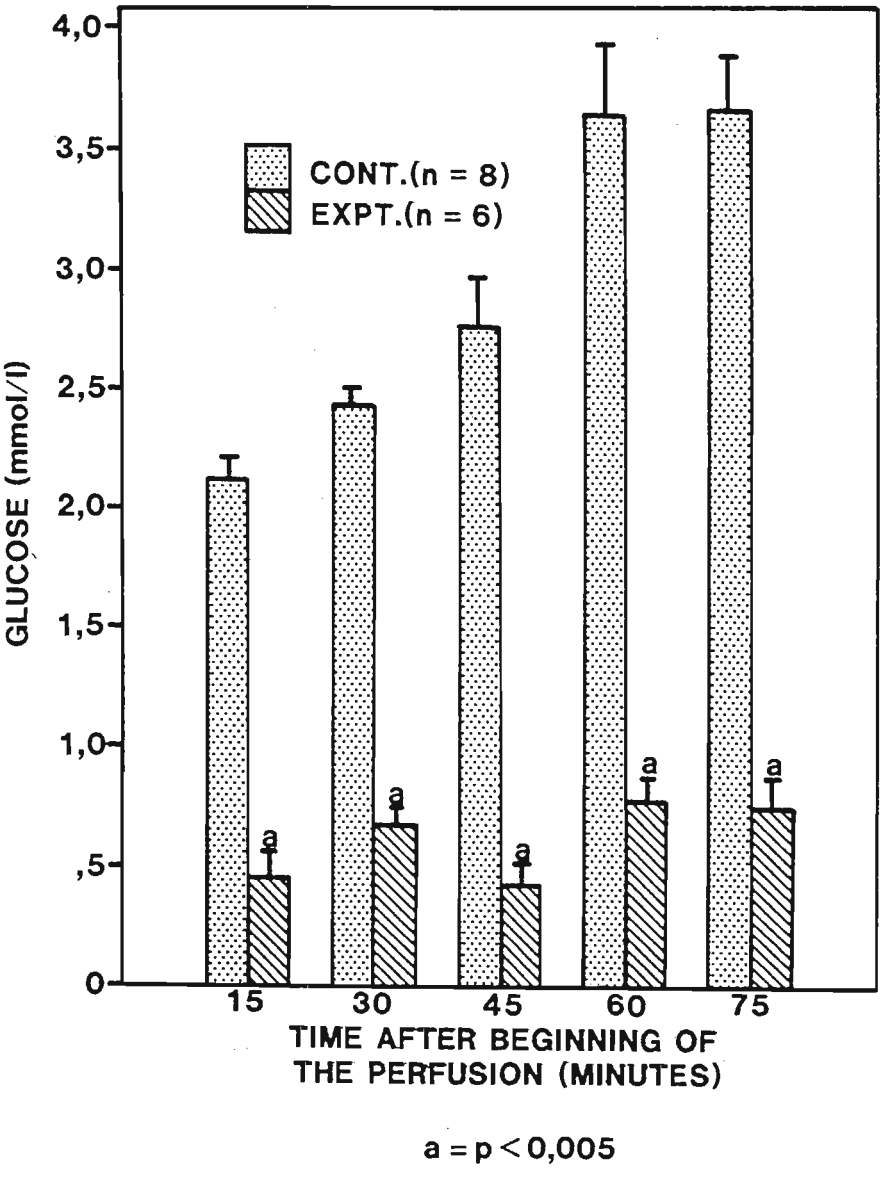


Fig. 25 Lactate concentrations derived from perfusate of isolated perfused livers of control and paracetamol-treated rats

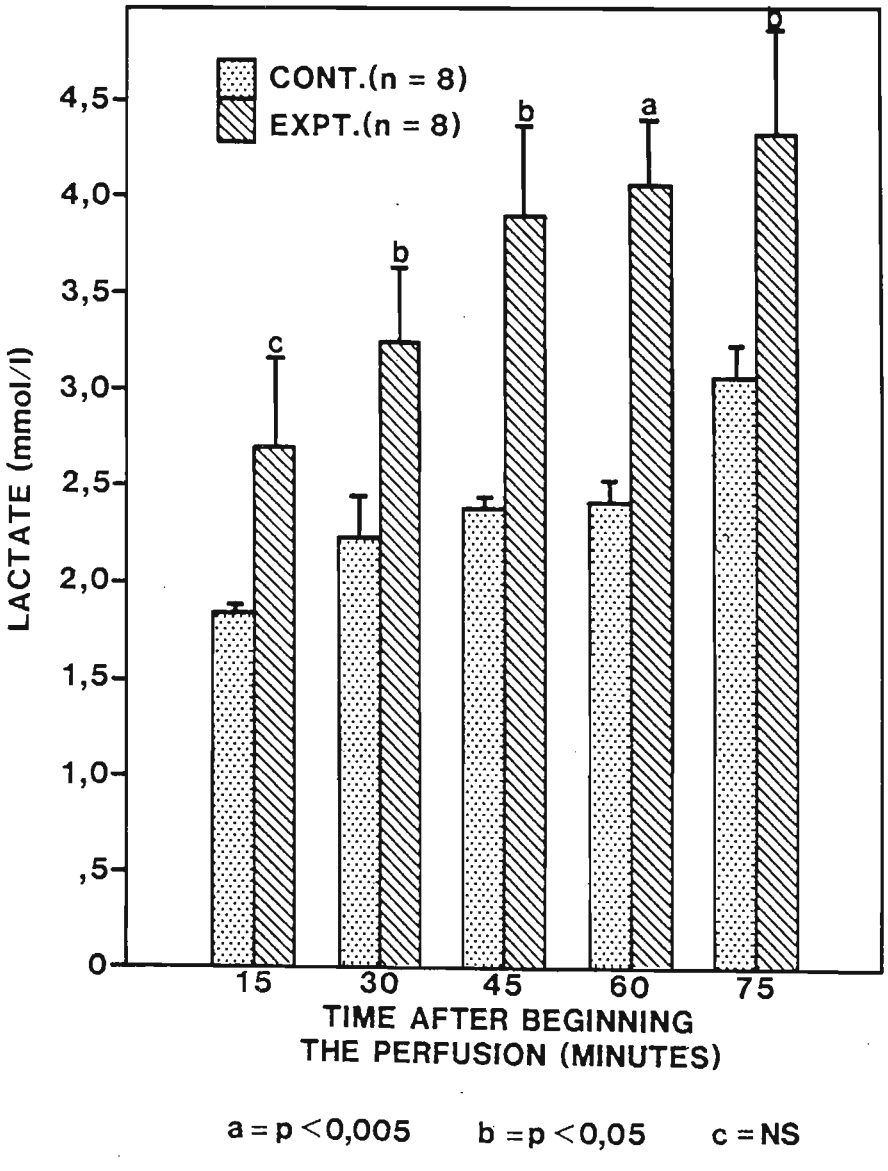


Fig. 26 Pyruvate concentrations derived from perfusate of isolated perfused livers of control and paracetamol-treated rats

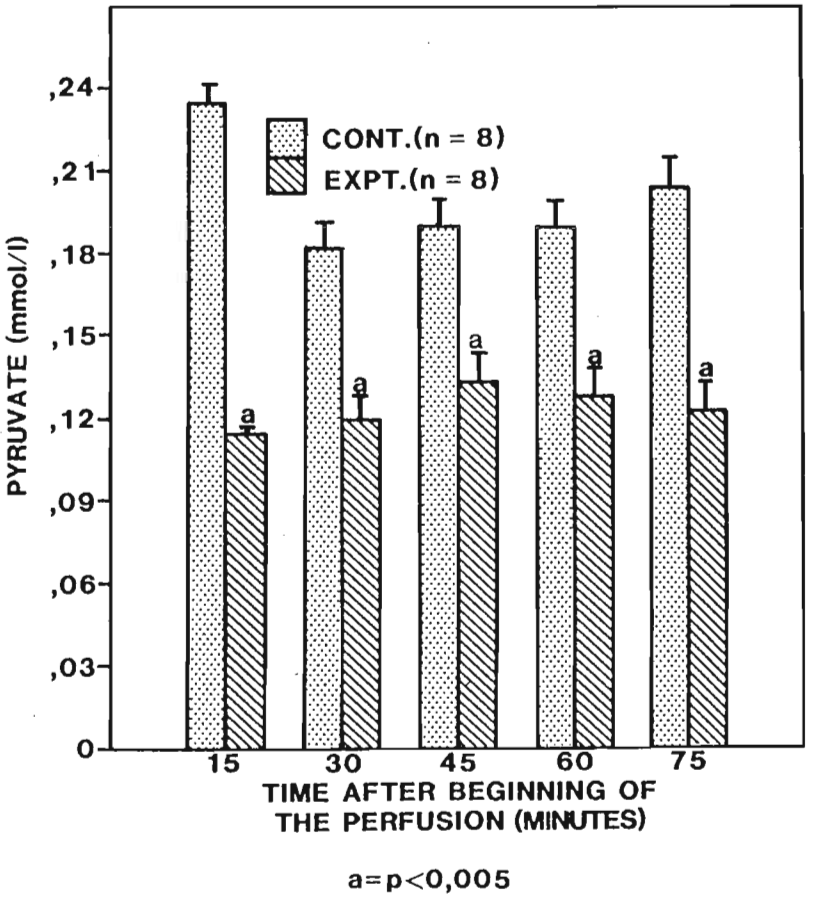


Fig. 27 Lactate:Pyruvate ratios derived from perfusate of isolated perfused livers of control and paracetamol-treated rats

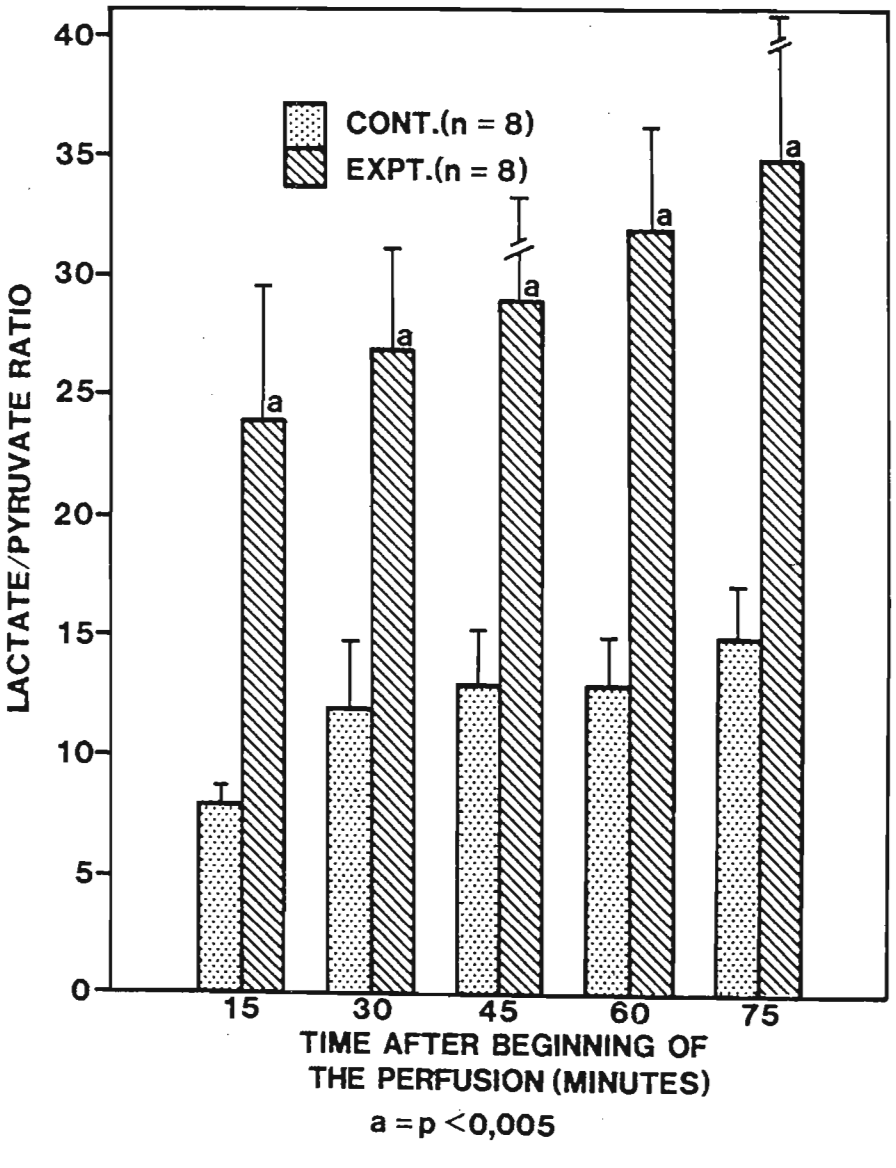


Fig. 28 The effect of methylene blue, a redox agent, on glucose concentrations derived from perfusate of isolated perfused livers of control (experimental group 1) and paracetamol-treated rats (experimental group 2).

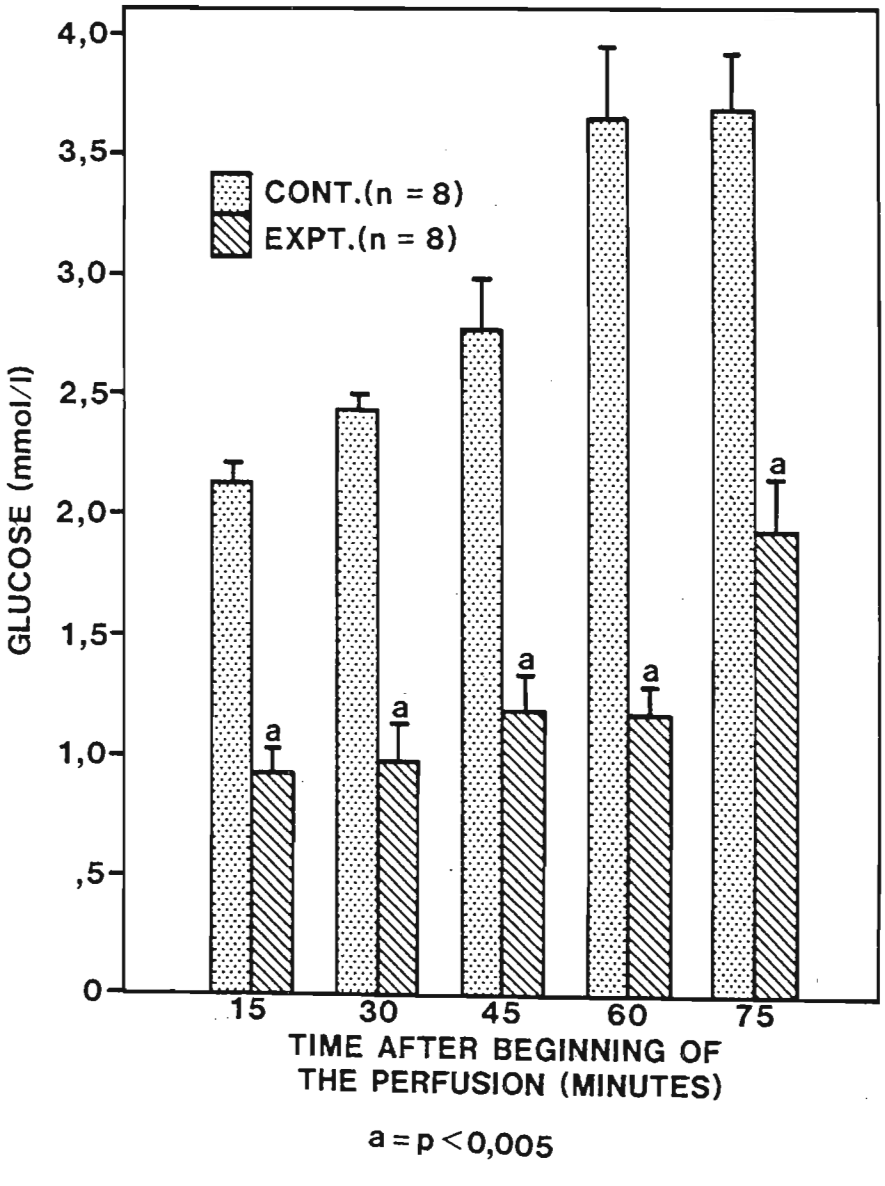


Fig. 29 Bromosulphthalein clearance derived from perfusate of isolated perfused livers of control and paracetamol-treated rats

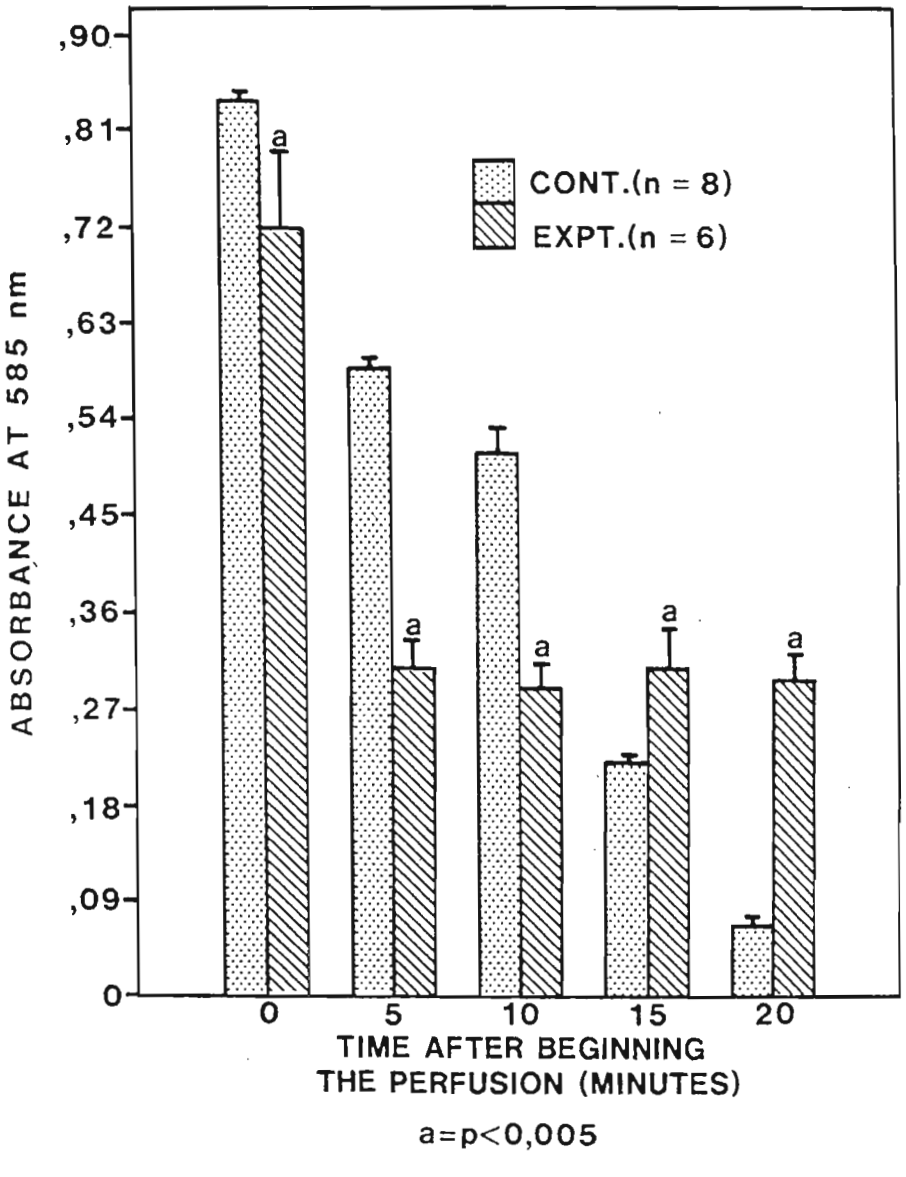
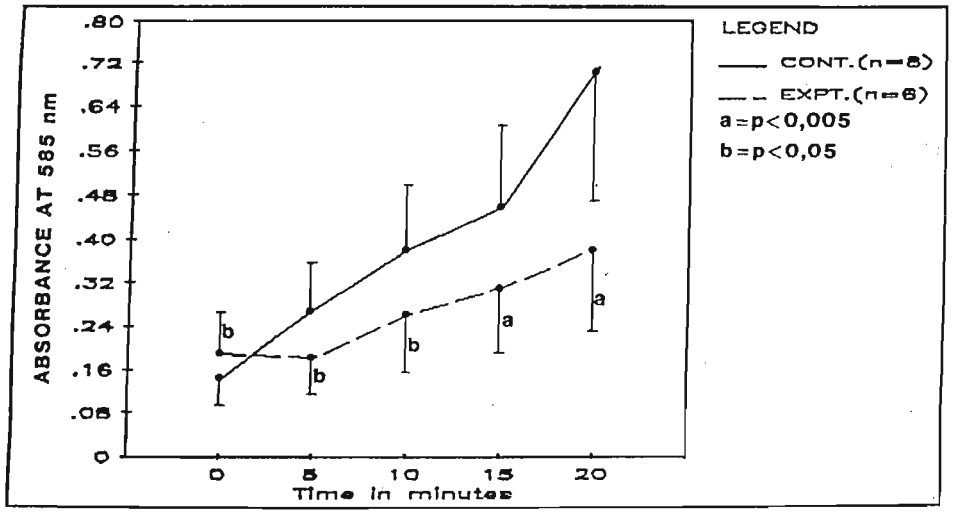


Fig. 30 Billary excretion of bromosulphthalein derived from bile of control and paracetamol-treated rats.



Discussion

Paracetamol, a widely used analgesic, is known to cause acute hepatic necrosis (Henry and Volans, 1984; Meredith et al, 1986), which is sometimes fatal in adults when taken in overdose (Clark et al, 1973; Davidson and Eastham, 1966; Koch-Weser, 1976; Prescott and Wright, 1973). The clinical course of patients with liver damage following paracetamol overdosage is often complicated by the development of hypoglycaemia 24 - 72 hours after overdose (Record et al, 1975). In a study of glucose metabolism in 14 patients with liver damage due to paracetamol overdose, impaired gluconeogenesis was suggested by mild fasting hypoglycaemia in 4 patients and raised fasting blood lactate levels. It is also recognised that in some patients chronic ingestion of paracetamol, even in low doses, may produce hepatic necrosis and hepatitis long after the drug has been discontinued (Barker et al, 1977; Bonkowsky et al, 1978; Canalese et al, 1981; Johnson and Tohman, 1977; Neuberger and Davis, 1981). The recommended maximum dose of paracetamol is 4 g daily.

In this study paracetamol, which was given in therapeutic doses, was found to inhibit gluconeogenesis. This was evidenced by decrease in glucose levels, an increase in the concentration of lactate and an increase in the lactate : pyruvate ratio in the perfusion medium of the experimental animals. The decreased glucose production by the experimental livers which occurred pari passu with an increased pyruvate utilization indicates that pyruvate in these animals was used for the production of lactate. In contrast the rate and amount of glucose production and of pyruvate utilization were increased in the control group indicating that pyruvate was used mainly for the production of glucose.

The increased lactate : pyruvate ratio, which reflects the increase in NADH : NAD^+ ratio, suggests that an alteration in the redox potential of the cytosol was responsible for the impaired gluconeogenesis. However when methylene blue (a redox dye capable of oxidising NADH to NAD^+) was added to the perfusion medium, the glucose output remained low in the experimental group. It is likely therefore that impaired gluconeogenesis is not directly related to an alteration in the NADH : NAD^+ ratio. It is possibly related to the non availability of oxaloacetate or the impairment of the activity of key enzymes of gluconeogenesis. (1) pyruvate carboxylase, 2) phosphoenol pyruvate carboxykinase, 3) fructose 1,6-diphosphatase and 4) glucose-6-phosphatase; Fig. 12).

In the present investigation, in rats treated with therapeutic doses of paracetamol, a marked bromosulphthalein retention in perfusate and decreased biliary excretion of bromosulphthalein was observed. During the twenty minute perfusion, most of the dye was cleared from perfusate with increased biliary excretion of the dye in the control animals whereas in the experimental animals after an initial high clearance from perfusate, concentration of the dye remained constant with concomitant decreased biliary excretion of the dye till the end of the experiment. The rate limiting step in the elimination of bromosulphthalein from plasma into the bile of man and experimental animals is probably the conjugation of the dye with hepatic glutathione (Combes, 1965).

Previous studies in rats have shown that impaired plasma clearance of bromosulphthalein is associated with low hepatic concentration of glutathione which results from the over-dose with paracetamol (Mitchell et al, 1973). It has also been reported that toxic doses of paracetamol suppress hepatic glutathione synthesis. It is likely therefore, that the impaired bromosulphthalein clearance from perfusate in this study was related to reduced glutathione levels.

However low concentration of hepatic glutathione are not soley responsible for the impaired bromosulphthalein clearance observed in rats. Another study has shown that the activity of glutathione transferase was also diminished in animals pre-treated with large doses of paracetamol (Davis et al, 1975). In addition to impairment of hepatic uptake and conjugation of the dye, paracetamol markedly inhibits biliary excretion of the dye, and this is manifested both at therapeutic and hepatotoxic doses of the drug in man (Davis et al, 1975). Studies in rats indicate that interaction between paracetamol and bromosulphthalein is at the canalicular membrane level, and appears to be competitive (Davis et al, 1975).

The present study demonstrates that paracetamol, when administered chronically in doses equivalent to therapeutic doses in the adult human, impairs hepatic function as evidenced by impaired gluconeogenesis and bromosulphthalein clearance, from perfusate and decreased biliary excretion of the dye and since it is difficult to apply animal data to primates; it is proposed to extend this study to include humans. Paracetamol is considered to be a safe analgesic but should the results obtained coincide with that of man; it would be important to monitor liver function tests in patients treated chronically with therapeutic doses of paracetamol.

CHAPTER 7

HEXANE

HEXANE

Introduction

The industrial solvent n-Hexane has been implicated as the agent responsible for the development of peripheral neuropathy in a variety of industrial workers and also in individuals who habitually inhale glue vapours for their euphoric properties. In view of the importance of n-Hexane to industry and the problem of glue-sniffing among the younger generation, the present study was carried out to investigate the chronic effects of the pure compound on experimental animals. The development of distinctive hepatic damage is reported for the first time.

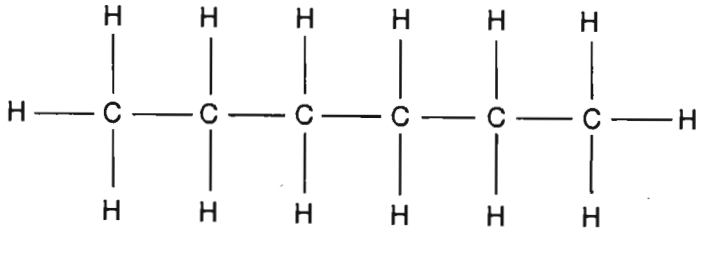
Physical and Chemical Properties

Hexane is a colourless, transparent, non-volatile, highly inflammable liquid with a characteristic odour. It is insoluble in water, soluble in dehydrated alcohol, miscible with chloroform, ether and most fixed and volatile oils. Vapour-air mixture is explosive. Boiling point ranges (95%) from 67°C to 70°C. Weight per ml at 20°C is 0.660g to 0.670g.

Metabolism

Hexane, a fraction from petroleum at 67°C to 70°C, is metabolised to 2,5 - hexanedione before exerting its hepatotoxic effect.

Structure of Hexane



Experimental Design

Glue and benzine, solvents which contain hexane in high concentrations, are often inhaled to produce a state of euphoria. The toxic effects of hexane on the nervous system are well known. In order to determine the influence of hexane on liver function and morphology, the effects of hexane on gluconeogenesis and bromosulphthalein clearance were studied using the isolated liver perfusion model and hepatic histology was examined using standard methods.

Materials and Methods

20 male Wistar rats (170 -180 g) of the University of Natal inbred strain were individually earmarked for identification and randomly assigned to 4 groups and kept in stainless steel cages with plastic bottoms. 3 groups were given subcutaneous injections of 0.2 ml hexane in olive oil (1:1v/v) and the control group 0.2 ml olive oil daily for 90 days. The livers of the control group and one of the experimental group were perfused for 75 minutes, with a medium containing pyruvate (500umoles), a gluconeogenic precursor, and subsequently these livers were perfused with a medium containing bromosulphthalein (1.25mg/100g rat weight) for an additional 20 minutes, while those of the 2nd group were perfused for 75 minutes with a medium containing pyruvate (500umoles) and 1ml 10% solution of methylene blue (a redox dye capable of oxidising NADH to NAD^+). The animals were randomly chosen for perfusion. Serial specimens were obtained for analysis. The livers of the third experimental group were subjected to histological examination.

Results

The results obtained indicate that the method of perfusion is satisfactory for the study of gluconeogenesis from pyruvate as the observed rates were higher than the normal rates measured in vivo. The time course of glucose and lactate production by the livers of control and hexane-treated rats, pyruvate concentrations and lactate:pyruvate ratios are shown in Tables 7a and 7b respectively and illustrated graphically (Figs. 31-34).

Gluconeogenesis

Pyruvate (500 umoles) was added ten minutes after the start of perfusion; 295 umoles of the added pyruvate was utilized after 75 minutes of perfusion by the control livers. Mean glucose concentration was 5.75 mmoles/l at the end of perfusion with glucose formation at an average rate of 0.07 mmoles/min g wet weight of liver. Lactate concentration was 3.12 mmoles/l and the average rate of lactate formation was 0.06 mmoles/min per g wet weight of liver such that the lactate:pyruvate ratio averaged 12.

Similarly in the hexane-treated rats, after 80 minutes of perfusion 340 umoles of pyruvate was utilised. The glucose concentration was 1.46 mmoles/l and the average glucose formation rate was 0.01 mmoles/min per g wet weight of liver. Lactate concentration was 2.83 mmoles/l and the average rate of formation was 0.01 mmoles/min per g wet weight of liver such that the lactate:pyruvate ratio averaged 18.

Glucose Formation

1ml 10% methylene blue (a redox agent capable of oxidising NADH to NAD^+) was added at the start of the perfusion. The glucose concentration obtained in the group perfused with pyruvate and methylene blue (1.34 mmoles/l) and pyruvate alone (1.46 mmoles/l) was more or less similar. Methylene blue had no effect on glucose output in the hexane-treated group (Fig. 35).

The rate of glucose formation and glucose concentration was decreased, lactate levels and lactate:pyruvate ratio increased in the experimental animals compared to controls ($p<0.05$ for all comparisons except 60 and 75 minute lactate levels).

Bromosulphthalein Clearance

Bromosulphthalein was added at a dosage of 1.25 mg/100g body weight of rat 75 minutes after beginning of the perfusion. The time course of bromosulphthalein clearance from perfusate and biliary excretion of dye are shown in Table 7c. The bromosulphthalein percentage retention was 10.5% in the control group compared to 41.26% in the experimental group. Bromosulphthalein clearance from perfusate ($p<0.05$; Fig. 36) and biliary excretion of the dye ($p<0.05$ for all comparisons except the 5 minute biliary excretion; Fig. 37) were impaired in the hexane-treated rats.

Microscopy

Histological examination of liver biopsy specimens from hexane-treated rats revealed severe fatty change (Fig. 38).

Table 7a

TIME-COURSE OF THE PRODUCTION OF LACTATE, AND GLUCOSE BY PERFUSED LIVERS OF CONTROL RATS (n=5)

UNDER AEROBIC CONDITIONS (500 umoles of pyruvate added to perfusion medium).

The results are expressed as MEAN ± SEM. The initial volume of the medium was 150 ml; it decreased every 15 minutes by 10 ml. (removed for analysis). The livers had a mean wet weight of 6.05g.

TIME (minutes)	15		30		45		60		75	
CONC. OF METABOLITE (mmoles/l)										
LACTATE	1.52	±0.21	2.31	±0.07	2.66	±0.19	2.98	±0.21	3.12	±0.33
PYRUVATE	0.204	±0.01	0.196	±0.01	0.210	±0.004	0.195	±0.01	0.201	±0.004
GLUCOSE	3.87	±0.30	4.35	±0.31	4.80	±0.46	5.24	±0.54	5.75	±0.49
LACTATE:PYRUVATE ratios	7.73	±1.5	11.95	±0.88	12.78	±1.20	15.44	±1.4	15.51	±1.6
METABOLIC RATES (mmoles / min per g wet weight of liver)										
GLUCOSE FORMATION			0.08		0.07		0.07		0.08	
LACTATE FORMATION			0.13		0.06		0.05		0.02	

Table 7b

TIME-COURSE OF THE PRODUCTION OF LACTATE, AND GLUCOSE BY PERFUSED LIVERS OF EXPERIMENTAL RATS (n=5)

UNDER AEROBIC CONDITIONS (500 umoles of pyruvate added to perfusion medium)

The results are expressed as MEAN ± SEM. The initial volume of the medium was 150ml; it decreased every 15 minutes by 10 ml. (removed for analysis). The livers had a mean wet weight of 6.01 g.

TIME (minutes)	15		30		45		60		75	
CONC. OF METABOLITE (mmoles/l)										
LACTATE	2.72	±0.11	2.63	±0.10	3.12	±0.08	3.12	±0.20	2.83	±0.19
PYRUVATE	0.146	±0.008	0.168	±0.02	0.155	±0.008	0.172	±0.01	0.166	±0.01
GLUCOSE	1.06	±0.15	1.22	±0.16	1.08	±0.12	1.28	±0.15	1.46	±0.16
LACTATE:PYRUVATE ratios	18.96	±1.5	16.3	±1.3	18.17	±1.3	18.59	±1.8	17.16	±1.2
METABOLIC RATES (mmoles/ min per g wet weight of liver)										
GLUCOSE FORMATION			0.03		0.02		0.03		0.03	
LACTATE FORMATION			0.01		0.08		0.00		−0.05	

Table 7c

TIME-COURSE OF BROMOSULPHTHALEIN CLEARANCE BY PERFUSED LIVERS OF CONTROL AND EXPERIMENTAL ANIMALS

The results are expressed as MEAN ± SEM. BSP was added, at a dosage of 1.25 mg / 100g body weight, to the perfusion medium

TIME (minutes)	0		5		10		15		20	
CONTROLS (5)	0.85	±0.004	0.61	±0.02	0.572	±0.004	0.25	±0.01	0.09	±0.004
EXPERIMENTALS (5)	0.63	±0.01	0.29	±0.01	0.31	±0.008	0.28	±0.008	0.26	±0.01

TIME-COURSE OF BILIARY EXCRETION OF BROMOSULPHTHALEIN

TIME (minutes)	0		5		10		15		20	
CONTROLS (n=5)	0.03	±0.06	0.30	±0.13	0.43	±0.19	0.56	±0.25	0.74	±0.25
EXPERIMENTALS (n=5)	0.27	±0.12	0.32	±0.14	0.35	±0.15	0.38	±0.17	0.39	±0.18

Fig. 31 Glucose concentrations derived from perfusate of isolated perfused livers of control and hexane-treated rats

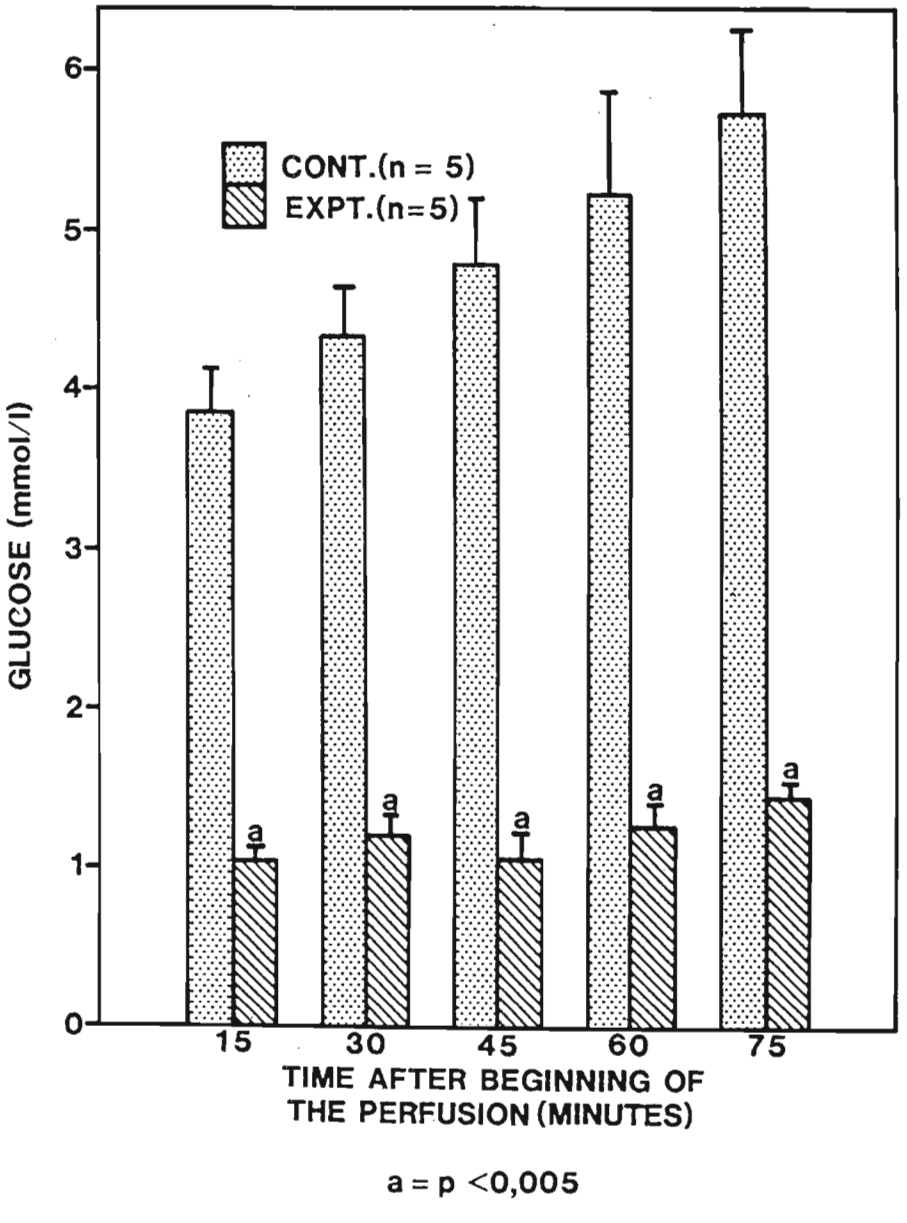
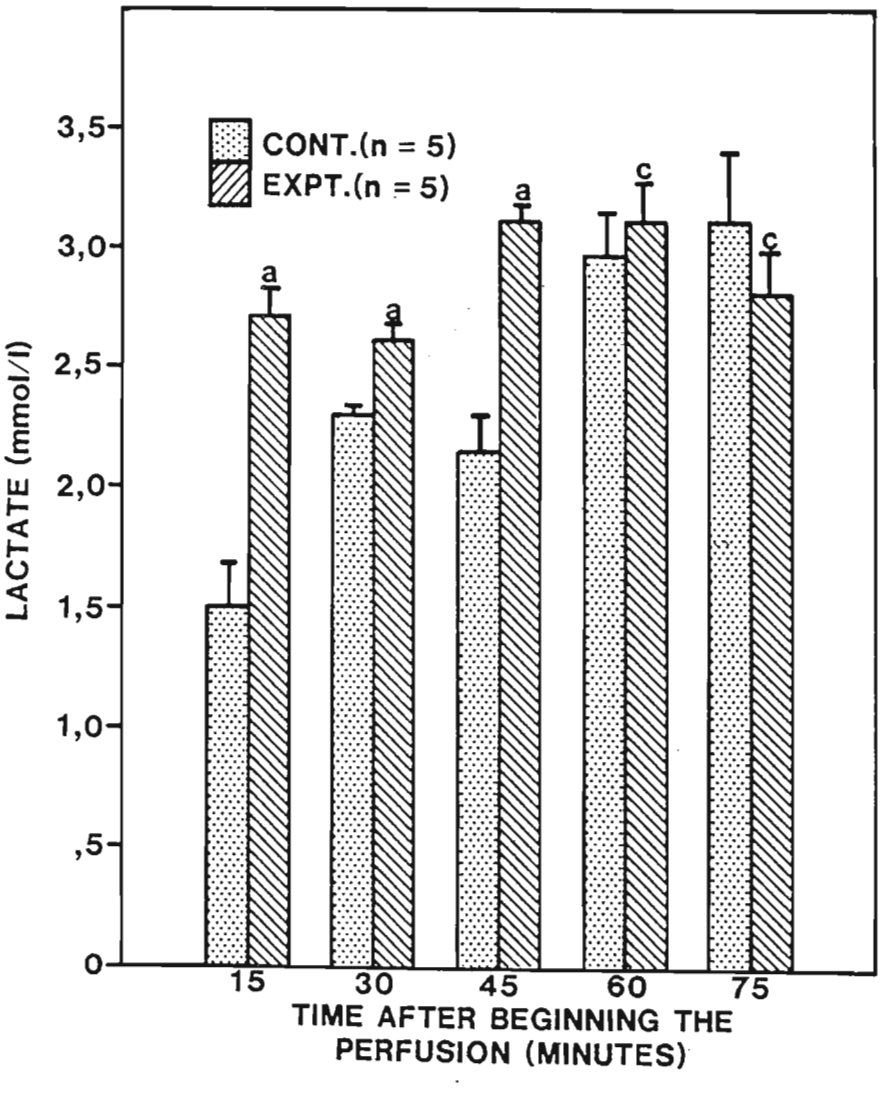


Fig. 32 Lactate concentrations derived from perfusate of isolated perfused livers of control and hexane-treated rats



a = p <0,005 c = NS

Fig. 33 Pyruvate concentrations derived from perfusate of isolated perfused livers of control and hexane-treated rats

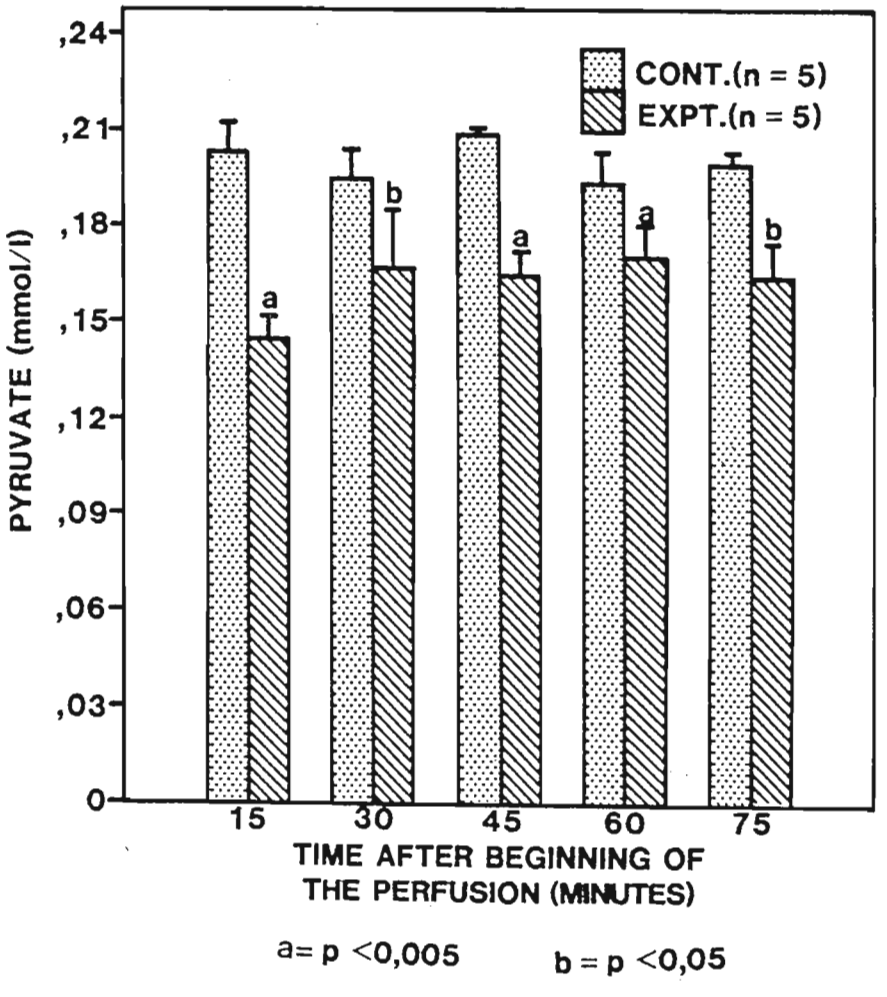
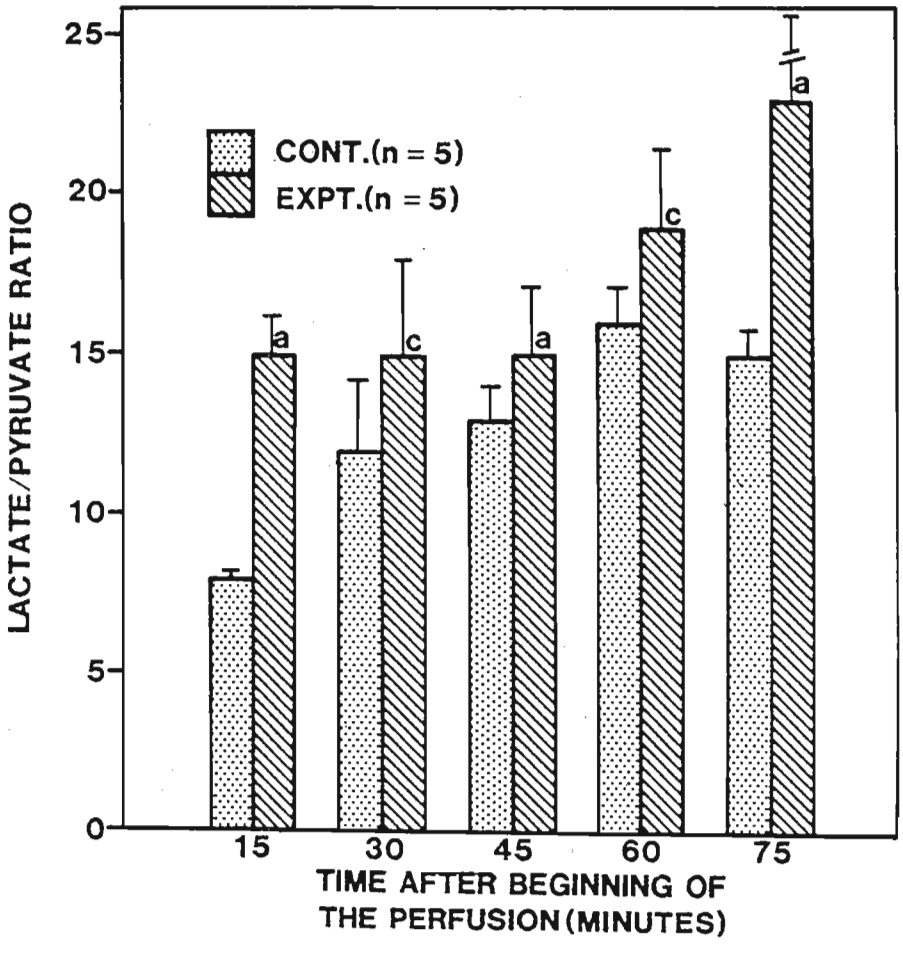


Fig. 34 Lactate:Pyruvate ratios derived from perfusate of isolated perfused livers of control and hexane-treated rats



a=p 0,005 c=NS

Fig. 35 The effect of methylene blue, a redox agent, on glucose concentrations derived from perfusate of isolated perfused livers of control (experimental group 1) and hexane-treated rats (experimental group 2)

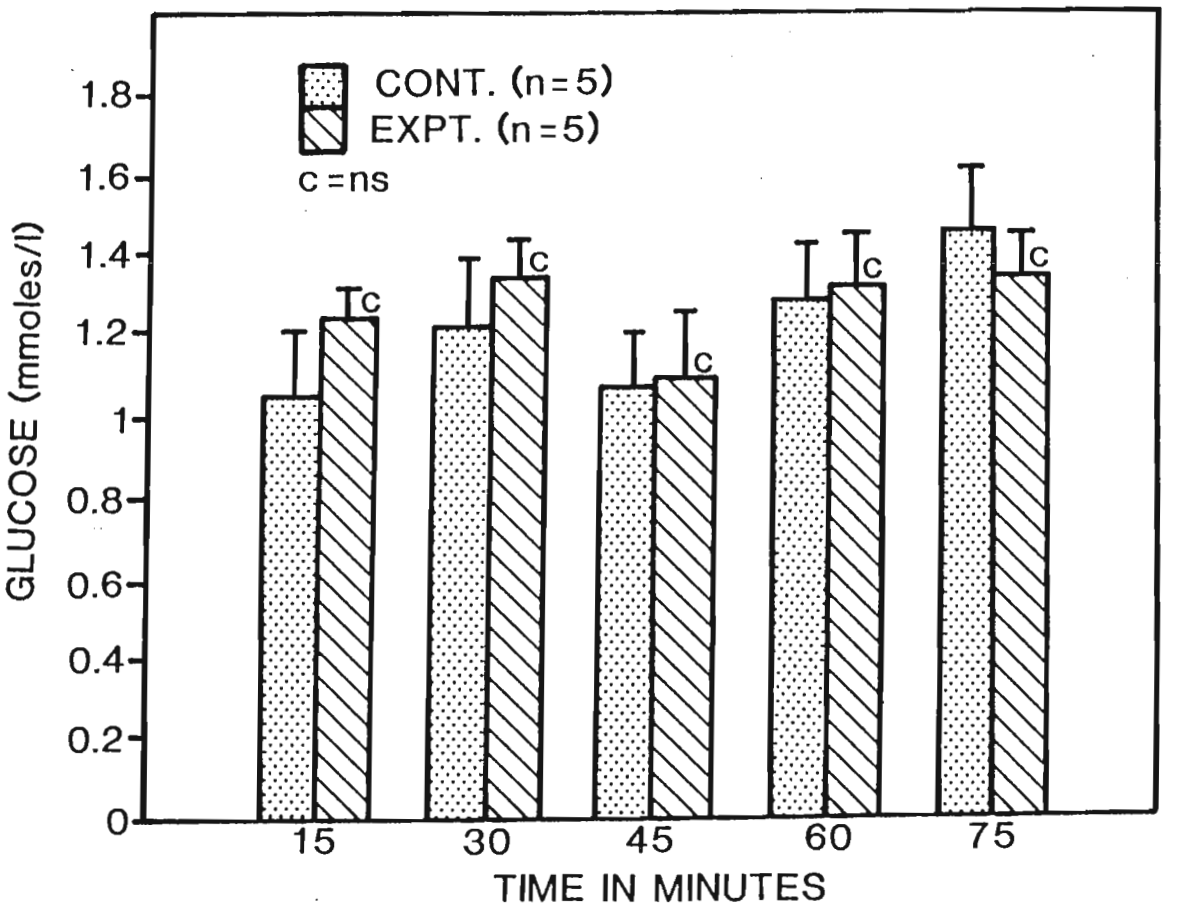


Fig. 36 Bromosulphthalein clearance derived from perfusate of isolated perfused livers of control and hexane-treated rats

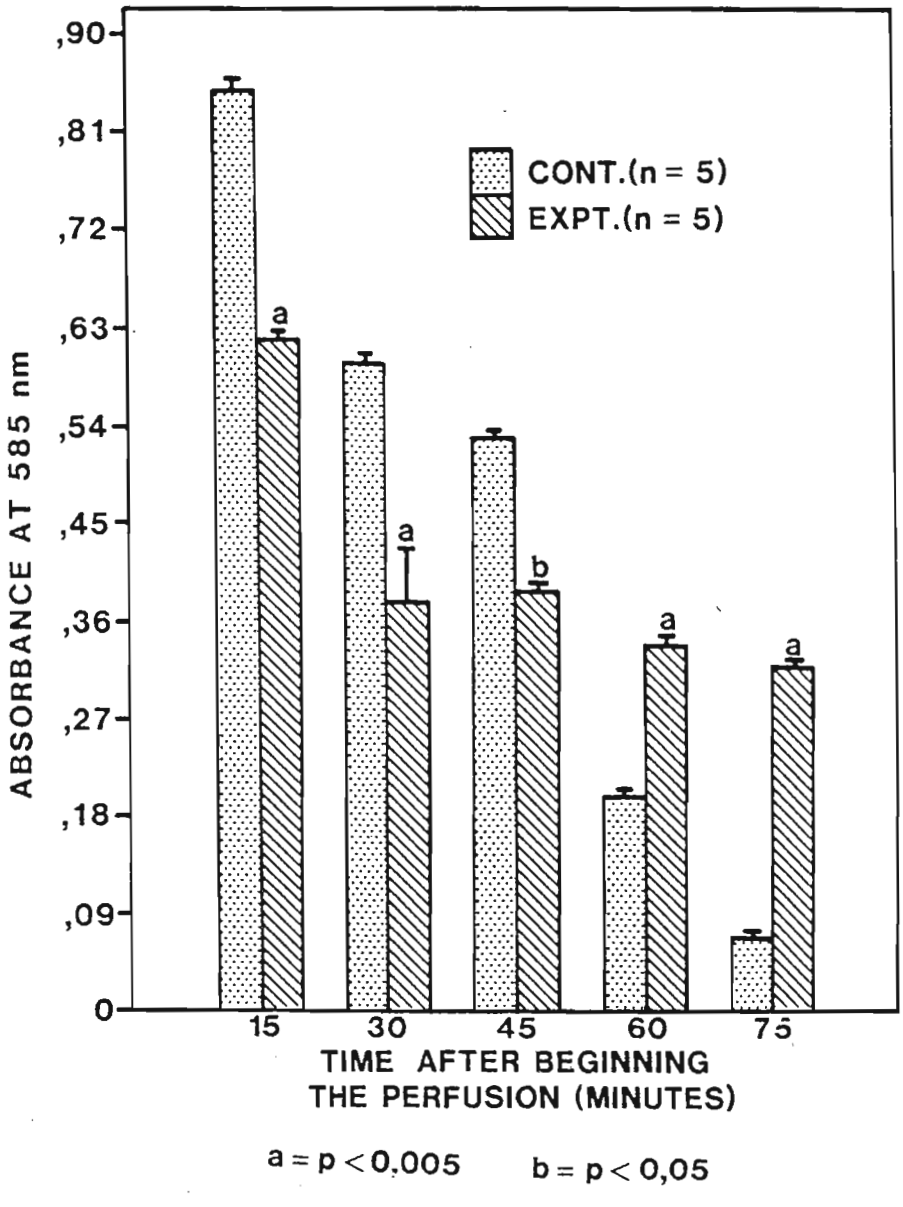


Fig. 37 Biliary excretion of bromosulphthalein derived from bile of control and hexane-treated rats.

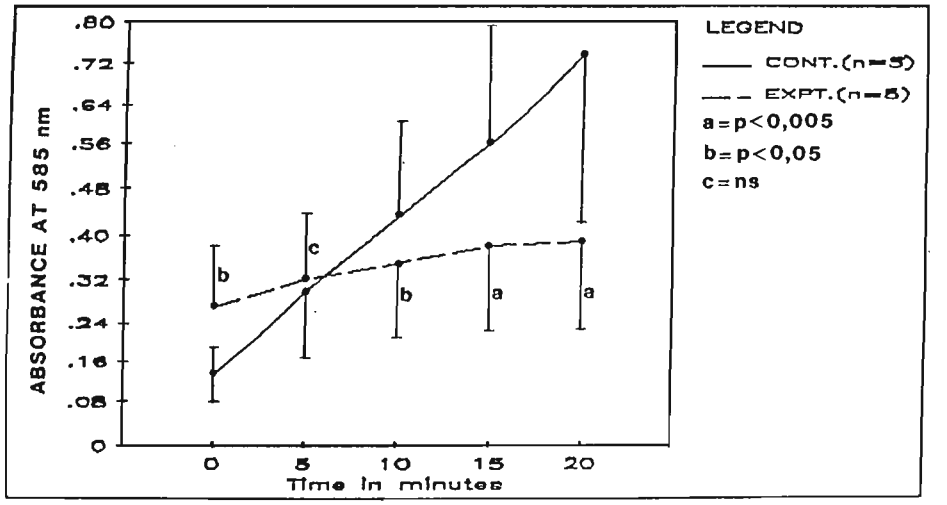
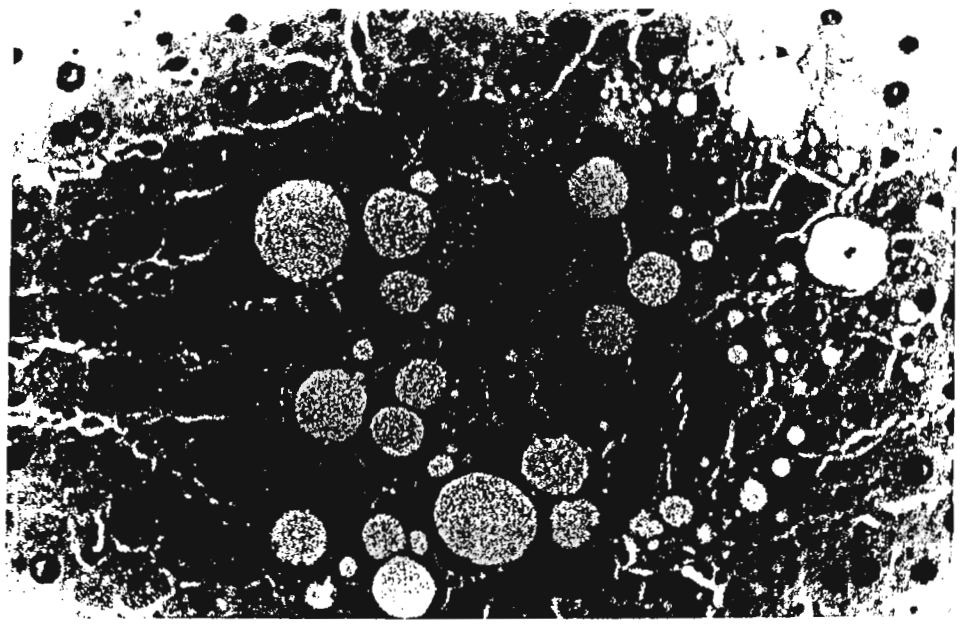


Fig. 38 Fatty change in hexane-treated rats x 450



Hepatocytes showing macro and micro vesicular fatty change

Discussion

A wide variety of substances have been abused by sniffing or inhalation in order to produce a state of euphoria (Anderson et al, 1982; Garriot and Petty, 1980; Kringsholm, 1980). Solvents in adhesives are the commonest, hence the term "glue sniffing", but many other substances are used including petrol, cleaning fluids, aerosol propellents, fire extinguishers and butane. Solvent or volatile substance abuse is therefore a more descriptive term.

The method of inhaling may include pouring or squirting the substance onto a rag and inhaling from the rag, or pouring or squirting the substance into a jar or plastic bag and then inhaled. Sometimes the plastic bag may be placed over the head to ensure a high concentration of the substance.

Benzine, a petroleum product consisting principally of hexane and n-pentane is bought freely by a large number of black children in Natal and sniffed to produce a state of euphoria. Glues containing hexane are also inhaled for this purpose. Long term inhalation of hexane has resulted in the development of progressive and predominantly motor polyneuropathy.

Most reports on solvent abuse are concerned with effects on the nervous system; little attention has been paid to other organs such as the liver. Some forms of solvent abuse may result in direct hepatotoxicity. A commercial cleaning fluid which contained trichloroethylene was at one time popular with solvent abusers. Baerg et al (1970) reported that this agent caused acute centrilobular necrosis progressing to centrilobular fibrosis, and permanent disturbance of liver architecture following repeated exposure. Another report describes a 45 yr old man who presented with intermitttent epistaxis and impaired liver function several days after his loghouse was coated with butyl caulk containing petroleum distillate and toluene (National Institute for Occupational Safety and Health report, 1982). Conroy et al (1984) reported transient ground glass hepatocellular change following a period of glue sniffing in a patient with underlying benign recurrent cholestatic jaundice. They postulate that the toluene base in glue was responsible for hepatotoxicity. There are no reports of liver damage resulting from hexane abuse.

In this study hexane was found to inhibit gluconeogenesis. This was evidenced by decrease in glucose levels, an increase in the concentration of lactate and an increase in the lactate : pyruvate ratios in the perfusion medium of experimental animals. The rate and the amount of glucose production and of pyruvate utilzation was increased in the control group indicating that the pyruvate was used mainly for the production of glucose. In contrast, the decreased glucose production by the experimental livers which occurred in the presence of increased pyruvate

utilization indicates that the pyruvate in these animals was used for the production of lactate. The increased lactate : pyruvate ratio, which reflects the increase in the NADH : NAD^+ ratio, suggests that an alteration in the redox potential of the cytosol was responsible for impaired gluconeogenesis. However, when methylene blue (a redox dye capable of oxidising NADH to NAD^+) was added to the perfusion medium, the output of glucose remained low in the experimental group indicating that the impaired gluconeogenesis was not directly related to the change in the NADH : NAD^+ ratio. It is probably related to the non availability of oxaloacetate or the impairment of the activity of key enzymes of gluconeogenesis. [1) pyruvate carboxylase, 2) phosphoenol pyruvate carboxykinase, 3) fructose 1,6-diphosphatase and 4) glucose-6-phosphatase, Fig. 12).

In the present investigation a marked retention of bromosulphthalein in perfusate with decreased biliary excretion of the dye was observed in the hexane-treated rats; after an initial high clearance, the concentration of th dye in the perfusate remained constant with decreased biliary excretion of the dye till the end of the experiment. Most of the dye was cleared from perfusate and into the bile in the control animals during the 20 minutes of perfusion. Our data suggest that in the isolated liver, hexane is capable, in toxic doses, of interfering with bromosulphthalein clearance from perfusate and the biliary excretion of the dye. The mechanism is presumed to be canalicular with transport defects in the hepatic cell. Histological examination of liver biopsy specimens from the hexane -treated rats revealed severe fatty change.

This study demonstrates that rats intoxicated by repetitive subcutaneous injections of pure hexane develop biochemical and pathological evidence of liver damage. Because of the difficulty in monitoring the dose of hexane used during sniffing by humans, the dose that was selected for this investigation was identical to that which resulted in the degeneration in the central and peripheral nervous system in an earlier experimental study involving rats (Schaumburg and Spencer, 1976). The fact that neurotoxicity has been documented in man, supports the view that this model is relevant to man (Clark and Tinston, 1982). Greater awareness of the risk of hepatotoxicity following hexane is needed.

Since benzine and glues have a fairly high concentration of hexane, an important cause of central and peripheral nervous system and liver damage, and since their abuse is common among school children every effort must be made to combat this form of solvent abuse through an educational programme involving the school and the media.

CHAPTER 8

CONCLUSION

CONCLUSION

This study was performed to determine whether the isolated rat liver perfusion technique could be used to measure the effect on liver function of therapeutic and supra-therapeutic doses of various drugs; some of which have been reported to be hepatotoxic in man. Alcohol and hexane were administered in toxic doses, rifampicin and isoniazid in high doses and paracetamol in therapeutic doses. Liver function was assessed by studying gluconeogenesis and bromosulphthalein clearance. The results obtained indicate that this technique is very satisfactory for this purpose.

Evidence of satisfactory functioning of the liver in this study was indicated by the absence of patchy colouring of the liver and swelling and formation of exudate on the surface after two hours of perfusion. Glucose synthesis was well maintained over two hours and the concentrations obtained were higher than those reported using other liver perfusion techniques (Garcia et al, 1966; Eisenstein et al, 1966) and several times higher than in liver slices (Hems et al, 1966).

Although the liver remained in situ, it was completely separated from other tissues, and the delay and trauma associated with removal from body and cannulation were avoided.
A major advantage of the modified method of perfusion is the shorter duration in the interruption of circulation, the interruption period being about three minutes in this study. In contrast Schimassek (1963) noted that after seven to nine minutes period during which the circulation was interrupted the concentration of lactate in the liver rose eightfold. Restoration to the initial state required about thirty minutes. A further advantage was the use of human red blood cells from the blood bank instead of rat blood because of the cost involved and the elimination of blood glycolysis.

All the drugs studied were found to inhibit gluconeogenesis. This was shown by a decrease in glucose levels and an increase in lactate : pyruvate ratios in the perfusion medium of experimental livers. The decreased glucose production by the experimental livers which occurred pari passu with an increased pyruvate utilization indicates that in these animals pyruvate was used for the production of other compounds such as lactate. In contrast glucose production and pyruvate utilization were increased in the control groups indicating that pyruvate was used mainly for the production of glucose.

In the ethanol group impaired gluconeogenesis was probably due to a change in the NADH : NAD^+ ratio; when methylene blue was introduced in the perfusion medium of this group the output of glucose was high. Impaired gluconeogenesis in the paracetamol and hexane-treated group was probably related to the non availability of oxaloacetate or the impairment of the activity of key enzymes involved in gluconeogenesis; when methylene blue was added to the perfusion medium of these animals the glucose output remained low.

Except for the rifampicin study bromosulphthalein clearance was impaired in all the experimental groups. Histological examination of liver tissue obtained from the hexane-treated animals demonstrated severe fatty change.

In conclusion this study demonstrates:

i) the isolated rat-liver perfusion technique is a valuable method of evaluating the effects of therapeutic and supra-therapeutic doses of drugs in vitro

ii) that rifampicin and isoniazid in supra-therapeutic doses and paracetamol in therapeutic doses alter liver function in rats.

Since the observations in the alcohol-treated rats are in keeping with those noted in man viz. that hypoglycaemia may complicate alcohol excess, no further studies using this compound are planned.

The following questions that arise from the present investigation, will need to be answered in future studies:

i) What are the effects of therapeutic doses of rifampicin and isoniazid on liver function when using the isolated rat-liver perfusion technique (The present study involved approximately doses twice the normal daily dosage)

ii) What are the effects of a) chronic administration of therapeutic doses of paracetamol, and b) hexane abuse on liver function in man.

REFERENCES

Ameer B. and Greenblatt D.J. (1977). Acetaminophen. Annals of Internal Medicine 87: 202-209.

Anderson H.R., Dick B., Macnair R.S., Palmer J.C., Ramsey J.D. (1982). An investigation of 140 deaths associated with volatile substance abuse in U.K. (1971-1981). Human Toxicology 1: 207-221.

Baerg R.D. and Kimberg D.V. (1970). Centrilobular hepatic necrosis and acute renal failure in "solvent sniffers". Annals of Internal Medicine 73: 713-720.

Bailey W.C., Weill H., De Rouen T.A., et al (1974). The effect of isoniazid on transaminase levels. Annals of Internal Medicine. 81: 200-202.

Barker J.D., DeCarle D.J., Anuras S. (1977). Chronic excessive acetaminophen use and liver damage. Annals of Internal Medicine. 87: 299-301.

Bassan H., Kendler J., Harinasuta U., Zimmerman H.J. (1971). Effects of an anabolic steroid (norbolethone) on the function of the isolated perfused liver. Biochemical Pharmacology 20: 1429-1435.

Biebyuck J.F., Saunder S.J., Harrison G.G., Bull A.B. (1970). Multiple halothane exposure and hepatic bromosulphthalein clearance. British Medical Journal 1: 668-671.

Bonkowsky H.L., Mudge G.H., McMurtry R.J. (1978). Chronic hepatic inflammation and fibrosis due to low doses of paracetamol. Lancet i: 1016-1018.

Canalese J., Gimson A.E.S., Davis M., Williams R. (1981). Factors contributing to mortality in paracetamol induced liver failure. British Medical Journal 282: 199-201.

Cannetti G., Le Lerzim M., Proveu G., Rist N., Grumbach F. (1968). Some comparative aspects of Rifampicin and Isoniazid. Tubercle 49: 367-375.

Capelle P., Dhumeaux D., Mora M., Feldmann G., Berthelot P.(1972). The effect of rifampicin on liver function in man. Gut 13: 366-372.

Clark R., Thompson R.P.H., Borirahchanyarat V., Widdop B., Davidson A.R., Goulding R., Williams R. (1973). Hepatic damage and death from overdose of paracetamol. Lancet i: 66-69.

Clark D.G. and Tinston D.J. (1982). Acute inhalation toxicity of some halogenated and non-halogenated hydrocarbons. Human Toxicology 1: 239-247.

Combes B. (1965). The importance of conjugation with glutathione for sulfobromophthalein sodium (BSP) transfer from blood to bile. Journal of Clinical Investigation 44: (7) 1214-1224.

Conroy B. and Pennington C.R. (1984). Transient ground glass hepatocellular change following a period of glue sniffing in a patient with underlying benign recurrent cholestatic jaundice. Medicine, Science and Law 24: (2) 102-106.

Corey E.L. and Britton S.W. (1941). Glycogen levels in the isolated liver perfused with cortico-adrenal extract, insulin and other preparations. American Journal of Physiology 131: 783-789.

Czok R. and Lamprecht W. (1974). Pyruvate, Phosphoenolpyruvate and D-Glycerate-2-phosphate. Page 1446 ff. in H.U. Bergmeyer, ed. Methods of Enzymatic Analysis, 2nd English ed. (Transl. from 3rd German ed) Verlag Chemie Weinheim and Academic Press, inc., New York and London. 4 vols.

Davidson D.G.D. and Eastham W.N. (1966). Acute liver necrosis following overdose of paracetamol. British Medical Journal 2: 497-499.

Davis M and Glaivinsbi J.J. (1961). Jaundice due to isoniazid. Tubercle 42: 504-506.

Davis M., Ideo G., Harrison N.G., Williams R. (1975). Hepatic glutathione depletion and impaired bromosulphthalein clearance early after paracetamol overdose in man and rat. Clinical Sciences and Molecular Medicine 49: 495-502.

Edward I.R. (1982). Solvent abuse. New England Medical Journal 95: 870-880.

Eisenstein A.B., Spencer S., Flatness S., Brodsky A. (1966). Carbohydrate synthesis in the isolated perfused rat liver. Role of adrenal cortex. Endocrinology 79: 182-186.

Exton J.H. and Park C.R. (1967). Control of gluconeogenesis in liver. General features of gluconeogenesis in the perfused livers of rats.
Journal of Biological Chemistry 242 : 2622-2636.

Exton J.H. (1972). Gluconeogenesis.
Metabolism 21: 945-990.

Field J.B., Williams H.E., Mortimore G.E. (1963). Studies on the mechanism of ethanol induced hypoglycemia. Journal of Clinical Investigation 42: (4) 497-506.

Forsander O.A., Raiha N., Salaspuro M., Maenpaa P. (1965). Influence of ethanol on liver metabolism of fed and starved rats. Biochemical Journal 94: 259-265.

Freinkel N., Arky R.A., Singer D.L., Cohen A.K., Bleicher S.J., Anderson J.B., Silbert C.K., Foster A.E. (1965). Alcohol hypoglycemia - IV Current concepts of its pathogenesis. Diabetes 14: 350-361.

Garcia A., Williamson J.R., Cahill G.F. Jr (1966). Studies on the perfused rat liver II. Effect of glucagon on gluconeogenesis. Diabetes 15: 188-193.

Garriot J. and Petty C.S. (1980). Death from inhalent abuse: Toxicological and pathological evaluations of 34 cases. Clinical Toxicology 16: 305-315.

Gartner U., Stockert R.J., Levine W.G., Wolkoff A.W. (1982). Effect of nafenopin on the uptake of bilirubin and sulfobromophthalein by isolated perfused rat liver. Gastroenterology 83: 1163-1169.

Gilman A.G., Goodman C.S., Gilman A. (Eds): The pharmacological basis of therapeutics ed.6 New York Macmillian 1980 pp 1200-1203.

Glaser H.H. and Massengale O.N. (1962). Glue-sniffing in children - deliberate inhalation of vaporised plastic cements. Journal of America Medical Association 181: 300-303.

Gronwall A. (1957). Dextran and its use in colloidal infusion solutions. Oxford:Blackwell Scientific Publications.

Gumucio D.L., Gumucio J.J., Wilson J.A.P., Cutter C., Krauss M., Caldwell R., Chen E. (1984). Albumin influences sulfobromophthalein transport by hepatocytes of each acinar-zone. American Journal of Physiology 246: G86-G95.

Gutmann I. and Wahlefeld A.W. (1974). L-(+)-Lactate. Determination with lactate dehydrogenase and NAD. Page 1464 ff. in H.U. Bergmeyer, ed. Methods of Enzymatic Analysis, 2nd ed. (Transl. from 3rd German ed.) Verlag Chemie Weinheim and Academic Press, Inc., New York and London 4 vols.

Hems R., Ross B.D., Berry M.N., Krebs H.A. (1966). Gluconeogenesis in the perfused rat liver. Biochemical Journal 101: 284-292.

Henry J. and Volans G. (1984). ABC of poisoning. Analgesics : 11 Paracetamol. British Medical Journal 289: 907-908.

Henry R.J., Chamori N., Ware A.G. (1959). Determination of bromosulphthalein in serum by means of acetone precipitation of proteins. American Journal of Clinical Pathology 32: 201-203.

Johnson G.K. and Tohman K.G. (1977). Chronic liver disease and acetaminophen. Annals of Internal Medicine 87: 302-304.

Johnson M.W., Friedman P.A., Mitch W.E. (1981). Alcoholism, non-prescription drugs and hepatotoxicity. American Journal of Gastroenterology 76: 530 - 533.

Kaden M., Oakley N.W., Field J.B. (1969). Effect of alcohol on gluconeogenesis using the isolated rat perfusion technique. American Journal of Physiology 216 : (4) 756-763.

Kendler J., Bowry S., Seeff L.B., Zimmerman H.J. (1971). Effect of chlorpromazine on the function of the perfused isolated liver. Biochemical Pharmacology 20: 2439-2445.

Kenwright S. and Levi A.J. (1974). Sites of competition in selective hepatic uptake of rifampisin - SV, flavaspidic acid, bilirubin and bromosulphthalein. Gut 15: 220-226.

Kirsch R.E., Frith L., Stead R.H., Saunders S.J. (1973). Effect of alcohol on albumin synthesis by the isolated perfused rat liver. American Journal of Clinical Nutrition 26: 1191-1194.

Koch-Weser J. (1976). Drug therapy: acetaminophen. New England Journl of Medicine 295: 1297-1300.

Krebs H.A. and Hensleit K. (1932). Untersuchungen uber die harnstoffbildung imm tierkorper. Hoppe-Seylers Z. Physiological Chemistry 210: 33-66.

Krebs H.A. and Hems R. (1970). Fatty acid metabolism in the perfused rat liver. Biochemical Journal 119: 525-533.

Krebs H.A., Freedland R.A., Hems R., Stubbs M. (1969). Inhibition of hepatic gluconeogenesis by ethanol. Biochemical Journal 12: 117-124.

Kreisberg R.A., Siegal A.M., Owen W.C. (1971). Glucose - lactate inter relationships: Effect of ethanol. Journal of Clinical Investigation 50: 175-185.

Kringsholm B. (1980). Sniffing-associated death in Denmark. Forensic Sciences International 15: 215-225.

Lauterberg B.H., Davies S., Mitchell J.R. (1984). Ethanol suppresses hepatic glutathione synthesis in vivo. Journal of Pharmacology and Experimental Therapeutics 230: (1) 7-11.

Litt I.F., Cohen M.I., McNamara H. (1976). Isoniazid-induced hepatitis in adolescents. Journal of Pediatrics 89: 133-135.

Lochner A., Wulff J., Madison L.L. (1967). Ethanol induced hypoglycemia. 1. Acute effects of alcohol on hepatic glucose output and peripheral glucose utilization in fasted dogs. Metabolism 16: 1-18.

MacSween R.N.M.: Alcoholic liver disease. (ED).. In H.C. Thomas, R.N.M. MacSween. Recent Advances in Hepatology. Edinburgh 1983 71-78.

Maddrey W.C. (1981). Isoniazid-induced liver disease. Seminars in Liver Disease 1: 129-133.

Madison L.L., Lochner A., Wulff J. (1967). Ethanol-induced hypoglycemia. II. Mechanism of suppression of hepatic gluconeogenesis. Diabetes 16: (4) 252-258.

Madison L.L. (1968). Ethanol induced hypoglycemia. Advance Metabolic Disorders. 3:85.

Martin C.E. and Arthaud J.B. (1970). Hepatitis after isoniazid administration. New England Journal of Medicine 282: 433-434.

McLain C.J., Kromhout J.P., Peterson F.J., Holzman J.L. (1980). Potentiation of acetaminophen hepatotoxicity by alcohol. Journal of American Medical Association 244: 251 - 253.

Meredith T.J., Prescott L.F., Vale J.A. (1986). Why do patients still die from paracetamol poisoning? British Medical Journal 293: 345-346.

Miller L.L., Bly C.G., Watson M.L., Bale W.F. (1951). The dominant role of the liver in plasma protein synthesis - a direct study of the isolated perfused rat liver with aid of lysine -C^{14}. Journal of Experimental Medicine 94: 431.

Mitchell J.R., Long M.W., Thorgeirsson U.P., Jollow D.J. (1975). Acetylation rates and monthly liver function tests during one year of isoniazid preventive therapy. Chest 68: 181-190.

Mitchell J.R., Thorgeirsson U.P., Black M., Timbrell J.A., Snodgrass W.R., Potter W.Z., Jollow D.J., Keiser H.R. (1975). Increased incidence of isoniazid hepatitis in rapid acetylators : possible relationship to hydrazine metabolites. Clinical Pharmacology and Therapeutics 8: 70-79.

Mitchell J.R., Zimmerman H.J., Ishak K.G., Thorgeirsson U.P., Snodgrass W.R., Nelson S.D. (1976). Isoniazid liver injury : clinical spectrum, pathology and probable pathogenesis. Annals of Internal Medicine 84: 181-192.

Mitchell J.R., Jollow, D.J., Potter, W.Z., Gillette, J.R., Brodie B.B. (1973). Acetaminophen-induced hepatic necrosis II. Protective role of glutathione. Journal of Pharmacology and Experimental Therapeutics 187: 211-212.

Monteagudo F.S.E. and Folb P.I. (1987). Paracetamol poisoning at Groot Schuur Hospital. South African Medical Journal 72: 773-776.

Mortimore C.E.(1963). Effect of insulin on release of glucose and urea by isolated rat liver. American Journal of Physiology 204: 699-704.

Mortimore C.E. (1961). The effect of insulin on potassium transfer in isolated rat liver. American Journal of Physiology 200: 1315-1319.

Morton S. and Mitchell M.C. (1985). Effect of chronic ethanol feeding on glutathione turnover in the rat. Biochemical Pharmacology 34: (9) 1559-1562.

National Institute for Occupational Safety and Health Report (1982). Epistaxis and liver - function abnormalities associated with exposure to "butyl" caulk. Kentucky Morbidity Mortality Weekly Review Report: 31, 251.

Neuberger J.M. and Davis M. (1981). Hepatic damage from lower doses of paracetamol. In: Davis M., Tredger J.M., Williams R. eds. Drug Reactions and the Liver. London : Pitman Medical 176-179.

Pine S.A. (1971). Rifampicin with ethambutol for drug resistant tuberculosis. British Journal of Diseases of the Chest 65: 178-181.

Prescott L.F. and Wright N. (1973). The effect of hepatic and renal damage on paracetamol metabolism and excretion following overdosage. A pharmacokinetic study. British Journal of Pharmacology 49: 602-613.

Record C.O., Chase R.A., Williams R., Appleton D. (1981). Disturbances in lactate metabolism in patients with liver disease due to paracetamol overdose. Metabolism 30: 638-643.

Record C.O., Chase R.A., Albert K.G.M.M., Williams R. (1975). Disturbances in glucose metabolism with liver damage due to paracetamol overdose. Clinical Sciences and Molecular Medicine 49: 473-479.

Ross B.D., Hems R., Freedland R.A., Krebs H.A. (1967). Carbohydrate metabolism of the perfused rat liver. Biochemical Journal 105: 869-875.

Ross B.D., Hems R., Krebs H.A. (1967). The rate of gluconeogenesis from various precursors in the perfused rat liver. Biochemical Journal 102: 942-951.

Saunders J.B., Wodak A.D., Williams R. (1984). What determines susceptibility to liver damage from alcohol. Journal of Royal Society of Medicine 77: 204-216.

Scheuer P.J., Summerfield J.A., Lal S., Sherlock S. (1974). Rifampicin hepatitis. Clinical and histological study. Lancet ii: 421-425.

Schimassek H. (1963). Der einflus der leber aufden extracellularem redox quotiented lactate:pyruvate. Versuche ander isoledt perfundisten ratten leber. Biochemical Z 336 : 468.

Schimassek H. (1963). Metabolites of carbohydrate metabolism in the isolated perfused rat liver. Biochemical Z 336: 460-467.

Schimassek H. (1962). Regulation of lactate and pyruvate levels in perfusion medium by isolated rat liver. Life Sciences 11: 635-638.

Schimassek H. (1962). Perfusion of isolated rat liver with a semi-synthetic medium and and control of liver function. Life Sciences 11: 629-634.

Schaumburg H.H. and Spencer P.S. (1976). Degeneration in central and peripheral nervous systems produced by pure n-hexane: An experimental study. Brain 99: 183-192.

Seeff L.B., Cuccherin B.S., Zimmerman H.J., Adler E., Benjamin S.B. (1986). Acetaminophen hepatotoxicity by alcohol. Jounal of American Medical Association 104: 399 - 404.

Shaw S., Rubin K.P., Lieber C.S. (1983). Depressed hepatic glutathione and increased diene conjugates in alcohol liver disease - Evidence of lipid peroxidation. Digestive Diseases and Sciences 28: (7) 585-589.

Smith M.E. and Newman H.W. (1959). The rate of ethanol metabolism in fed and fasting animals. Journal of Biological Chemistry 234: 1544-1549.

Sokal J.E., Miller L.L., Sarcione E.J. (1958). Glycogen metabolism in the isolated liver. American Journal of Physiology 195: 295-300.

Speisky H., MacDonald A., Giles W., Orrego R., Israel Y. (1985). Increased loss and decreased synthesis of hepatic glutathione after acute ethanol administration. Biochemical Journal 225: 565-572.

Spencer P.S., Schumburg H.H., Sabri M.I., Veronesi V. (1980). The enlarging view of hexacarbon neurotoxicity. CRC Critical Reviews in Toxicology 7: 279-345.

Trowell O.A. (1942). Urea formation in the isolated perfused liver of rat. Journal of Physiology 100: 432-458.

Tygstrup N., Winkler K., Lundquist F. (1965). The mechanism of fructose effect on the ethanol metabolism of the human liver. Journal of Clinical Investigation 44: (5) 817-830.

Vall-Spinosa A., Lester W., Moulding T., Davidson P.T. McClatchy J.K. (1970). Rifampisin in the treatment of drug resistant Mycobacterium tuberculosis infection. New England Journal of Medicine 283: 616-621.

Watson J.M. (1979). Morbidity and Mortality statistics on solvent abuse. Medical Science and Law 19: 246-252.

Wilson N.M., Brown P.M., Juul S.M., Prestwich S.A., Sonksen P.H. (1981). Glucose turnover and metabolic and hormonal changes in ethanol induced hypoglycaemia. British Medical Journal 282: 849-853.

Woods H.F. and Krebs H.A. (1971). Lactate production in the perfused rat liver. Biochemical Journal 125: 129-139.

Woods H.F. and Krebs H.A. (1973). Xylitol metabolism in the isolated perfused rat liver. Biochemical Journal 134: 437-443.

Zimmerman H.J. (1978). In: Hepatotoxicity , New York Appleton Century-Crofts p510.

List of papers emanating from this study which was presented at a local congress. (Annual Congress of the Pharmacological Society of South Africa, Cape Town, October 1987 and Port Elizabeth, October 1988.)

1. *THE EFFECT OF THERAPEUTIC DOSES OF PARACETAMOL ON LIVER FUNCTION IN THE PERFUSED RAT LIVER.*
   Khedun SM, Maharaj B, Leary WP.
   Department of Experimental and Clinical Pharmacology, Natal Medical School, Durban.

2. *THE EFFECT OF HEXANE ON LIVER FUNCTION AND LIVER MORPHOLOGY IN THE RAT.*
   Khedun SM, Maharaj B, Leary WP.
   Department of Experimental and Clinical Pharmacology, Natal Medical School, Durban.

3. *THE EFFECT OF SUPRA-THERAPEUTIC DOSES OF ISONIAZID ON LIVER FUNCTION IN THE PERFUSED RAT LIVER.*
   Khedun SM, Maharaj B, Leary WP.
   Department of Experimental and Clinical Pharmacology, Natal Medical School, Durban.

THE EFFECT OF THERAPEUTIC DOSES OF PARACETAMOL ON LIVER FUNCTION IN THE PERFUSED RAT LIVER.

Khedun SM, Maharaj B, Leary WP.

Department of Experimental and Clinical Pharmacology, Natal Medical School, Durban.

Background

Paracetamol, a widely used analgesic, can produce hepatic damage which can be fatal when taken in overdose. In some patients chronic ingestion of paracetamol, even in therapeutic doses, may produce hepatic necrosis and hepatitis.

Aim

This study was conducted to determine the influence of therapeutic doses of paracetamol on liver function (gluconeogenesis and bromosulphthatlien clearance) in the rat.

Methods

Three groups of rats were studied; those in the two experimental groups were given 0.005 g paracetamol per os daily for 90 days. The isolated liver perfusion technique was used in the study. The livers of the control and one of the experimental groups were perfused with a medium containing pyruvate, a substrate for gluconeogenesis. A perfusion medium containing both pyruvate and methylene blue, a redox agent capable of oxidising NADH to NAD^+, was used in the second experimental group. Serial specimens were obtained for analysis.

Results

In the experimental livers glucose production was decreased, lactate production was increased, pyruvate ratios were increased, methylene blue had no effect on glucose production, and bromosulphthalein clearance was impaired.

Conclusion

This study demonstrates that paracetamol, when administered over a prolonged period in doses equivalent to therapeutic doses in adult humans, impairs function of the isolated perfused rat liver as evidenced by impaired gluconeogenesis and bromosulphthalein clearance.

References

Barker JD, DeCarle DJ, Anuras S. (1977). Chronic excessive acetaminophen use and liver damage. Annals of Internal Medicine. 87: 299-301.

Bonkowsky HL, Mudge GH, McMurtry GH. (1978). Chronic hepatic inflammation and fibrosis due to low doses of paracetamol. Lancet i: 1016-1018.

Canalese J, Gimson AES, Davis M, Williams R. (1981). Factors contributing to mortality in paracetamol induced liver failure. British Medical Journal 282: 199-201.

Clark R, Thompson RPH, Borirahchanyarat V, Widdop B, Davidson AR, Goulding R, Williams R. (1973). Hepatic damage and death from overdose of paracetamol. Lancet i: 66-69.

Combes B. (1965). The importance of conjugation with glutathione for sulfobromphthalien sodium (BSP) transfer from blood to bile. Journal of Clinical Investigation 44: (7) 1214-1224.

Davidson DGD and Eastham WN. (1966). Acute liver necrosis following overdose of paracetamol. British Medical Journal 2: 497-499.

Davis M, Ideo G, Harrison NG, Williams R. (1975). Hepatic glutathione depletion and impaired bromosulphthalein clearance early after paracetamol overdose in man and rat. Clinical Sciences and Molecular Medicine 49: 495-502.

Johnson GK and Tohman KG. (1977). Chronic liver disease and acetaminophen. Annals of Internal Medicine 87: 302-304.

Koch-Weser J (1976). Drug therapy: acetaminophen. New England Journal of Medicine 295: 1297-1300.

Meredith TJ, Prescott LF, Vale JA. (1980). Why do patients still die from paracetamol poisoning. British Medical Journal 293: 345-346.

Mitchell JR, Jollow DJ, Potter WZ, Gillette Jr, Brodie BB. (1973). Acetaminophen-induced hepatic necrosis II. Protective role of glutathione. Journal of Pharmacology and Experimental Therapeutics 187: 211-212.

Neuberger JM and Davis M (1981). Hepatic damage from lower doses of paracetamol. In: Davis M, Tredger JM, Williams R eds. Drug reactions and the Liver. London: Pitman Medical 176-179.

Prescott LF and Wright N (1973). The effect of hepatic and renal damage on paracetamol metabolism and excretion following overdosage. A pharmacokinetic study. British Journal of Pharmacology 49: 602-613.

Record CO, Chase RA, Albert KGMM, Williams R (1975). Disturbances in glucose metabolism with liver damage due to paracetamol overdose. Clinical Sciences and Molecular Medicine 49: 473-479.

THE EFFECT OF HEXANE ON LIVER FUNCTION AND LIVER MORPHOLOGY IN THE RAT.

Khedun SM, Maharaj B, Leary WP.
Department of Experimental and Clinical Pharmacology, Natal Medical School, Durban.

Background

Little attention has been paid to the possible effect of solvent abuse on the liver.

Aim

This study was conducted to determine the influence of hexane, one of the major components of benzine and glue, on liver function (bromosulphthalein clearance and gluconeogenesis) and liver morphology in rats.

Methods

Four groups of rats were studied; three groups were given subcutaneous injections of 0.2ml hexane daily for 90 days. The effects of hexane on gluconeogenesis and bromosulphthalein clearance were studied using the isolated liver perfusion technique. The livers of control group and one experimental group were perfused with a medium containing pyruvate, a substrate for gluconeogenesis, while those of the second group were perfused with a medium containing pyruvate and methylene blue (a redox agent capable of oxidising NADH to NAD^+). Serial specimens were obtained for analysis. The livers of the third experimental group were subjected to histological examination using standard methods.

Results

In the hexane-treated livers bromosulphthalein clearance was impaired, glucose and pyruvate concentrations were decreased, lactate concentrations and lactate : pyruvate ratios were increased. Methylene blue had no effect on glucose production. In addition severe fatty change was noted in the experimental livers.

Conclusion

This study demonstrates that hexane is hepatotoxic; it alters liver function (bromosulphthalein clearance and gluconeogenesis were impaired) and produces histological changes which are indicative of hepatic damage. In addition to central and

peripheral nervous system damage, it is likely that hexane abuse could also result in hepatotoxicity in humans. This hypothesis needs to be confirmed. Educational programmes designed to discourage abuse of benzine and glue are urgently needed.

References

Anderson HR, Dick B, Macnair RS, Palmer JC, Ramsey JD. (1982). An investigation of 140 deaths associated with volatile substance abuse in U.K. (1971-1981). Human Toxicology 1: 201-221.

Baerg RD and Kimberg DV. (1970). Centrilobular hepatic necrosis and acute renal failure in "solvent sniffers". Annals of Internal Medicine 73: 713-720.

Clark DG and Tinston DJ. (1982). Acute inhalation toxicity of some halogenated and non-halogenated hydrocarbons. Human Toxicology 1: 239-247.

Conroy B and Pennington CR. (1984). Transient ground glass hepatocellular change following a period of glue sniffing in a patient with underlying benign recurrent cholestatic jaundice. Medicine, Science and Law 24: (2) 102-106.

Garriot J and Petty CS. (1980). Death from inhalent abuse: Toxicological and pathological evaluations of 34 cases. Clinical Toxicology 16: 305-315.

Kringsholm B. (1980). Sniffing-associated death in Denmark. Forensic Sciences International 15: 215-225.

National Institute for Occupational Safety and Health Report (1982). Epistaxis and liver - function abnormalities associated with exposure to "butyl" caulk. Kentucky Morbidity Weekly Review Report: 31, 251.

Schaumburg HH and Spencer PS. (1976). Degeneration in central and peripheral nervous systems produced by pure n-hexane: An experimental study. Brain 99: 183-192.

THE EFFECT OF SUPRA-THERAPEUTUC DOSES OF ISONIAZID ON LIVER FUNCTION IN THE PERFUSED RAT LIVER.

Khedun SM, Maharaj B, Leary WP.

Department of Experimental and Clinical Pharmacology, Natal Medical School, Durban.

Background

Isoniazid has been the most widely used drug in the treatment of tuberculosis with excellent therapeutic efficacy and good patient compliance.

Aim

This study was conducted to determine the influence of supra-therapeutic doses of isoniazid on liver function (gluconeogenesis and bromosulphthalein clearance from perfusate and biliary excretion of the dye) in the rat.

Methods

Two groups of rats were studied; those in the experimental group were given 2.5 mg isoniazid per os daily for 90 days. The effects of isoniazid on gluconeogenesis and bromosulphthalein clearance from perfusate and biliary excretion of the dye were studied using the isolated liver perfusion model. The livers of the control and the experimental group were perfused with a medium containing pyruvate, a substrate fro gluconeogenesis, and subsequently these livers were perfused with a medium containing bromosulphthalein. Serial specimens were obtained for analysis.

Results

In the isoniazid-treated livers glucose and pyruvate concentrations were decreased, lactate concentrations and lactate:pyruvate ratios were increased, and bromosulphthalein clearance from perfusate and biliary excretion of the dye was impaired.

Conclusion

This study demonstrates that isoniazid, when administered over a prolonged period in doses equivalent to two times the therapeutic dose in adult humans, impairs the function of the isolated perfused rat liver as evidenced by impaired gluconeogenesis and bromosulphthalein clearance from perfusate and biliary excretion of the dye.

References

Davies D and Glowinsbi JJ. (1961). Jaundice due to isoniazid. Tubercle 42: 504-506.

Exton JH. (1972). Gluconeogenesis. Metabolism 21: 945-990.

Gilman AG, Goodman LS, Gilman A. (Eds): The pharmacological basis of Therapeutics ed 6. New York, Macmillan 1980 pp 1200-1203.

Maddrey WC. (1981). Isoniazid-induced liver disease. Seminars in Liver Disease 1: 129-133.

Mitchell JR, Zimmerman HJ, Ishak KG, Thorgeirsson UP, Snodgrass WR, Nelson Sd. (1976). Isoniazid liver injury : clinical spectrum, pathology and probable pathogenesis. Annals of Internal Medicine 84: 181-192.

Zimmerman HJ: In Hepatotoxicity, New York Appleton Century-Crofts 1978 p510.

APPENDIX A

CONSTITUENTS OF 1 LITRE PERFUSION MEDIUM:

NaCl	6.9 g
KCL	303.9 mg
KH_2PO_4	157.7 mg
$MgSO_4$	292.3 mg
$CaCL_2$	115.4 mg
$NaHCO_3$	2.1 g
pH adjusted to	7.4

APPENDIX B

GLUCOSE CONCENTRATIONS IN CONTROL AND ALCOHOL-TREATED RATS

CONTROLS

Time in Minutes

No.	15	30	45	60	75
1.	2.65	3.47	3.55	5.64	6.84
2.	2.83	3.21	4.52	4.85	5.62
3.	2.87	4.43	4.53	4.32	6.65
4.	3.79	3.59	4.17	4.28	6.41
5.	3.29	4.28	7.51	7.51	8.17
6.	2.85	3.15	4.12	5.89	4.31
$\overline{X}$	3.05	3.69	4.73	5.42	6.33
SEM	0.17	0.22	0.57	0.49	0.52

EXPERIMENTALS

1.	1.50	1.90	2.90	3.10	1.50
2.	1.30	1.70	1.70	1.90	2.00
3.	1.20	1.10	1.60	2.30	2.00
4.	1.30	1.10	1.80	1.80	2.00
5.	1.20	1.70	0.90	1.80	1.80
6.	1.20	1.50	1.60	1.90	2.50
$\overline{X}$	1.28	1.50	1.75	2.13	1.97
SEM	0.04	0.13	0.26	0.20	0.13

THE EFFECTS OF METHYLENE BLUE, A REDOX AGENT, ON GLUCOSE CONCENTRATIONS IN CONTROL (EXPERIMENTAL GROUP 1) AND ALCOHOL-TREATED RATS (EXPERIMENTAL GROUP 2)

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	1.50	1.90	2.90	3.10	1.50
2.	1.30	1.70	1.70	1.90	2.00
3.	1.20	1.10	1.60	2.30	2.00
4.	1.30	1.10	1.80	1.80	2.00
5.	1.20	1.70	0.90	1.80	1.80
6.	1.20	1.50	0.60	1.90	2.50
$\overline{X}$	1.28	1.50	1.75	2.13	1.97
SEM	0.04	0.13	0.26	0.20	0.13

EXPERIMENTALS

1.	8.10	20.30	10.2	9.50	9.70
2.	6.60	4.80	4.80	4.30	7.90
3.	8.70	5.20	18.70	14.40	23.00
4.	13.40	8.10	3.40	9.90	4.30
5.	2.20	5.20	10.80	10.10	9.00
6.	7.60	5.50	14.20	7.80	14.10
$\overline{X}$	7.77	8.18	10.35	9.33	11.33
SEM	1.47	2.46	2.33	1.34	2.66

THE EFFECT OF FRUCTOSE, A NON-NAD$^+$ DEPENDENT PRECURSOR, ON GLUCOSE CONCENTRATIONS IN CONTROL (EXPERIMENTAL GROUP 1) AND ALCOHOL-TREATED RATS (EXPERIMENTAL GROUP 3)

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	1.50	1.90	2.90	3.10	1.50
2.	1.30	1.70	1.70	1.90	2.00
3.	1.20	1.10	1.60	2.30	2.00
4.	1.30	1.10	1.80	1.80	2.00
5.	1.20	1.70	0.9	1.80	1.80
6.	1.20	1.50	1.60	1.90	2.50
$\overline{X}$	1.28	1.50	1.75	2.13	1.97
SEM	0.04	0.13	0.26	0.20	0.13

EXPERIMENTALS

1.	5.10	9.50	11.30	16.20	14.00
2.	11.40	9.90	7.70	7.10	6.20
3.	12.80	6.40	11.60	10.50	7.20
4.	9.50	12.90	10.50	6.50	5.50
5.	12.90	12.60	9.60	7.40	5.40
6.	9.80	6.50	10.80	6.60	6.30
$\overline{X}$	10.25	9.63	10.25	9.05	7.43
SEM	1.18	1.15	0.58	1.55	1.32

LACTATE CONCENTRATIONS IN CONTROL AND ALCOHOL-TREATED RATS

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	1.93	2.11	2.83	2.25	2.95
2.	2.21	2.45	2.81	2.99	2.83
3.	2.41	2.81	2.21	2.89	2.15
4.	2.35	2.35	2.48	2.79	3.15
5.	1.67	2.13	2.19	2.59	2.26
6.	1.98	1.85	2.13	2.55	2.43
$\overline{X}$	2.09	2.28	2.44	2.68	2.63
SEM	0.11	0.13	0.13	0.11	0.16

EXPERIMENTALS

1.	3.60	4.30	2.80	3.60	2.90
2.	2.80	3.20	2.90	3.10	2.50
3.	1.80	4.10	3.20	3.60	3.20
4.	2.80	3.50	3.90	3.10	4.40
5.	2.60	1.90	2.80	1.80	2.40
6.	3.10	1.80	1.90	2.60	3.20
$\overline{X}$	2.78	3.13	2.92	2.97	3.10
SEM	0.24	0.43	0.26	0.27	0.29

PYRUVATE CONCENTRATIONS IN CONTROL AND ALCOHOL-TREATED RATS

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	0.156	0.221	0.218	0.221	0.195
2.	0.222	0.204	0.201	0.181	0.203
3.	0.214	0.208	0.151	0.195	0.159
4.	0.158	0.219	0.222	0.161	0.185
5.	0.241	0.195	0.218	0.144	0.201
6.	0.151	0.201	0.206	0.221	0.201
$\bar{X}$	0.190	0.208	0.202	0.187	0.190
SEM	0.010	0.040	0.010	0.010	0.008

EXPERIMENTALS

1.	0.103	0.130	0.101	0.107	0.120
2.	0.115	0.100	0.130	0.100	0.109
3.	0.105	0.120	0.104	0.100	0.106
4.	0.115	0.115	0.102	0.120	0.200
5.	0.102	0.107	0.110	0.106	0.101
6.	0.108	0.104	0.120	0.113	0.130
$\bar{X}$	0.108	0.112	0.111	0.107	0.127
SEM	0.004	0.004	0.004	0.004	0.010

LACTATE:PYRUVATE RATIOS IN CONTROL AND ALCOHOL-TREATED RATS

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	12.37	9.54	12.98	10.18	15.12
2.	9.95	12.00	13.98	16.51	13.94
3.	11.26	13.50	14.63	14.82	13.52
4.	14.87	10.73	11.17	17.32	17.02
5.	6.92	10.92	10.04	17.98	11.24
6.	13.11	9.20	10.33	11.53	12.08
$\overline{X}$	11.41	10.98	12.19	14.72	13.82
SEM	1.10	0.65	0.79	1.30	0.85

EXPERIMENTALS

1.	34.95	33.07	27.72	33.64	24.16
2.	24.34	32.00	22.30	31.00	22.93
3.	17.14	34.16	30.76	36.00	30.18
4.	24.34	30.43	38.23	25.83	22.00
5.	25.49	17.75	25.45	16.98	23.76
6.	28.70	17.30	15.83	23.00	24.61
$\overline{X}$	25.83	27.45	26.72	27.77	24.72
SEM	2.40	3.10	3.10	2.90	1.10

BROMOSULPHTHALEIN CLEARANCE IN CONTROL AND ALCOHOL-TREATED RATS

CONTROLS

Time in minutes

No.	0	5	10	15	20
1.	0.849	0.588	0.598	0.193	0.085
2.	0.884	0.629	0.638	0.151	0.086
3.	0.842	0.582	0.514	0.172	0.093
4.	0.852	0.608	0.618	0.181	0.062
5.	0.846	0.603	0.522	0.196	0.082
6.	0.848	0.621	0.584	0.186	0.091
$\overline{X}$	0.850	0.610	0.579	0.180	0.080
SEM	0.008	0.008	0.010	0.008	0.004

EXPERIMENTALS

1.	1.266	0.619	0.621	0.633	0.667
2.	1.135	0.466	0.503	0.410	0.444
3.	0.876	0.573	0.138	0.160	0.252
4.	0.768	0.366	0.445	0.399	0.525
5.	1.196	0.486	0.717	0.756	0.529
6.	0.943	0.536	0.520	0.377	0.427
$\overline{X}$	1.030	0.510	0.490	0.460	0.470
SEM	0.080	0.030	0.080	0.080	0.050

BILIARY EXCRETION OF BROMOSULPHTHALEIN IN CONTROL AND ALCOHOL-TREATED RATS

CONTROL

Time in minutes

No.	0	5	10	15	20
1.	0.071	0.315	0.521	0.598	0.814
2.	0.069	0.291	0.501	0.614	0.793
3.	0.085	0.381	0.623	0.625	0.821
4.	0.058	0.216	0.598	0.618	0.770
5.	0.081	0.312	0.498	0.591	0.765
6.	0.069	0.290	0.613	0.621	0.815
$\bar{X}$	0.070	0.300	0.550	0.610	0.790
SEM	0.030	0.120	0.230	0.250	0.330

EXPERIMENTALS

1.	0.281	0.295	0.316	0.295	0.305
2.	0.239	0.306	0.215	0.331	0.321
3.	0.301	0.289	0.313	0.401	0.298
4.	0.281	0.293	0.285	0.351	0.415
5.	0.237	0.314	0.273	0.291	0.321
6.	0.314	0.308	0.293	0.358	0.318
$\bar{X}$	0.270	0.300	0.280	0.330	0.320
SEM	0.110	0.120	0.110	0.140	0.130

APPENDIX C

GLUCOSE CONCENTRATIONS IN CONTROL AND RIFAMPICIN-TREATED RATS

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	2.850	2.930	3.850	3.210	3.930
2.	3.050	3.770	4.710	4.940	5.160
3.	2.950	3.270	3.620	17.78	18.25
4.	2.960	2.990	3.640	3.480	7.430
5.	2.860	3.150	3.150	3.280	3.630
6.	2.350	2.170	3.120	2.930	3.710
$\overline{X}$	2.840	3.050	3.680	3.570	4.770
SEM	0.100	0.210	0.230	0.320	0.650

EXPERIMENTALS

1.	1.889	2.022	1.997	1.542	2.427
2.	1.917	1.156	1.013	2.118	1.701
3.	1.583	1.707	1.917	2.032	1.214
4.	1.564	1.678	1.688	1.841	1.554
5.	13.029	2.609	2.198	2.366	1.526
6.	1.946	1.784	2.089	1.242	2.253
$\overline{X}$	1.990	1.830	1.820	1.860	1.780
SEM	0.220	0.190	0.170	0.160	0.190

PYRUVATE CONCENTRATIONS IN CONTROL AND RIFAMPICIN-TREATED RATS

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	0.269	0.249	0.222	0.213	0.201
2.	0.201	0.165	0.151	0.221	0.204
3.	0.195	0.222	0.214	0.215	0.201
4.	0.186	0.201	0.227	0.209	0.195
5.	0.193	0.221	0.224	0.201	0.193
6.	0.156	0.214	0.213	0.203	0.183
$\bar{X}$	0.200	0.212	0.211	0.210	0.196
SEM	0.010	0.010	0.010	0.004	0.004

EXPERIMENTALS

1.	0.106	0.130	0.105	0.104	0.155
2.	0.120	0.105	0.101	0.159	0.161
3.	0.111	0.122	0.110	0.102	0.115
4.	0.102	0.114	0.125	0.107	0.121
5.	0.105	0.107	0.110	0.136	0.154
6.	0.118	0.114	0.100	0.103	0.109
$\bar{X}$	0.110	0.115	0.108	0.118	0.135
SEM	0.004	0.004	0.004	0.008	0.008

LACTATE CONCENTRATIONS IN CONTROL AND RIFAMPICIN-TREATED RATS

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	2.80	2.60	3.20	2.80	2.60
2.	1.40	2.60	2.80	2.90	2.70
3.	3.10	2.90	2.60	2.80	2.90
4.	3.80	2.30	2.80	1.90	2.50
5.	2.20	2.60	2.90	2.10	2.50
6.	2.30	2.90	2.80	2.10	2.60
$\overline{X}$	2.60	2.65	2.85	2.43	2.63
SEM	0.33	0.09	0.08	0.18	0.06

EXPERIMENTALS

1.	2.34	1.89	2.45	3.15	2.26
2.	2.12	2.26	2.52	2.85	3.86
3.	2.95	2.89	3.81	3.21	2.41
4.	2.62	3.56	2.86	2.81	3.26
5.	3.16	2.29	3.56	3.21	2.18
6.	2.31	2.42	2.19	3.18	4.21
$\overline{X}$	2.58	2.55	2.73	3.07	3.03
SEM	0.16	0.24	0.23	0.07	0.35

LACTATE : PYRUVATE RATIOS IN CONTROL AND RIFAMPICIN-TREATED RATS

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	10.40	10.44	14.41	13.41	12.93
2.	6.96	15.75	18.54	13.12	13.23
3.	15.89	13.06	12.14	13.02	14.42
4.	20.43	11.44	12.33	9.09	12.82
5.	11.39	11.76	12.94	10.44	12.95
6.	14.74	13.55	13.14	10.34	14.20
$\overline{X}$	13.30	12.67	13.92	11.53	13.43
SEM	1.90	0.78	0.98	0.72	0.28

EXPERIMENTALS

1.	22.07	14.53	23.33	30.28	14.58
2.	17.66	21.52	24.95	17.92	23.97
3.	26.57	23.68	34.63	31.47	20.95
4.	25.68	31.22	22.88	26.26	26.94
5.	30.09	21.40	23.27	23.60	14.15
6.	19.57	21.22	21.90	30.87	38.62
$\overline{X}$	23.61	22.26	25.16	26.73	23.20
SEM	1.90	2.20	1.90	2.10	3.70

BROMOSULPHTHALEIN CLEARANCE IN CONTROL AND RIFAMPICIN-TREATED RATS

CONTROLS

Time in minutes

No.	0	5	10	15	20
1.	0.862	0.498	0.478	0.252	0.088
2.	0.841	0.602	0.503	0.197	0.014
3.	0.849	0.542	0.491	0.302	0.062
4.	0.795	0.621	0.501	0.308	0.059
5.	0.843	0.596	0.422	0.258	0.043
6.	0.825	0.613	0.412	0.215	0.083
$\overline{X}$	0.840	0.580	0.467	0.250	0.050
SEM	0.008	0.020	0.030	0.010	0.010

EXPERIMENTALS

1.	0.784	0.694	0.681	0.202	0.085
2.	0.871	0.557	0.471	0.139	0.043
3.	0.725	0.487	0.504	0.337	0.042
4.	0.871	0.551	0.551	0.376	0.052
5.	0.790	0.763	0.688	0.295	0.061
6.	0.761	0.676	0.625	0.237	0.043
$\overline{X}$	0.800	0.620	0.580	0.260	0.050
SEM	0.020	0.040	0.030	0.100	0.020

APPENDIX D

GLUCOSE CONCENTRATIONS IN CONTROL AND ISONIAZID-TREATED RATS

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	2.820	3.010	3.810	4.200	4.010
2.	2.690	3.400	3.840	3.870	3.890
3.	2.580	3.810	4.660	5.380	6.260
4.	2.770	3.420	3.870	4.240	4.970
5.	2.610	3.120	5.430	6.930	7.990
$\overline{X}$	2.690	3.350	4.320	4.920	5.420
SEM	0.040	0.130	0.310	0.560	0.760

EXPERIMENTALS

1.	1.499	1.399	1.359	1.699	1.369
2.	1.058	1.458	2.827	2.426	2.716
3.	1.459	1.739	2.008	2.188	2.717
4.	1.619	1.721	2.088	2.428	2.158
5.	1.439	1.359	1.349	1.689	1.369
$\overline{X}$	1.410	1.540	1.930	2.090	2.070
SEM	0.090	0.080	0.270	0.160	0.300

LACTATE CONCENTRATIONS IN CONTROL AND ISONIAZID-TREATED RATS

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	2.14	2.25	2.38	3.49	3.51
2.	2.16	2.88	2.56	2.38	2.65
3.	2.23	2.56	2.95	3.21	3.41
4.	1.85	1.98	1.99	2.15	2.75
5.	2.31	3.13	2.18	3.45	2.65
$\bar{X}$	2.14	2.56	2.41	2.94	2.99
SEM	0.07	0.20	0.16	0.28	0.19

EXPERIMENTALS

1.	3.81	3.15	2.26	2.45	3.41
2.	2.86	2.47	2.52	3.81	3.16
3.	2.26	2.58	2.89	2.39	2.62
4.	2.57	2.63	3.17	3.21	3.15
5.	3.14	2.95	2.49	2.95	2.73
$\bar{X}$	2.93	2.76	2.67	2.96	3.01
SEM	0.26	0.12	0.16	0.26	0.14

PYRUVATE CONCENTRATIONS IN CONTROL AND ISONIAZID-TREATED RATS

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	0.281	0.243	0.122	0.201	0.196
2.	0.272	0.129	0.204	0.120	0.210
3.	0.291	0.222	0.219	0.198	0.251
4.	0.210	0.256	0.189	0.201	0.183
5.	0.285	0.225	0.214	0.210	0.151
$\bar{X}$	0.267	0.215	0.189	0.186	0.198
SEM	0.010	0.020	0.010	0.010	0.010

EXPERIMENTALS

1.	0.209	0.236	0.204	0.215	0.106
2.	0.180	0.215	0.198	0.171	0.104
3.	0.212	0.225	0.123	0.141	0.101
4.	0.201	0.182	0.155	0.163	0.177
5.	0.201	0.101	0.119	0.107	0.171
$\bar{X}$	0.201	0.191	0.179	0.159	0.131
SEM	0.004	0.020	0.010	0.010	0.010

LACTATE : PYRUVATE RATIOS IN CONTROL AND ISONIAZID-TREATED RATS

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	7.61	9.25	19.50	17.36	17.90
2.	7.94	22.32	12.54	19.83	12.61
3.	7.66	11.53	13.47	16.21	13.58
4.	8.80	7.73	10.52	10.69	15.02
5.	8.10	13.91	10.18	16.42	17.54
$\overline{X}$	8.20	13.00	13.40	16.20	15.60
SEM	0.20	2.40	1.70	1.40	1.00

EXPERIMENTALS

1.	18.22	13.34	11.24	11.39	32.16
2.	15.88	11.48	12.72	22.28	30.38
3.	10.66	11.46	23.49	16.95	25.94
4.	12.78	14.45	20.45	19.69	17.79
5.	15.62	29.02	20.92	27.57	15.96
$\overline{X}$	14.63	15.95	17.76	19.61	24.39
SEM	1.30	3.30	2.40	2.70	3.20

BROMOSULPHTHALEIN CLEARANCE IN CONTROL AND ISONIAZID-TREATED RATS

CONTROLS

Time in minutes

No.	0	5	10	15	20
1.	0.842	0.596	0.533	0.198	0.067
2.	0.851	0.667	0.521	0.179	0.083
3.	0.851	0.542	0.606	0.212	0.041
4.	0.849	0.612	0.589	0.241	0.095
5.	0.846	0.598	0.408	0.199	0.065
$\bar{X}$	0.850	0.600	0.531	0.200	0.070
SEM	0.010	0.010	0.010	0.004	0.008

EXPERIMENTALS

1.	0.671	0.270	0.367	0.342	0.308
2.	0.589	0.519	0.360	0.345	0.360
3.	0.601	0.392	0.410	0.343	0.319
4.	0.629	0.422	0.405	0.351	0.314
5.	0.614	0.316	0.402	0.339	0.294
$\bar{X}$	0.620	0.380	0.390	0.340	0.320
SEM	0.010	0.040	0.008	0.010	0.008

BILIARY EXCRETION OF BROMOSULPHTHALEIN IN CONTROL AND ISONIAZID-TREATED RATS

CONTROLS

Time in minutes

No.	0	5	10	15	20
1.	0.122	0.298	0.491	0.561	0.851
2.	0.091	0.302	0.388	0.626	0.839
3.	0.084	0.256	0.522	0.613	0.792
4.	0.141	0.239	0.498	0.597	0.776
5.	0.059	0.258	0.567	0.618	0.693
$\bar{X}$	0.090	0.270	0.490	0.620	0.790
SEM	0.040	0.120	0.220	0.280	0.350

EXPERIMENTALS

1.	0.287	0.339	0.389	0.318	0.406
2.	0.113	0.317	0.321	0.298	0.392
3.	0.198	0.295	0.289	0.315	0.388
4.	0.217	0.319	0.301	0.328	0.379
5.	0.188	0.401	0.256	0.403	0.368
$\bar{X}$	0.200	0.330	0.310	0.330	0.380
SEM	0.090	0.150	0.140	0.150	0.170

APPENDIX E

GLUCOSE CONCENTRATIONS IN CONTROL AND PARACETAMOL-TREATED RATS

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	2.49	2.31	3.41	2.89	3.82
2.	2.58	2.45	3.13	4.13	3.89
3.	1.81	2.87	2.66	5.79	4.07
4.	1.89	2.01	2.82	3.50	3.20
5.	1.89	2.73	3.81	4.03	4.65
6.	1.92	2.34	2.85	3.20	3.80
7.	2.43	2.49	1.57	3.25	4.16
8.	2.13	2.42	1.96	2.46	1.98
$\bar{X}$	2.14	2.45	2.78	3.66	3.69
SEM	0.10	0.09	0.25	0.36	0.28

EXPERIMENTALS

1.	0.60	1.40	0.60	1.00	1.40
2.	1.20	0.40	1.50	0.60	1.80
3.	1.20	1.10	1.60	1.60	3.00
4.	0.40	0.50	1.70	1.90	2.70
5.	0.30	1.80	1.00	1.20	1.40
6.	1.20	0.60	0.70	1.20	2.70
7.	1.30	1.50	1.80	0.90	1.20
8.	1.20	0.60	0.70	1.10	1.40
$\bar{X}$	0.93	0.99	1.20	1.19	1.95
SEM	0.14	0.18	0.17	0.14	0.25

THE EFFECT OF METHYLENE BLUE, A REDOX AGENT, ON GLUCOSE CONCENTRATIONS IN CONTROL (EXPERIMENTAL GROUP 1) AND PARACETAMOL-TREATED RATS (EXPERIMENTAL GROUP 2)

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	0.60	1.40	0.60	1.00	1.40
2.	1.20	0.40	1.50	0.60	1.80
3.	1.20	1.10	1.60	1.60	3.00
4.	0.40	0.50	1.70	1.90	2.70
5.	0.30	1.80	1.00	1.20	1.40
6.	1.20	0.60	0.70	1.20	2.70
7.	1.30	1.50	1.80	0.90	1.20
8.	1.20	0.60	0.70	1.10	1.40
$\overline{X}$	0.93	0.99	1.20	1.19	1.95
SEM	0.14	0.18	0.17	0.14	0.25

EXERIMENTALS

1.	0.50	0.80	1.00	0.80	0.40
2.	0.80	0.70	0.50	0.70	1.10
3.	0.30	0.60	0.20	0.40	0.80
4.	0.50	0.70	0.30	1.20	1.20
5.	0.70	0.40	0.50	0.60	0.30
6.	0.10	1.00	0.20	1.10	0.90
$\overline{X}$	0.47	0.70	0.45	0.80	0.78
SEM	0.11	0.08	0.12	0.12	0.15

LACTATE CONCENTRATIONS IN CONTROL AND PARACETAMOL-TREATED RATS

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	1.95	1.89	2.30	2.46	3.19
2.	1.91	2.30	2.10	2.48	2.95
3.	1.63	2.14	2.73	2.41	3.08
4.	2.30	2.41	2.98	2.48	2.52
5.	1.82	2.43	2.52	2.15	3.89
6.	1.71	2.81	2.10	2.07	3.21
7.	1.68	1.94	2.15	2.15	2.83
8.	1.72	1.92	2.14	3.14	2.85
$\bar{X}$	1.84	2.23	2.38	2.42	3.07
SEM	0.07	0.22	0.11	0.12	0.14

EXPERIMENTALS

1.	2.50	1.80	2.10	4.00	2.30
2.	1.30	2.10	1.90	2.80	2.90
3.	1.70	3.20	4.00	5.40	6.90
4.	1.80	3.20	4.40	5.00	4.50
5.	5.40	4.10	4.90	4.40	4.70
6.	3.80	4.90	3.90	4.20	3.40
7.	2.50	3.80	4.50	3.00	5.10
8.	2.70	3.00	5.60	3.80	4.90
$\bar{X}$	2.71	3.26	3.91	4.08	4.34
SEM	0.47	0.36	0.46	0.31	0.51

LACTATE : PYRUVATE RATIOS IN CONTROL AND PARACETAMOL-TREATED RATS

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	9.70	8.79	10.79	9.53	24.72
2.	7.79	17.96	9.30	16.42	13.84
3.	6.36	9.95	12.75	11.53	12.57
4.	9.12	16.97	23.28	16.42	10.90
5.	7.58	11.35	11.94	11.87	18.00
6.	6.86	26.01	10.76	10.29	15.28
7.	7.80	8.85	16.79	9.72	13.34
8.	8.00	8.64	9.90	21.07	15.40
$\bar{X}$	7.90	13.57	13.19	13.36	15.51
SEM	0.38	2.20	1.60	1.40	1.50

EXPERIMENTALS

1.	20.83	16.98	20.79	19.13	20.90
2.	12.38	20.38	17.75	26.16	23.96
3.	14.16	29.90	19.80	53.46	53.48
4.	13.83	16.00	20.00	24.39	38.13
5.	49.54	38.67	40.83	42.71	40.86
6.	29.45	44.14	32.50	40.00	18.47
7.	24.03	31.40	43.26	29.41	46.36
8.	25.00	27.57	53.30	35.18	47.57
$\bar{X}$	21.01	28.12	31.03	33.81	36.22
SEM	4.50	3.50	4.70	3.90	4.70

PYRUVATE CONCENTRATIONS IN CONTROL AND PARACETAMOL-TREATED RATS

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	0.201	0.215	0.213	0.258	0.129
2.	0.245	0.128	0.225	0.151	0.213
3.	0.256	0.215	0.214	0.209	0.245
4.	0.252	0.142	0.128	0.151	0.231
5.	0.240	0.214	0.211	0.181	0.216
6.	0.249	0.108	0.195	0.201	0.210
7.	0.215	0.219	0.128	0.221	0.212
8.	0.215	0.222	0.216	0.149	0.185
$\overline{X}$	0.235	0.182	0.191	0.190	0.205
SEM	0.070	0.010	0.010	0.010	0.010

EXPERIMENTALS

1.	0.120	0.106	0.101	0.209	0.110
2.	0.105	0.103	0.107	0.107	0.121
3.	0.120	0.200	0.202	0.101	0.129
4.	0.130	0.107	0.220	0.205	0.118
5.	0.109	0.106	0.120	0.103	0.115
6.	0.129	0.111	0.120	0.105	0.184
7.	0.104	0.121	0.104	0.102	0.110
8.	0.108	0.109	0.105	0.108	0.103
$\overline{X}$	0.115	0.120	0.134	0.129	0.123
SEM	0.003	0.010	0.010	0.010	0.010

BROMOSULPHTHALEIN CLEARANCE IN CONTROL AND PARACETAMOL-TREATED RATS

CONTROLS

Time in minutes

No.	0	5	10	15	20
1.	0.847	0.593	0.512	0.223	0.059
2.	0.798	0.489	0.414	0.219	0.061
3.	0.853	0.582	0.514	0.264	0.082
4.	0.847	0.631	0.606	0.214	0.062
5.	0.847	0.531	0.498	0.220	0.052
6.	0.857	0.641	0.503	0.194	0.089
7.	0.846	0.598	0.601	0.197	0.086
8.	0.855	0.622	0.489	0.218	0.073
$\overline{X}$	0.840	0.590	0.517	0.220	0.070
SEM	0.007	0.010	0.020	0.007	0.003

EXPERIMENTALS

1.	0.671	0.371	0.301	0.251	0.251
2.	0.429	0.181	0.195	0.226	0.234
3.	0.873	0.341	0.316	0.336	0.355
4.	0.807	0.358	0.317	0.329	0.345
5.	0.708	0.329	0.311	0.284	0.362
6.	0.809	0.303	0.290	0.460	0.266
$\overline{X}$	0.720	0.310	0.290	0.310	0.300
SEM	0.060	0.020	0.020	0.030	0.020

BILIARY EXCRETION OF BROMOSULPHTHALEIN IN CONTROL AND PARACETAMOL-TREATED RATS

CONTROLS

Time in minutes

No.	0	5	10	15	20
1.	0.125	0.191	0.398	0.388	0.594
2.	0.213	0.234	0.402	0.295	0.621
3.	0.301	0.287	0.385	0.423	0.588
4.	0.156	0.299	0.374	0.517	0.713
5.	0.082	0.310	0.421	0.601	0.820
6.	0.063	0.285	0.285	0.481	0.798
7.	0.114	0.277	0.391	0.492	0.815
8.	0.106	0.302	0.415	0.516	0.793
$\bar{X}$	0.140	0.270	0.380	0.460	0.710
SEM	0.050	0.090	0.130	0.160	0.250

EXPERIMENTALS

1.	0.231	0.201	0.314	0.3987	0.428
2.	0.228	0.131	0.295	0.321	0.491
3.	0.191	0.285	0.214	0.201	0.308
4.	0.185	0.101	0.231	0.385	0.316
5.	0.179	0.291	0.287	0.375	0.491
6.	0.158	0.980	0.271	0.228	0.288
$\bar{X}$	0.190	0.180	0.260	0.310	0.380
SEM	0.080	0.070	0.110	0.130	0.160

APPENDIX F

GLUCOSE CONCENTRATIONS IN CONTROL AND HEXANE-TREATED RATS

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	4.66	5.38	6.26	6.84	6.98
2.	3.31	3.63	4.90	5.70	6.17
3.	4.50	4.69	4.80	5.30	6.00
4.	3.75	4.20	4.70	4.90	5.60
5.	3.13	3.85	3.32	3.47	3.99
$\overline{X}$	3.87	4.35	4.80	5.24	5.75
SEM	0.30	0.31	0.46	0.54	0.49

EXPERIMENTALS

1.	1.00	1.50	1.40	1.50	1.60
2.	1.20	0.70	1.10	1.60	1.90
3.	0.50	1.50	0.70	0.90	1.20
4.	1.30	1.00	0.90	0.90	1.00
5.	1.30	1.40	1.30	1.50	1.60
$\overline{X}$	1.06	1.22	1.08	1.28	1.46
SEM	0.15	0.16	0.12	0.15	0.16

THE EFFECTS OF METHYLENE BLUE, A REDOX AGENT, ON THE GLUCOSE CONCENTRATIONS IN CONTROL (EXPERIMENTAL GROUP 1) AND HEXANE-TREATED RATS (EXPERIMENTAL GROUP 2)

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	1.00	1.50	1.40	1.50	1.60
2.	1.20	0.70	1.10	1.60	1.90
3.	0.50	1.50	0.70	0.90	1.20
4.	1.30	1.00	0.90	0.90	1.00
5.	1.30	1.40	1.30	1.50	1.60
$\bar{X}$	1.06	1.22	1.08	1.28	1.46
SEM	0.15	0.16	0.15	0.15	0.16

EXPERIMENTALS

1.	1.10	1.40	1.40	1.30	1.40
2.	1.20	0.90	1.00	1.70	1.70
3.	1.10	1.80	0.70	0.90	1.20
4.	1.50	1.20	0.90	1.20	1.10
5.	1.30	1.40	1.50	1.50	1.30
$\bar{X}$	1.24	1.34	1.10	1.32	1.34
SEM	0.07	0.14	0.15	0.13	0.10

LACTATE CONCENTRATIONS IN CONTROL AND HEXANE-TREATED RATS

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	1.95	2.14	2.95	3.53	3.14
2.	2.13	2.43	1.91	2.26	3.85
3.	1.21	2.16	2.83	3.18	2.65
4.	1.16	2.33	2.63	2.79	2.13
5.	1.13	2.51	2.99	3.12	3.85
$\bar{X}$	1.52	2.31	2.66	2.98	3.12
SEM	0.21	0.07	0.19	0.21	0.33

EXPERIMENTALS

1.	3.12	2.35	2.82	3.42	2.56
2.	2.83	2.92	3.15	2.67	3.19
3.	2.53	2.46	3.32	2.56	3.07
4.	2.53	2.59	3.15	3.62	2.18
5	2.57	2.83	3.15	3.32	3.14
$\bar{X}$	2.72	2.63	3.12	3.12	2.83
SEM	0.11	0.10	0.08	0.20	0.19

PYRUVATE CONCENTRATIONS IN CONTROL AND HEXANE-TREATED RATS

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	0.159	0.198	0.201	0.201	0.212
2.	0.201	0.219	0.225	0.215	0.191
3.	0.221	0.201	0.215	0.218	0.188
4.	0.221	0.151	0.222	0.151	0.201
5.	0.221	0.215	0.191	0.194	0.215
$\overline{X}$	0.204	0.196	0.210	0.195	0.201
SEM	0.010	0.010	0.004	0.010	0.004

EXPERIMENTALS

1.	0.160	0.125	0.196	0.152	0.180
2.	0.136	0.227	0.173	0.141	0.152
3.	0.109	0.137	0.148	0.224	0.162
4.	0.172	0.140	0.188	0.178	0.133
5.	0.155	0.211	0.165	0.168	0.206
$\overline{X}$	0.146	0.168	0.155	0.172	0.166
SEM	0.008	0.020	0.008	0.010	0.010

LACTATE : PYRUVATE RATIOS IN CONTROL AND HEXANE-TREATED RATS

CONTROLS

Time in minutes

No.	15	30	45	60	75
1.	12.26	10.80	14.76	17.56	14.81
2.	10.59	11.09	8.48	10.51	20.15
3.	5.47	10.74	13.16	14.58	14.09
4.	5.24	15.43	11.84	18.47	10.59
5.	5.11	11.67	15.65	16.08	17.90
$\overline{X}$	7.73	11.95	12.78	15.44	15.51
SEM	1.50	0.88	1.20	1.40	1.60

EXPERIMENTALS

1.	19.50	18.80	14.38	22.50	14.22
2.	20.80	12.86	18.20	18.93	20.98
3.	23.21	17.95	22.43	11.42	18.95
4.	14.70	18.50	16.75	20.33	16.39
5.	16.58	13.41	19.09	19.76	15.24
$\overline{X}$	18.96	16.30	18.17	18.59	17.16
SEM	1.50	1.30	1.30	1.80	1.20

BROMOSULPHTHALEIN CLEARANCE IN CONTROL AND HEXANE-TREATED RATS

CONTROLS

Time in minutes

No.	0	5	10	15	20
1.	0.856	0.523	0.523	0.304	0.066
2.	0.844	0.6222	0.601	0.285	0.086
3.	0.841	0.610	0.521	0.212	0.090
4.	0.849	0.621	0.693	0.218	0.098
5.	0.852	0.682	0.524	0.245	0.096
$\bar{X}$	0.850	0.610	0.572	0.250	0.090
SEM	0.004	0.020	0.004	0.010	0.004

EXPERIMENTALS

1.	0.656	0.253	0.332	0.295	0.287
2.	0.573	0.315	0.326	0.303	0.292
3.	0.633	0.270	0.291	0.280	0.252
4.	0.621	0.322	0.310	0.259	0.254
5.	0.642	0.314	0.302	0.243	0.218
$\bar{X}$	0.630	0.290	0.310	0.280	0.260
SEM	0.010	0.010	0.008	0.008	0.010

BILIARY EXCRETION OF BROMOSULPHTHALEIN IN CONTROL AND HEXANE-TREATED RATS

CONTROLS

Time in minutes

No.	0	5	10	15	20
1.	0.131	0.298	0.391	0.523	0.701
2.	0.091	0.325	0.388	0.618	0.823
3.	0.180	0.316	0.439	0.493	0.719
4.	0.203	0.293	0.516	0.591	0.693
5.	0.078	0.285	0.435	0.602	0.813
$\overline{X}$	0.130	0.300	0.430	0.560	0.740
SEM	0.060	0.130	0.190	0.250	0.340

EXPERIMENTALS

1.	0.281	0.315	0.381	0.392	0.406
2.	0.313	0.287	0.325	0.356	0.392
3.	0.215	0.303	0.369	0.392	0.408
4.	0.320	0.351	0.358	0.408	0.352
5.	0.251	0.362	0.319	0.356	0.428
$\overline{X}$	0.270	0.320	0.350	0.380	0.390
SEM	0.120	0.140	0.150	0.170	0.180