UNIVERSITY OF SOUTHAMPTON

LACTATE METABOLISM IN THE RAT

A thesis submitted for the degree of Master of Philosophy

by Guy Alexander Hewitt Johnson

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UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF MEDICINE

CHEMICAL PATHOLOGY AND HUMAN METABOLISM

MASTER OF PHILOSOPHY

LACTATE METABOLISM IN THE RAT

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Increases in blood lactate concentrations in man may give rise to a severe acidosis. This occurs particularly in patients with diabetes mellitus treated with the oral hypoglycaemic agent phenethylbiguanide (PBG). Lactic acidosis has also been reported in patients receiving parenteral nutrition with solutions containing fructose. The present study was designed to evaluate the use of dichloroacetate, an activator of pyruvate dehydrogenase, in reversing hyperlactataemia in such situations. The work is concerned with experimental elevation of lactate concentrations *in vivo* and in the isolated perfused rat liver, and the subsequent effects of DCA treatment.

Chapter 1 begins with general comments on lactate metabolism and a survey of the current metabolic effects of DCA and also of the three hyperlactataemic agents used in this study, PBG, fructose and galactosamine. Chapter 2 outlines the methods used in this study.

Chapter 3 reports the results of experiments with PBG and DCA in the isolated perfused rat liver. Although both agents had considerable metabolic effects when administered separately, DCA was not effective in reversing the PBG-induced inhibition of lactate clearance.

Chapter 4 is an account of the metabolic effects of fructose in the isolated perfused rat liver. Fructose caused a rapid release of lactate from the liver; however, in these conditions DCA was effective in preventing the rapid generation of lactate.

In Chapter 5 hyperlactataemia in the 24 h starved rat was established by the administration of galactosamine, an amino sugar that causes experimental hepatitis in the rat. The infusion of DCA into these galactosamine treated animals was totally effective in reversing the hepatitis-induced hyperlactataemia, even when fructose was administered in order to raise blood lactate concentrations even further.

The general conclusions are presented in Chapter 6.

ABBREVIATIONS

ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
CoA	coenzyme A
DCA	dichloroacetate
FAD	flavin adenine dinucleotide
GalN	D-galactosamine
GalN-1-P	D-galactosamine-1-phosphate
Gluc	D-glucose
Gluc-1-P	D-glucose-1-phosphate
IMP	inosine monophosphate
NAD	nicotinamide adenine dinucleotide (oxidised)
NADH	nicotinamide adenine dinucleotide (reduced)
NADP	nicotinamide adenine dinucleotide phosphate (oxidised)
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
NEFA	non-esterified fatty acid
PBG	phenethylbiguanide
PDH	pyruvate dehydrogenase
PDHP	pyruvate dehydrogenase phosphate (inactive)
Pi	inorganic phosphate
RNA	ribonucleic acid
SEM	standard error of the mean
TPP	thiamine pyrophosphate
UDP-GalN	uridine diphosphate D-galactosamine
UDP-GalNAc	uridine diphosphate N-acetyl-D-galactosamine
UDP-Gluc	uridine diphosphate D-glucose
UDP-GlucN	uridine diphosphate D-glucosamine
UDP-GlucNAc	uridine diphosphate N-acetyl-D-glucosamine
UMP	uridine monophosphate
UTP	uridine triphosphate
VLDL	very low density lipoprotein

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CHAPTER 1

INTRODUCTION

A. General Comments

B. Regulation of Lactate Metabolism

C. Pharmacological Agents

i) Dichloroacetate
ii) Phenethylbiguanide
iii) Fructose
iv) Galactosamine

INTRODUCTION

A. General Comments

The work presented here represents part of a longer-term project to find out whether dichloroacetate (DCA), an activator of pyruvate dehydrogenase (Whitehouse et al., 1974), may be of use in moderating hyperglycaemia or hyperlactataemia, both of which may occur in certain pathological conditions. In this particular work attempts have been made to set up models of impaired lactate metabolism in the isolated perfused rat liver and whole rat using fructose, galactosamine, and the oral hypoglycaemic agent phenethylbiguanide. These models have been used to investigate the effects of DCA on impaired lactate metabolism. Previous work in our department indicates that DCA may reverse hyperlactataemia caused acutely by phenethylbiguanide infusion into whole rats (Holloway & Alberti, 1975), and also chronically by intraperitoneal injections of phenethylbiguanide (Man & Alberti, 1976). We have also shown it to be hypolactataemic in normal and severely diabetic rats (Blackshear et al., 1974; 1975), and in normal man (Mattrass & Alberti, unpublished work). The four pharmacological agents used are described in more detail in the following sections of this chapter.

B. Regulation of lactate metabolism

The formation of lactate from pyruvate in mammals is an offshoot of the glycolytic pathway and is catalysed by lactate dehydrogenase, requiring NADH as a cofactor. The various reactions by which pyruvate may be formed and disposed of are outlined in Figure 1.1. The lactate dehydrogenase reaction lies close to equilibrium, and the relative amounts of pyruvate and lactate present will therefore depend on the ratio of the concentrations of NAD and NADH, which in turn reflect the redox state of the cell (Williamson et al., 1967a). The formation of lactate during glycolysis enables the regeneration of NAD for the triose phosphate

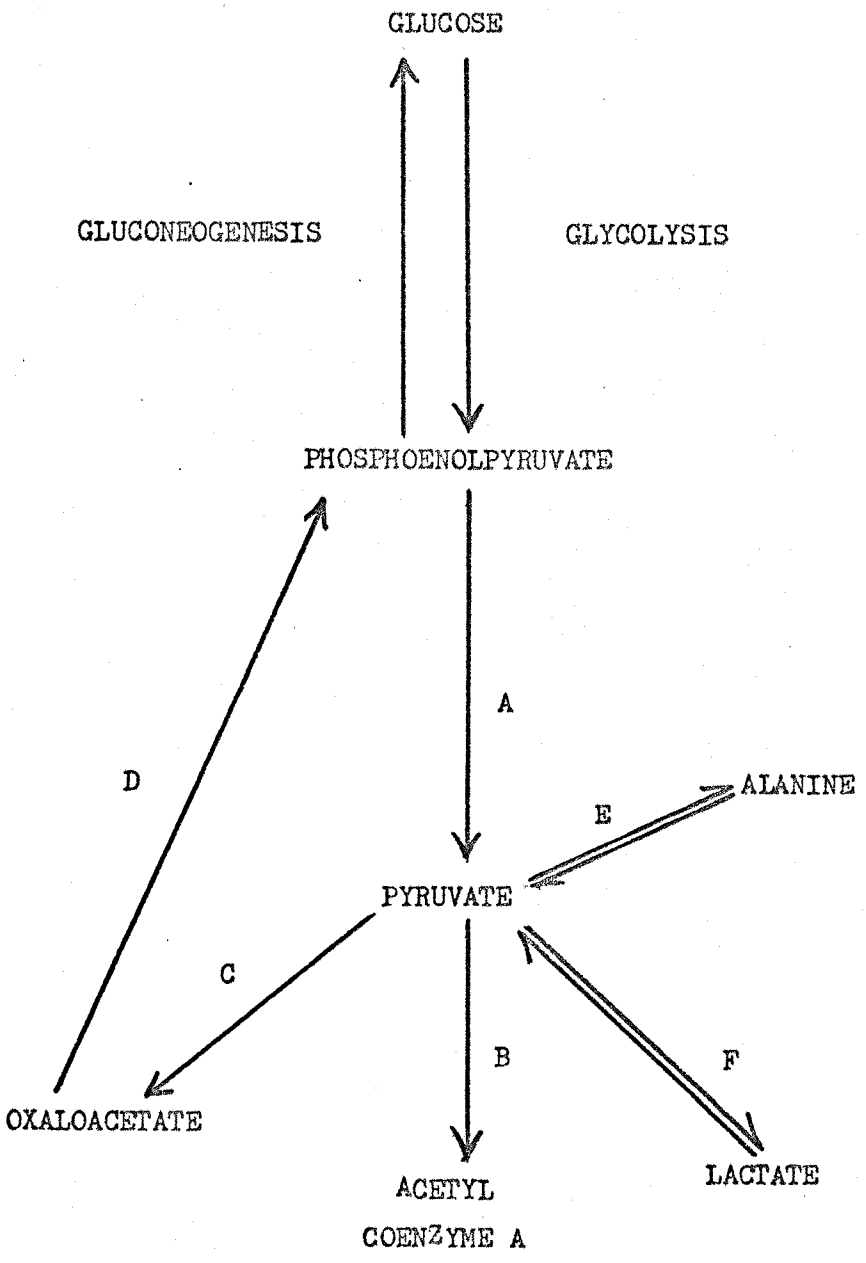


Figure 1.1 The metabolic fates of pyruvate

The reactions by which pyruvate is formed and disposed of in mammalian systems. The enzymes are: A, pyruvate kinase (EC 2.7.1.40); B, pyruvate dehydrogenase (EC 1.2.4.1); C, pyruvate carboxylase (EC 6.4.1.1); D, phosphoenolpyruvate carboxykinase (EC 4.1.1.32); E, alanine aminotransferase (EC 2.6.1.2); F, lactate dehydrogenase (EC 1.1.1.27)

dehydrogenase reaction, and therefore the continuation of glycolysis in anaerobic states.

Lactate is produced by all mammalian tissues, the most important being erythrocytes, brain, skeletal muscle, skin, renal medulla and intestinal mucosa (Kreisberg, 1972). Lactate may be utilised by gluconeogenesis in liver and kidney, and there is a constant cycle (the Cori cycle) involving oxidation of glucose by extrahepatic tissues, release of lactate into the circulation, resynthesis of glucose from lactate in the liver, and the release of glucose into the circulation. Although liver and kidney cortex may produce lactate in certain conditions, they are the only tissues capable of gluconeogenesis, the synthesis of glucose from 3-carbon precursors such as lactate, pyruvate and alanine. Lactate may also be utilised by oxidation through pyruvate dehydrogenase, and this may occur in heart and striated muscle, liver, kidney and brain when lactate concentrations in the blood rise, such as in severe exercise (Krebs, 1972).

Ruderman et al. (1976) have recently reviewed the hormonal control of gluconeogenesis in man. During the postabsorptive period insulin levels fall, and blood glucose concentrations are maintained by the hydrolysis of liver glycogen. In the early phases of starvation glucagon levels rise and the capacity of the liver for gluconeogenesis rises. Glucocorticoids may also play a role in this increase. At the same time the degradation of muscle protein due to decreased insulin and increased cortisol levels provides a supply of lactate, pyruvate and alanine for hepatic gluconeogenesis. Degradation of muscle glycogen may also provide 3-carbon precursors for gluconeogenesis. The synthesis of glucose from lactate, pyruvate and alanine therefore plays a vital role in the maintenance of blood glucose levels, essential for the brain and central nervous system at this stage. As the period of starvation continues the brain is able to cut down its glucose requirement by increasing its capacity for oxidation of an alternative fuel, ketone bodies. There is, however, a continued requirement of glucose by the erythrocyte.

Once oxidised through pyruvate dehydrogenase it is not possible to recover carbohydrate since there can be no net synthesis

of pyruvate from acetyl coenzyme A. In the starved condition the body must therefore conserve as much carbohydrate as possible and this is achieved by two major changes. Firstly, an increase in the rate of lipolysis and ketogenesis provides two alternative fuels, non-esterified fatty acids (NEFA) and ketone bodies. The energy required for hepatic gluconeogenesis is provided by fatty acid oxidation (Parrilla et al., 1976), the rate of which is known to rise in starvation (McGarry et al., 1975). Secondly, the activity of pyruvate dehydrogenase (PDH) in muscle and liver decreases (Wieland et al., 1973), thus sparing pyruvate for gluconeogenesis. PDH is a complex enzyme, its subunit composition is described in Figure 1.2 and its regulation in Figure 1.3. In mammalian systems two types of regulation of the PDH system have been described (Denton & Pogson, 1976): end product inhibition by acetyl CoA and NADH, and the interconversion of a phosphorylated (inactive) and dephosphorylated (active) form by a specific kinase and phosphatase (see Fig. 1.3).

(a) Subunit composition of the PDH complex

60 E_1: Pyruvate decarboxylase each with 2 subunits, α and β

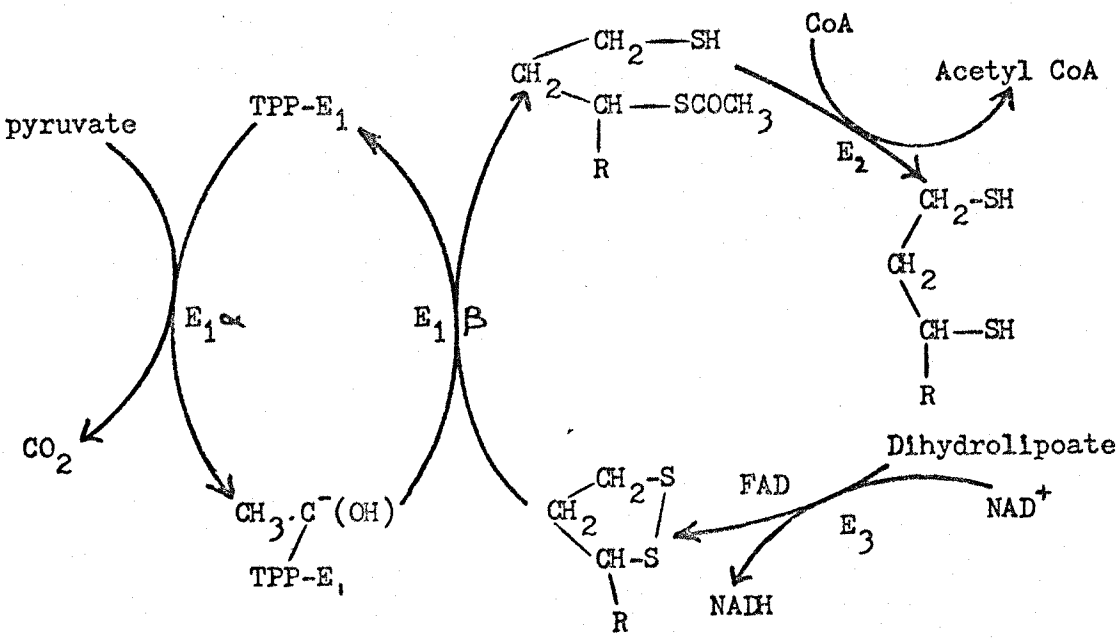
60 E_2: Dihydrolipoate transacetylase

12 E_3: Dihydrolipoate dehydrogenase

PDHP Phosphatase - rather loosely bound

PDH Kinase - tightly bound to E_2, phosphorylates the α-subunit of E_1

(b) The PDH reaction sequence



Net reaction: Pyruvate + NAD^+ + CoA → Acetyl CoA + CO_2 + NADH + H^+

Figure 1.2. The mammalian pyruvate dehydrogenase complex

Data from Denton & Pogson (1976).
(a) Subunit composition and (b) details of the reaction sequence. TPP is thiamine pyrophosphate. Dihydrolipoate is covalently bound to E_2, and visits the active centres of E_1, E_2 and E_3 in turn.
R = $(CH_2)_4COOH$

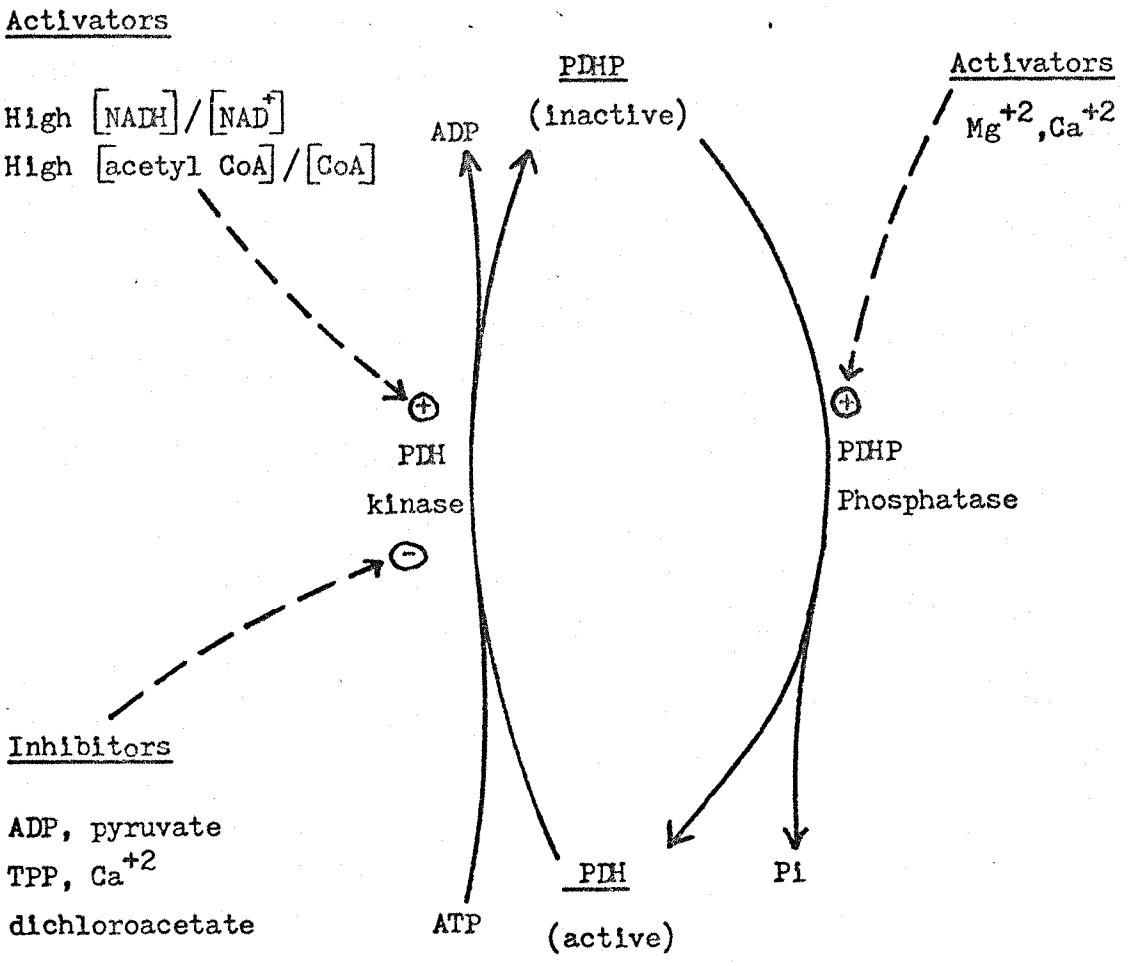


Figure 1.3. Regulation of the mammalian pyruvate dehydrogenase complex

Data from Denton & Pogson (1976) and Whitehouse et al. (1974)

C. Pharmacological agents

(i) Dichloroacetate

The metabolic effects of dichloroacetate (DCA) were first observed by Lorini and Ciman (1962), who injected di-isopropyl-ammonium dichloroacetate into alloxan diabetic rats and found decreased blood glucose concentrations. Stacpoole and Felts (1971) showed that dichloroacetate was the active ingredient, stimulating glucose oxidation and inhibiting oleate oxidation in diaphragms from alloxan diabetic rats. Subsequent experiments on the isolated perfused rat heart (Whitehouse et al., 1973) suggested that DCA activates pyruvate dehydrogenase, and this has now been confirmed by Whitehouse et al. (1974) who found that DCA inhibits pyruvate dehydrogenase kinase from pig and rat heart (Fig. 1.3). DCA increased the proportion of active pyruvate dehydrogenase in the perfused rat heart, isolated rat diaphragm and in rat epidydymal fat pads. Similar effects were observed in mitochondria from rat heart, kidney and fat cells and in extracts of rat heart, psoas muscle, adipose tissue, kidney and liver.

In the 24-hour starved rat DCA lowers blood concentrations of glucose and lactate when injected intraperitoneally (Whitehouse et al., 1974), or into the femoral vein (Blackshear et al., 1974). The hypoglycaemic action of this compound is thought to be due to an interruption of the Cori and alanine cycles (Blackshear et al., 1974; Whitehouse et al., 1974), resulting in deprivation of gluconeogenic precursors for the liver. Direct evidence supporting this idea of precursor deprivation has come from Goodman et al. (1976). Working on the perfused rat hindquarter they observed that DCA inhibits the release of lactate, alanine and pyruvate from hindquarters of fed, starved and diabetic rats. Recent work *in vivo* indicates that DCA lowers blood glucose in the depancreatised dog (Searle et al., 1976) and in diabetic man (Stacpoole et al., 1976).

DCA infusion into the rat (Blackshear et al., 1974) lowers insulin concentrations in blood and increases concentrations of ketone bodies in blood and liver. This hyperketonaemia may be the

result of increased ketogenesis by the liver, secondary to pyruvate dehydrogenase activation, or may be due to the inhibition of peripheral ketone body uptake observed in functionally hepatectomised animals infused with DCA (Blackshear et al., 1974).

Anderson et al. (1975) administered DCA orally and intraperitoneally to normal and diabetic rats over a period of seven days and observed decreased hepatic activities of glucokinase and pyruvate kinase; however, activities of glucose-6-phosphate dehydrogenase and malic enzyme rose. This increase in the capacity for NADPH generation and the increased liver weight observed suggest that lipogenesis may have increased. Evidence to support this comes from the work of Crabb et al. (1976) on isolated rat hepatocytes. They found increased fatty acid synthesis and decreased [1-^{14}C] oleate oxidation to $^{14}CO_2$ in hepatocytes from fed rats. In these experiments DCA did not influence net glucose utilisation in hepatocytes from fed or starved rats. In spite of this gluconeogenesis from lactate was slightly inhibited by DCA in hepatocytes from fasted rats, a result also found by Stacpoole (1977).

Several problems still remain to be resolved. For example it is not yet known whether DCA affects the activity of any other enzyme, and it is also not yet clear how the activation of PDH can result in the inhibition of fatty acid oxidation in isolated hepatocytes (Crabb et al., 1976) or the lowering of blood triglyceride and cholesterol levels in diabetic man (Stacpoole et al., 1976).

(ii) Phenethylbiguanide

The hypoglycaemic properties of guanidine derivatives were first reported by Watanabe (1918) who found that guanidine itself lowered blood sugar levels in laboratory animals and man. Guanidine, however, had intolerable side effects, and so over the years substituted guanidines, diguanides and biguanides have been developed as hypoglycaemic agents (see Schäfer, 1976a). Phenethylbiguanide (PGB) was reported to possess hypoglycaemic properties by Ungar et al. (1957) and in the same year Tyberghein & Williams (1957) discovered that PBG raises blood lactate concentrations in animals. It is now

known that in man this side effect can give rise to a fatal condition, lactic acidosis (see Cohen & Woods, 1976)

Studies in man have shown that PBG therapy can cause hyperlactataemia in normal (Lyngsoe et al., 1972) and diabetic subjects (Nattrass et al., 1977). PBG also increases blood concentrations of other gluconeogenic precursors such as pyruvate, alanine and glycerol in man (Nattrass et al., 1977).

The studies of Ruggles et al. (1968) confirmed that PBG causes hyperlactataemia in animals, while Meyer et al. (1967) found that PBG inhibits gluconeogenesis from pyruvate in rats and guinea pigs in vivo. Wick et al. (1960) demonstrated that ^{14}C-PBG accumulates mainly in liver, muscle and the gastrointestinal tract, and subsequent work has indicated that PBG does act in these tissues. Caspary & Creutzfeld (1968) for example, showed that PBG inhibits glucose absorption by the hamster small intestine, and Czyzyk et al. (1968) have demonstrated the same effect in man. Jangaard et al. (1967) observed that PBG inhibits pyruvate oxidation but stimulates glucose oxidation by minced guinea-pig diaphragms, and more recently Polacek (1974) has shown that PBG lowers blood glucose concentrations in hepatectomised rats infused with glucose, suggesting that the liver and intestine are not the only sites of action of PBG in vivo.

There have been many reports of the effects of PBG on the liver. PBG inhibits gluconeogenesis from lactate in the isolated perfused rat liver (Altschuld & Kruger, 1968) and guinea-pig liver (Haeckel & Haeckel, 1972). Inhibition of gluconeogenesis from fructose and glycerol in the perfused rat liver (Medina et al., 1971) has also been reported. PBG inhibits the Krebs cycle in the perfused rat liver, as reported by Toews et al. (1970), who observed a decrease in $^{14}CO_2$ release from [2 - ^{14}C] pyruvate after PBG administration. Although gluconeogenesis was inhibited in this work, this was not a direct consequence of reduced energy supply since ATP concentrations were not significantly altered.

PBG is an inhibitor of the respiratory chain, and work on isolated mitochondria summarised recently by Schäfer (1976a,b) indicates that PBG exerts its effects on respiration by causing a change in the mitochondrial membrane potential, also resulting in

the inhibition of cation uptake during respiration.

It seems probable that PBG causes lactic acidosis by increasing lactate production by liver and muscle, and also by interfering with hepatic gluconeogenesis. However, there is still some doubt about the mechanism of its interference with gluconeogenesis, possibly because the role of cation transport across the mitochondrial membrane in gluconeogenesis is not yet fully understood.

(iii) Fructose

Fructose metabolism in the rat takes place primarily in the liver, where the enzyme fructokinase rapidly phosphorylates the sugar to fructose-1-phosphate. This compound is then converted to dihydroxyacetone phosphate and glyceraldehyde by aldolase (see Newsholme & Start, 1973). This pathway by-passes phosphofructokinase which is considered to be the major regulatory point in glycolysis, and fructose therefore rapidly gives rise to lactate and pyruvate. The accumulation of lactate and pyruvate after fructose administration has in fact been observed in the isolated perfused rat liver (Woods et al., 1970; Topping & Mayes, 1972; Johnson & Man, 1975), and in the rat *in vivo* (Rawat & Menahan, 1975).

Kidney and intestine also possess some fructokinase activity and may contribute to fructose metabolism. Although some fructose may be converted to fructose-6-phosphate by hexokinase in peripheral tissues, the Km of hexokinase for fructose is considerably higher than that for glucose, and therefore tissues such as muscle are unlikely to metabolise fructose at a significant rate. This may also be because muscle aldolase is only able to split fructose-1-phosphate at 1/50 of the rate of liver aldolase (Freedland & Briggs, 1977).

In man Dodds et al. (1960) discovered that sucrose administration elevates blood lactate and pyruvate concentrations compared with dextrose treated controls, while Woods & Alberti (1972) have reported lactic acidosis in patients receiving parenteral nutrition with solutions containing fructose. The results of fructose administration to man, plus the observations in the rat mentioned

above, led to the choice of fructose as a hyperlactataemic agent in this work.

Fructose also has other effects on the liver. Fructose injection in the rat results in inhibition of protein synthesis, and a loss of ATP and of total adenine nucleotides (Mäenpää et al., 1968). Inhibition of RNA synthesis, an increase in the number of lysozomes, inhibition of bile flow and degranulation of the endoplasmic reticulum have also been observed after intraportal fructose infusions (Bode et al., 1974). Fructose metabolism is also of interest from the nutritional point of view. Fructose feeding to rats increases the activities of the lipogenic enzymes ATP- citrate lyase and glucose-6-phosphate dehydrogenase (Kornacker et al., 1965), and results in hypertriglyceridaemia (Macdonald, 1965). In the isolated perfused rat liver fructose addition results in increased hepatic lipid content (Wimshurst & Manchester, 1973) and increased secretion of triglyceride in VLDL (Topping & Mayes, 1972). Finally, in man sucrose consumption has been linked with hypertriglyceridaemia (Naismith, 1971) and also with hyperinsulinism and coronary heart disease (Yudkin, 1969).

(iv) Galactosamine

The intraperitoneal administration of D(+)-galactosamine (GalN) to the rat results in changes in liver histology that resemble human viral hepatitis (Keppler et al., 1968). GalN is very specific in provoking hepatitis, and of all the rat organs examined only the liver appears to be involved (Keppler et al., 1968). Acute hepatitis develops twenty-four hours after GalN administration. Discontinuation of GalN treatment results in complete recovery within one week, but prolonged treatment over a period of months may result in cirrhosis and hepatoma (Decker et al., 1973).

GalN hepatitis causes increased glutamate dehydrogenase, aspartate aminotransferase and alanine aminotransferase activities and bilirubin concentrations in serum (Keppler et al., 1968). There is also fat accumulation in the liver (Koff et al., 1971a) and impairment of protein synthesis. Bauer et al. (1974) have observed impairment of hepatic protein synthesis and inhibition of

the Golgi complex galactosyltransferases by GalN metabolites; this interferes with glycoprotein secretion.

GalN is converted in the liver to GalN-1-phosphate and to UDP-GalN by galactokinase and galactose-1-phosphate uridylyl-transferase respectively (Fig. 1.4). UDP-GalN is then acetylated and incorporated into glycoproteins. Work on the isolated perfused rat liver (Keppler & Decker, 1969) reveals that GalN treatment causes a build-up of UDP-N-Acetyl hexosamines, and that GalN-1--phosphate inhibits UDP-glucose pyrophosphorylase, resulting in a fall in hepatic levels of UDP-glucose and glycogen.

The primary change after GalN administration is the trapping of uridine phosphates as sugar derivatives (Fig. 1.4), resulting in dramatic falls in hepatic UTP levels (Keppler et al., 1970). The administration of orotate, a precursor of uracil, prevents GalN hepatitis, suggesting that this liver damage may be a consequence of deranged pyrimidine metabolism (Keppler et al., 1970). More recent studies (Hoffman et al., 1975) have confirmed the above findings in isolated hepatocytes.

Monier & Wagle (1971) observed impaired gluconeogenesis in liver slices from rats twenty-four hours after GalN administration, as well as decreased activities of glucose-6-phosphatase, fructose diphosphatase, phosphoenolpyruvate carboxykinase and pyruvate carboxylase. This loss of hepatic phosphoenolpyruvate carboxykinase activity has also been observed by Record et al. (1972). The perfused livers of these GalN-treated rats showed impaired lactate clearance and glucose production. Record et al. (1972) also observed fasting hyperlactataemia in rats treated with GalN. GalN was chosen in the present work because of its ability to impair lactate metabolism by the rat liver.

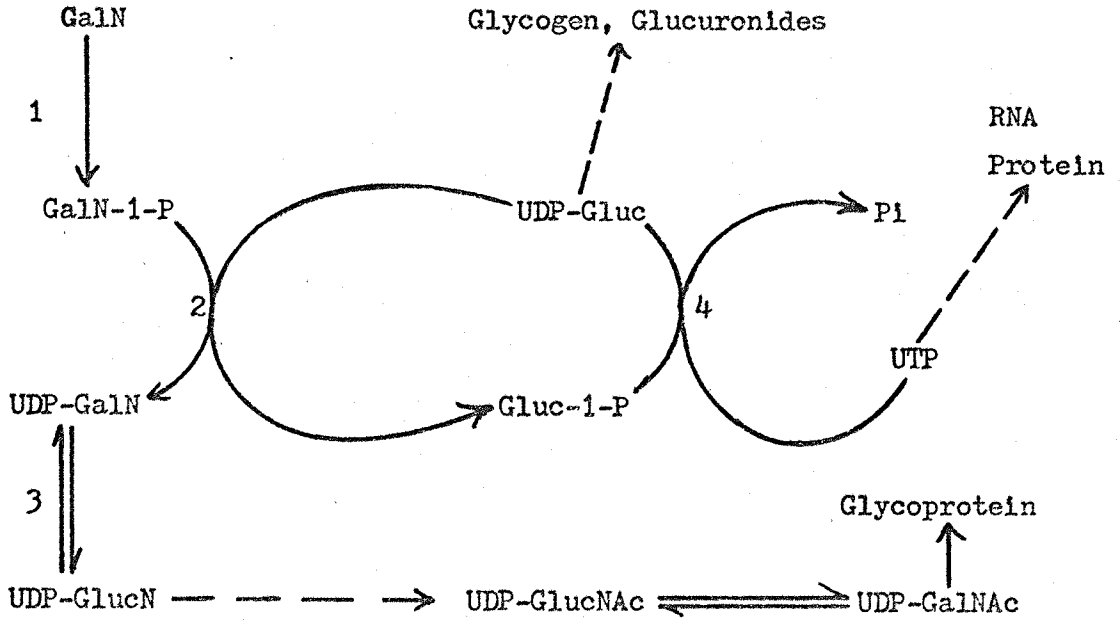


Figure 1.4. D-Galactosamine metabolism in the rat liver

Data from Decker et al. (1973). The numbered reactions are catalysed by the following enzymes. 1: Galactokinase (E.C. 2.7.1.6). 2: UDP-glucose: galactose-1-phosphate uridylyltransferase (E.C. 2.7.7.12). 3: UDP-galactose 4'-epimerase (E.C. 5.1.3.2). 4: UDP-glucose pyrophosphorylase (E.C. 2.7.7.9).

CHAPTER 2

MATERIALS AND METHODS

1. Animal Experiments
   - (a) Rats
   - (b) Liver perfusion
   - (c) Femoral cannulations and infusions
   - (d) Blood sampling
   - (e) Liver sampling

2. Chemical Methods
   - (a) Determination of metabolites
   - (b) Special chemicals
   - (c) Calculations and statistics

METHODS

1. Animal Experiments

(a) Rats. Male Wistar rats weighing 150 to 350 g were used for all experiments and were allowed free access to water and food (Labshure Diet PRD, Christopher Hill Ltd., Poole, Dorset), or were deprived of food, but not water, for 24 or 48 h.

(b) Liver perfusion. Rats were anaesthetised with sodium pentobarbitone (60 mg/kg body wt, intraperitoneally) and livers were perfused in situ via the portal vein by the method of Hems et al. (1966) (see Fig. 2.1). A semi-synthetic perfusion medium containing bovine serum albumin (26 g/l), aged human erythrocytes (12 ml/100 ml) and bicarbonate-buffered saline was used (Krebs & Henseleit, 1932); the composition of this is given in Table 2.1. Livers of 48 h starved rats were perfused with medium alone for 38 minutes, substrates and inhibitors (where used) were then added, three minutes were allowed for mixing, and perfusate samples were taken every ten minutes for one hour. In perfusion of livers from fed rats substrates and inhibitors were added before the start of perfusion, and samples were taken every fifteen minutes for two hours. On certain occasions livers were freeze-clamped at the end of perfusion by the method of Wollenberger et al. (1960) for determination of hepatic metabolites.

(c) Femoral cannulations and infusions. Fed rats were anaesthetised with sodium pentobarbitone (60 mg/kg body wt, intraperitoneally) and polythene cannulae (No. 1619R, Bardic I-Catheter, C.R. Bard International Ltd., Clacton-on-Sea, Essex, U.K.; No. 2FG Intravenous Cannula, Portex Ltd., Hythe, Kent, U.K.) were inserted into the left femoral artery and vein. The rats were then placed in restraining cages (see Fig. 2.2) and deprived of food, but not water, for 24 h. The next day the arterial cannula was flushed with saline (NaCl, 9 g/l) and heparin (100units), and 15 minutes later infusions were begun. NaCl (9 g/l) or dichloroacetate (0.5 M, pH 7.4) were infused at a rate of approximately

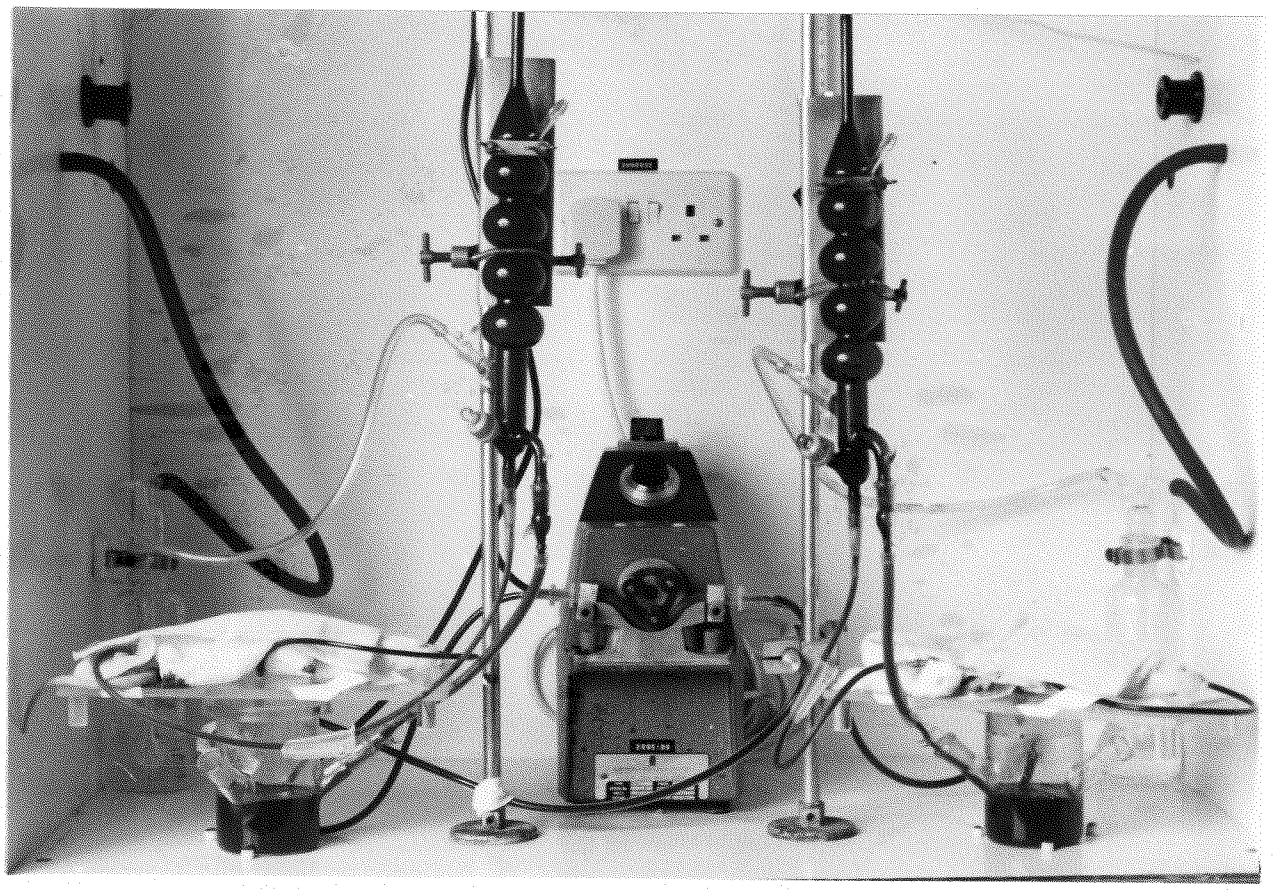


Fig. 2.1. Liver perfusion

The apparatus for liver perfusion as used by Hems et al. (1966)

Table 2.1. Composition of bicarbonate saline

The constituents of the bicarbonate saline used in all perfusion experiments are listed below. This 'saline' was also used in the dialysis of bovine serum albumin and in the washing of red cells. It was gassed with 95% O_2:5% CO_2 before mixing with the albumin and blood cells prior to perfusion.

Constituent	Concentration (mM)
NaCl	119
KCl	4.75
KH_2PO_4	1.19
$MgSO_4$	1.19
$CaCl_2$	2.54
$NaHCO_3$	25.0

Fig. 2.2. Rats in restraining cages

Cannulae were placed in the femoral vessels for infusion and blood sampling

1 ml/h. Where used fructose (1 g per kg body wt) was given as a 2.8 M solution in saline (NaCl, 9 g/l) over a period of two minutes immediately after the basal (zero time) sample was taken. Samples were then taken at 15, 30, 60, 90 and 120 minutes after the start of infusions. The volume of solution infused after 2 hours (2 ml) was approximately equal to the volume of blood drawn from each animal.

(d) Blood sampling. In the whole animal studies arterial blood was drawn into a syringe and a portion of this (0.2 ml) was immediately deproteinised in 2 ml of ice-cold 0.3 M $HClO_4$. In the perfision studies 0.5 ml of perfusate were deproteinised in 4 ml of 0.3 M $HClO_4$. These samples were then centrifuged, the supernatant was neutralised with 1.8 M KOH in the presence of universal indicator, and the $KClO_4$ precipitate was removed by recentrifugation.

(e) Liver sampling. At the end of infusions rats were killed by cervical dislocation, and their livers were rapidly removed and freeze-clamped by the method of Wollenberger et al. (1960). The frozen tissue was transferred to a mortar precooled in liquid nitrogen and ground to a fine powder, 2.5 g of which were homogenised in 10 ml of 0.6 M $HClO_4$ using a Teflon motor driven pestle. A portion (0.5 g) of the frozen powder was shaken with 8 ml of chloroform-methanol (2:1, v/v) for triglyceride determination. The perchloric acid homogenate was centrifuged and the supernatant neutralised with 3.6 M KOH in the presence of universal indicator. This method is described in detail by Williamson et al. (1967a).

2. Chemical Methods

(a) Determination of metabolites. All metabolites were determined by enzymatic methods. These are based on the absorption of the reduced nicotinamide adenine dinucleotides (NADH and NADPH) at 340 nm. Extinctions were measured with a Zeiss PMQ III spectrophotometer using glass cuvettes with a 1 cm light path. The change in extinction observed after addition of an enzyme will be a measure of the NADH (or NADPH) consumed or produced, and this is directly related to the concentration of the metabolite concerned.

The following methods were used: glucose (Slein, 1963); ATP (Lamprecht & Trautschold, 1963); acetoacetate and 3-hydroxybutyrate (Williamson et al., 1962); glycerol (Eggstein & Kreutz, 1966); lactate (Hohorst et al., 1959); pyruvate (Bücher et al., 1963); ADP and AMP (Adam, 1963); glycerol-3-phosphate (Hohorst, 1963a); phosphoenolpyruvate, 2-phosphoglycerate and 3-phosphoglycerate (Czok & Eckert, 1963); citrate (Gruber & Möllering, 1966); 2-oxoglutarate (Bergmeyer & Bernt, 1963); malate (Hohorst, 1963b); L-alanine (Williamson et al., 1967b); L-glutamate (Bernt & Bergmeyer, 1963); L-aspartate (Pfleiderer, 1963); glucose-6-phosphate, fructose-6-phosphate and fructose-1,6-diphosphate (Racker, 1963). Liver triglyceride was measured by the method of Eggstein & Kreutz (1966) and glycogen was assayed as glucose after incubation with α-amylo-1,6-glucosidase (Keppler & Decker, 1974).

(b) Special chemicals. All enzymes and coenzymes were supplied by the Boehringer Corporation Ltd., Lewes, Sussex. D(+)-galactosamine was supplied by the Sigma Chemical Co. Ltd., Kingston-upon-Thames, Surrey. Pentobarbitone sodium (Nembutal) was supplied by Abbot Laboratories Ltd., Queensborough, Kent. Heparin (5000 U/ml) was from Weddell Pharmaceuticals, London. Dichloroacetic acid and Florisil were from B.D.H. Chemicals Ltd., Poole, Dorset. Bovine serum albumin was from Miles Laboratories Ltd., Stoke Poges, Bucks, and was dialysed against 3 volumes of bicarbonate saline at 4°C.

(c) Calculations and statistics. Assessment of significance between means was performed using Students' t-test for unpaired populations. Results are expressed as means ± SEM.

CHAPTER 3

PHENETHYLBIGUANIDE

1. Results

Livers from 48 hour starved rats: perfusate concentrations

(a) Dichloroacetate
(b) Phenethylbiguanide
(c) Dichloroacetate plus phenethylbiguanide

Livers from 48 hour starved rats: liver metabolite concentrations

(a) Dichloroacetate
(b) Phenethylbiguanide
(c) Dichloroacetate plus phenethylbiguanide

Livers from fed rats: perfusate concentrations

(a) Dichloroacetate
(b) Phenethylbiguanide
(c) Dichloroacetate plus phenethylbiguanide

Livers from fed rats: liver metabolite concentrations

(a) Dichloroacetate

2. Discussion

(a) Dichloroacetate
(b) Phenethylbiguanide
(c) Dichloroacetate plus phenethylbiguanide

PHENETHYLBIGUANIDE AND DICHLOROACETATE

Isolated livers of fed rats were perfused with 2 mM phenethylbiguanide in order to increase lactate production. Attempts were made to reverse this effect by the addition of 10 mM dichloroacetate (DCA). Livers of 48 hour starved rats were perfused with 10 mM sodium L-lactate. Phenethylbiguanide (PBG) was added (1 or 2 mM) in order to inhibit lactate uptake by the liver, and dichloroacetate (10 mM) was added alone and with PBG to try to prevent the inhibition of lactate uptake. At the end of perfusions livers were freeze-clamped for the determination of hepatic metabolites. These experiments were done to see whether DCA exerts any of its in vivo effects through the liver.

1. RESULTS

Livers from 48 hour starved rats: perfusate concentrations

(a) Dichloroacetate. When 10 mM L-lactate was used as substrate, DCA (10 mM) caused a 33% increase in the rate of lactate removal compared with control livers (Table 3.1, Fig. 3.1), and the appearance of pyruvate in the perfusion medium was markedly decreased (Fig. 3.2). Glucose production from lactate did not increase, but there was a threefold increase in ketone body production (Fig. 3.1; Table 3.1). Changes in the perfusate concentrations of lactate, glucose and ketone bodies are shown in Figures 3.3, 3.4 and 3.5 respectively. The [lactate]/[pyruvate] ratio in the perfusate increased significantly with DCA (Fig. 3.6), and there was also a small increase in the [3-hydroxybutyrate]/[acetoacetate] ratio (Fig. 3.7).

(b) Phenethylbiguanide (Table 3.1; Figs. 3.1, 2, 3, 4 and 5) PBG (1 mM) had no significant effect on lactate uptake or glucose production although ketone body appearance in the perfusion medium increased. The perfusate [lactate]/[pyruvate] ratio increased (Fig. 3.6), as did the [3-hydroxybutyrate]/[acetoacetate] ratio (Fig. 3.7).

Table 3.1. Effect of DCA and PBG on lactate uptake and glucose and ketone body production by perfused livers from 48 h starved rats

All perfusions were given a 10 mM lactate load and perfused for 60 minutes, DCA and/or PBG were added with the lactate. See the text for details. Results are means ± S.E.M., with the number of livers in parentheses. No differences between groups D and F were significant. ***, $p < 0.001$; **, $p < 0.01$ versus group A. ††, $p < 0.01$; †, $p < 0.05$ versus group C.

Group	Additions (mM)		Metabolite changes (μmol/h per g fresh liver)		
	DCA	PBG	Lactate uptake	Glucose production	Ketone body production
A	0	0	101 ± 3 (12)	36.7 ± 3.2(12)	3.8 ± 0.5(7)
B	10	0	134 ± 6 (11)***	40.3 ± 6.7(11)	11.4 ± 1.5(9)***
C	0	1	92.8 ± 8.6(12)	27.8 ± 3.3(12)	9.1 ± 1.6(12)**
D	0	2	55.5 ± 4.7 (5)***	6.5 ± 2.8 (5)***	13.7 ± 2.6(5)**
E	10	1	90.3 ± 9.4 (7)	16.0 ± 4.1 (7)†	20.5 ± 2.4(7)††
F	10	2	50.6 ± 6.5 (4)	0.0 ± 2.6 (4)	20.7 ± 7.2(3)

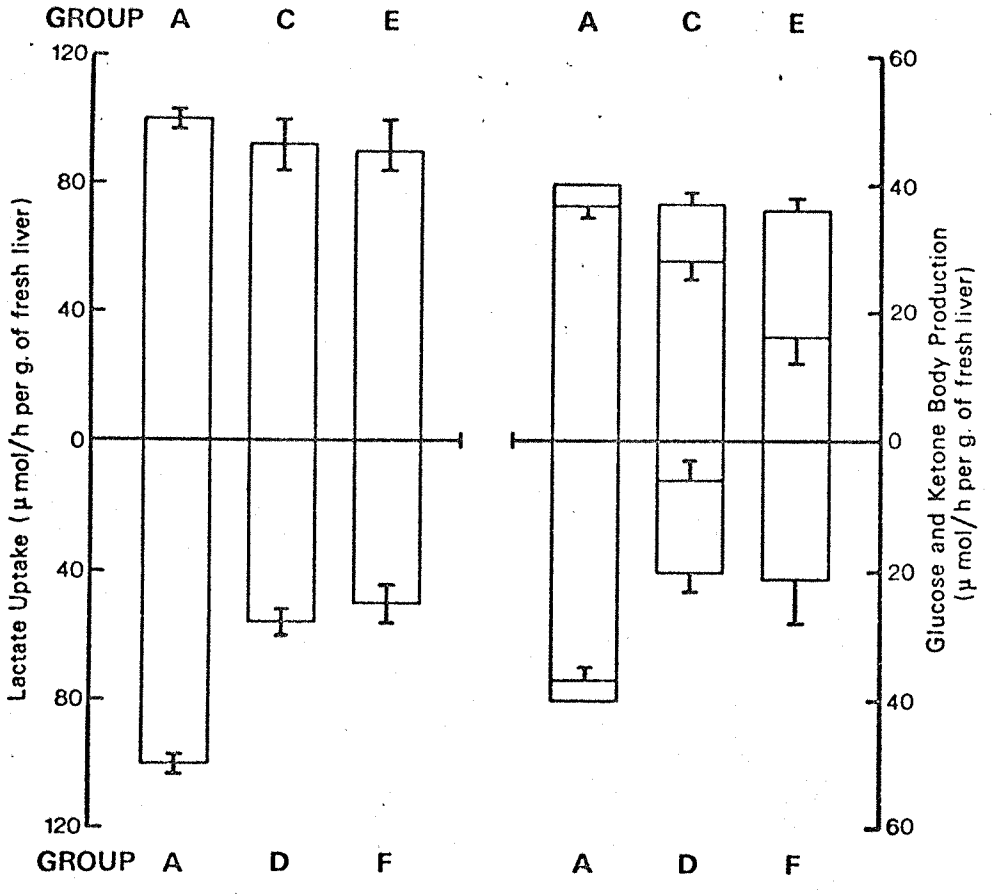


Figure 3.1. Effect of DCA and PBG on lactate uptake and glucose and ketone body production by perfused livers from 48 h starved rats.

The data are derived from Table 1 and are presented as a histogram to show the effects of DCA and PBG on the fate of the lactate load. The results are means ± S.E.M., the groups are as defined in Table 1. On the right hand side of the diagram, the areas with standard error bars pointing towards the horizontal axis represent glucose production.

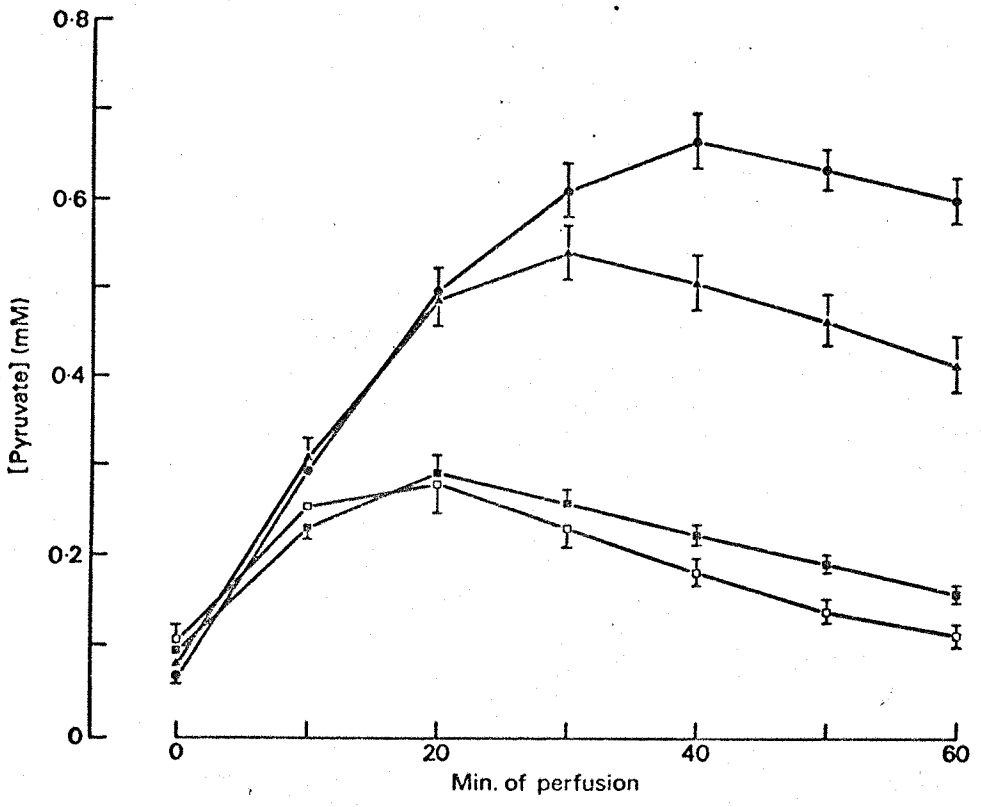


Figure 3.2. The effects of DCA and PBG on pyruvate concentrations in the perfusion medium of livers from 48 h starved rats

The lactate load was given 3 mins before zero time, details as in Table 3.1. The groups are: ●, controls; □, 10 mM DCA; ▲, 1 mM PBG; ■, 1 mM PBG plus 10 mM DCA. Probabilities of statistical significance are presented as percentages below.

Time →	20	30	40	50	60
DCA vs. control	0.1	0.1	0.1	0.1	0.1
PBG vs. control	NS	NS	1	1	1
PBG + DCA vs. PBG	0.1	0.1	0.1	0.1	0.1

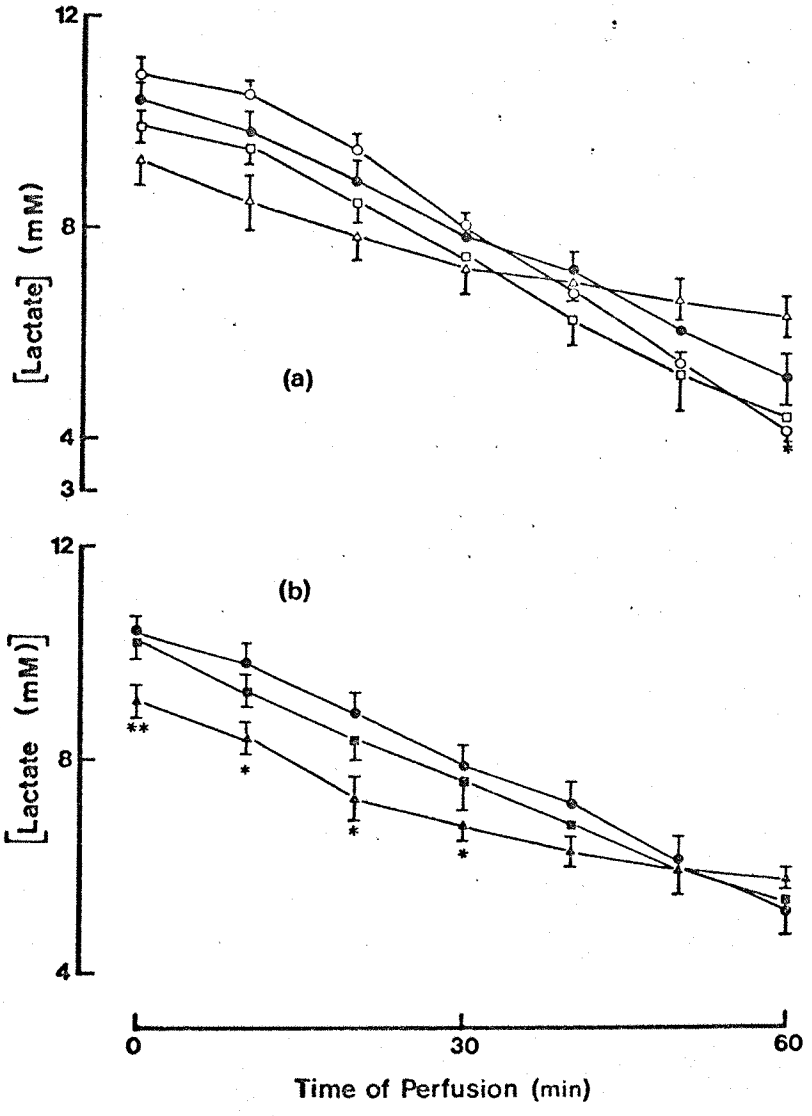


Figure 3.3. The effects of DCA and PBG on lactate uptake by perfused livers of 48 h starved rats

Experimental conditions are described in Table 3.1.
(a) ●, controls (10 mM lactate alone); ○, 10 mM DCA (*, $p < 0.05$ vs. control); □, 1 mM PBG plus 10 mM DCA; △, 2 mM PBG plus 10 mM DCA. (b) ●, controls; ■, 1 mM PBG; ▲, 2 mM PBG (*, $p < 0.05$; **, $p < 0.01$ vs. control)

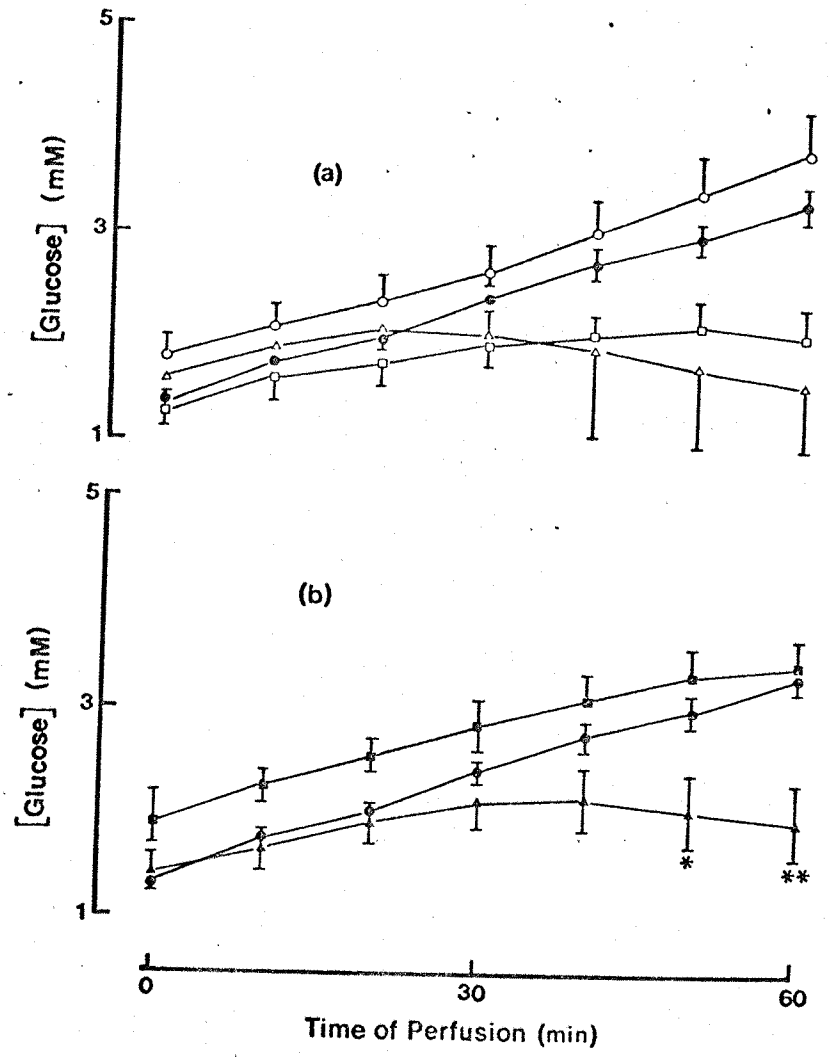


Figure 3.4. The effects of DCA and PBG on glucose production by perfused livers of 48 h starved rats

Experimental conditions are described in Table 3.1. Symbols as in Figure 3.3; *, $p < 0.05$ and **, $p < 0.01$ vs. control.

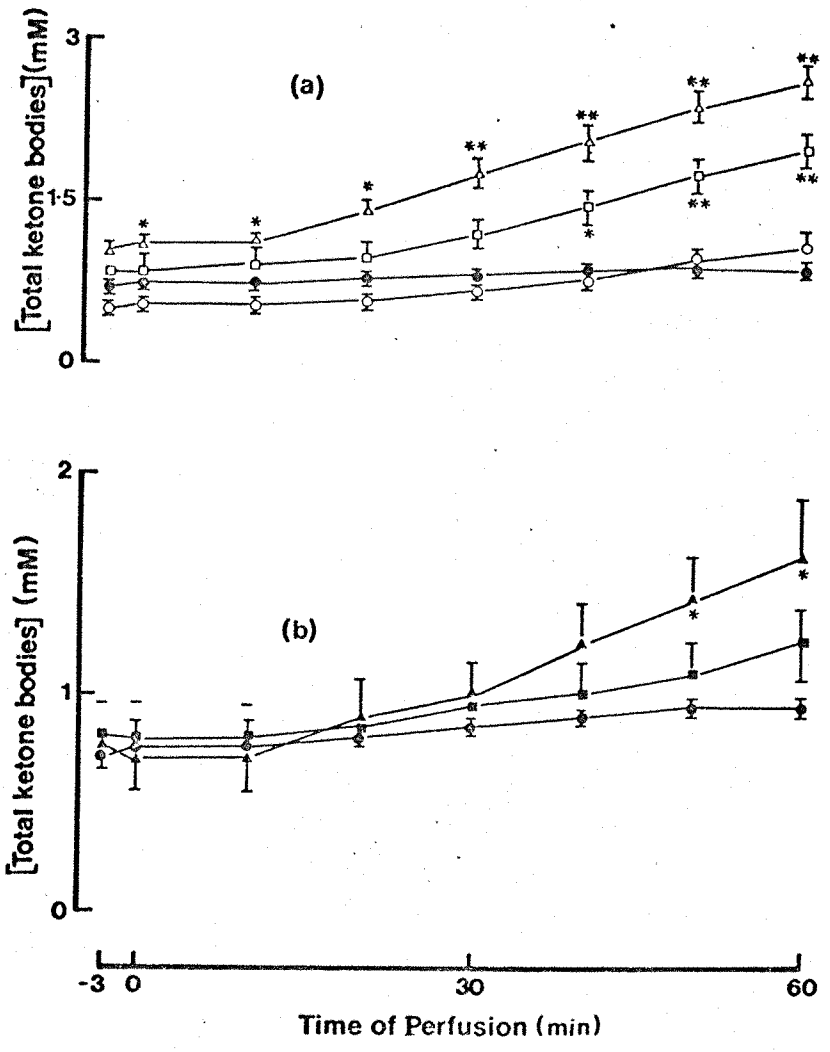


Figure 3.5. The effects of DCA and PBG on ketone body production by perfused livers of 48 h starved rats

Experimental conditions are as in Table 3.1, symbols and groups as in Figure 3.3. *, $p < 0.05$; **, $p < 0.01$ vs. controls (●) in both (a) and (b).

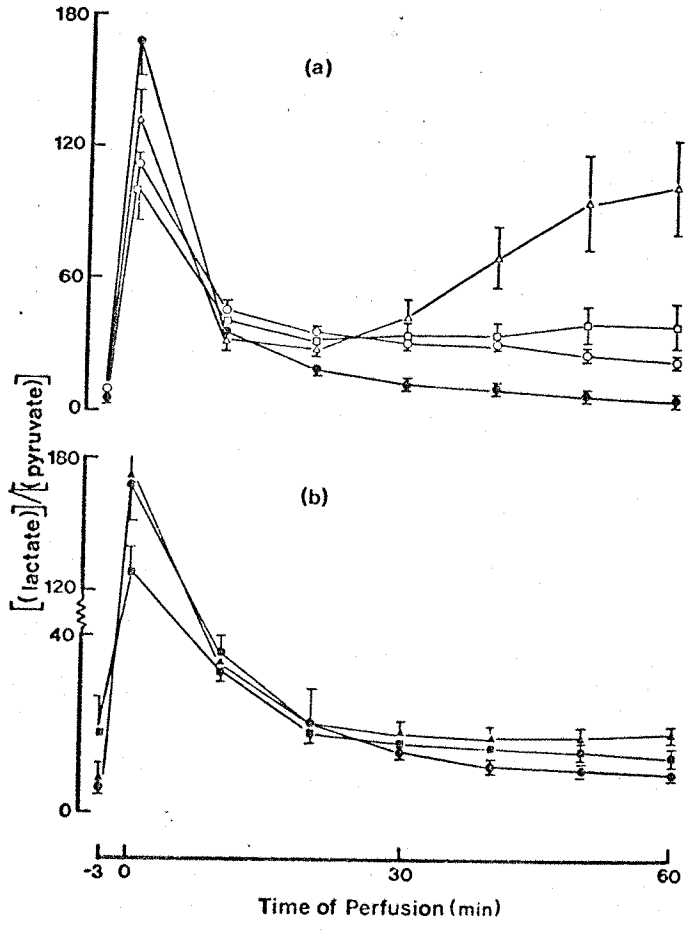


Figure 3.6. The effects of DCA and PBG on the [lactate]/[pyruvate] ratio in the perfusion medium of livers from 48 h starved rats

Experimental conditions as in Table 3.1, symbols as in Figure 3.3

Probabilities of statistical significance are presented below.

Time →	30	40	50	60
○ DCA vs control ●	0.001	0.001	0.001	0.001
■ 1 mM PBG vs control ●	NS	0.05	0.05	0.05
▲ 2 mM PBG vs control ●	NS	NS	NS	0.05
1 mM PBG + DCA vs 1 mM PBG	0.01	0.01	0.01	0.01
2 mM PBG + DCA vs 2 mM PBG	NS	0.01	0.01	0.01

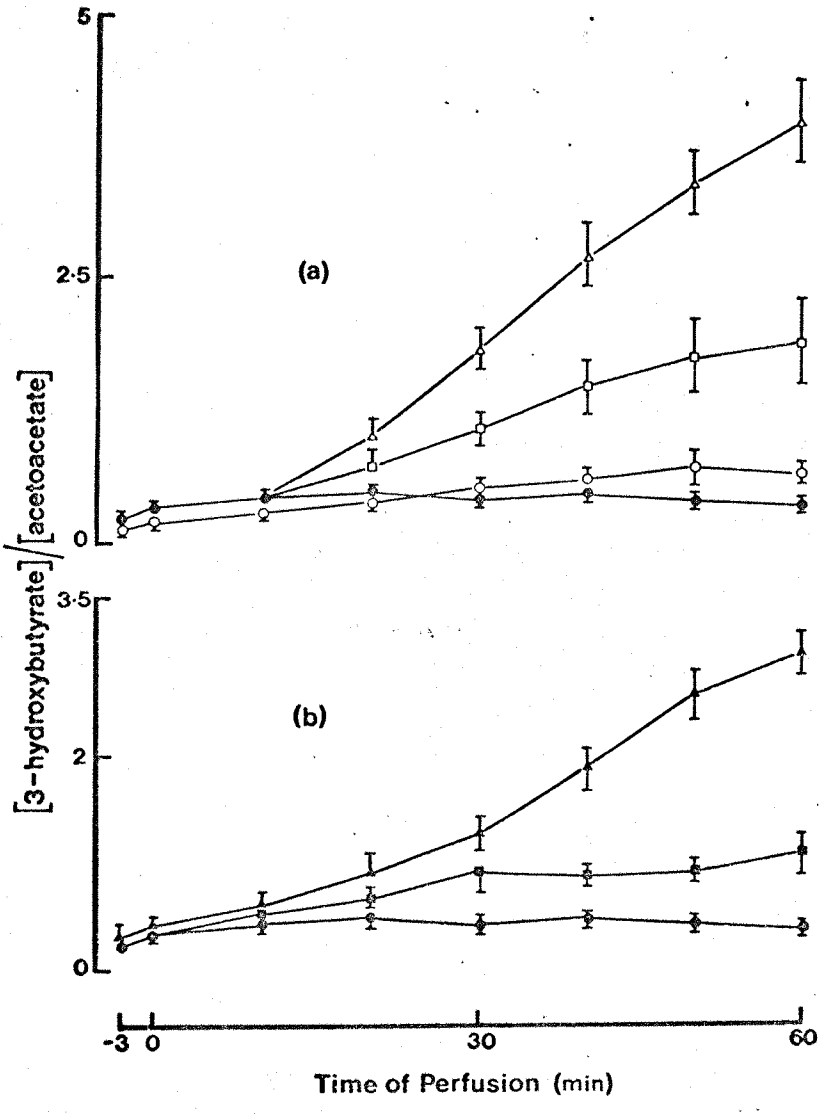


Figure 3.7 The effects of DCA and PBG on the [3-hydroxybutyrate]/[acetoacetate] ratio in the perfusion medium of livers from 48 h starved rats

Experimental conditions as in Table 3.1, symbols as in Figure 3.3
Probabilities of statistical significance are presented below.

Time →	30	40	50	60
○ DCA vs control ●	NS	NS	0.05	0.05
■ 1 mM PBG vs control ●	NS	0.01	0.01	0.01
▲ 2 mM PBG vs control ●	0.001	0.001	0.001	0.001
□ 1 mM PBG + DCA vs 1 mM PBG	NS	NS	0.05	NS
△ 2 mM PBG + DCA vs 2 mM PBG	NS	NS	NS	0.05

Table 3.2. The effects of DCA and PBG on concentrations of metabolic intermediates in livers of 48 h starved rats

Livers were given a lactate load 3 min before zero time, and were freeze-clamped after 60 min of perfusion, as described in the text. DCA (10 mM) and PBG (1 or 2 mM) were added where appropriate, results are expressed as μmol/g fresh liver ± SEM, and the significance of differences between means is presented in Table 3.3. Glycogen content is expressed as μmol of glucose per g fresh liver

	Group					
Metabolite	A	B	C	D	E	F
Glycogen	0.96 ± 0.24	1.86 ± 0.60	2.45 ± 1.46	0.08 ± 0.04	0.34 ± 0.06	2.39 ± 1.14
Glucose	2.50 ± 0.05	2.96 ± 0.16	2.78 ± 0.42	1.99 ± 0.20	1.82 ± 0.41	4.09 ± 0.89
Glucose-6-phosphate	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01
Glycerol-1-phosphate	0.24 ± 0.06	0.32 ± 0.05	0.29 ± 0.04	0.19 ± 0.01	0.54 ± 0.05	0.32 ± 0.03
3-Phosphoglycerate	0.38 ± 0.04	0.16 ± 0.01	0.56 ± 0.04	0.87 ± 0.07	0.24 ± 0.03	0.31 ± 0.09
2-Phosphoglycerate	0.07 ± 0.01	0.03 ± 0.00	0.07 ± 0.01	0.16 ± 0.02	0.03 ± 0.01	0.06 ± 0.01
Phosphoenolpyruvate	0.17 ± 0.02	0.08 ± 0.00	0.27 ± 0.03	0.72 ± 0.11	0.10 ± 0.01	0.15 ± 0.04
Pyruvate	0.25 ± 0.03	0.12 ± 0.01	0.15 ± 0.02	0.25 ± 0.02	0.11 ± 0.01	0.13 ± 0.02
Lactate	2.25 ± 0.24	1.77 ± 0.32	2.61 ± 0.42	4.52 ± 0.12	3.00 ± 0.52	5.79 ± 0.47
Alanine	1.37 ± 0.10	0.51 ± 0.10	1.42 ± 0.15	0.58 ± 0.15	2.78 ± 0.07	1.21 ± 0.08
Citrate	0.63 ± 0.07	0.91 ± 0.09	0.38 ± 0.09	0.13 ± 0.01	0.32 ± 0.07	0.05 ± 0.01
2-Oxoglutarate	0.29 ± 0.03	0.39 ± 0.09	0.12 ± 0.01	0.11 ± 0.01	0.08 ± 0.02	0.04 ± 0.01
Glutamate	2.57 ± 0.21	2.45 ± 0.07	2.11 ± 0.06	1.93 ± 0.15	1.78 ± 0.28	0.70 ± 0.03
Malate	0.45 ± 0.06	0.38 ± 0.06	0.25 ± 0.02	0.31 ± 0.03	0.45 ± 0.04	0.19 ± 0.02
Acetoacetate	0.45 ± 0.06	0.38 ± 0.06	0.25 ± 0.02	0.31 ± 0.03	0.45 ± 0.04	0.19 ± 0.02
3-Hydroxybutyrate	0.41 ± 0.06	0.36 ± 0.04	0.28 ± 0.02	0.49 ± 0.04	0.87 ± 0.09	0.81 ± 0.03
Total ketone bodies	0.86 ± 0.08	0.74 ± 0.06	0.52 ± 0.02	0.80 ± 0.04	1.32 ± 0.09	1.00 ± 0.05
$\frac{[3\text{-Hydroxybutyrate}]}{[\text{Acetoacetate}]}$	1.00 ± 0.2	1.1 ± 0.2	1.1 ± 0.1	1.6 ± 0.2	2.0 ± 0.3	4.4 ± 0.3
$\frac{[\text{Lactate}]}{[\text{Pyruvate}]}$	8.8 ± 0.6	14 ± 2	16 ± 3	19 ± 2	28 ± 6	48 ± 7
AMP	0.61 ± 0.07	0.49 ± 0.08	0.53 ± 0.07	0.68 ± 0.04	0.69 ± 0.07	1.04 ± 0.05
ADP	1.63 ± 0.15	1.41 ± 0.07	1.55 ± 0.05	2.01 ± 0.08	1.71 ± 0.04	1.52 ± 0.17
ATP	1.43 ± 0.09	1.56 ± 0.18	1.33 ± 0.16	0.76 ± 0.10	1.21 ± 0.19	0.27 ± 0.06
Total adenine nucleotides	3.67 ± 0.20	3.47 ± 0.17	3.41 ± 0.13	3.46 ± 0.16	3.60 ± 0.25	2.83 ± 0.19
[ATP] / [ADP]	0.91 ± 0.12	1.12 ± 0.16	0.86 ± 0.11	0.37 ± 0.04	0.71 ± 0.12	0.18 ± 0.02
Triglyceride	7.84 ± 0.86	7.33 ± 0.57	8.80 ± 0.68	6.78 ± 0.65	9.96 ± 1.13	8.39 ± 1.08
n	5	5	4	4	4	4
Additions DCA	0	10	0	0	10	10
(mM) PBG	0	0	1	2	1	2

Table 3.3. Probabilities of significance of the differences in the values presented in Table 3.2

*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

	AvsB	AvsC	AvsD	AvsE	AvsF	BvsE	CvsE	BvsF	DvsF
Glycogen			**	*	**	*			
Glucose	*		*			*			
Glucose-6-phosphate			**			*	*	*	
Glycerol-1-phosphate				**		*	**		**
3-Phosphoglycerate	**	*	**	*		*	***		**
2-Phosphoglycerate	**		**	**			***	*	**
Phosphoenolpyruvate	**	*	**	*			***		**
Pyruvate	**	*		**	**				**
Lactate			***		***			***	*
Alanine	***		***	***			**	**	***
Citrate	*		***	*	***	**		***	***
2-Oxoglutarate		**	***	***	***	*		**	***
Glutamate			*		***			***	***
Malate	***	**			**			***	**
Acetoacetate		**			**		**	*	**
3-Hydroxybutyrate				**	***	**	***	***	***
Total ketone bodies		**		**		***	***	**	*
$\frac{[\text{3-Hydroxybutyrate}]}{[\text{Acetoacetate}]}$				*	***	*	*	***	***
$\frac{[\text{Lactate}]}{[\text{Pyruvate}]}$		*	***	*	***			**	**
AMP					**			***	***
ADP						**	*		*
ATP			**		***			***	**
Total adenine nucleotides					*			*	*
[ATP]/[ADP]			**		***			***	**
Triglyceride						*			

In contrast 2 mM PBG inhibited both lactate uptake and glucose production, and ketogenesis increased still further. The [lactate]/pyruvate and [3-hydroxybutyrate]/[acetoacetate] ratios in the perfusate also increased (Figs. 3.6 and 3.7 respectively).

(c) Dichloroacetate plus phenethylbiguanide (Table 1; Figs. 3.1, 2, 3, 4 and 5). In the presence of 1 mM PBG, DCA (10 mM) no longer increased lactate uptake. Glucose production decreased compared with PBG alone, DCA alone and controls, while the increase in ketogenesis was greater than that observed with 1 mM PBG or 10 mM DCA alone. Figure 3.1 shows that although total lactate removal accountable as ketone bodies plus glucose remains constant, there appears to be some diversion of carbon from gluconeogenesis to ketogenesis.

When livers were perfused with 2 mM PBG and 10 mM DCA lactate removal was the same as with 2 mM PBG alone, glucose production decreased yet further, and ketone body production increased (Table 3.1; Fig. 3.1). The [lactate]/[pyruvate] and [3-hydroxybutyrate]/[acetoacetate] ratios were greater than those observed with 10 mM DCA or 2 mM PBG alone.

Livers from 48 hour starved rats: liver metabolite concentrations

(a) Dichloroacetate. DCA (10 mM) (Table 3.2) caused a slight increase in hepatic glucose concentration compared with control perfusions, but concentrations of pyruvate, alanine, phosphoenolpyruvate, 2-phosphoglycerate and 3-phosphoglycerate fell. Malate and citrate concentrations increased but there was no change in those of adenine nucleotides, triglyceride or glycogen.

(b) Phenethylbiguanide (Table 3.2) PBG administration (1 mM) increased hepatic concentrations of phosphoenolpyruvate, 3-phosphoglycerate and malate, and the [lactate]/[pyruvate] ratio also rose. The concentration of 2-oxoglutarate fell, but there was no change in [ATP] or the [ATP]/[ADP] ratio.

More marked changes were seen with 2 mM PBG (Table 3.2). In addition to the changes observed with 1 mM PBG, there were increases in the hepatic concentrations of 2-phosphoglycerate, lactate and

alanine while those of glucose, glucose-6-phosphate, citrate glutamate and ATP, and the [ATP]/[ADP] ratio decreased.

(c) Dichloroacetate plus phenethylbiguanide (Table 3.2). When 10 mM DCA was used together with 1 mM PBG, concentrations of phosphoenolpyruvate, 2-phosphoglycerate and 3-phosphoglycerate decreased, but to a lesser extent than with DCA alone. Levels of glucose-6-phosphate and alanine decreased compared with controls and DCA alone, while [pyruvate] was unchanged, and [citrate] and [2-oxoglutarate] decreased as with PBG alone.

In the presence of 2 mM PBG and 10 mM DCA (Table 3.2) hepatic concentrations of citrate, 2-oxoglutarate, malate, glutamate and ATP were all extremely low, [lactate] increased, and [pyruvate] decreased. Concentrations of phosphoenolpyruvate, 2-phosphoglycerate and 3-phosphoglycerate were similar to the control values, i.e. midway between PBG and DCA alone.

Livers from fed rats: perfusate concentrations

(a) Dichloroacetate. In the presence of 10 mM DCA concentrations of lactate and pyruvate in the medium (Fig. 3.8) fell dramatically compared with controls, while glucose and ketone body levels did not change significantly (Fig. 3.9). DCA initially increased the [lactate]/[pyruvate] and [3-hydroxybutyrate]/[acetoacetate] ratios (Fig. 3.10), but at the end of perfusions these ratios fell to below control levels.

(b) Phenethylbiguanide. PBG (2 mM) treatment resulted in a considerable increase in perfusate lactate (Fig. 3.8), glucose and ketone body (Fig. 3.9) concentrations as well as substantial increases in the [lactate]/[pyruvate] and [3-hydroxybutyrate]/[acetoacetate] ratios (Fig. 3.10).

(c) Dichloroacetate and phenethylbiguanide (Table 3.4). In the presence of 2 mM PBG, DCA (10 mM) did not significantly alter lactate or glucose concentrations or the [3-hydroxybutyrate]/[acetoacetate] ratio compared with 2 mM PBG alone. Pyruvate

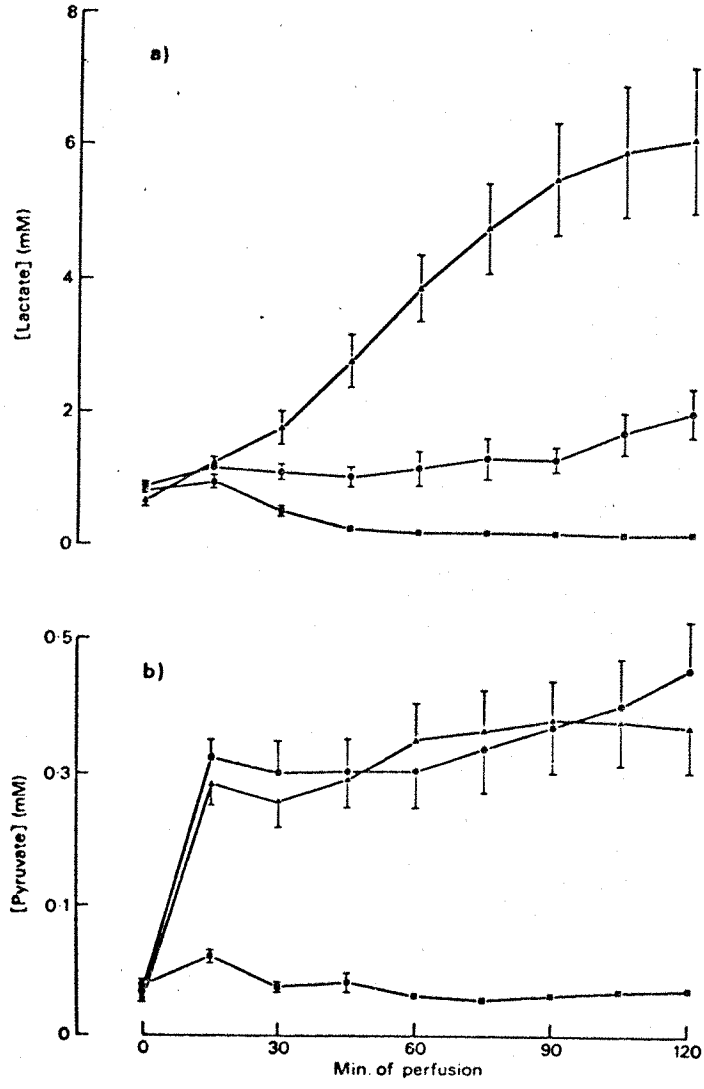


Figure 3.8. Effect of DCA and PBG on (a) lactate and (b) pyruvate concentrations in the perfusion medium: fed rats

Livers from fed rats were perfused with no addition (●, n = 10), 10 mM DCA (■, n = 9) or 2 mM PBG (▲, n = 9). See text for experimental details. On (a) all points from 45 min onwards are significant ($p < 0.001$) when comparing the DCA or PBG groups with the control group. On (b) all points from 15 min onwards are significant ($p < 0.001$) when comparing the DCA group with controls. None of the PBG values are significant compared with controls.

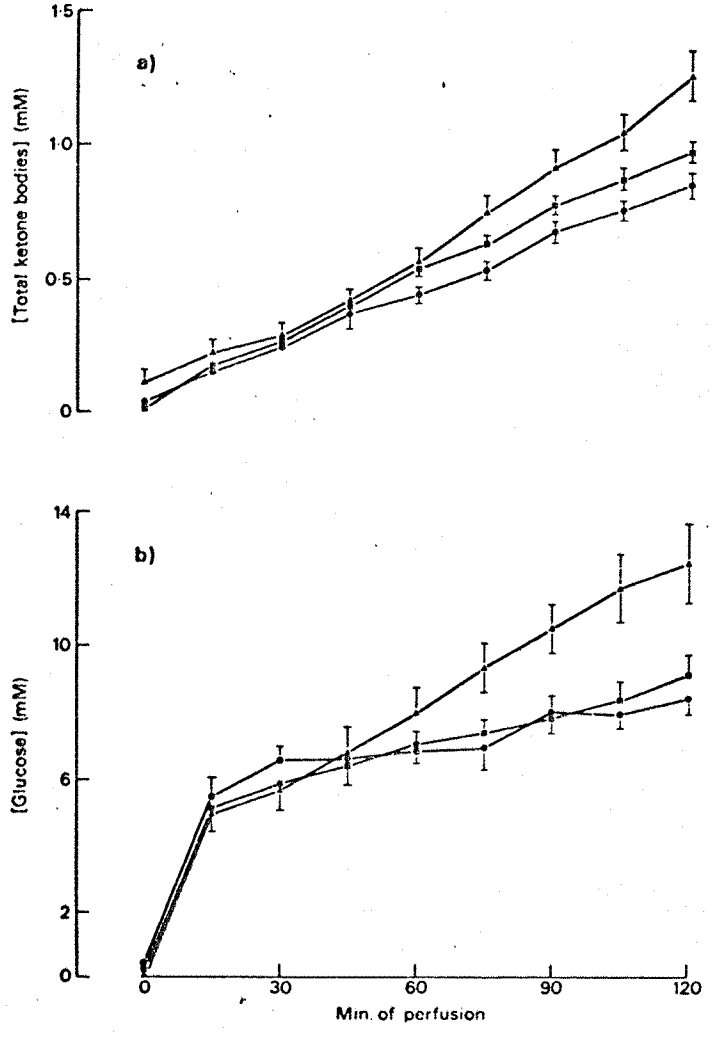


Figure 3.9. Effect of DCA and PBG on (a) ketone body and (b) glucose concentrations in the perfusion medium: fed rats

Livers from fed rats were perfused as described in Figure 3.8. In (a) for the control groups n = 5 and for the DCA group n = 4. In both (a) and (b) all points from 75 min onwards are significant ($p < 0.05$) when comparing PBG (▲) with controls (●). No DCA (■) points are significant compared with controls in (a) or (b).

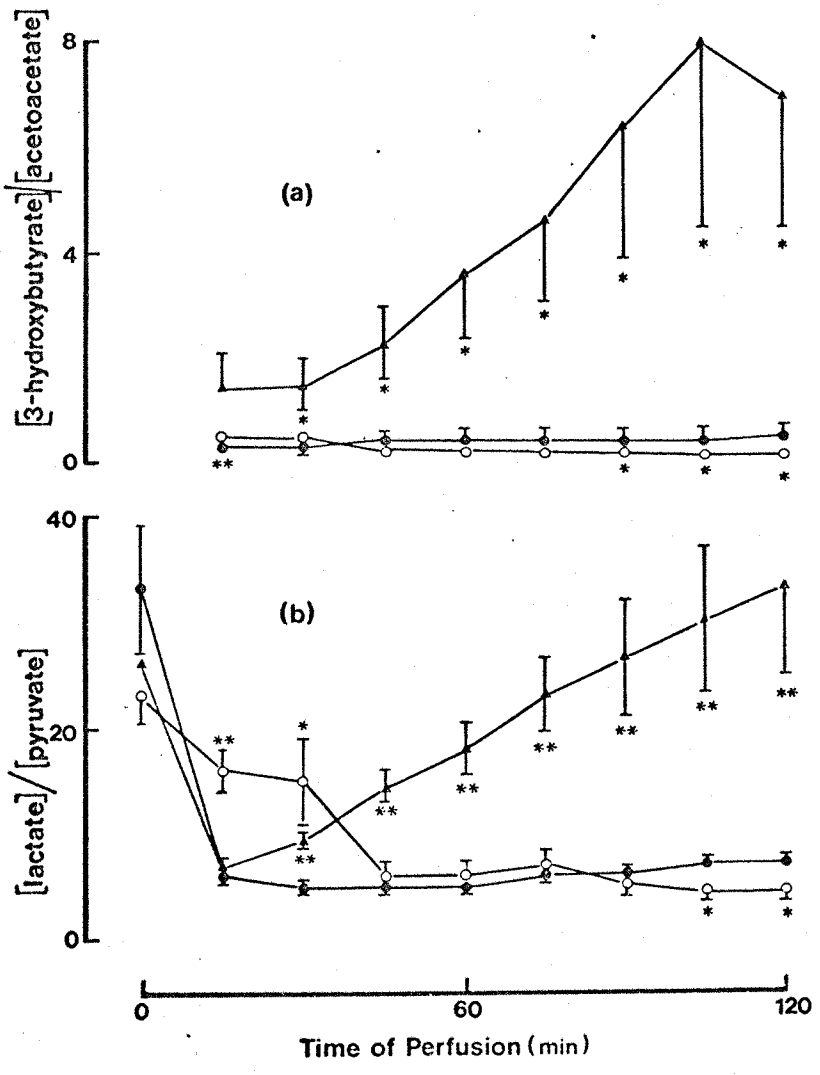


Figure 3.10. The effects of DCA and PBG on (a) the [3-hydroxybutyrate]/[acetoacetate] and (b) the lactate / pyruvate ratio in the perfusion medium of livers from fed rats.

Livers were perfused as described in Figure 3.8. The 2 mM PBG group (▲; n = 9) was compared with the control group (●; n = 5 in (a), n = 10 in (b)). The 10 mM DCA group (○; n = 4 in (a), n = 9 in (b)) was also compared with the control group; *, $p < 0.05$; **, $p < 0.01$

Table 3.4 The effect of DCA and PBG on concentrations of metabolites in the medium after perfusion of livers from fed rats: 120 min values.

Livers were perfused for two hours, see the text for details. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. The groups with DCA and PBG separately were compared with the control group (no additions), and the PBG + DCA group was compared with the PBG group. The number of perfusions is shown in parentheses. Data from Figs. 3.8, 9 and 10.

	Concentrations of metabolites and additions (mM)			
DCA	0	10	0	10
PBG	0	0	2	2
Lactate	1.98 ± 0.36(10)	0.14 ± 0.01(9)***	6.09 ± 1.07(9)**	6.18 ± 1.97(5)
Pyruvate	0.28 ± 0.04(10)	0.04 ± 0.00(9)***	0.23 ± 0.03(9)	0.09 ± 0.02(5)**
Glucose	8.40 ± 0.46(10)	9.14 ± 0.57(9)	12.5 ± 1.2 (9)**	17.0 ± 3.2 (5)
Ketone bodies	0.86 ± 0.05(5)	0.98 ± 0.04(4)	1.26 ± 0.09(9)**	2.74 ± 0.41(5)**
$\frac{[\text{3-Hydroxybutyrate}]}{[\text{Acetoacetate}]}$	0.5 ± 0.1 (5)	0.1 ± 0.0 (4)*	7.0 ± 2.5 (9)**	9.1 ± 1.4 (5)
$\frac{[\text{Lactate}]}{[\text{Pyruvate}]}$	6.9 ± 0.6 (10)	4.5 ± 0.7 (9)*	33 ± 8 (9)	63 ± 8 (5)*

Table 3.5 Concentrations of metabolic intermediates in livers of fed rats perfused with DCA

Livers were perfused for 120 min with and without DCA (10 mM), and were then freeze-clamped, see the text for details; 4 observations in each group. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Glycogen content is expressed as μmol of glucose/g fresh liver.

	μmol/g fresh liver	
Metabolite	Control	DCA
Glycogen	203 ± 21	155 ± 41
Glucose	5.84 ± 0.77	8.19 ± 0.66
Glucose-6-phosphate	0.09 ± 0.02	0.11 ± 0.02
Fructose-6-phosphate	0.04 ± 0.01	0.03 ± 0.00
Fructose-1,6-diphosphate	0.03 ± 0.01	0.04 ± 0.01
Glycerol-1-phosphate	0.38 ± 0.04	0.41 ± 0.06
3-Phosphoglycerate	0.28 ± 0.02	0.40 ± 0.05*
2-Phosphoglycerate	0.08 ± 0.01	0.06 ± 0.01
Phosphoenolpyruvate	0.12 ± 0.01	0.19 ± 0.02*
Pyruvate	0.31 ± 0.03	0.09 ± 0.01***
Lactate	1.68 ± 0.11	0.60 ± 0.03***
Alanine	0.86 ± 0.20	0.19 ± 0.02*
Citrate	0.37 ± 0.02	0.23 ± 0.01***
2-Oxoglutarate	0.64 ± 0.03	0.33 ± 0.02***
Glutamate	2.19 ± 0.29	2.16 ± 0.12
Malate	0.19 ± 0.02	0.10 ± 0.01**
Aspartate	0.48 ± 0.06	0.42 ± 0.05
Acetoacetate	0.51 ± 0.02	0.74 ± 0.04***
3-Hydroxybutyrate	0.17 ± 0.02	0.16 ± 0.01
$\frac{[\text{3-Hydroxybutyrate}]}{[\text{Acetoacetate}]}$	0.3 ± 0.0	0.2 ± 0.0*
$\frac{[\text{Lactate}]}{[\text{Pyruvate}]}$	5.6 ± 0.3	6.4 ± 0.5
AMP	0.27 ± 0.02	0.23 ± 0.00
ADP	1.01 ± 0.10	1.19 ± 0.03
ATP	2.23 ± 0.09	2.13 ± 0.10
Total adenine nucleotides	3.51 ± 0.19	3.55 ± 0.13
[ATP] / [ADP]	2.25 ± 0.16	1.80 ± 0.04*
Triglycerides	7.51 ± 0.77	9 32 ± 0.33
Total ketone bodies	0.68 ± 0.03	0.90 ± 0.04**

concentrations fell compared with the 2 mM PBG group but ketone body concentrations and the [lactate]/[pyruvate] ratio rose.

Livers from fed rats: liver metabolite concentrations

(a) Dichloroacetate (Table 3.5). DCA (10 mM) resulted in a marked fall in the concentrations of pyruvate, lactate, alanine, citrate, 2-oxoglutarate and malate. In contrast the concentrations of phosphoenolpyruvate and 3-phosphoglycerate rose with 10 mM DCA. There were decreases in the [ATP]/[ADP] and [3-hydroxybutyrate]/[acetoacetate] ratios as well as in total ketone body concentrations.

2. DISCUSSION

(a) Dichloroacetate. Whitehouse et al. (1974) showed that dichloroacetate (DCA) activates pyruvate dehydrogenase in rat liver, and recently Crabb et al. (1976) demonstrated that DCA increases the activity of pyruvate dehydrogenase (PDH) five-fold in isolated hepatocytes from starved rats. The present results agree with the conclusions of Crabb et al. (1976) that the activation of PDH has little effect on gluconeogenesis; however, this may only apply to isolated liver preparations with a copious supply of a gluconeogenic substrate. These results confirm that the hypoglycaemic effects of DCA *in vivo* are due to a decrease in hepatic gluconeogenesis secondary to inhibition of precursor release from the periphery (Blackshear et al., 1974; see also Ch. 1(b)), and are not the result of a direct effect on hepatic gluconeogenesis itself.

In livers from fasted rats the increased citrate concentrations may inhibit phosphofructokinase, which could explain the increased glucose levels observed in these conditions. The inhibition of this enzyme together with the activation of PDH may explain the depletion of phosphoglycerates and phosphoenolpyruvate, and also of alanine and pyruvate. The increase in acetyl CoA production that must result from PDH activation may explain the increased ketogenesis

observed after DCA addition.

Although Crabb et al. (1976) state that PDH activation has little effect on gluconeogenesis, they observed that DCA causes a slight but significant decrease in the rate of gluconeogenesis from lactate, a result also found by Stacpoole (1977). The results presented here indicate that lactate utilisation by, and glucose levels in the perfused liver increase with DCA treatment, and there is also a slight although not significant increase in the glucose content of the perfusion medium.

The changes in the lactate/pyruvate and 3-hydroxybutyrate/acetoacetate ratios observed here after DCA addition also differ in some respects to the results of Crabb et al. (1976) and Stacpoole (1977). In isolated hepatocytes from starved rats Crabb and coworkers found an increase in the lactate/pyruvate ratio, in agreement with the present results; however, Stacpoole (1977) found a decrease in the 3-hydroxybutyrate/acetoacetate ratio, a result not found in this work. The reasons for this are not clear.

In isolated hepatocytes from fed rats Crabb et al. (1976) observed no significant change in the ketone body ratio but an increase in the lactate/pyruvate ratio. In the present work both these ratios rose initially in the perfusate but fell at the end of perfusion. The activation of PDH may explain the initial increase in the ketone body ratio, since NADH is a product of the PDH reaction. The inhibition of fatty acid oxidation may explain the eventual decrease in the ketone body ratio; however, this effect did not achieve significance in the work of Crabb et al. (1976).

In the present work levels of phosphoenolpyruvate and of 3-phosphoglycerate rose after perfusion of fed livers with DCA, while those of citrate fell. This contrasts with the results found in livers from starved rats. The same pattern occurs when DCA is added to perfused livers of fed rats given fructose as substrate (see Table 4.3). This may be due to inhibition of pyruvate kinase; however, the levels of the known allosteric modifiers of this enzyme (ATP, alanine and fructose-1,6-diphosphate) did not change in the right direction to explain this effect. One possibility is that the decreased citrate levels may activate phosphofructokinase, thus speeding up glycolysis to such an extent that the activity of

pyruvate kinase is insufficient to metabolise the increase in phosphoenolpyruvate supply.

(b) Phenethylbiguanide. The inhibitory effect of PBG on hepatic gluconeogenesis is well known (Altschuld & Kruger, 1968; Haeckel & Haeckel, 1972). Although species such as the rat and guinea pig differ in their sensitivity to the drug, much of this may be explained by the differing activities of the detoxifying enzymes (Cook et al., 1973a).

In this work 1mM PBG did not affect gluconeogenesis significantly although other metabolic changes were apparent: ketogenesis increased, and there may have been a slight interference with respiration, since the mitochondrial redox state increased, as judged by the 3-hydroxybutyrate/acetoacetate ratio. PBG at 2 mM proved to be completely successful in inhibiting gluconeogenesis and lactate uptake, and in this situation (as well as with 1 mM PBG) there appears to have been a substantial diversion of 3-carbon units to ketone bodies (Fig. 3.1).

Although the present results indicate that 1 mM PBG did not affect gluconeogenesis from lactate, lactate uptake or ATP concentrations in the liver, there was an accumulation of gluconeogenic intermediates prior to the triose phosphate dehydrogenase/phosphoglycerate kinase reaction. Haeckel & Haeckel (1972) studied the effects of PBG on gluconeogenesis from lactate in the isolated perfused guinea pig liver, and observed a 'cross-over' between 3-phosphoglycerate and glyceraldehyde-3-phosphate. The results published by Toews et al. (1970) and by Cook et al. (1973b) suggest that the same phenomenon is happening in the rat liver, and the present results support this view. The mechanism of this effect has never been satisfactorily explained. Although Cook et al. (1973b) found a slight fall in the ATP/ADP ratio, Toews et al. (1970) found no change in [ATP], therefore the 'cross-over' is unlikely to be the result of an ATP shortage in the cytosol. The rise in the cytosol $NADH/NAD^+$ ratio found here is likely to be the result of, rather than the reason for inhibition of gluconeogenesis at the triose phosphate dehydrogenase step, since an increase in the above ratio might be expected to favour the synthesis of glyceraldehyde-3-phosphate from 1,3-diphosphoglycerate. It therefore appears that

PBG may be causing an alteration in the concentration of an unknown factor which influences the activities of phosphoglycerate kinase or glyceraldehyde-3-phosphate dehydrogenase, either directly or indirectly.

The possibility that PBG alters the distribition of cations between mitochondrion and cytosol merits further investigation. Davidoff (1974) points out the possible interference of PBG with gluconeogenesis by alterations in calcium distribution in the hepatocyte. Recently Schäfer (1976a,b) has summarised many years of work with PBG *in vitro*, and concludes that PBG interferes with respiration by inhibiting the transport of H^+, K^+ and Ca^{+2} across the mitochondrial membrane, and also that PBG can inhibit gluconeogenesis when the energy supply is not rate limiting.

The present results indicate that whereas only 2 mM PBG was effective in inhibiting gluconeogenesis from lactate, both 1 and 2 mM PBG increased the rate of ketogenesis. Toews et al. (1970) also observed increased ketogenesis with PBG and the pattern of hepatic metabolites they observed is similar to that found in the present study, and the pattern reported by Cook et al. (1973b). In addition Toews et al. (1970) observed a decrease in $^{14}CO_2$ production from [2-^{14}C] pyruvate and three-fold elevation of acetyl-CoA concentrations with PBG, supporting their hypothesis that PBG inhibits the Krebs cycle, causing an accumulation of acetyl-CoA and increasing ketogenesis. In a separate series of perfusions it has been found (results not shown here) that 1 mM PBG does not significantly increase the rate of uptake of 1 mM oleate although ketogenesis still increases. The ketogenic effect of PBG is therefore unlikely to be the result of an increase in NEFA uptake by the liver.

Previously it was thought that the mitochondrial NADH/NAD^+ ratio influenced ketogenesis by altering oxaloacetate availability for the citrate synthase reaction (Wieland, 1965). Recently techniques have been developed that permit very rapid separation of mitochondria from hepatocytes (Siess et al., 1976). In these experiments oxaloacetate concentrations in mitochondria from hepatocytes metabolising oleate were found to be considerably higher than the Km of citrate synthase. PBG would therefore seem unlikely to increase ketogenesis by altering the mitochondrial NADH/NAD^+ ratio. It seems that PBG raises the mitochondrial NADH/NAD^+ ratio,

and inhibits the Krebs cycle leading to an accumulation of acetyl--CoA which results in increased ketogenesis. Holland et al. (1973) have observed increased ketone body levels in the livers of rats treated with diphenyleneiodonium, a potent hypoglycaemic agent and inhibitor of the respiratory chain. It would be of interest to find out whether increasing ketogenesis is a property of respiratory inhibitors in general.

When added to the perfusate of livers from fed rats 2 mM PBG appears to bring about changes that resemble those that occur in anoxic conditions (Woods & Krebs, 1971). There is a release of glucose and lactate into the perfusion medium in these conditions, possibly the result of glycogenolysis. Unfortunately glycogen was not measured in these livers; however, evidence for respiratory inhibition is provided by the large increase in the mitochondrial $NADH/NAD^+$ ratio, as judged by the ten-fold increase in the 3-hydroxybutyrate/acetoacetate ratio (Williamson et al., 1967a).

(c) Phenethylbiguanide plus dichloroacetate. Recent reports indicate that DCA can reverse PBG induced hyperlactataemia in the rat (Holloway & Alberti, 1975) and dog (Loubatieres et al., 1976). The present results indicate that DCA is not effective in reversing the PBG effects on the metabolism of lactate by the perfused rat liver. Other tissues must therefore be involved in vivo, this is discussed in the concluding remarks presented in Chapter 6.

PBG plus DCA addition to perfused livers from both fed and starved rats results in the diversion of carbon to ketogenesis, more than that observed with either agent alone. This effect may be of use in the rat with lactic acidosis, for the following reasons: firstly, the oxidation of 2 moles of lactate through PDH can only give rise to 1 mole of acetoacetate (or 3-hydroxybutyrate), so the number of carboxyl groups involved is halved. Secondly, the ketone bodies appear to be more readily oxidised than lactate in extrahepatic tissues, especially in diabetes mellitus when PDH activity in many tissues is known to be low.

CHAPTER 4

FRUCTOSE

1. Results

Livers from 48 hour starved rats: perfusate concentrations

(a) Fructose

(b) Fructose plus dichloroacetate

Livers from 48 hour starved rats: liver metabolite concentrations

(a) Fructose

(b) Fructose plus dichloroacetate

Livers from fed rats: perfusate concentrations

(a) Fructose

(b) Fructose plus dichloroacetate

Livers from fed rats: liver metabolite concentrations

(a) Fructose

(b) Fructose plus dichloroacetate

2. Discussion

(a) Fructose

(b) Fructose and ketogenesis

(c) Fructose plus dichloroacetate

FRUCTOSE

Livers of fed and 48 hour starved rats were perfused with 10 mM fructose to establish a system with increased lactate production. DCA (10 mM) was then added with fructose in an attempt to decrease this production. At the end of perfusion livers were freeze-clamped for the determination of hepatic metabolites.

1. RESULTS

Livers from 48 h starved rats: perfusate concentrations

(a) Fructose. Although perfusions of livers from starved rats without added substrate were not carried out, it is of interest to compare the 10 mM fructose results with those obtained in perfusion of livers from starved rats with 10 mM lactate (Table 4.1). Fructose (10 mM) was taken up more rapidly by the liver than 10 mM lactate (Table 4.1, Figs. 3.3 and 4.1). Although glucose production from fructose was correspondingly greater, ketone body production from fructose was not significantly different to that observed after 10 mM lactate addition (Table 4.1). Fructose addition resulted in a rapid release of lactate and pyruvate into the perfusion medium (Fig. 4.4).

(b) Fructose plus dichloroacetate (Table 4.1). The addition of 10 mM DCA with 10 mM fructose did not affect the uptake of fructose by the liver (Fig. 4.1), the production of glucose (Fig. 4.2) or ketone body production (Fig. 4.3). After DCA addition, however, lactate and pyruvate production by the liver fell dramatically (Fig. 4.4). There was a transient decrease in the [3-hydroxybutyrate]/[acetoacetate] ratio and a temporary rise in the [lactate]/[pyruvate] ratio after DCA addition (Fig. 4.5). These results can be compared with the effects of DCA in perfusions with 10 mM lactate, a summary of which is presented in Appendix A, located in the folder inside the back cover of this thesis.

Table 4.1. Uptake or production of metabolites by perfused livers of 48 h starved rats

Livers were perfused for 38 minutes without substrate; substrates ± DCA were added as indicated and perfusions were continued for one hour. Results are expressed as μmol/h per g fresh liver; minus signs indicate uptake. The perfusions with fructose plus DCA were compared with perfusions with fructose alone. The 10 mM lactate data are included for comparison and are taken from Table 3.1. ***, $p < 0.001$ versus fructose alone.

Metabolic change	Substrates ± DCA (all 10 mM)		
	Lactate	Fructose	Fructose + DCA
Fructose uptake	-	142 ± 7 (9)	152 ± 8 (9)
Glucose production	36.7 ± 3.2 (12)	76.0 ± 11.3 (9)	83.4 ± 13.2 (9)
Ketone body production	3.76 ± 0.45 (7)	2.23 ± 1.19(9)	5.71 ± 2.37(9)
Lactate production	-101 ± 3 (12)	27.9 ± 5.6 (9)	3.12 ± 1.50(9)***
Pyruvate production	10.9 ± 0.4 (12)	3.93 ± 0.99(9)	−0.15 ± 0.07(9)***

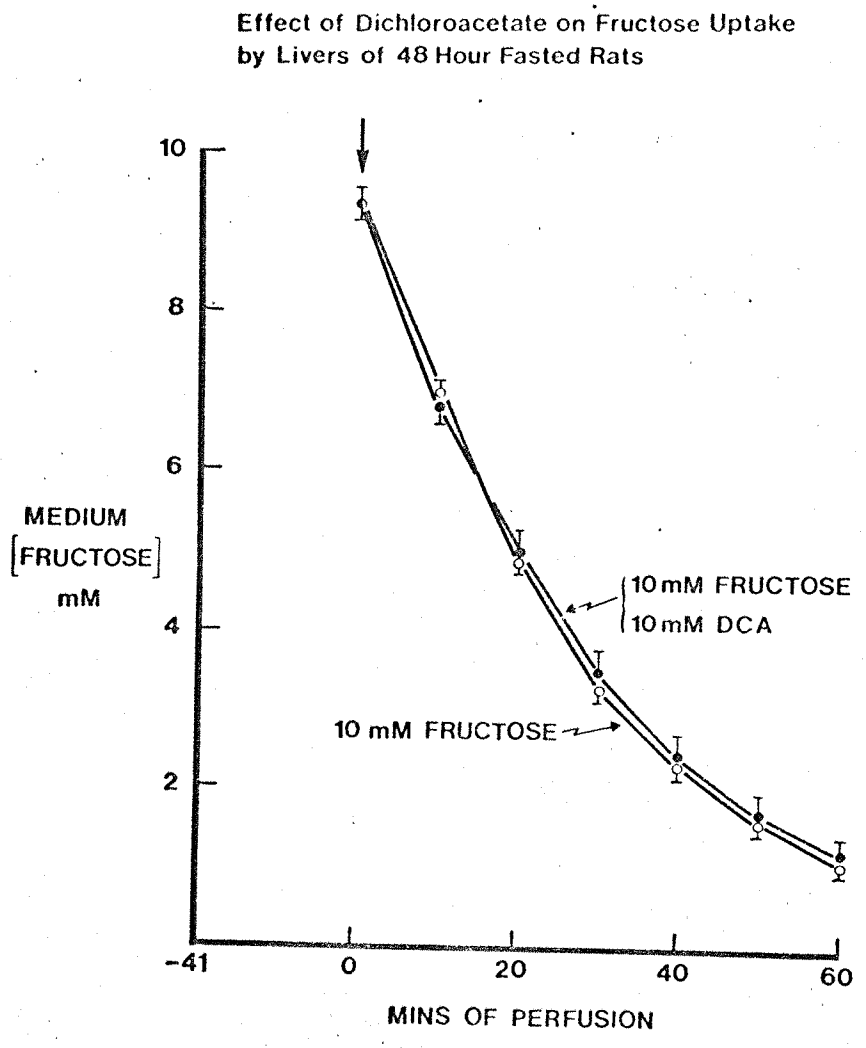


Figure 4.1. The effect of DCA on fructose uptake by perfused livers of 48 h starved rats

The experimental details are given in Table 4.1. There are no significant differences between the two groups. ○, 10 mM fructose; ●, 10 mM fructose plus 10 mM DCA

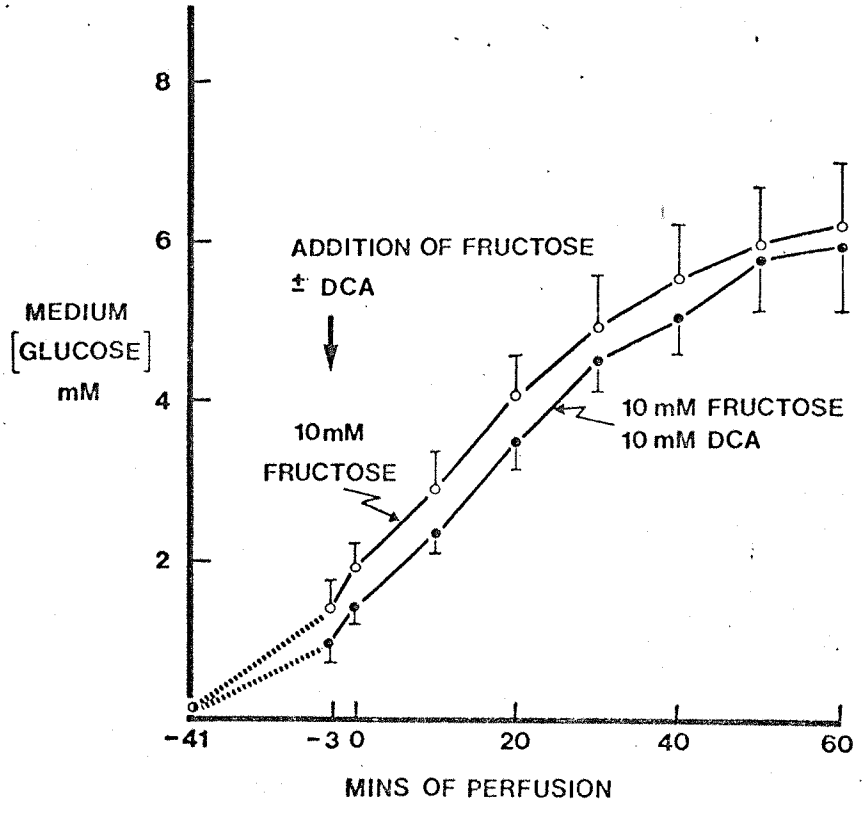


Figure 4.2. The effect of DCA on glucose production by perfused livers of 48 h starved rats

Fructose was used as substrate, experimental details are given in Table 4.1. There are no significant differences between the two groups.

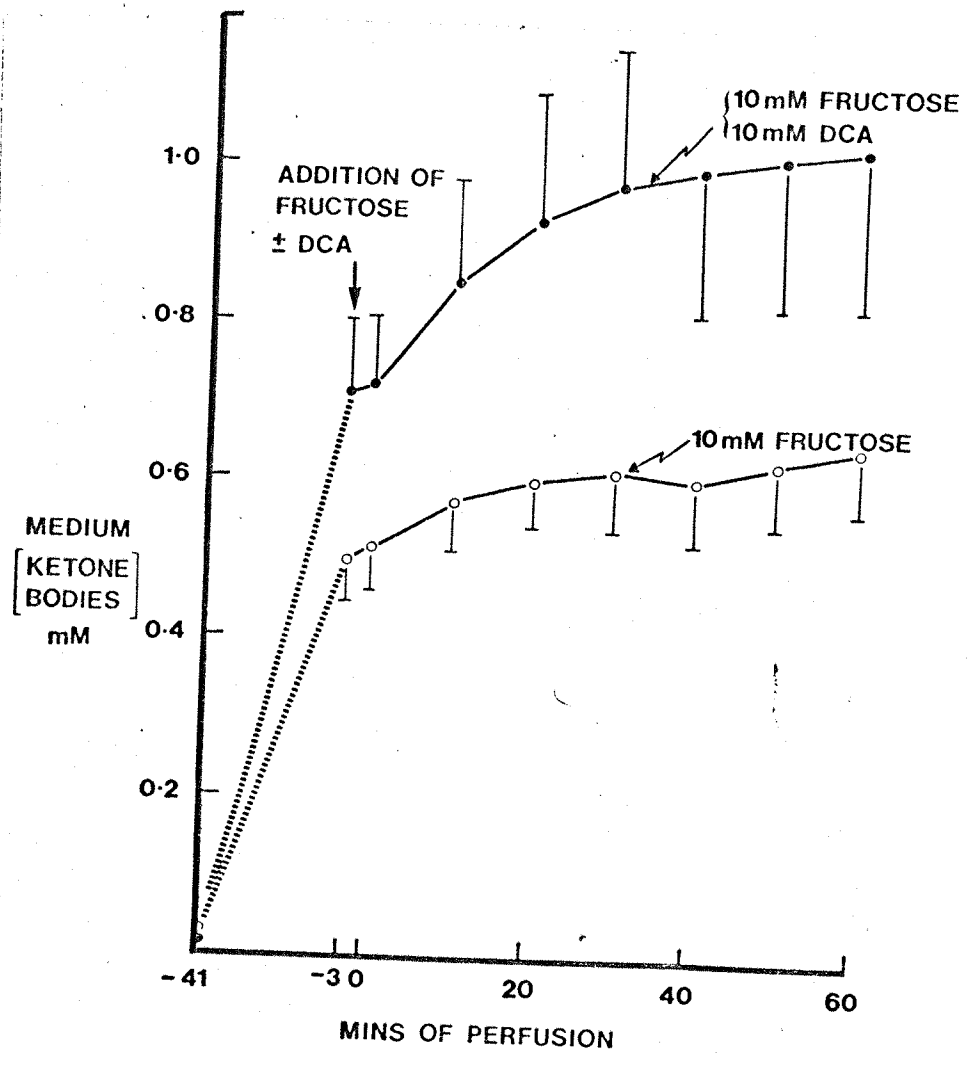


Figure 4.3. The effect of DCA on ketogenesis by perfused livers of 48 h starved rats

Fructose was used as substrate, experimental details as in Table 4.1. There are no significant differences between the two groups.

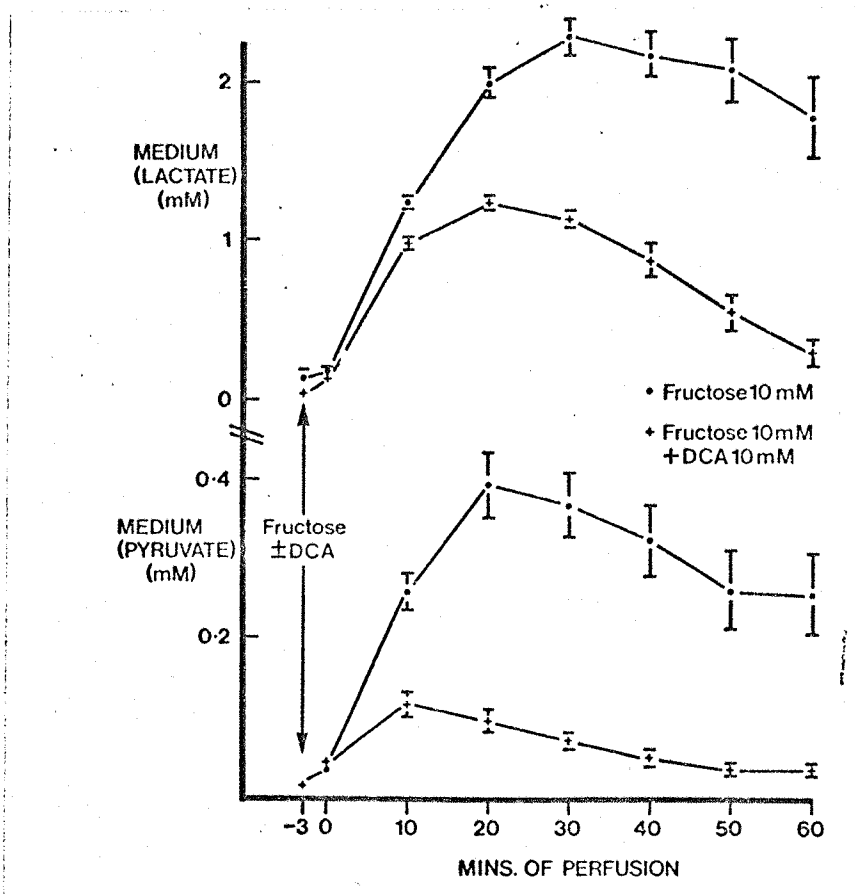


Figure 4.4. The effect of DCA on lactate and pyruvate production by perfused livers of 48 h starved rats

Fructose was used as substrate, experimental details as in Table 4.1. On the pyruvate graph, fructose alone values are significantly higher from 10 min onwards, $p < 0.001$. On the lactate graph, fructose alone values are significantly higher, $p < 0.01$ at 10 min and $p < 0.001$ from 20 min onwards.

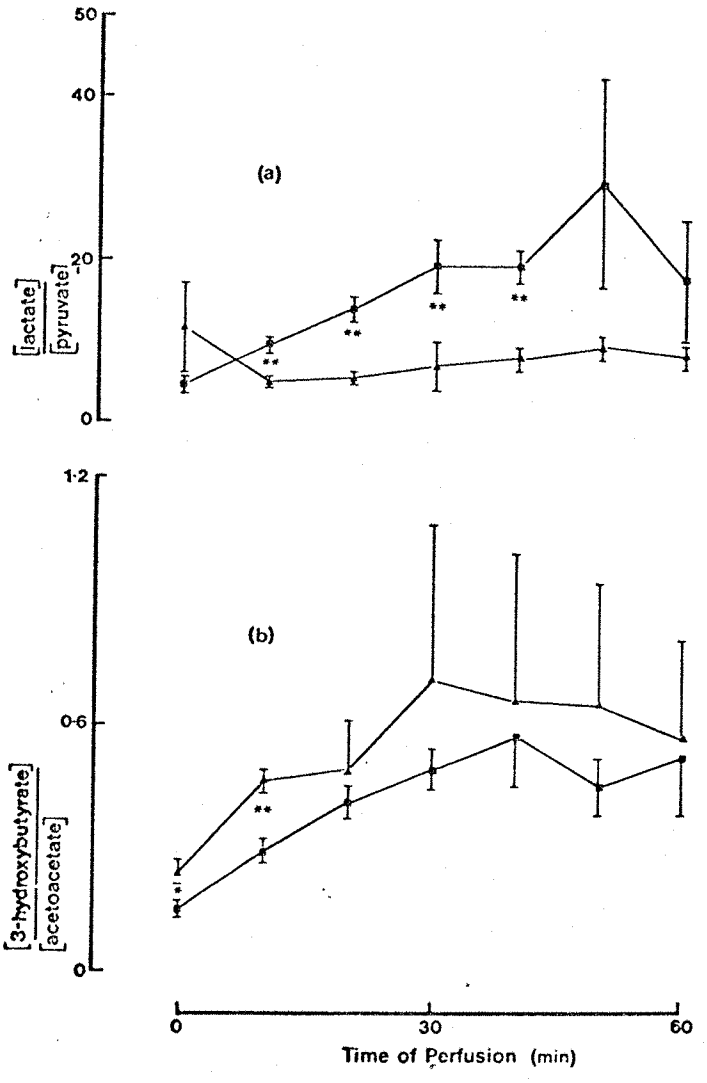


Figure 4.5. The effects of DCA and fructose on (a) the [lactate]/[pyruvate] and (b) the [3-hydroxybutyrate]/[acetoacetate] ratio in the perfusion medium of livers from 48 h starved rats.

Fructose was used as substrate, experimental details as in Table 4.1. In both (a) and (b) ▲, 10 mM fructose and ■, 10 mM fructose plus 10 mM dichloroacetate. *, $p < 0.05$; **, $p < 0.01$

Table 4.2 The effects of DCA on concentrations of metabolic intermediates in livers from 48 h starved rats

Livers were given a fructose load 3 min before zero time and were freeze clamped after 60 min of perfusion as described in the text. Results are expressed as μmol/g fresh liver ± SEM; *, $p < 0.05$; **, $p < 0.01$; glycogen content is expressed as μmol of glucose per g fresh weight. Four livers in each group.

Metabolite	10mM fructose	10mM fructose + 10mM DCA
Glycogen	15.5 ± 1.5	12.9 ± 3.8
Glucose	5.64 ± 0.57	4.99 ± 0.65
Glucose-6-phosphate	0.10 ± 0.00	0.09 ± 0.01
Fructose-6-phosphate	0.03 ± 0.00	0.03 ± 0.00
Glycerol-1-phosphate	0.33 ± 0.02	0.48 ± 0.09
3-phosphoglycerate	0.58 ± 0.08	0.32 ± 0.06*
2-phosphoglycerate	0.08 ± 0.00	0.04 ± 0.01
Phosphoenolpyruvate	0.24 ± 0.04	0.18 ± 0.04
Pyruvate	0.24 ± 0.05	0.08 ± 0.00*
Lactate	1.84 ± 0.22	0.50 ± 0.09**
Alanine	1.04 ± 0.20	0.17 ± 0.04**
Citrate	0.54 ± 0.06	0.49 ± 0.04
2-oxoglutarate	0.34 ± 0.05	0.27 ± 0.05
Glutamate	1.90 ± 0.07	1.41 ± 0.21
Malate	0.18 ± 0.02	0.18 ± 0.01
Acetoacetate	0.55 ± 0.08	0.55 ± 0.11
3-hydroxybutyrate	0.26 ± 0.05	0.37 ± 0.04
Total ketone bodies	0.81 ± 0.05	0.93 ± 0.13
$\frac{[\text{3-hydroxybutyrate}]}{[\text{acetoacetate}]}$	0.55 ± 0.19	0.74 ± 0.16
$\frac{[\text{Lactate}]}{[\text{Pyruvate}]}$	9.15 ± 2.02	5.93 ± 1.01
AMP	0.30 ± 0.02	0.33 ± 0.03
ADP	1.04 ± 0.07	1.01 ± 0.09
ATP	1.41 ± 0.05	1.14 ± 0.14
ATP/ADP	1.39 ± 0.15	1.14 ± 0.11
Total adenine nucleotides	2.74 ± 0.07	2.51 ± 0.06*
Triglyceride	9.60 ± 1.03	6.64 ± 0.88

Livers from 48 hour starved rats: liver metabolite concentrations

(a) Fructose. A formal comparison of the fructose group with the group of livers from starved rats that received 10 mM lactate has not been attempted here, since the latter is not the appropriate control group. As mentioned in Section 1(a) of this chapter, perfusions of livers from starved rats without added substrate were not carried out.

(b) Fructose plus dichloroacetate (Table 4.2). Hepatic concentrations of 3-phosphoglycerate, pyruvate, lactate and alanine decreased after DCA addition, as did those of total adenine nucleotides which were already abnormally low after fructose administration. There were no other significant changes compared with the group where 10 mM fructose alone was added.

These results may be compared with the effects of DCA on hepatic metabolites after 10 mM lactate addition, a summary of which is presented in Appendix B located in the folder inside the back cover.

Livers from fed rats: perfusate concentrations

(a) Fructose. Fructose (10 mM) was rapidly metabolised by livers from fed rats (Fig. 4.6) but this did not result in a significant increase in glucose production compared with controls (Fig. 4.7). In contrast ketone body production fell (Fig. 4.8) while there was a considerable output of lactate after fructose addition (Fig. 4.9). There were transient increases in the [lactate]/[pyruvate] and [3-hydroxybutyrate]/[acetoacetate] ratios (Fig. 4.10).

(b) Fructose plus dichloroacetate. When added with fructose, 10 mM DCA had no effect on the rate of fructose uptake by the liver (Fig. 4.6), while glucose production was not significantly affected (Fig. 4.7). Ketone body concentrations in the perfusate rose compared with fructose alone (Fig. 4.8), while lactate production fell dramatically after DCA addition (Fig. 4.9). There was a temporary rise in the [lactate]/[pyruvate] ratio, while the [3-hydroxybutyrate]/[acetoacetate] ratio fell at the end of perfusion (Fig. 4.10). The reader is again referred to Appendix A, for the

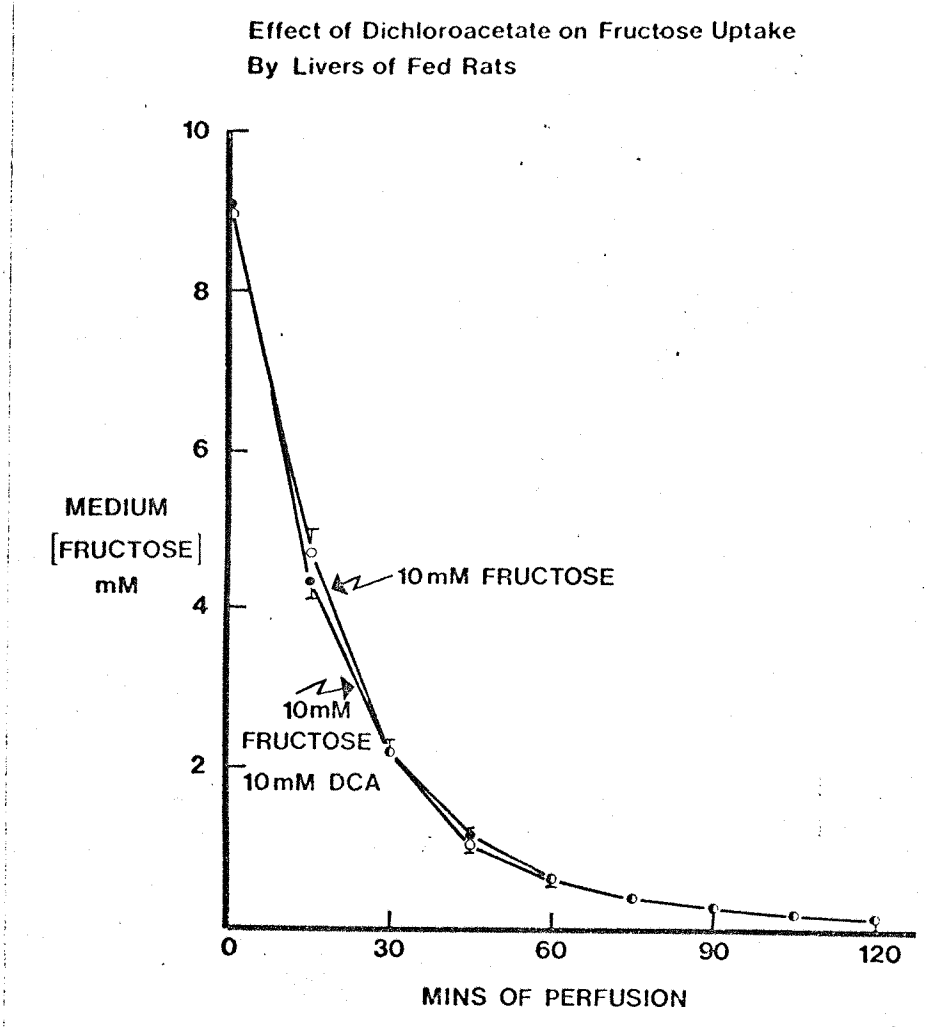


Figure 4.6. The effects of DCA on fructose uptake by perfused livers of fed rats.

Fructose (10 mM) was added to the medium before the start of perfusion, and livers were perfused for 120 min. DCA had no significant effect on fructose uptake. See the text for further details. ○, 10 mM fructose; ●, 10 mM fructose plus 10 mM DCA.

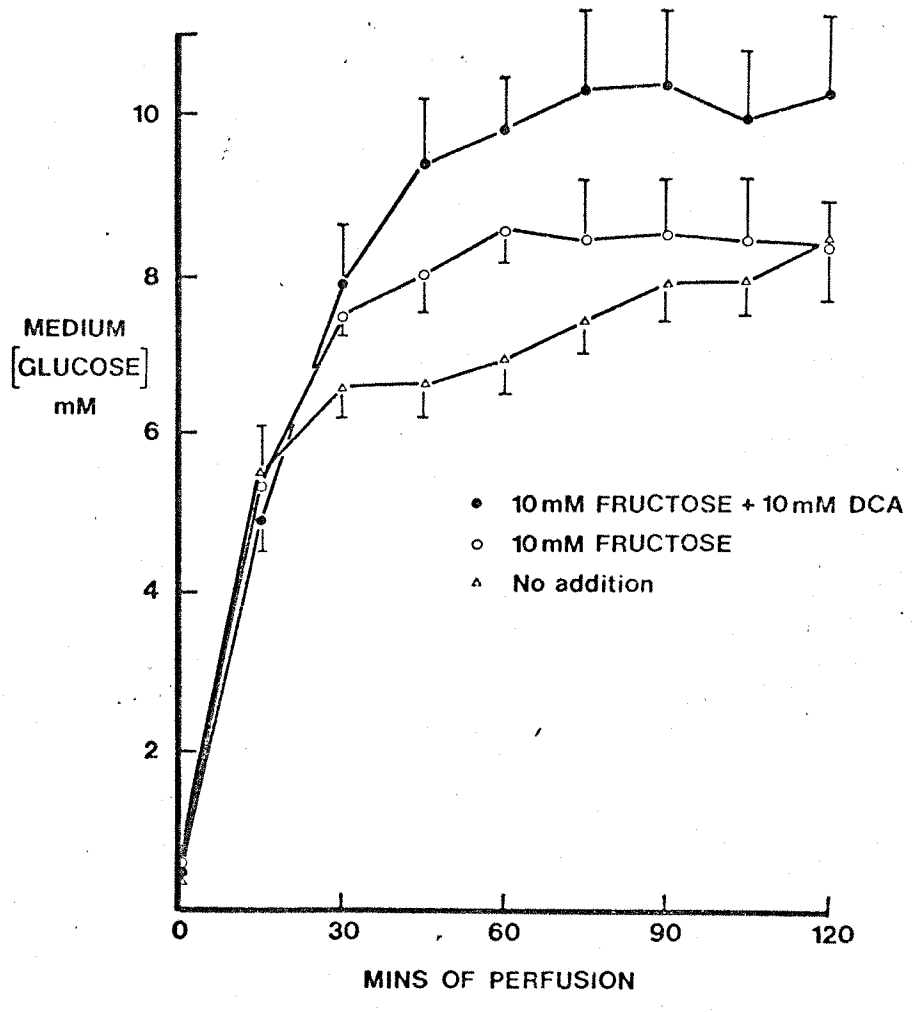


Figure 4.7. The effects of DCA on glucose production by perfused livers of fed rats.

Fructose was used as substrate, experimental details as in Figure 4.6, except △, no substrates or inhibitors added. The fructose group (○) was compared with the control group (△), and the fructose + DCA group (●) was compared with the fructose group. The only significant difference was the fructose (○) value versus control at 60 min ($p < 0.05$).

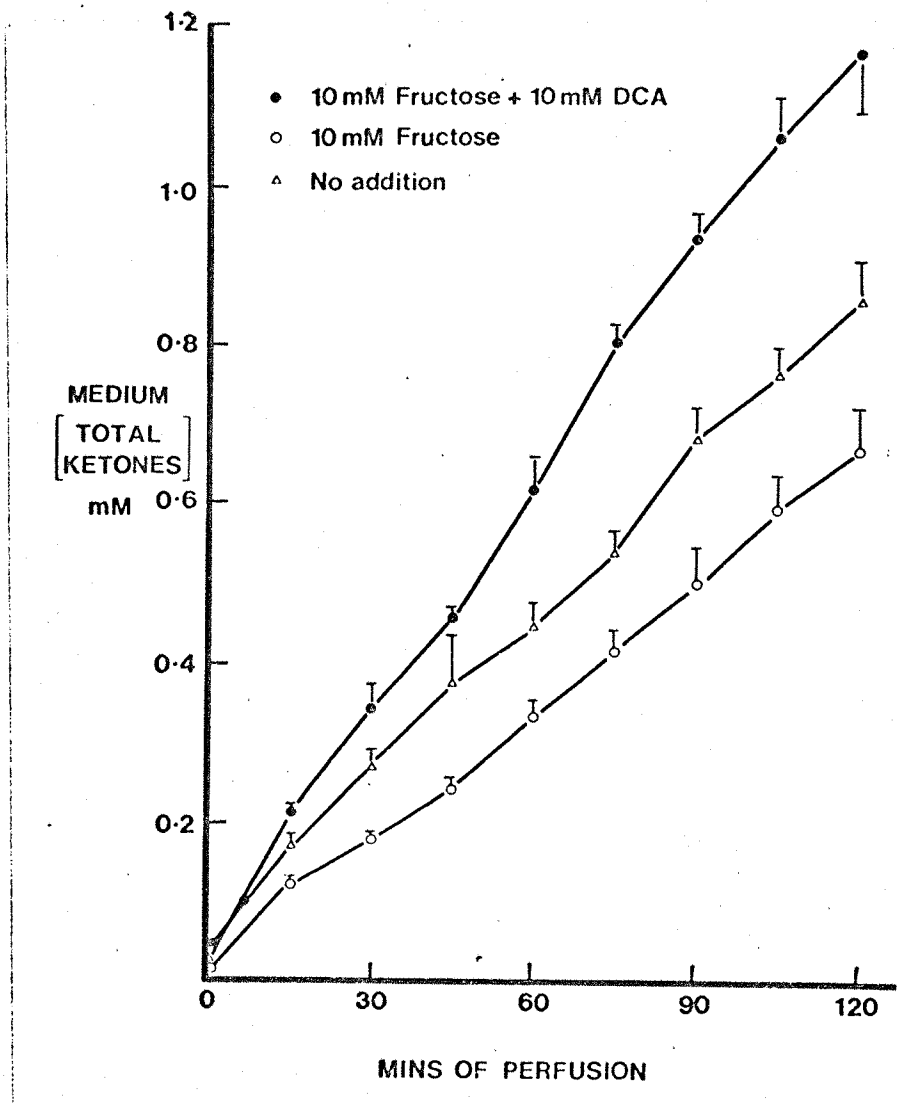


Figure 4.8. The effect of DCA on ketogenesis by perfused livers of fed rats

Fructose was used as substrate, experimental and other details as in Fig. 4.6. For the fructose group (o) $p < 0.05$ from 60 min onwards, and $p < 0.02$ at 30 min compared with controls (Δ). For the fructose plus DCA group (●) $p < 0.01$ from 15 min onward compared with fructose alone.

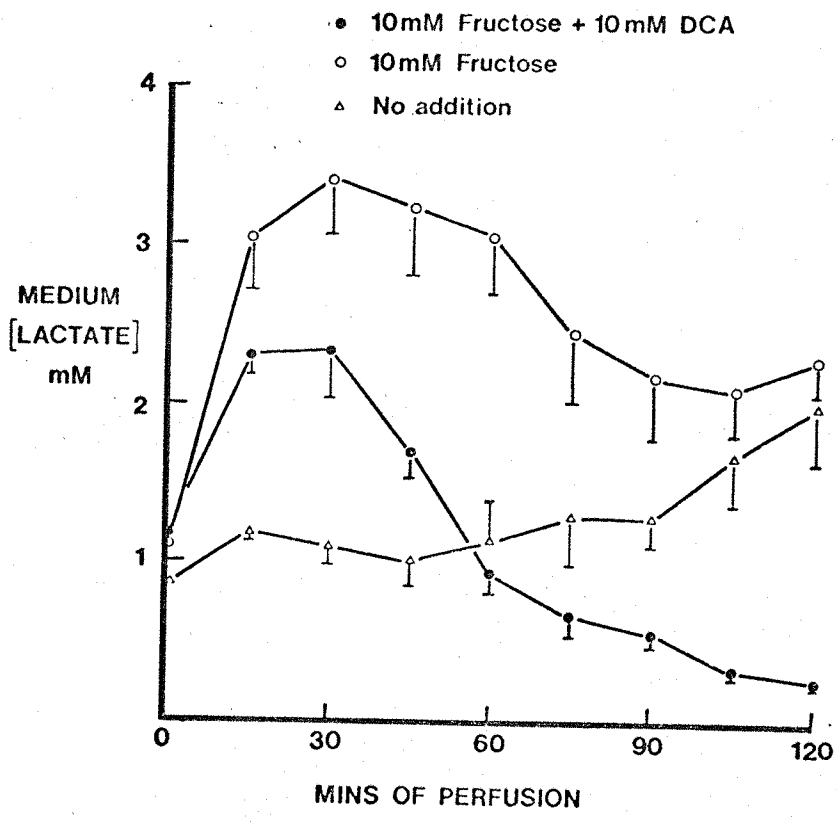


Figure 4.9. The effect of DCA on lactate production by perfused livers of fed rats.

Fructose was used as substrate, other details as in Fig. 4.6. For the fructose group (●) values from 15 to 60 min inclusive are significantly different from △ ($p < 0.001$). For the fructose + DCA group all points from 45 min onward are significantly different from fructose values, $p < 0.01$ from 45 min onwards.

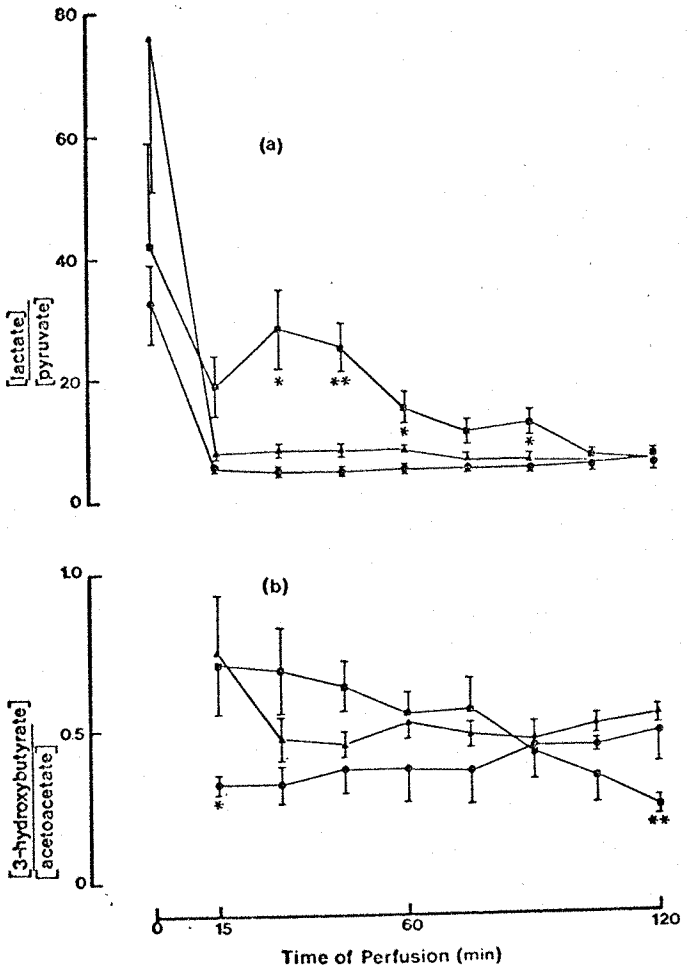


Figure 4.10. The effects of fructose and DCA on (a) the [lactate]/[pyruvate] and (b) [3-hydroxybutyrate]/[acetoacetate] ratios in the perfusion medium of livers from fed rats

Experimental details as in Fig. 4.6. The fructose group (▲, n = 5) was compared with the control group (●; n = 10 in (a), n = 5 in (b)). The fructose + DCA group (■, n = 5) was compared with the fructose group; *, $p < 0.01$; **, $p < 0.05$. Also the fructose alone values at 30, 45 and 60 mins are significantly higher than control values on graph (a), $p < 0.01$.

Table 4.3. Concentrations of metabolic intermediates in livers of fed rats after perfusion with fructose ± DCA

Livers were perfused for 120 min and freeze-clamped as described in the text. Results are expressed as μmol/g fresh wt ± SEM, glycogen content is expressed as μ-mol of glucose/g fresh wt. The 10 mM fructose group was compared with controls (no additions), the fructose (10 mM) + DCA (10 mM) group was compared with the fructose group. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.01$

No. of livers	CONTROL 4	Fructose 5	Fructose + DCA 5
Metabolite			
Glycogen	203 ± 21	222 ± 29	197 ± 22
Glucose	5.84 ± 0.76	11.4 ± 1.6 *	11.7 ± 1.1
Glucose-6-phosphate	0.09 ± 0.01	0.15 ± 0.02*	0.15 ± 0.01
Fructose-6-phosphate	0.04 ± 0.01	0.05 ± 0.00	0.04 ± 0.00
Fructose-1,6-diphosphate	0.03 ± 0.01	0.05 ± 0.00**	0.05 ± 0.01
Glycerol-1-phosphate	0.38 ± 0.04	0.71 ± 0.10*	0.54 ± 0.05
3-phosphoglycerate	0.28 ± 0.02	0.32 ± 0.02	0.50 ± 0.04**
2-phosphoglycerate	0.08 ± 0.00	0.06 ± 0.01*	0.09 ± 0.01**
Phosphoenolpyruvate	0.12 ± 0.00	0.12 ± 0.01	0.24 ± 0.02***
Pyruvate	0.31 ± 0.03	0.27 ± 0.01	0.11 ± 0.01***
Lactate	1.68 ± 0.11	3.55 ± 0.47**	0.91 ± 0.08***
Alanine	0.86 ± 0.19	1.98 ± 0.19**	0.40 ± 0.04***
Citrate	0.37 ± 0.02	0.45 ± 0.01*	0.30 ± 0.02***
2-oxoglutarate	0.63 ± 0.03	0.38 ± 0.01***	0.33 ± 0.03
Glutamate	2.19 ± 0.28	2.56 ± 0.42	2.14 ± 0.33
Malate	0.19 ± 0.02	0.34 ± 0.05*	0.14 ± 0.01**
Acetoacetate	0.51 ± 0.01	0.39 ± 0.04*	0.75 ± 0.03***
3-hydroxybutyrate	0.17 ± 0.02	0.35 ± 0.07	0.28 ± 0.04
Total ketone bodies	0.68 ± 0.03	0.73 ± 0.07	1.02 ± 0.05**
[3-hydroxybutyrate] / [Acetoacetate]	0.33 ± 0.02	0.99 ± 0.25*	0.37 ± 0.05*
[Lactate] / [Pyruvate]	5.55 ± 0.34	13.2 ± 1.8 **	8.66 ± 0.79
AMP	0.27 ± 0.02	0.33 ± 0.05	0.26 ± 0.03
ADP	1.01 ± 0.09	0.95 ± 0.05	0.95 ± 0.08
ATP	2.23 ± 0.08	1.69 ± 0.08**	1.54 ± 0.03
ATP/ADP	2.25 ± 0.16	1.81 ± 0.15	1.68 ± 0.17
Total adenine nucleotides	3.51 ± 0.19	2.97 ± 0.07*	2.75 ± 0.10
Triglyceride	7.51 ± 0.68	7.91 ± 0.59	8.09 ± 0.35

metabolic effects of DCA alone in these conditions.

Livers from fed rats: liver metabolite concentrations

(a) Fructose (Table 4.3). Fructose addition resulted in increases in hepatic concentrations of glucose, glucose-6-phosphate, lactate, alanine, citrate and malate. Concentrations of 2-phosphoglycerate, 2-oxoglutarate, acetoacetate, ATP and total adenine nucleotides fell. The [lactate]/[pyruvate] and [3-hydroxybutyrate]/[acetoacetate] ratios in the liver increased as a result of fructose addition.

(b) Fructose plus dichloroacetate (Table 4.3). The addition of DCA (10 mM) with fructose (10 mM) resulted in decreased hepatic concentrations of pyruvate, lactate, alanine, citrate and malate compared with fructose alone; however, those of 3-phosphoglycerate, 2-phosphoglycerate and phosphoenolpyruvate increased. Concentrations of acetoacetate and of total ketone bodies also increased, while there was a fall in the [3-hydroxybutyrate]/[acetoacetate] ratio. Appendix B lists the effects of DCA alone on hepatic metabolite concentrations in perfused livers of fed rats, and it is of interest to note that DCA has similar effects in these conditions with and without fructose addition.

2. DISCUSSION

(a) Fructose. Fructose is rapidly taken up by the liver and this results in the release of considerable quantities of lactate into the perfusate, an effect noted by many authors (Woods et al., 1970; Topping & Mayes, 1972; Wimshurst & Manchester, 1973). This rapid release of lactate is probably due to the by-pass of phosphofructokinase in the liver (Chapter 1(d)).

Fructose administration lowers adenine nucleotide levels in the liver, an effect which is also well known (Mäenpää et al., 1968; Woods et al., 1970). In the present work, the perfused livers of fed rats showed an adenine nucleotide deficit two hours after the addition of 10 mM fructose. Fructose is rapidly phosphorylated to fructose-1-phosphate in the liver resulting in a decrease in ATP and

Pi concentrations both of which are essential in stabilising AMP and therefore the total adenine nucleotide content of this tissue. They inhibit the enzymes that cause the irreversible degradation of AMP. ATP inhibits 5'-nucleotidase (Baer et al., 1966) and Pi inhibits AMP deaminase (Nikiforuk & Cdowick, 1966), therefore when the inhibition of AMP deaminase becomes less effective hepatic IMP concentrations rise. Both IMP and AMP are dephosphorylated to adenine and hypoxanthine, resulting in the appearance of allantoin and uric acid in the perfusion medium or plasma (Mäenpää et al., 1968; Raivio et al., 1969). A further complication arises since IMP is a potent inhibitor of fructose-1-phosphate aldolase (Woods et al., 1970), so the increased IMP concentrations in the liver after fructose loading cause an accumulation of fructose-1-phosphate, and this may trap inorganic phosphate.

(b) Fructose and ketogenesis. The mechanism of the antiketogenic effect of fructose (Rawat & Menahan, 1975) is still not clear. In studying the antiketogenic effects of fructose in the isolated perfused rat liver it would seem relevant to include NEFA in the perfusion medium if one is attempting to relate to the situation in vivo. Rawat & Menahan (1975) observed that fructose is antiketogenic in vivo and obviously NEFA was present in the plasma of both fed (0.23 mM) and starved (0.75 mM) rats. Analyses of NEFA in the present perfusate revealed initial concentrations of 0.2 mM.

Previous work in the isolated perfused rat liver indicates that fructose addition without exogenous NEFA may actually increase the rate of ketogenesis (Söling et al., 1970; Söling & Willms, 1971; Papenberg, 1971). Söling et al. (1970) also pointed out that fructose addition activates pyruvate dehydrogenase (PDH); however, the addition of oleate with fructose prevents this activation (Patzelt et al., 1973). Furthermore, D(+)-decanoylcarnitine, an inhibitor of long chain fatty acid oxidation (McGarry et al., 1973), reverses the oleate inhibition of PDH activation by fructose (Patzelt et al., 1973), indicating that fatty acid oxidation is necessary for the inhibition of PDH by oleate in these conditions.

In perfusions where fructose is added with oleate McGarry and Foster (1971) found that the rate of ketogenesis and acetyl-CoA

accumulation decreased, and suggested that fatty acids are diverted from oxidation to esterification after fructose addition. Indeed Topping and Mayes (1972) found that fructose did not affect NEFA uptake by the isolated perfused rat liver but increased the secretion of triglyceride in VLDL.

It therefore appears that an increase in fatty acid oxidation inhibits PDH and that fructose activates PDH but inhibits fatty acid oxidation. Whether or not fructose is antiketogenic would seem to depend on the relative quantities of fatty acids and fructose present, as pointed out by Krebs and Hems (1970). It is of interest to note that DCA, an activator of PDH, reverses the antiketogenic effects of fructose in the present perfusions.

The concentration of glycerol-phosphate has been thought to be important in the regulation of triglyceride synthesis (Rawat & Menahan, 1975); however, McGarry and Foster (1973) have pointed out that glycerol-1-phosphate concentrations rise in conditions where triglyceride synthesis falls. In the present work glycerol-1--phosphate concentrations did not correlate with the rates of ketogenesis.

McGarry and Foster (1975) have provided evidence that the carnitine acyl transferase system may regulate the rate of fatty acid oxidation in the liver, and they propose that there is an intermediate of carbohydrate metabolism that inhibits carnitine acyl transferase. It is possible that fructose is converted to this unknown intermediate, inhibiting carnitine acyl transferase and therefore fatty acid oxidation, acetyl-CoA production and ketogenesis. The intermediate has recently been identified as malonyl-CoA (McGarry et al., 1977).

(c) Fructose plus dichloroacetate. Fructose is thought to activate PDH in the isolated perfused rat liver by lowering the ATP/ADP ratio, and possibly also by increasing pyruvate concentrations (Söling et al., 1970). In the presence of oleate and fructose this enzyme is inhibited (Patzelt et al., 1973). However, DCA appears to reverse this inhibition resulting in decreases in lactate and pyruvate concentrations in the liver and perfusate, as well as a considerable increase in ketogenesis. As discussed in

Section 3.2(c) the accumulation of 3-phosphoglycerate and of phosphoenolpyruvate is possibly due to the activation of phospho-fructokinase that may result from lower hepatic citrate concentrations in perfused livers from fed rats.

The marked effects of DCA on hepatic lactate output after fructose administration to the isolated perfused rat liver suggest that DCA is well worth trying in the reversal of lactic acidosis in patients receiving paranteral nutrition with solutions containing fructose. However, DCA had no effect on the low ATP levels observed after perfusion of livers with fructose, and it is possible that the liver requires the addition of adenine and phosphate to restore adenine nucleotide levels after perfusion with fructose.

CHAPTER 5

GALACTOSAMINE

1. Results

Blood Metabolites

(a) Galactosamine
(b) Galactosamine plus dichloroacetate
(c) Galactosamine plus fructose
(d) Galactosamine plus fructose and dichloroacetate

Liver Metabolites

(a) Galactosamine
(b) Galactosamine plus dichloroacetate
(c) Galactosamine plus fructose
(d) Galactosamine plus fructose and dichloroacetate

2. Discussion

(a) Galactosamine
(b) Fructose
(c) Dichloroacetate

GALACTOSAMINE

Rats were anaesthetised, and cannulae were placed in their femoral veins and arteries. Animals were then placed in restraining cages and food was withdrawn. Galactosamine (GalN) was administered intravenously (1 g/kg body wt) in order to impair the ability of the liver to metabolise lactate. In certain experiments fructose was injected into GalN treated animals to raise blood lactate concentrations even further (see Ch. 1(d)). Dichloroacetate was infused (300 mg/kg body wt per h) in an attempt to reverse the hyperlactataemic effects of GalN and fructose. Experimental details are given in Chapter 2.1(c).

1. RESULTS

Blood Metabolites

(a) Galactosamine (Figs. 5.1, 2 & 3). The administration of 1 g galactosamine/kg body wt resulted in an increase in arterial lactate and pyruvate concentrations, and the [3-hydroxybutyrate]/[acetoacetate] ratio also rose compared with controls. In contrast arterial concentrations of glucose and total ketone bodies were unaffected by galactosamine treatment.

(b) Galactosamine plus dichloroacetate (Figs. 5.1, 2 & 3). The infusion of DCA (300 mg/kg body wt per hour) for 120 mins into GalN treated rats resulted in decreased arterial concentrations of lactate, pyruvate and glucose compared with saline infused GalN treated rats. In these conditions concentrations of total ketone bodies, 3-hydroxybutyrate and acetoacetate all increased, and the [3-hydroxybutyrate]/[acetoacetate] ratio was unaltered.

(c) Galactosamine plus fructose (Figs. 5.4 & 5.5). The injection of fructose (1 g/kg body wt) into GalN treated rats resulted in immediate increases in arterial lactate, pyruvate and glucose concentrations, and equally sudden decreases in total ketone body concentrations and in the [3-hydroxybutyrate]/[acetoacetate] ratio. The [lactate]/[pyruvate] ratio was unchanged.

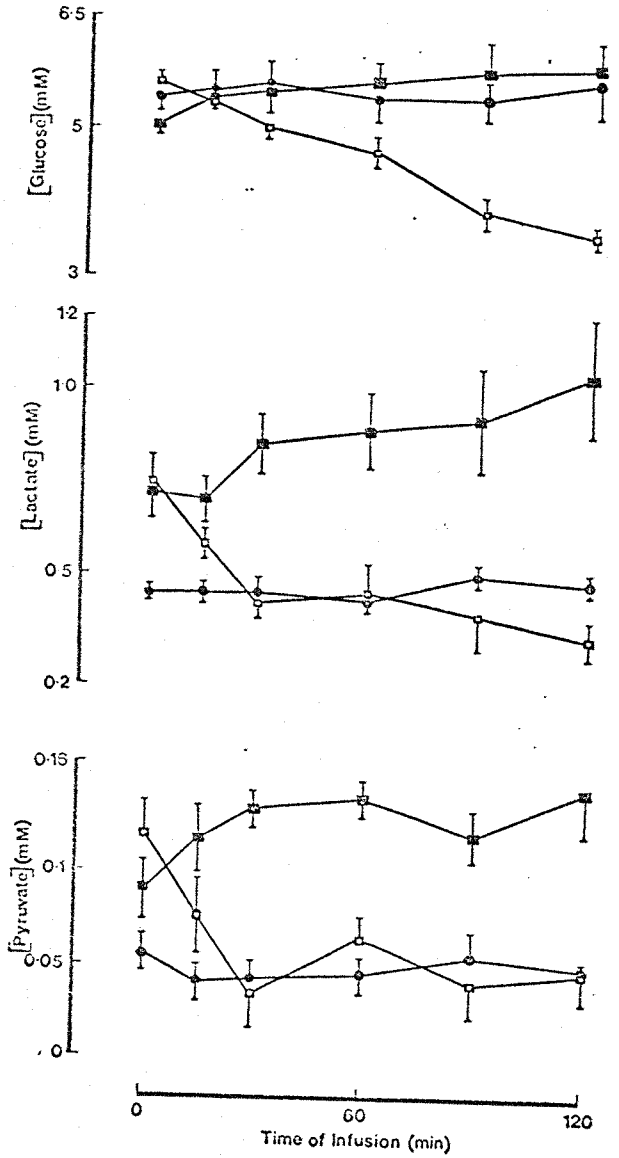


Figure 5.1 The effects of galactosamine injection and DCA on blood concentrations of glucose, lactate and pyruvate in 24 h starved rats.

The groups are defined as follows: ●, saline infused controls (n = 7) ■, galactosamine injection and saline infusion (n = 5); □, galactosamine injection and DCA infusion (n = 5). The statistical significance of the various differences is shown below.

	Control vs GalN			GalN vs GalN + saline		
Sample time(mins)	Glucose	Lactate	Pyruvate	Glucose	Lactate	Pyruvate
0	NS	0.01	NS	NS	NS	NS
15	NS	0:01	0.01	NS	NS	NS
30	NS	0.001	0.001	NS	0.01	0.01
60	NS	0.01	0.001	0.05	0.02	0.01
90	NS	0.01	0.01	0.01	0.02	0.01
120	NS	0.01	0.01	0.001	0.01	0.02

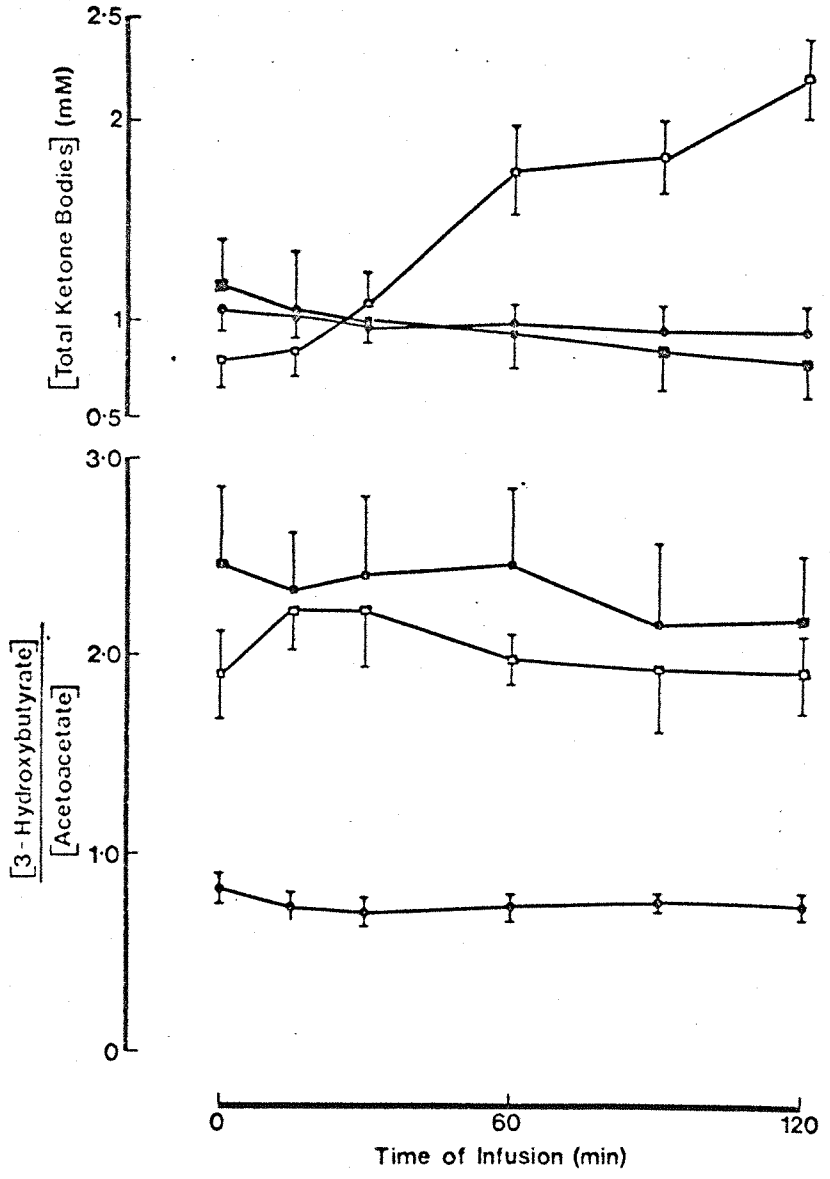


Figure 5.2. The effects of galactosamine injection and DCA infusion on blood total ketone body concentrations and the [3-hydroxybutyrate]/[acetoacetate] ratio in 24 h starved rats

The groups are as in Fig. 5.1. ●, saline infused controls; ■, galactosamine injection and saline infusion; □, galactosamine injection and DCA infusion. The significance of the various differences is listed below.

Sample time (mins)	Control vs GalN		GalN vs GalN + saline	
	Total Ketones	[3-OHB] / [Acac]	Total Ketones	[3-OHB]/[Acac]
0	NS	0.01	NS	NS
15	NS	0.001	NS	NS
30	NS	0.01	NS	NS
60	NS	0.01	0.05	NS
90	NS	0.01	0.02	NS
120	NS	0.01	0.001	NS

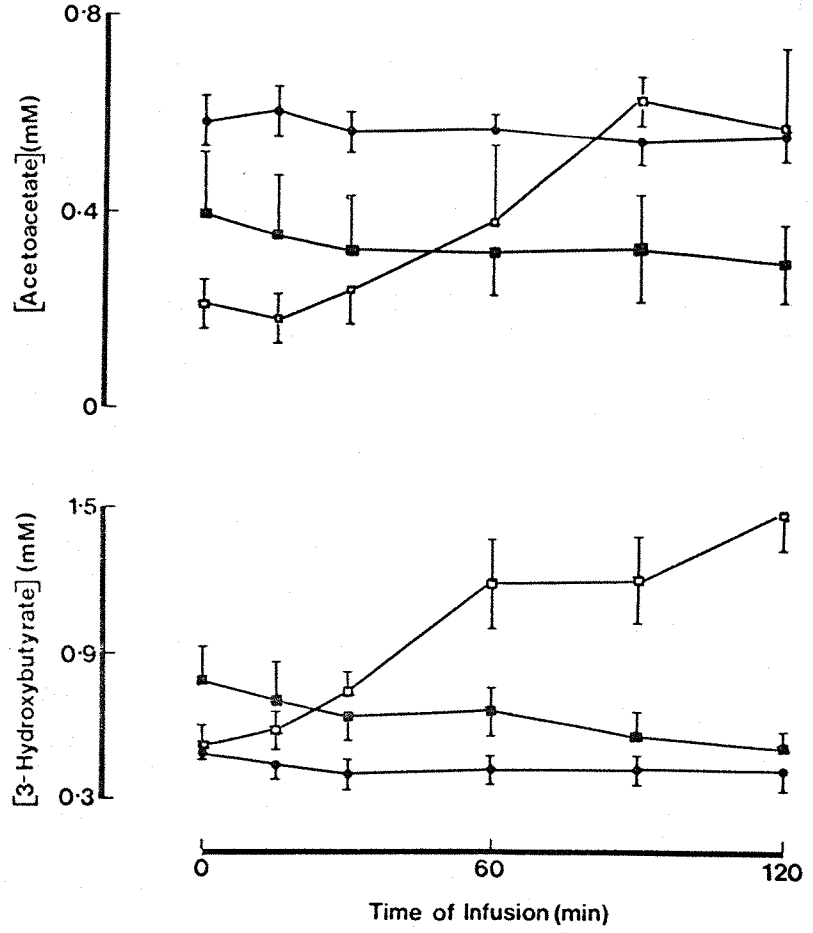


Fig. 5.3 The effect of galactosamine injection and DCA infusion on blood acetoacetate and 3-hydroxybutyrate concentrations in 24 h starved rats

The groups are as inFig. 5.1. ●, saline infused controls; ■, galN injection and saline infusion; □, galN injection and DCA infusion. The significance of the various differences is listed below; comparing galN injection and saline infusion with controls no differences are significant.

		Sample Time (mins)					
		0	15	30	60	90	120
Comparing galN + saline with galN + DCA	Acetoacetate	NS	NS	NS	NS	0.05	NS
	3-hydroxybutyrate	NS	NS	NS	0.05	0.02	0.001

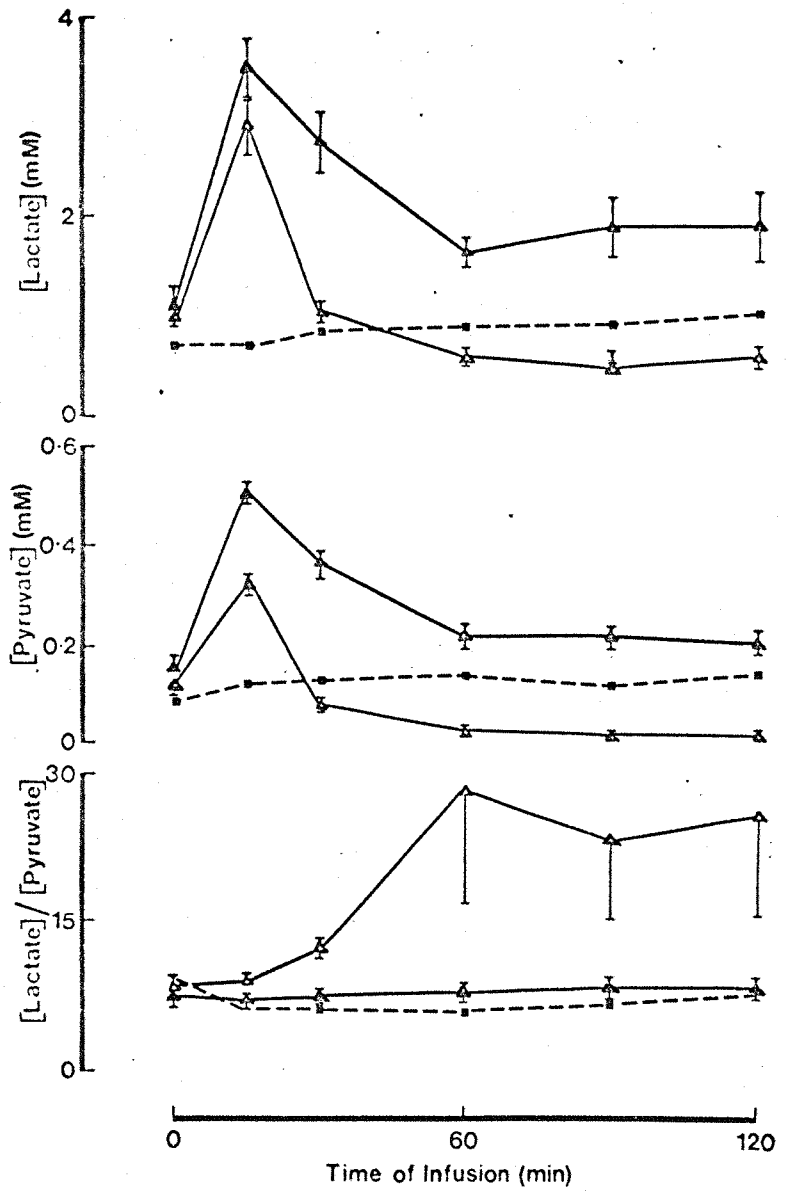


Figure 5.4. The effect of fructose injection and DCA infusion on blood lactate and pyruvate concentrations and on the blood [lactate]/[pyruvate] ratio in 24 h starved rats with galactosamine hepatitis

Fructose was injected immediately after the zero time sample, and all rats received galactosamine injections. The groups are as follows: ■, saline infusion (n = 5, as in Figs. 5.1, 2 & 3); ▲, fructose injection plus saline infusion (n = 5); △, fructose injection plus DCA infusion (n = 5). Statistical significances are presented below.

Sample time (min)	Saline vs fructose			Fructose vs fructose + DCA		
	Lactate	Pyruvate	Lac/pyr	Lactate	Pyruvate	Lac/pyr
0	NS	NS	NS	NS	NS	NS
15	0.001	0.001	NS	NS	0.001	0.02
30	0.001	0.001	NS	0.001	0.001	0.001
60	0.01	0.05	NS	0.001	0.001	NS
90	0.05	0.01	NS	0.01	0.001	NS
120	NS	NS	NS	0.01	0.001	NS

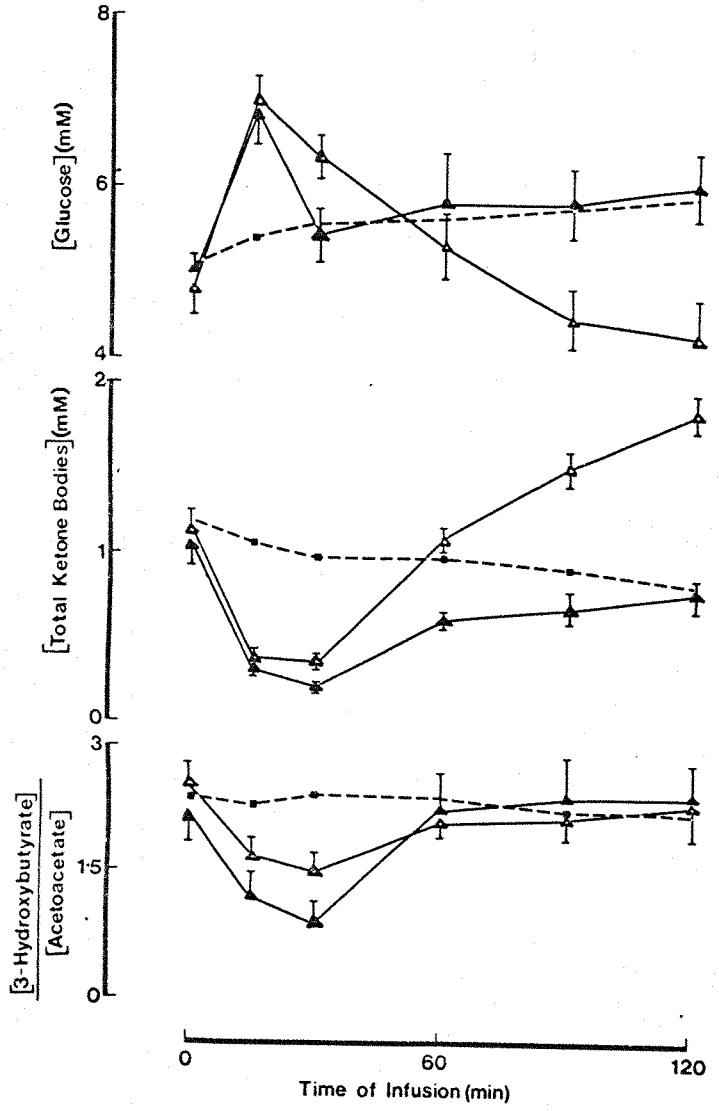


Figure 5.5 The effect of fructose injection and DCA infusion on blood glucose and total ketone body concentrations and on the blood [3-hydroxybutyrate]/[acetoacetate] ratio in 24 h starved rats with galactosamine hepatitis

All rats received galactosamine injections, the groups are as defined in Fig. 5.4. ■ , saline infusion; △, fructose injection plus saline infusion; ▲, fructose injection plus DCA infusion. Statistical significances are presented below

	Saline vs fructose			Fructose vs fructose + DCA		
Sample time (mins)	Glucose	Total Ketones	3-OHB/Acac	Glucose	Total Ketones	3-OHB/Acac
0	NS	NS	NS	NS	NS	NS
15	0.01	0.05	0.05	NS	NS	NS
30	NS	0.01	0.02	0.02	0.01	NS
60	NS	NS	NS	NS	0.01	NS
90	NS	NS	NS	0.02	0.01	NS
120	NS	NS	NS	0.01	0.01	NS

Table 5.1. The effects of galactosamine hepatitis, fructose and DCA on concentrations of metabolites in freeze-clamped livers from 24 h starved rats.

Experimental details as in Figs. 5.1 and 5.4. Results are expressed as μmol/g fresh wt ± SEM. Comparisons: B vs A, C vs B, D vs B and E vs D. *, $p < 0.05$; **, $p < 0.01$.

Treatment	Group				
Saline	+	+	-	+	-
Galactosamine	-	+	+	+	+
Fructose	-	-	-	+	+
Dichloroacetate	-	-	+	-	+
	A(n = 5)	B(n = 5)	C(n = 4)	D(n = 5)	E(n = 4)
Metabolite					
Glycogen	27.8 ± 7.0	8.23 ± 0.90*	13.5 ± 4.3	-	-
Glucose	9.73 ± 0.66	5.17 ± 0.35**	3.26 ± 0.22**	5.34 ± 0.45	3.80 ± 0.29**
Glucose-6-phosphate	0.53 ± 0.08	0.09 ± 0.01**	0.06 ± 0.01**	0.14 ± 0.05	0.05 ± 0.01
Fructose-6-phosphate	0.13 ± 0.02	0.02 ± 0.00**	0.01 ± 0.01	0.04 ± 0.01	0.02 ± 0.00
Fructose-1,6-diphosphate	0.04 ± 0.00	0.03 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Glycerol-1-phosphate	0.27 ± 0.05	0.22 ± 0.01	0.15 ± 0.02*	0.22 ± 0.03	0.27 ± 0.03
3-phosphoglycerate	0.40 ± 0.03	0.31 ± 0.03	0.17 ± 0.02**	0.27 ± 0.04	0.16 ± 0.02*
2-phosphoglycerate	0.05 ± 0.00	0.05 ± 0.01	0.04 ± 0.00	0.04 ± 0.01	0.03 ± 0.01
Phosphoenolpyruvate	0.15 ± 0.01	0.13 ± 0.02	0.08 ± 0.01*	0.11 ± 0.02	0.07 ± 0.01
Pyruvate	0.05 ± 0.00	0.08 ± 0.01*	0.08 ± 0.02	0.07 ± 0.01	0.06 ± 0.00
Lactate	0.60 ± 0.12	0.84 ± 0.27	0.41 ± 0.05*	1.30 ± 0.28	0.47 ± 0.10*
Alanine	0.19 ± 0.03	0.40 ± 0.10	0.10 ± 0.03*	0.57 ± 0.09	0.02 ± 0.01**
Citrate	0.36 ± 0.03	1.18 ± 0.22**	0.75 ± 0.08	1.79 ± 0.34	1.09 ± 0.30
Glutamate	1.88 ± 0.21	3.03 ± 0.40*	1.81 ± 0.14*	3.47 ± 0.55	1.42 ± 0.44*
2-oxoglutarate	0.05 ± 0.01	0.16 ± 0.03*	0.19 ± 0.01	0.28 ± 0.09	0.38 ± 0.18
Malate	0.34 ± 0.05	0.89 ± 0.18*	0.58 ± 0.05	1.09 ± 0.16	0.77 ± 0.17
Acetoacetate	0.16 ± 0.04	0.28 ± 0.09	0.60 ± 0.07*	0.15 ± 0.04	0.28 ± 0.03*
3-hydroxybutyrate	0.70 ± 0.05	0.59 ± 0.04	1.26 ± 0.22*	0.51 ± 0.12	0.90 ± 0.08*
Total ketone bodies	0.85 ± 0.06	0.87 ± 0.11	1.85 ± 0.27*	0.66 ± 0.14	1.17 ± 0.11*
$\frac{[3\text{-hydroxybutyrate}]}{[\text{acetoacetate}]}$	5.49 ± 1.51	2.89 ± 0.66	2.13 ± 0.32	3.84 ± 0.99	3.31 ± 0.23
[Lactate]/[Pyruvate]	13.6 ± 2.8	11.2 ± 3.1	5.72 ± 1.44	18.3 ± 3.0	7.30 ± 1.57*
AMP	1.61 ± 0.08	0.59 ± 0.06**	0.43 ± 0.06	0.82 ± 0.14	0.72 ± 0.10
ADP	2.43 ± 0.06	1.82 ± 0.15**	1.55 ± 0.10	1.76 ± 0.10	1.70 ± 0.27
ATP	1.66 ± 0.08	1.62 ± 0.23	1.68 ± 0.12	1.07 ± 0.21	0.88 ± 0.21
[ATP]/[ADP]	0.69 ± 0.05	0.88 ± 0.07*	1.11 ± 0.14	0.61 ± 0.11	0.51 ± 0.06
Total adenine nucleotides	5.16 ± 0.09	4.03 ± 0.35*	3.66 ± 0.13	3.65 ± 0.23	3.31 ± 0.57
Triglyceride	23.4 ± 1.4	64.3 ± 15.4*	92.3 ± 16.8	60.0 ± 5.5	58.1 ± 3.7

(d) Galactosamine plus fructose and dichloroacetate (Figs. 5.4 & 5.5). Rats were treated with GalN plus fructose as in the previous section. In these circumstances DCA infusion (300 mg/kg body wt per h) rapidly decreased arterial lactate, pyruvate and glucose concentrations compared with animals that received fructose loads and saline infusions. In contrast total ketone body concentrations rose, although DCA did not entirely reverse the immediate antiketogenic effects of fructose. There was a transient rise in the [lactate]/[pyruvate] ratio, but the [3-hydroxybutyrate]/[acetoacetate] ratio remained unaltered.

Liver Metabolites

(a) Galactosamine (Table 5.1). GalN administration resulted in a decrease in hepatic concentrations of glycogen, glucose, glucose-6-phosphate, fructose-6-phosphate, AMP, ADP and total adenine nucleotides; however, [ATP] was not significently affected. Hepatic concentrations of pyruvate, citrate, glutamate, 2-oxo-glutarate, triglyceride and the [ATP]/[ADP] ratio all increased.

(b) Galactosamine plus dichloroacetate (Table 5.1). DCA infusion into GalN treated rats resulted in further falls in hepatic concentrations of glucose and glucose-6-phosphate, while glycerol-1-phosphate, 3-phosphoglycerate, phosphoenolpyruvate, lactate, alanine and glutamate were lower than in GalN treated saline infused rats, and in controls. In contrast there was an increase in hepatic concentrations of acetoacetate, 3-hydroxybutyrate and total ketone bodies after DCA infusion.

(c) Galactosamine plus fructose (Table 5.1). Galactosamine treated rats injected with fructose showed no significant changes in the hepatic metabolites measured after two hours of saline infusion compared with uninjected GalN treated animals. However, lactate, pyruvate and alanine concentrations were now significantly higher than in saline treated animals (Group A).

(d) Galactosamine plus fructose and dichloroacetate (Table 5.1). In rats treated with GalN and fructose, DCA infusion caused a decrease in hepatic concentrations of glucose, 3-phosphoglycerate, lactate, alanine and glutamate, and in the [lactate]/[pyruvate] ratio. Lactate and alanine were now lower than in saline treated controls. Hepatic concentrations of acetoacetate, 3-hydroxybutyrate and total ketone bodies rose. The changes observed here in concentrations of 3-carbon glycolytic intermediates are similar to those observed in perfused livers treated with fructose and DCA (Table 4.2).

2. DISCUSSION

(a) Galactosamine. The dose of galactosamine used in this work (1 g per kg body wt) is slightly higher than that used by other authors to obtain experimental hepatitis. Record et al. (1972) observed considerable leakage of aspartate aminotransferase from the liver to serum, triglyceride accumulation and adenine nucleotide depletion in the liver, as well as histological evidence of liver damage, using 0.5 to 0.75 g of galactosamine per kg body weight. In earlier studies on galactosamine hepatitis doses of 1.5 g/kg body weight were used (Keppler et al., 1968; Koff et al., 1971a). The triglyceride and adenine nucleotide data presented here agree with the results of Record et al. (1972). The accumulation of triglyceride may be the result of impaired lipoprotein synthesis; protein synthesis is known to be impaired in galactosamine hepatitis (Koff et al., 1971b).

Galactosamine administration resulted in elevated blood lactate concentrations, as previously noted by Record et al. (1972), and Record and Alberti (1973). These authors also found decreased ketone body concentrations after galactosamine administration, a result not found in the present study. The perfused livers of galactosamine treated rats show impaired gluconeogenesis and lactate uptake (Record et al., 1972) and this is probably the cause of hyperlactataemia in this condition. Record et al. (1972) demonstrated that the impairment of lactate uptake was at least partly due to a loss of phosphoenolpyruvate carboxykinase from the

liver, and the pattern of hepatic metabolites they observed resembles that presented here. Although hepatic concentrations of glycogen, glucose and hexose monophosphates decreased, this did not result in lower blood glucose concentrations, as noted by Record et al. (1972). The accumulation of gluconeogenic precursors prior to the phosphoenolpyruvate step supports the proposed loss of phosphoenolpyruvate carboxykinase activity in galactosamine hepatitis in the present work.

(b) Fructose. The administration of fructose to man results in a rapid rise in blood lactate concentrations (Dodds et al., 1960; Woods & Alberti, 1972). Rawat and Menahan (1975) have also observed this phenomenon in the rat, and the present results indicate that the same effect occurs in rats with galactosamine hepatitis. The source of this excess lactate is probably the liver, and the mechanism of this effect is described in Chapter 1(d).

Fructose is a potent antiketogenic agent in the rat in vivo (Rawat & Menahan, 1975), a result also found in the present work. The antiketogenic effects of fructose in the perfused liver are probably due to the diversion of fatty acids from oxidation to esterification in the liver. The mechanism of this effect in the isolated perfused liver is discussed in Chapter 4.2(a). The situation in vivo may be further complicated by changes in the supply of NEFA to the liver. Fructose is known to stimulate insulin secretion and there could therefore be an inhibition of lipolysis after fructose administration; however, Rawat and Menahan (1975) found minimal changes in plasma NEFA concentrations after fructose administration to the starved rat.

Surprisingly there were no significant changes in liver metabolite concentrations in the fructose injected rats with galactosamine hepatitis compared with galactosamine injected controls. This finding contrasts with the perfused liver experiments in which concentrations of glucose, lactate and alanine rose while those of total adenine nucleotides and ATP fell. In the whole animal experiments fructose did not lower ATP or total adenine nucleotide levels, which were already low after galactosamine administration. Also, levels of lactate, alanine and glucose were not significantly affected in these conditions.

The total amount of fructose added to the perfused liver (270 mg/150 ml perfusate, 10 mM) is approximately the same as that administered in the whole animal experiments; i.e. 270 mg for a 270 g rat. In the perfusions the fructose reaches the liver directly via the portal vein, whereas in the whole animal the fructose is injected into the femoral vein, and therefore has ample time to mix with body water and possibly enter other tissues. It may also be excreted in the urine. The maximum arterial fructose concentration was 2.25 mM 15 minutes after injection, and this may explain the difference in the effects of fructose on the liver in vivo and in the perfusion experiments.

(c) Dichloroacetate. Dichloroacetate (DCA) was effective in reversing the hyperlactataemia that resulted from galactosamine and fructose administration. The data presented in Chapter 4.1 indicate that the activation of pyruvate dehydrogenase by DCA in the perfused liver is sufficient to remove the lactate produced by the liver after fructose addition. Work on functionally hepatectomised rats infused with DCA (Blackshear et al., 1974) suggests that extrahepatic tissues contribute to the hypolactataemic effects of DCA in vivo. The hyperketonaemic effects of DCA in vivo may be the result of the inhibition of 3-hydroxybutyrate uptake by extrahepatic tissues (Blackshear et al., 1974) as well as the increased rate of ketogenesis by the liver (see Chapter 3.1).

The effect of DCA on liver metabolite concentrations after fructose injection into galactosamine treated rats resembles the metabolic changes observed in liver perfusions with fructose after the addition of DCA. Levels of lactate and alanine fell, probably the result of PDH activation, while ketogenesis appears to increase. Glucose levels fell in the whole animal experiments, a result not found in the liver perfusion experiments. This is probably due to the lack of effect of DCA on gluconeogenesis in the presence of a copious supply of precursors (e.g. fructose in the perfused liver), whereas in vivo the work of Blackshear and his colleagues (1974) indicates that DCA inhibits gluconeogenesis by depriving the liver of lactate, alanine and pyruvate. The data do, however, suggest that the activation of hepatic pyruvate dehydrogenase by DCA is playing a role in the disposal of the lactate accumulating in blood and liver as a result of GalN and fructose administration.

CHAPTER 6

GENERAL DISCUSSION

As outlined in the Introduction, the basic aims of this work were firstly to set up models of impaired lactate metabolism in the isolated perfused rat liver and whole rat, and secondly to determine whether dichloroacetate, an activator of pyruvate dehydrogenase, could reverse these effects. The first aim has been achieved in each preparation. Fructose or phenethylbiguanide did cause an impairment of lactate metabolism in the isolated perfused rat liver, and galactosamine injection into whole rats caused hyperlactataemia. The second aim has partly been achieved, DCA does reverse the dramatic increases in lactate concentrations observed in the fructose perfusions, and also reverses hyperlactataemia in the whole animal experiments with galactosamine. However, the impairment of lactate clearance caused by PBG addition to the isolated perfused liver was unaffected by treatment with DCA. In these conditions PBG and DCA addition resulted in inhibition of glucose production and increased ketogenesis.

DCA is a potent hypoglycaemic agent in normal starved rats and in starved rats with galactosamine hepatitis. Blackshear et al. (1974) and Whitehouse et al. (1974) have suggested that the hypoglycaemic effects of DCA are due to the inhibition of the release of gluconeogenic precursors from extrahepatic tissues. In the case of skeletal muscle this has been confirmed by the work of Goodman et al. (1976) on the isolated perfused rat hindquarter. It was found that DCA inhibits the release of lactate, pyruvate and alanine by this preparation.

Hypoglycaemia due to a direct inhibition of hepatic gluconeogenesis is ruled out by the results presented in Section 3.1. In addition, Blackshear et al. (1974) observed that there was no accumulation of any gluconeogenic precursor in the livers of starved rats infused with DCA.

Hypoglycaemia due to an insulin mediated increase in glucose utilisation is ruled out since insulin levels fall after DCA infusion (Blackshear et al., 1974). Finally, hypoglycaemia due to a direct effect of DCA on peripheral glucose utilisation is ruled

out by the experiments of Blackshear et al. (1974) on functionally hepatectomised rats. In this preparation DCA infusion decreases the rate of accumulation of lactate and pyruvate but the rate of glucose disappearance is unaffected.

DCA reverses PBG hyperlactataemia in the whole rat (Holloway & Alberti, 1975) but is unable to reverse the impairment of lactate metabolism in the isolated perfused rat liver, therefore other tissues must be involved. In vitro studies have shown that PBG increases lactate production by perfused rat hearts (Williamson et al., 1963), epidydymal fat pads (Pereira et al., 1967) and kidney slices (Patrick, 1966); however, Wick et al. (1960) observed that very small amounts of ^{14}C-PBG accumulate in heart, kidney and adipose tissue compared with liver, skeletal muscle and the gastrointestinal tract. It therefore seems questionable whether PBG can act on heart, kidney and adipose tissue in vivo. Whitehouse et al. (1974) found that DCA activates PDH in heart, kidney and adipose tissue, and so it is possible that in DCA reversed PBG hyperlactataemia these tissues may play a role in the disposal of the excess lactate present in blood. The role of skeletal muscle in this situation is also not clear. Although DCA inhibits lactate production by the isolated perfused rat hindquarter (Goodman et al., 1976), it remains to be seen whether this effect occurs in the presence of PBG.

Dichloroacetate is therefore an interesting drug which may be useful in the treatment of lactic acidosis and hyperglycaemia in diabetes mellitus in man.

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APPENDIX A

A summary of the metabolic effects of DCA (10 mM) in the isolated perfused rat liver. Results are expressed as mmol/l at the end of perfusion, except for the underlined values which represent production in μmol/g wet wt per hour. Negative signs indicate uptake; the starved group received 10 mM lactate as substrate, other details as in Table 3.1. The fed group received no substrate; details as in Table 3.4.
**, $p < 0.01$; *, $p < 0.05$ versus controls. Number of observations in parentheses.

		Lactate	Glucose	Total Ketone Bodies	Pyruvate	Lactate / Pyruvate	3-hydroxybutyrate / acetoacetate
48 h starved	CONTROL	-101 ± 3 (12)	36.7 ±3.2 (12)	3.76±0.45(7)	0.60±0.03(12)	8.81±0.86(12)	0.38±0.04(7)
	DCA	-134 ± 6 (11)**	40.3 ±6.7 (11)	11.4 ±1.5 (9)**	0.16±0.01(11)**	26.1 ±1.1 (11)**	0.70±0.09(9)**
Fed	CONTROL	1.98±0.36(10)	8.40±0.46(10)	0.86±0.05(5)	0.28±0.04(10)	6.9 ±0.6(10)	0.5 ±0.1 (5)
	DCA	0.14±0.01 (9)**	9.14±0.57 (9)	0.98±0.04(4)	0.04±0.00 **	4.5 ±0.7 (9)*	0.1 ±0.0 (4)*

APPENDIX B

Concentrations of metabolic intermediates in perfused livers of fed and 48 h starved rats with and without 10 mM DCA. The starved group received 10 mM lactate as substrate, other details as in Table 3.2. For the fed group details as in Table 3.5. Results expressed as μmol/g fresh liver ± S.E.M.

	48 h starved		Fed	
Metabolite	Control	DCA	Control	DCA
Glycogen	0.96±0.21	1.86±0.60	203 ± 21	155 ± 41
Glucose	2.50±0.05	2.96±0.16*	5.84±0.77	8.19±0.66
Glucose-6-phosphate	0.04±0.01	0.05±0.01	0.09±0.02	0.11±0.02
Fructose-6-phosphate	ND	ND	0.04±0.01	0.03±0.00
Fructose-1,6-diphosphate	ND	ND	0.03±0.01	0.04±0.01
Glycerol-1-phosphate	0.24±0.06	0.32±0.05	0.38±0.04	0.41±0.06
3-phosphoglycerate	0.38±0.04	0.16±0.01**	0.28±0.02	0.40±0.05*
2-phosphoglycerate	0.07±0.01	0.03±0.00**	0.08±0.01	0.06±0.01
Phosphoenolpyruvate	0.17±0.02	0.08±0.00**	0.12±0.01	0.19±0.02*
Pyruvate	0.25±0.03	0.12±0.01**	0.31±0.03	0.09±0.01**
Lactate	2.25±0.24	1.77±0.32	1.68±0.11	0.60±0.03**
Alanine	1.37±0.10	0.51±0.10**	0.86±0.20	0.19±0.02*
Citrate	0.63±0.07	0.91±0.09*	0.37±0.02	0.23±0.01**
2-oxoglutarate	0.27±0.03	0.39±0.09	0.64±0.03	0.33±0.02**
Glutamate	2.57±0.21	2.45±0.07	2.19±0.29	2.16±0.12
Malate	0.24±0.02	0.44±0.03**	0.19±0.02	0.10±0.01**
Acetoacetate	0.45±0.06	0.38±0.06	0.51±0.02	0.74±0.04**
3-hydroxybutyrate	0.41±0.05	0.36±0.04	0.17±0.02	0.16±0.01
Total ketone bodies	0.86±0.08	0.74±0.06	0.68±0.03	0.90±0.04**
$\frac{[\text{3-hydroxybutyrate}]}{[\text{acetoacetate}]}$	1.0 ±0.2	1.1 ±0.2	0.3 ±0.0	0.2 ±0.0 *
[Lactate]/[Pyruvate]	8.8 ±0.6	14 ±2	5.6 ±0.3	6.4 ±0.5
AMP	0.61±0.07	0.49±0.08	0.27±0.02	0.23±0.00
ADP	1.63±0.15	1.41±0.07	1.01±0.10	1.19±0.03
ATP	1.43±0.09	1.56±0.18	2.23±0.09	2.13±0.10
Total adenine nucleotides	3.67±0.20	3.47±0.17	3.51±0.19	3.55±0.13
[ATP]/[ADP]	0.91±0.12	1.12±0.16	2.25±0.16	1.80±0.04*
Triglyceride	7.84±0.86	7.33±0.57	7.51±0.77	9.32±0.33
Number of observations	5	5	4	4