STUDIES OF THE ALTERATION OF CARBOHYDRATE, FAT AND
BRAIN AMINE METABOLISM IN MAN IN HEALTH AND DISEASE.

by

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BIBLIOGRAPHY

PUBLICATIONS RESULTING FROM THIS WORK

Abbreviations used in this thesis

ADP	adenosine diphosphate
ADH	alcohol dehydrogenase
ATP	adenosine triphosphate
ACTH	adrenocorticotrophic hormone
c.s.f.	cerebro-spinal fluid
E.C.G.	electrocardiograph
FFA	free fatty acids
HIAA	5-hydroxy-3-indole acetic acid
HVA	homovanillic acid
HBDH	hydroxybutyrate dehydrogenase
HGH	human growth hormone
IRI	immuno reactive insulin
LDH	lactate dehydrogenase
NAD(H)	nicotinamide adenine dinucleotide (reduced)
MIN.	minutes
Hr.	hours
PCA	Perchloric acid
PEP	Phospheenol pyruvate
PK	Pyruvate kinase
R.P.M.	revolutions per minute
SEM	standard error of the mean
UV	ultra violet

Units used

l	Litre
ml	Millilitre − 10^{-3} l
μl	Microlitre − 10^{-6} l
Kg	Kilogram
g	Gram
mg	Milligram − 10^{-3} g
Kp	Kilopond
m	Meter

INTRODUCTION

This thesis is based on work carried out by myself while working as a research student in the Department of Neurology of the University of Glasgow. The thesis has been organised into three sections: studies of the metabolic effects of alcohol, exercise studies of patients and studies of brain transmitter metabolites in the c.s.f. of patients. Each section is composed of a number of chapters which deal with individual aspects of the appropriate study. The results are given and discussed in each chapter separately.

The thesis has a major division between studies of the effects of alcohol and disease on fat and carbohydrate metabolism and the cerebrospinal fluid studies. It had originally been intended to develop the studies of the effects of alcohol by examining its action upon brain transmitters. The investigations of transmitter metabolites in the c.s.f. of patients with neurological diseases were developed to gain experience in the measurement of normal levels and the way they could be affected by disease. These studies in Parkinsonism and Huntington's chorea were completed as they proved very successful and have resulted in publications. Acceptance of an appointment in the Civil Service prevented the further development of the work along the original plan and the studies of the effect of alcohol upon c.s.f. were therefore not completed.

1. THE EFFECTS OF ALCOHOL

Many compounds are alcohols; however ethanol is
the principal alcohol pound in alcoholic drinks.   In
the scientific literature on the effects of alcohol, the
terms alcohol, ethanol and ethyl alcohol have been variously
used to describe the same compound.   In this thesis, the
term alcohol is used exclusively and is taken to mean
ethanol.

a. Historical background

Man is likely to have experienced the effects of
alcohol consumption for centuries.   Fermented liquors
were known in very early times but the effects of
drinking alcoholic beverages became important with the
discovery of the distillation process and the production
of spiritous liquors which contained high concentrations
of alcohol.   Morewood (cited by Muspratt, 1860) has
speculated about the introduction of spiritous liquors
into England.   It is said that the art of distillation
was first introduced to Ireland from Spain or Italy where
the liquor was known as acque vite.   The monasteries being
the archives of science and the original dispensaries of
medicine probably corrupted the term acque vite into the
latin and universal appellation aqua vitae from its
beneficial effects as a medicine.   The dissolution of
the monasteries gave the secret of this to the public
and the elixir of the ambelic soon attained the summit
of popular regard.   In the time of Henry II, the English
found that an alcoholic liquor was being made and consumed

in Ireland and imported the process to their own country.

Cultural factors have played an important part in the spread of alcohol drinking and alcoholism. An important element in the spread of drinking was the habit of spirit drinking which became common towards the end of the 17th Century, the start of the gin era of cheap spirits which reached its peak in 1720-1750. The habit of drinking alcoholic beverages has remained with society whether it be to increase social discourse or to forget the miseries of life and is still popular today.

b. Metabolic Effects

By medical and legal definition, alcohol may be classified as a food. It supplies energy which the body can use; one gramme supplies 7 calories compared with 4 and 9 calories for 1g. of carbohydrate and fat respectively. Alcohol is however an inadequate food because too much of it may induce intoxication and because it lacks vitamins, proteins and minerals. Alcohol has been described as having the pharmacological properties of a sedative, tranquillizer, hypnotic or anaesthetic depending on the quantity consumed.

Traces of endogenous ethanol are found in all individuals and it is one of the many endogenous alcohols found in the body. Endogenous alcohols serve useful metabolic functions and enzyme systems are present for their production and disposal. Higher alcohols are formed in the process of fat metabolism e.g. cholesterol, glycerol and vitamin A.

Ingested alcohol is absorbed rapidly by virtue of its small size and high solubility. After absorption into

the blood, each tissue takes up alcohol according to its
water content and organs which have a very rich blood
supply such as brain, liver and kidney quickly reach
equilibrium with circulating blood alcohol.

Alcohol is metabolised principally by the hepatic
NAD dependent alcohol dehydrogenase (ADH) (Thompson, 1956).
Individuals have been found to vary in their rates of
alcohol oxidation and this has been found to be due to
the amount and activity of liver ADH (Westerfield, 1961).
The increased rate of alcohol oxidation in some alcoholics
has been attributed to the induction of a microsomal
ethanol oxidising system associated with the smooth
endoplasmic reticulum of the liver cell and inducible
by alcohol or barbiturates (Lieber and De Carli, 1970).
Figure (I,1) shows the pricipal steps in the metabolism
of alcohol. Alcohol is metabolised to acetaldehyde
which appears to be rapidly converted to Acetyl-Co-A
as only small amounts of acetaldehyde can be detected in
the blood. However, acetaldehyde has powerful pharmaco-
logical properties and it could be that even small
concentrations are very important. Acetaldehyde is
converted to acetyl coenzyme A which undergoes oxidation
to CO_2 and water via the Krebs cycle.

Alcohol is preferentially metabolised when available
but the rate of oxidation is limited by the availability of
NAD. NADH competes with NAD for a binding site on ADH
and if present in sufficient concentration NADH will inhibit
ADH. The oxidation of alcohol gives rise to a decrease in

FIG I.1
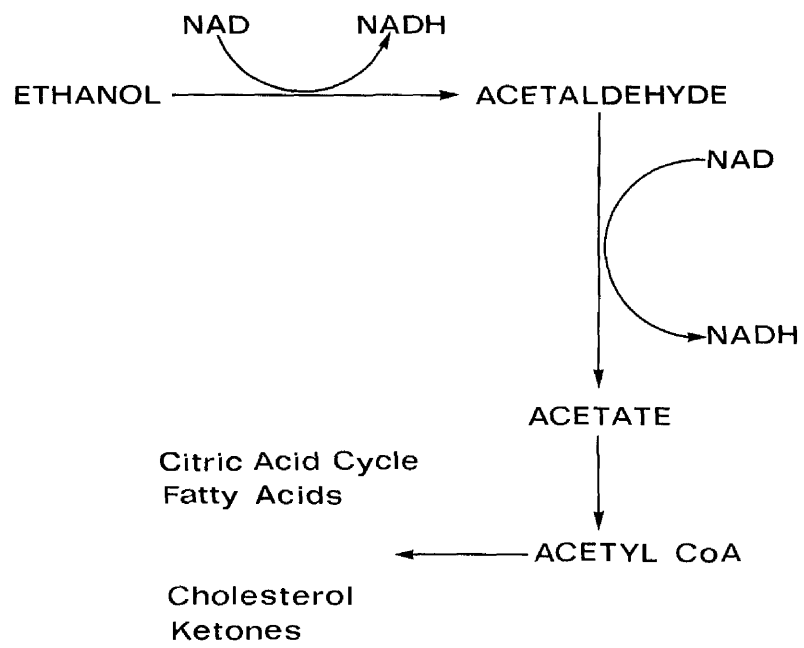
Hepatic Metabolism of Ethanol

the NAD/NADH ratio. The shift in the NAD/NADH ratio
gives rise to altered levels of blood metabolites that
are also linked to the NAD − NADH system. It was the
intention of this thesis to study the alteration of blood
metabolites produced by the redox change associated with
alcohol metabolism. Exercise is also associated with
a decrease in the NAD/NADH ratio and it was decided to
study the combined effect of alcohol and exercise to see
if the pattern of fuel utilisation was altered. Alcohol
has been reported to produce hypoglycaemia under certain
conditions (Madison, 1968) and it was hoped that a general
study of the metabolic effects of alcohol would aid an
understanding of the production of hypoglycaemia. It
was also hoped to extend the study of the effects of alcohol
to include a consideration of alcohol induced glucose
intolerance and the acceleration of the rate of alcohol
oxidation by fructose. The results of these investi-
gations are described in this thesis.

In summary, the effects of alcohol on man can be
grouped under three broad headings:-

1. Alteration of NAD/NADH ratio (redox effect)

2. Pharmacological action

3. Chronic effect: chronic effects of 1 and 2
   and also nutritional effect since alcohol
   is an inadequate food.

The studies carried out in section 1 of this thesis
are an attempt to examine some of the altered metabolite
levels associated with the decrease in the NAD/NADH ratio
when alcohol is metabolised. The second section includes

a consideration of the study of the chronic alcoholic who might well be considered to manifest the effects of chronic alcohol ingestion.

2. EXERCISE TESTING OF PATIENTS

A considerable amount of information is currently available concerning the normal metabolite changes associated with exercise. The research group of which I was a member had gained considerable expertise and knowledge of the physiological and biochemical changes produced during exercise in a variety of subjects. The exercise test therefore provided a useful method for studying the effects of drugs and disease on certain blood metabolites. Research involving such a simple test as exercise has the attraction that it may be of use in the diagnosis of certain diseases.

a. Alcoholic patients

Alcoholism results from the chronic intake of alcohol and it seems likely that alcohol is a weak drug of dependence compared with heroin for example and if enough is taken for a long time certain biochemical changes in the body may occur. Alcoholism has been defined in terms of alcohol's adverse effects on the drinker, his family or society; in terms of getting drunk; in terms of the compulsive nature of drinking and also in terms of recognisable physical or psychological symptoms. This

apparent lack of a firm definition has been a stumbling block to a better understanding of the disease.

Heredity or congenital factors, deficiency of specific nutrients, brain pathology and endocrine dysfunction have all been suggested as possible causes of alcoholism. Research so far has given little indication of any physiological or genetic factors that specifically cause alcoholism although the possibility that such factors exist cannot be ruled out and further research is essential.

Alcoholism is often associated with neuromuscular disorders and weakness and therefore alcoholics are a good group of patients in whom to study the metabolic response to exercise. On the basis of studies of the effects of acute alcohol ingestion, it may be possible to explain some of the effects of chronic alcohol ingestion.

b. Coronary patients

The exercise test is in current use clinically and is of particular value in the identification of abnormal electrocardiograph recordings in coronary subjects. Exercise has been reported to remove the symptoms of angina in certain coronary subjects but this conflicts with present ideas advocating prolonged immobilisation of patients (Lancet: Leader, 1972). A period of training produced striking improvement in coronary subjects and it has been suggested that this was due to improved myocardial oxygen supply, also training of coronary patients

appears to be associated with lower mortality rates
(Redwood et al, 1972)   To clarify the situation,
controlled trails of exercise programmes are now required
to assess the benefit of training coronary subjects.

An opportunity arose to carry out such a study in
collaboration with the Coronary Care Unit of the Victoria
Infirmary, Glasgow.   Initially, it was hoped to demonstrate
any abnormal metabolite changes associated with exercise in
coronary subjects and to determine the relationship of
any such changes to the occurence of an abnormal e.c.g..
It was hoped to re-assess the subjects after a period of
training to detect any improvement.However within the
time available it was not possible to complete this study
but what results were obtained are given and discussed
in this thesis.

3. STUDIES OF BRAIN TRANSMITTER METABOLITES IN c.s.f.

As an extention of the studies of alcohol, it was
originally planned to study the release of transmitter
substances by alcohol as this is important in the study
of neurological disorders associated with chronic alcoholism.
Because of difficulties encountered in the assay of
catecholamines, I decided to measure the concentrations
of the acid metabolites of transmitter substances in the
c.s.f.

The acid metabolites, homovanillic acid (HVA) and

5-hydroxy-3-indolacetic acid (5-HIAA) are the pricipal
metabolites of dopamine and serotinin respectively.
It has been demonstrated that changes in the concentration
of these metabolites in the c.s.f. give an indication of
the turnover rates of transmitters in the brain (Ashcroft,
et al, 1968).

Several studies of these acid metabolites have been
made in certain neurological diseases principally Parkinson's
disease and have helped to give an understanding of the
biochemical basis of the appropriate drug therapy. Having
developed methods and established control levels of HVA
and HIAA, I was able to follow and measure these metabolites
in patients with neurological disease undergoing drug
therapy. Such studies are extremely valuable in that they
give an objective measurement of the effects of the drug
and can be used to interpret clinical findings. The
results of these drug studies are given and discussed in
this thesis.

METHODS

This section gives details of the methods used
in the various investigations described in this thesis.
The section is divided into three parts and details the
methods appropriate to each part

    1.   Exercise

    2.   Oral Tolerance Tests

    3.   Biochemical Analyses.

1.   EXERCISE

Ergometer

Subjects were exercised on an electric, variable
load, Elema Schonander bicycle ergometer type EH369.
This machine allowed exercise to be performed at a
constant known work load. Subjects maintained a
relatively steady rate of exercise by monitoring the
r.p.m. of the bicycle at intervals. The exercise test
performed in this manner was both quantitative and
reproducible.

A work load of 600 k p m /min. was found by
experience to be suitable for all control subjects
and patients. An exercise duration of thirty minutes
was sufficient to produce changes in blood metabolites
and was convenient for the design of an experiment.
Consequently, the exercise test was standardised at a
load of 600 k p m /min. for thirty minutes.

Treadmill

In the study of Coronary patients and controls, a multistage treadmill test was performed. Subjects exercised at a series of stages each stage being of three minutes duration. At the end of each stage, blood samples and an electrocardiogram recording were taken.

The speed and gradient of the treadmill were varied to give a series of stages of exercise. To allow comparison with other published work, the approximate energy requirement of each stage was calculated in terms of metabolic equivalents (METS).

STAGE	$\frac{1}{2}$	I	II	III	IV	V
M.P.H.	1.7	1.7	2.5	3.4	4.2	5.0
GRADE	5%	10%	12%	14%	16%	18%
METS.	3	5	7	9.5	13.5	15

The exercise was discontinued when the subjects attained an age-related target heart rate because of coronary symptoms or falling blood pressure. Because of the danger in exercising Coronary patients, two cardiologists were present at all times. Implicit in this experimental design is the weakness that each subject tended to have different exercise regimes and consequently a large number of subjects had to be studied.

2. ORAL TOLERANCE TESTS

Glucose Tolerance Test

A standard oral glucose tolerance test was performed by giving the subject 50g. of glucose dissolved in 200 ml. of water. Venous blood samples were taken at 30 minute intervals after oral glucose for $2\frac{1}{2}$ hours.

Acetoacetate Tolerance Test

Acetoacetate was prepared by the hydrolysis of ethyl acetoacetate with a slight excess of NaOH. The resulting solution was vacuum distilled to remove contaminant ethanol and unreacted ester(Davies, 1943, Krebs and Eggleston, 1945). The concentration of acetoacetate was determined and the solution adjusted to 0.4 M.. An oral acetoacetate tolerance test was performed by giving the subject 200 ml. of the 0.4 M. acetoacetate solution and taking venous blood samples at 30 minute intervals for $2\frac{1}{2}$ hours,

Alcohol administration

To study the effect of alcohol, pure ethanol (Burroughs Ltd.) was given orally in a weight related dose (0.5 g./Kg. body weight) and made up to 200 ml. with water.

This corresponds approximately to a solution 22° proof or is equivalent in alcohol content to 60 ml. of 70° proof spirit (for 100 Kg. body weight).

Fructose administration

Because of the danger of giving alcohol/fructose mixtures which promote lactic acidosis, only small quantities were given and all administrations were oral. Fructose was given as a solution of 50g. fructose in 200 ml. of water.

3. BIOCHEMICAL ANALYSIS

Sampling technique

Venous blood samples were withdrawn at appropriate intervals of time from a polythene cannula in an ante-cubital vein. A period of at least 10 minutes was allowed to lapse between insertion of the cannula and the withdrawal of the first resting sample.

For each sample taken, 4 ml. of blood was added to 5 ml. of 10% (^{W}/v) ice cold perchloric acid in a pre-weighed tube for the determination of glucose, lactate, pyruvate, acetoacetate, β-hydroxy-butyrate, glycerol and where appropriate, alcohol. An additional 10 ml. of blood was added to a heparinised container for the estimation of plasma free fatty acids, and Human Growth hormone and insulin if required. Immediately after an investigation, samples were weighed, centrifuged at 3,000 r.p.m. for 10 minutes, separated and stored at -10°C. until required.

Treatment of denatured blood

The perchloric acid extract was centrifuged (2,500 r.p.m. for 10 mins.) poured into a graduated tube and the volume noted. The extract was neutralised with 20% potassium hydroxide using B.D.H. universal indicator and centrifuged (2,500 r.p.m. for 10 minutes) to remove potassium chlorate precipitate. The volume of the neutralised extract was noted. Biochemical analysis of glucose, lactate, pyruvate, β-hydroxy-butyrate, acetoacetate, glycerol and alcohol was carried out on a known volume of neutralised extract.

A dilution factor was calculated from the volume
of blood added to 5 ml. of perchloric acid and the change
in volume after neutralisation.

Spectrophotometric measurements were carried out on
either a Hilger & Watts 'Uvispek' H700307 or a Hilger &
Watts 'Spectrochem' H 840 301.

Methods

Enzymatic methods were used for the estimation of
the metabolites in deprotemised extracts of blood.
Enzymes and co-enzymes were purchased from the Boehringer
Corporation (London) Ltd.. All other chemicals used
were of analytical or aristar grade and were purchased
from B.D.H. (Poole) Ltd..

Pyruvate

Pyruvate was estimated by the enzymic method of
Hohorst, Kreutz and Bucher (1959). This assay measures
the decreased optical density due to the oxidation of
reduced nicotinamide adenine dinucleotide (NADH) at
340nm, following the reduction of pyruvate to lactate
by lactate dehydrogenase (LDH) at pH 7.0 in the 0.1 M.
phosphate buffer:-

$$\text{Pyruvate + NADH + H}^+ \underset{}{\overset{\text{LDH}}{\rightleftharpoons}} \text{lactate + NADH}$$

Lactate

Lactate was estimated by the enzymic method of Hohorst,
Kreutz and Bucher (1959). This assay measures the increase
in optical density due to the reduction of nicotinamide

adenine dinucleotide (NAD) at 340 nm following the
oxidation of lactate to pyruvate by lactate dehydrogenase
at pH 9.5.. Pyruvate was removed in the form of its
hydrozone. The conditions of this assay favour the
formation of pyruvate:-

$$\text{lactate} + \text{NAD} \underset{\longleftarrow}{\xrightarrow{\text{LDH}}} \text{pyruvate} + \text{NADH} + \text{H}^+$$
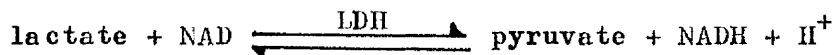
Acetoacetate

Acetoacetate was determined by the enzymic method of
Williamson, Mellanby and Krebs (1962). This assay
measures the decrease in optical density due to the
oxidation of NADH at 340 nm following the reduction of
acetoacetate to - hydroxybutyrate by -hydroxy-butyrate
dehydrogenase (HBDH) at pH 7.0:-

$$\text{acetoacetate} + \text{NADH} \underset{\longleftarrow}{\xrightarrow{\text{HBDH}}} \beta\text{-hydroxy-butyrate} + \text{NAD}$$
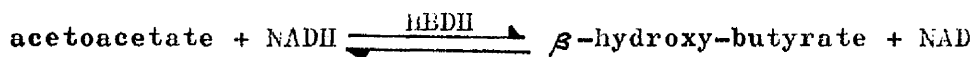
In practice, the determination of acetoacetate was
carried out in the same cuvette following the determination
of pyruvate. Acetoacetate is slowly decarboxylated in
neutral solution so the assay was carried out immediately
after samples were neutralised.

β-hydroxy-butyrate

β-hydroxy-butyrate was determined by the enzymic
method of Williamson, Mellanby and Krebs (1962).

This assay measures the increased optical density due
to the reduction of NAD at 340 nm following the oxidation
of β-hydroxy-butyrate to acetoacetate by β-hydroxy-butyrate
dehydrogenase at pH 9.5.. Acetoacetate is removed in the
form of its hydrazone. The condition of this

reaction favour the formation of acetoacetate.

$$\beta\text{-hydroxy-butyrate} + NAD \xrightleftharpoons{\text{HBDH}} \text{acetoacetate} + NADH + H^+$$
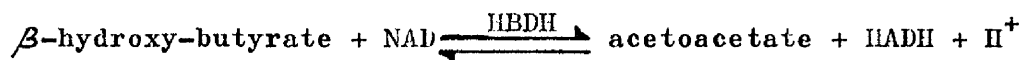
Glycerol

Glycerol was determined by the enzymic method of Kreutz (1962). Glycerokinase (GK) was used to catalyse the phosphorylation of glycerol to glycerol - 1 - phosphate from adenosine triphosphate (ATP):-

$$\text{glycerol} + ATP \xrightleftharpoons{\text{GK}} \text{glycerol} - 1 - \text{phosphate} + ADP$$
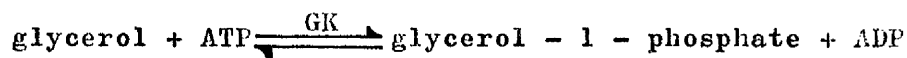
The adenosine diphosphate was rephosphorylated to ATP from phosphoenol pyruvate (PEP) by pyruvate kinase (PK):-

$$ADP + PEP \xrightleftharpoons{} ATP + \text{pyruvate}$$
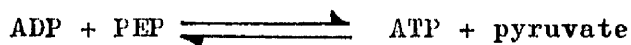
Pyruvate was then reduced to lactate with lactate dehydrogenase and the decrease in optical density due to the coupled oxidation of NADH measured at 340 nm:-

$$\text{pyruvate} + NADH + H^+ \xrightleftharpoons{\text{LDH}} \text{lactate} + NAD$$

The overall reaction is :-

$$\text{glycerol} + PEP + NADH + H^+ = \text{glycerol} - 1 - \text{phosphate} + \text{lactate} = NAD.$$
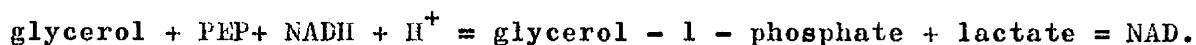
Blood alcohol

Blood alcohol was determined by the enzymic method of Bonnichsen and Theorell (1951). This method measures the increase in optical density due to the reduction of NAD at 340 nm following the oxidation of alcohol to acetaldehyde by alcohol dehydrogenase (ADH):-

$$\text{ethanol} + NAD \xrightleftharpoons{\text{ADH}} \text{acetaldehyde} + NADH + H^+$$
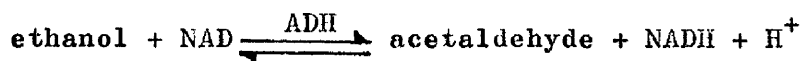
The reaction is made irreversibly by the addition of

semi carbazide which reacts with acetaldehyde as it is formed.

Great difficulty was found with this assay due to the high level of alcohol in the atmosphere. (The laboratory was situated next to a pathology laboratory). It was only with great difficulty that low enough control readings could be produced to allow the assay to be used.

Plasma free fatty acids

Free fatty acids were estimated in plasma by the colorimetric method of Itaya and Ui (1965) with the modification of Dalton and Kowalski (1967) for an automated procedure.

This method involves the extraction of free fatty acids (FFA) firstly into aqueos buffer then into chloroform. The FFA is estimated in the chloroform extract as the copper soap with diethyldithiocarbamate.

Glucose

Blood glucose was estimated by the enzymatic method of Werner et al (1970) using a Boehringer Biochemical Test Combination (No. 5755).

Glucose is oxidised by glucose oxidase to gluconolactone which in aqueous solution is converted to gluconic acid:-

$$\text{glucose} + O_2 + H_2O \rightleftharpoons \text{gluconic acid} + H_2O_2.$$
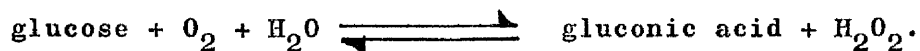
In the presence of peroxidase, the hydrogen peroxide oxidised the chromogen with the formation of a dye:-

$$H_2O_2 \quad + \quad chromogen \rightleftharpoons dye \qquad H_2O.$$
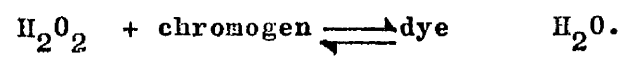
The intensity of the dye is proportional to the concentration of glucose and was measure at 640 nm.

CHAPTER 1

THE EFFECT OF ALCOHOL ON BLOOD METABOLITES AT REST

INTRODUCTION

Alcohol has been reported to cause a number of
alterations of blood metabolites.   Alcohol oxidation
in the liver has been shown to cause an increase in the
lactate/pyruvate and β-hydroxy-butyrate/acetoacetate
ratios in hepatic venous blood (Arthy et al, 1968, and
Tygstrup et al, 1965).

Many published reports of alcohol induced hypoglycaemia
are available (for example, Tucker and Porter, 1942;
Field et al, 1962,.   Moynihan, 1965, suggested that
alcohol induced hypoglycaemia was due to a direct metabolic
effect of alcohol and not, as earlier authors have suggested,
due to toxic products in denatured alcohol (Brown and
Harvey, 1941; Tucher and Porter, 1942).   It has also
been demonstrated, (Freinkel et al, 1962) that alcohol
induced hypoglycaemia could be produced after a forty-
eight hour fast.

It was hoped that a general study of the metabolic
effects of alcohol would aid an understanding of the
direct effects of alcohol on gluconeogenesis and explain
the origin of alcohol induced hypoglycaemia.   In order
to interpret later experiments on the effect of alcohol
on the metabolic changes normally associated with exercise
it was necessary to study the effect of alcohol on blood
metabolites at rest and the results are reported in this
chapter.

METHODS

Subjects

Five male subjects aged 25-38 years were given alcohol orally in a weight related dose (0.5g/Kg body weight). The subjects were relatively uniform with respect to weight 75.2 $\pm$ 6.7 Kg (mean $\pm$ SEM) and height, 174.4 $\pm$ 3.5 cm (mean $\pm$ SEM). The subjects had fasted overnight and none of them had taken alcohol on the day before the investigation.

Procedure

The subjects remained at rest throughout the investigation. Blood samples were taken from a catheter placed in an antecubital vein. Samples were taken before alcohol and at 15, 30, 45, 60, 90, 120 and 150 min. after alcohol had been taken.

RESULTS

Blood alcohol (Fig. 1:1)

The level of blood alcohol increased rapidly after
oral alcohol reaching a peak level (41 mg%) in the sample
taken at 45 min..   Alcohol levels remained high
throughout the experiment and after 150 min. following
oral alcohol the level was still 38% of the peak value.
The rate of alcohol disappearance from the blood was
approximately 14.3 mg/100ml/hr..

Blood lactate (Fig. 1:2)

Alcohol rapidly increased blood lactate which reached
a maximum level in the sample taken 90 min. after alcohol
ingestion.   Thereafter the blood lactate level returned
towards the resting level.   Samples taken at 15, 30, 60 and
90 min. had blood lactate significantly higher ($P < .05$)
than the pre-alcohol level.

Blood pyruvate (Fig. 1:2)

Alcohol rapidly decreased the level of blood pyruvate
which reached a minimum level 45 min. after alcohol.
There was little return towards the pre-alcohol level
within the duration of the experiment.   In samples taken
at 15, 45, 60 and 90 min. the pyruvate level was significantly
smaller ($P < .05$) than the pre-alcohol level.   Samples
taken at 120 and 150 min. had mean pyruvate levels lower
than the pre-alcohol level but the difference was not
significant.

Fig. 1 – 1.   Blood alcohol (mg/100 ml, mean $\pm$ SEM)

in five normal subjects at rest after an

oral dose of alcohol (0.5g/Kg. body weight).
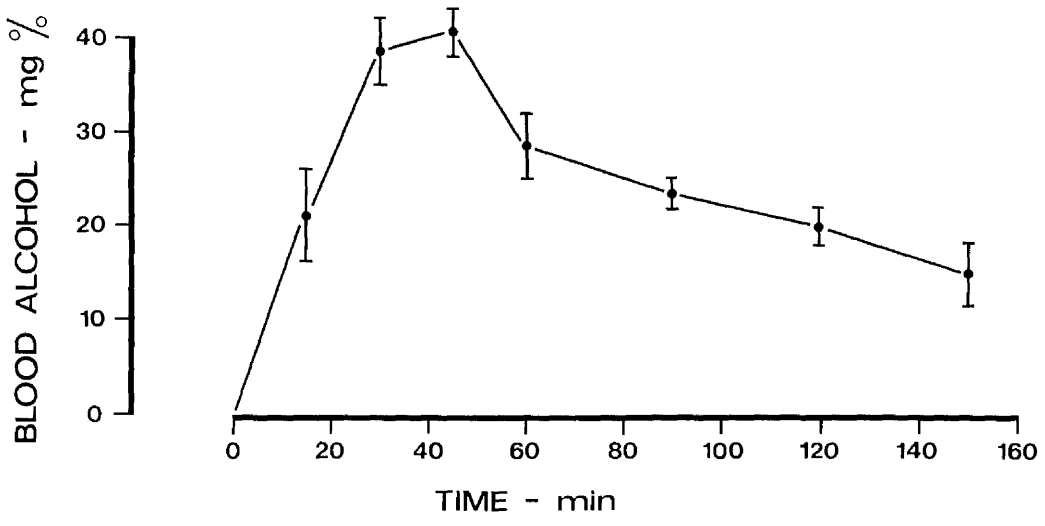
Fig. 1 - 2.   Blood lactate and blood pyruvate

(μ mol./ml., mean $\pm$ SEM) in five

normal subjects at rest before and

after an oral dose of alcohol (0.5g/

Kg. body weight).
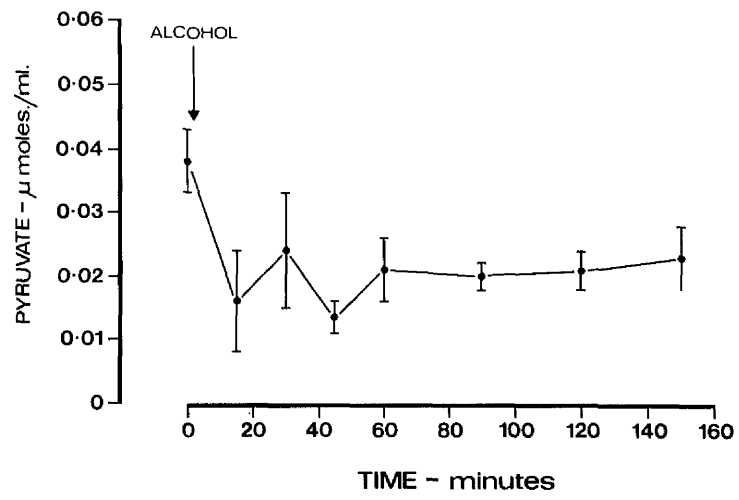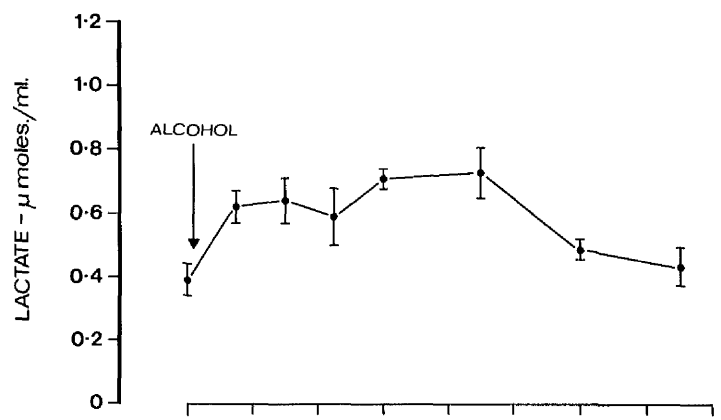
Lactate/pyruvate ratio (Fig. 1:4)

Alcohol rapidly increased the lactate/pyruvate ratio which reached a maximum value 15 min. after alcohol and remained elevated for the duration of the experiment. At 15, 30, 45, 60, 90 and 120 min. the lactate/pyruvate ratio was significantly higher ($P < .05$) than the pre-alcohol ratio.

Blood β-hydroxy-butyrate (Fig. 1:3)

Alcohol produced an increase in the blood β-hydroxy-butyrate level which reached a maximum level at 15 min.. The levels in samples taken at 15, 30, 45, 60 and 90 min. were significantly greater ($P < .01$) than the pre-alcohol level.

Blood acetoacetate (Fig. 1:3)

Alcohol produced only a small late decrease in the acetoacetate level. The levels in samples taken at 60 and 90 min. being significantly lower ($P < .05$) than the pre-alcohol level.

β-hydroxy-butyrate/acetoacetate ratio (Fig. 1:4)

Alcohol caused a rapid increase in the β-hydroxy-butyrate/acetoacetate ratio which was maintained for 90 min. following alcohol. The β-hydroxy-butyrate/acetoacetate ratio was significantly increased ($P < .05$) in samples taken at 15, 30, 45, 60 and 90 min. after alcohol had been taken. At 120 and 150 min. the ratio was not significantly different from the initial pre-alcohol ratio.

Fig. 1 - 3.   Blood β-hydroxy-butyrate and

acetoacetate (μ mol./ml., mean $\pm$ SEM)

in five normal subjects at rest before

and after an oral dose of alcohol (0.5g/
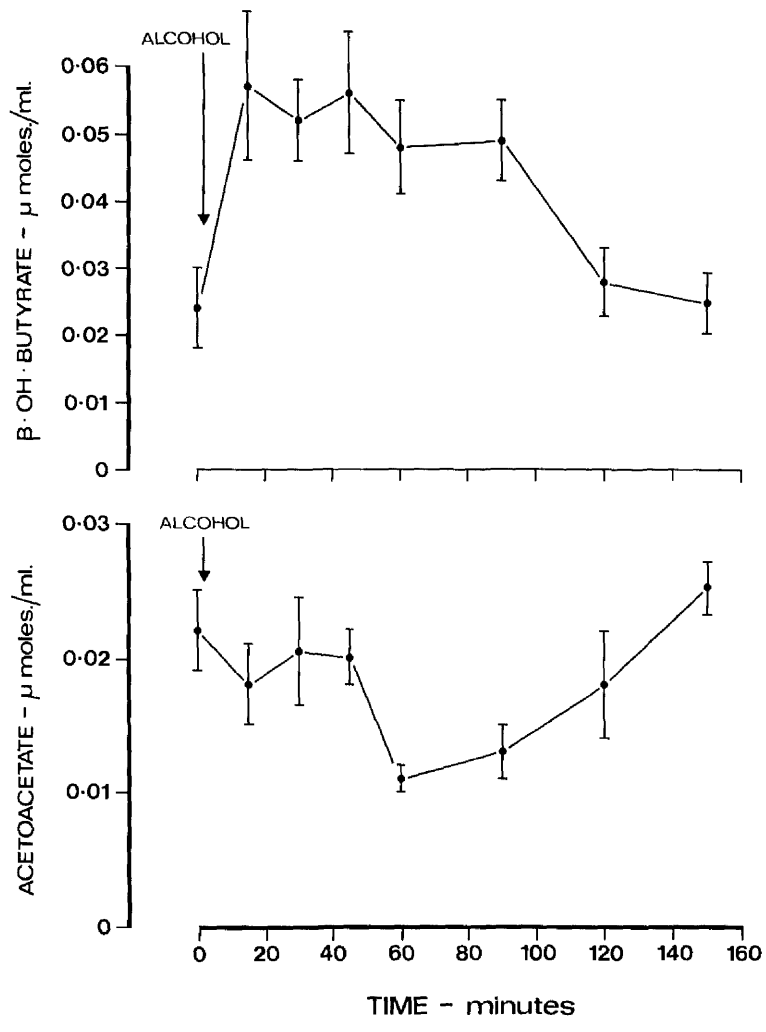
Kg body weight).

Fig. 1 - 4.   Lactate/pyruvate ratio and β -hydroxy-
butyrate ratio in five normal subjects
at rest before and after an oral dose
of alcohol (0.5g/Kg body weight).

TIME – min
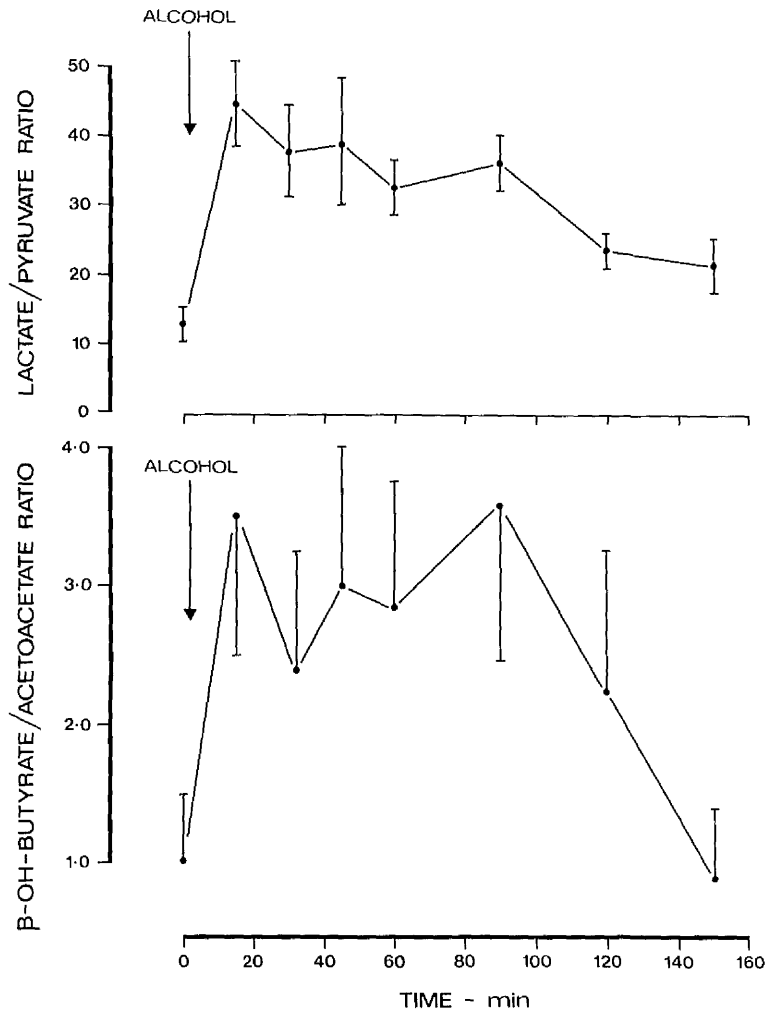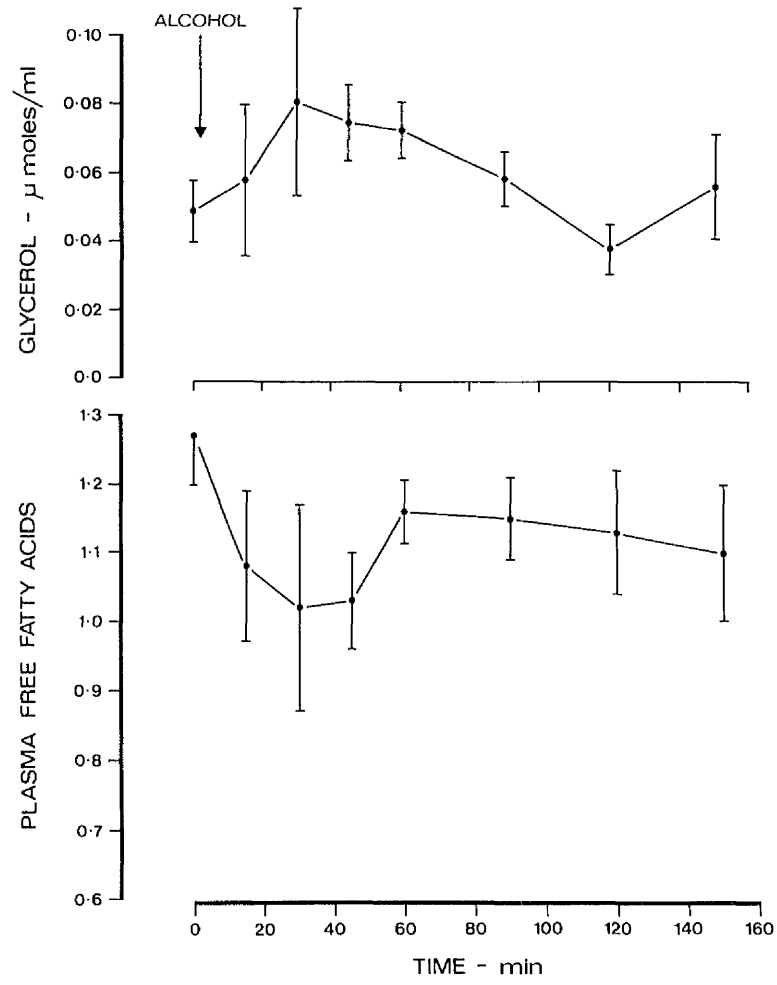
Fig. 1 – 5.   Blood glycerol (μ mol/ml., mean $\pm$ SEM)

and plasma free fatty acids (in equiv./

ml., mean $\pm$ SEM) in five normal subjects

before and after an oral dose of alcohol

(0.5g/Kg body weight).

Plasma free fatty acids (Fig. 1:5)

Alcohol produced a rapid depression in FFA, the FFA level being significantly lower ($P < .05$) than the .pre-alcohol level in the 45 min. sample.   Thereafter the FFA returned towards the initial pre-alcohol level.

Blood glucose

Alcohol was associated with a small rise in blood glucose but the difference was not significant.

DISCUSSION

The measurements of blood alcohol showed the levels attained after an oral dose of 0.5g/Kg.. A peak level of approximately 40 mg/100 ml. was reached 45 min. after alcohol ingestion and this level is half the present legal driving limit. The rate of removal of alcohol from the blood was approximately linear at 14 mg/100ml/hr. which is in good agreement with the figure of 17 mg/100ml/hr given by Patel et al (1969).

Alcohol is known to be metabolised principally by the NAD$^+$ dependent alcohol dehydrogenase system located mainly in the liver. A considerable amount of information is already available concerning the effect of alcohol on blood metabolites. Thus the increase in lactate/pyruvate ratio following alcohol and reported here is consistent with the results of Seligson et al (1953), Mendeloff (1954), Leiber (1967) and Krebs et al (1969). Similarly, the increase in the ratio of β-hydroxy-butyrate/acetoacetate reported here has been previously described by Gordon (1972) and Leiber (1967).

Bucher and Russman (1964) have shown that the ratio of lactate/pyruvate in tissue reflects the cytoplasmic NAD/NADH ratio. The NAD$^+$ linked β-hydroxy-butyrate dehydrogenase system has been found by Borst (1963) and Klinenberg (1963) to be a reliable index of the mitochondrial NAD/NADH ratio. It is reasonable to conclude that metabolite changes in the blood will follow closely those in tissue and that the increase in lactate/pyruvate ratio and β-hydroxy-butyrate/acetoacetate ratio in the blood

described in this chapter reflects the levels of these metabolites in the liver cell. It appears that alcohol is metabolised rapidly when available and leads to a reduced redox state in both the cytoplasm and mitochondria of the liver cell. The altered metabolite levels produced are due to the reduction of the redox state. Of particular importance is the observation that the increased lactate/pyruvate ratio is partly due to a fall in pyruvate levels which is in agreement with the results of Seligson et al (1953). It follows that we might expect alcohol to be associated with a reduced rate of hepatic gluconeogenesis from pyruvate.

The effect of alcohol on fat mobilisation has been studied in the rat; Mallow (1961) and Brodie and Maickel (1963) have observed increases in plasma free fatty acids following an acute dose of alcohol. However, Elko, Wooles and Deluzio (1961) failed to observe any significant changes in free fatty acids after alcohol. In human alcoholic subjects with blood alcohol levels greater than 200 mg% large changes in free fatty acids have been demonstrated (Schapiro, 1963). There appear to be discrepancies in reported changes in serum or plasma FFA possibly related to variation in the doses of alcohol and duration of experiments.

The transient fall in plasma free fatty acids following alcohol reported in this thesis is similar to that described by Lieber (1962) in acute experiments with blood alcohol levels of 90 mg/100ml.. The relatively low levels of blood alcohol in my experiments may explain why the initial fall in plasma free fatty acids was not significant.

SUMMARY

1. The effect of alcohol on blood metabolites was studied in a group of six normal healthy subjects by giving an oral dose of alcohol (0.5 g/Kg body weight) and taking blood samples at intervals before and after alcohol.

2. Blood alcohol measurements showed that a dose of 0.5 g/Kg produced maximum blood levels of approximately 40 mg/100 ml which is half the present legal driving limit.

3. Relatively small amounts of alcohol produced a substantial decrease in the NAD/NADH ratio as measured by the ratios of lactate/pyruvate and β-hydroxy-butyrate/acetoacetate as reported previously by many workers.

4. Alcohol produced a dramatic fall in the level of blood pyruvate an observation also previously reported.

5. Control levels were established and since the results confirmed other published results it was possible to proceed with other aspects of the study.

CHAPTER 2

THE EFFECT OF ALCOHOL ON THE METABOLIC RESPONSE TO EXERCISE

INTRODUCTION

There is a considerable amount of information available on the normal metabolic response to exercise and it was therefore considered that a study of the effect of alcohol on the normal response would be valuable in examining what effects alcohol had on glucose and fat metabolism. In general, there have been few studies of the effect of alcohol on the metabolic response to exercise despite the obvious importance of the subject. From the evidence that is available, it appears that even small amounts of alcohol produce significant alterations in blood metabolites at rest (previous chapter) and it would be surprising to find that alcohol had no effect on athletic performance.

Prolonged, near maximal, exercise can cause a considerable decrease in glycogen stores and under these conditions we might expect alcohol to produce hypoglycaemia due to the inhibition of gluconeogenesis. It is known from studies of perfused rat liver that low concentrations of alcohol can inhibit the conversion of lactate to glucose (Krebs, 1969). During exercise, there is a considerable rise in blood lactate, and since the liver is the main site of reconversion of lactate to carbohydrate we might expect a delayed removal of lactate during and after exercise with alcohol.

It was therefore decided to study the effect of alcohol on the metabolic changes produced during exercise in normal subjects and to examine to what extent carbohydrate and fat metabolism were altered.

METHODS

Subjects

Five normal male subjects aged 25 - 38 years performed 30 minutes of exercise on a bicycle ergometer at 200 kpm. The subjects were relatively uniform with respect to weight 71.2 $\pm$ 5.3 Kg (mean $\pm$ SEM) and height 176 $\pm$ 3 cm (mean $\pm$ SEM). The subjects had fasted overnight and none of them had taken alcohol on the day before the investigations.

Procedure

The subjects performed the exercise test on two occasions with and without an oral dose of alcohol (0.5 g/Kg body weight). Blood samples were taken from a catheter placed in an antecubital vein. Samples were taken at rest before alcohol, at rest after alcohol, at ten minute intervals during exercise and at 5, 15, 30, 60 and 90 minutes from the end of exercise. The exercise test commenced 15 minutes after oral alcohol.

RESULTS

<u>Blood Lactate</u>   (Fig. 2:1)

There was no significant difference in the lactate
levels at rest or during exercise on the two occasions with
and without alcohol.   The blood lactate level fell more
slowly during the post exercise period when alcohol had
been taken, samples taken at 80 and 110 minutes having
significantly greater (P $<$ .05) blood lactate levels than
in the corresponding samples in the control experiment.

<u>Blood pyruvate</u>   (Fig. 2:2)

With alcohol, the blood pyruvate rose initially during
exercise reaching a peak level after 15 minutes.   However,
all the blood pyruvate levels during exercise with alcohol
were significantly lower (P $<$ .05) than the corresponding
levels in the control samples without alcohol.   After
exercise, the pyruvate levels were significantly lower
(P $<$ .01) than the corresponding levels in control samples
taken at 35, 50 and 80 minutes.   At the end of the experiment,
there was no significant difference between the samples
taken at 150 minutes on the two occasions.

It is apparent from the figure 2:2 that there is a small
difference between the resting samples on the two occasions
the difference being significant (P $<$ .05).

Fig. 2 - 1

Blood lactate (μ mol/ml, mean $\pm$ SEM) during and after 30 minutes of exercise in 6 normal subjects with an oral dose of alcohol (0.5 g/Kg body weight) (●——————●) and without alcohol (■——————■).
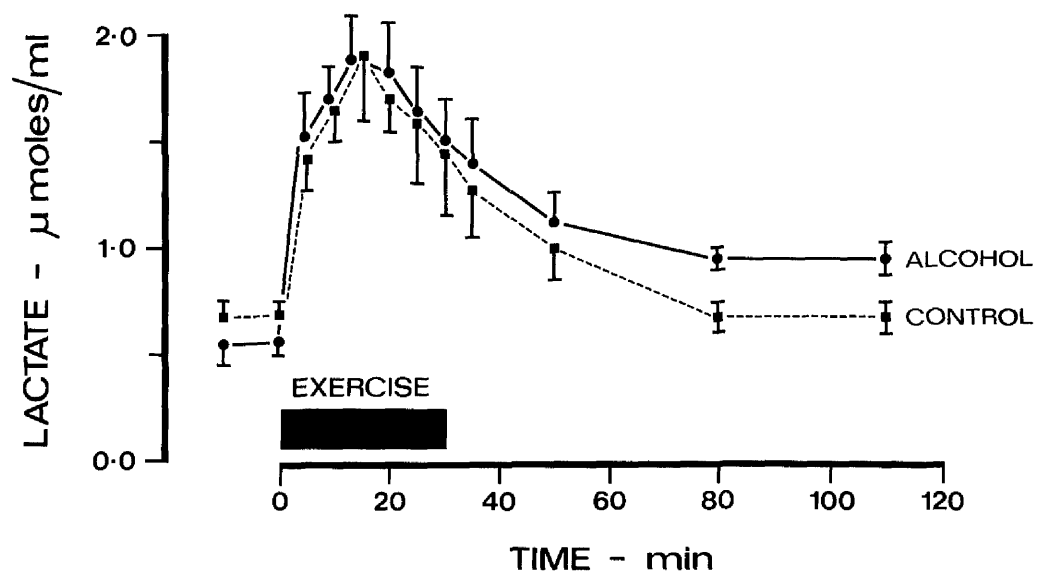
Lactate/pyruvate ratio   (Fig. 2:2)

In the control investigation the lactate/pyruvate ratio increased during the first 15 minutes of exercise reaching a peak value at 15 minutes, thereafter the ratio returned towards the resting value.   At the end of exercise the lactate/pyruvate ratio was not significantly different from the ratio at rest.

Alcohol significantly increased ($P < .05$) the lactate/pyruvate ratio in all samples during and after exercise. The most obvious effect of alcohol on the lactate/pyruvate ratio, appart from increasing the ratio, was to decrease the rate of recovery of the ratio towards the resting pre-alcohol level such that the last sample taken still had a significantly greater ratio than the corresponding control sample.

Blood acetoacetate

The acetoacetate level tended to increase initially and then to decrease towards the end of exercise.   During the post exercise period the sample taken at 80 minutes had an acetoacetate level significantly greater than the resting level ($P < .05$).

With alcohol, acetoacetate rose initially during exercise and samples taken at 15, 20 and 25 minutes during exercise had significantly greater ($P < .05$) acetoacetate levels than the corresponding control samples without alcohol. With alcohol, all the samples taken during the post exercise period were significantly greater ($P < .05$) than the resting

Fig. 2 - 2

Lactate/pyruvate ratio and blood pyruvate $(\mu$ mol/ml,

mean $\pm$ SEM) during and after 30 minutes of exercise in

6 normal subjects with an oral dose of alcohol (0.5 g/Kg

body weight) (●━━━●) and without alcohol (■━━━■)
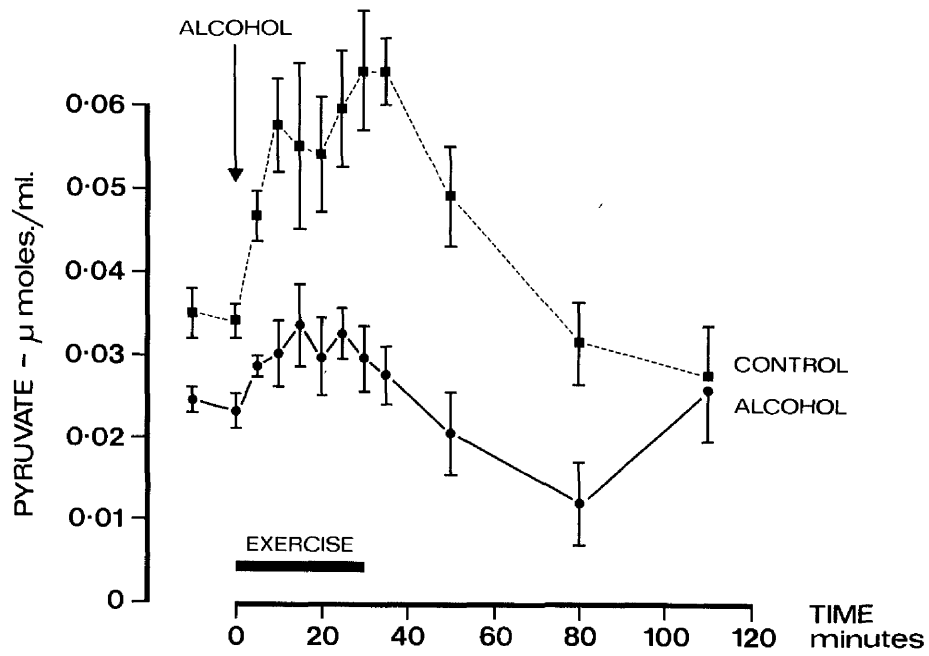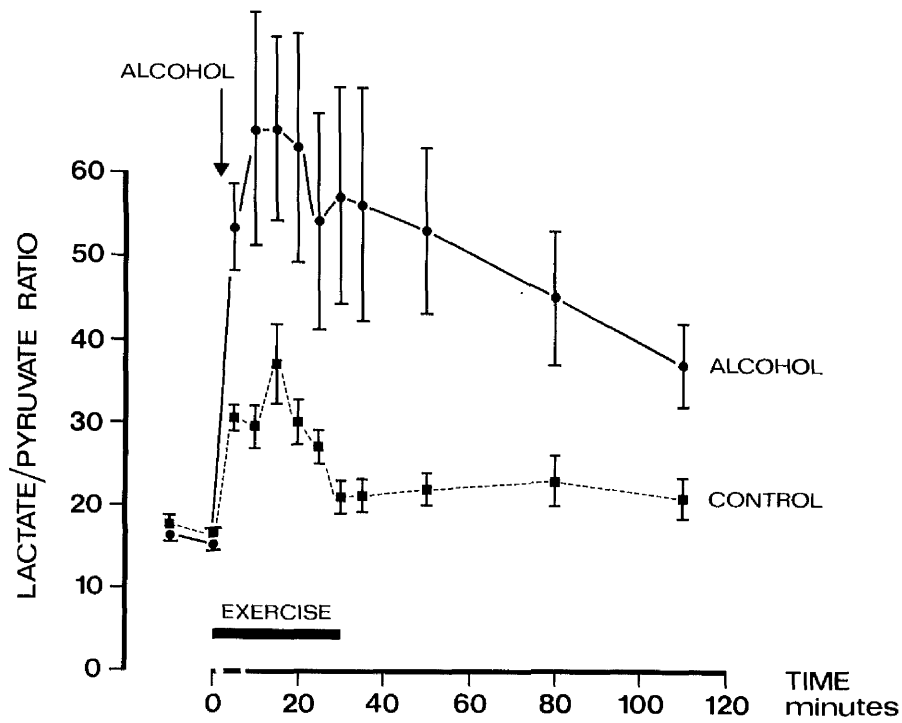
sample but were not significantly different from the corresponding control samples.

Blood β-hydroxy-butyrate (Fig. 2:3)

Without alcohol, β-hydroxy-butyrate did not change during exercise. After exercise, the level increased but it was not significantly greater than the resting level.

Alcohol produced a significant rise in β-hydroxy-butyrate level during exercise the level being significantly greater ($P < .05$) than both the corresponding control sample and the resting level. The levels of β-hydroxy-butyrate during exercise after alcohol were very similar in value suggesting a 'plateau' value during exercise. After exercise, the β-hydroxy-butyrate levels continued to rise reaching a maximum value in the sample taken at 60 minutes. Thereafter the levels declined. All the post exercise samples after alcohol were significantly greater ($P < .01$) than the corresponding control levels.

β-hydroxy-butyrate/Acetoacetate ratio (Fig. 2:3)

A small increase in β-hydroxy-butyrate/acetoacetate ratio occurred during exercise. During the post exercise period, the ratio initially increased then decreased towards the resting level. However, the changes during and after exercise were not significant.

Alcohol significantly increased the β-hydroxy-butyrate/acetoacetate ratio above the resting level ($P < .01$) and above the control level ($P < .05$) during exercise.

Fig. 2 - 3

Blood β-hydroxy-butyrate (μmol/ml, mean $\pm$ SEM) and

β-hydroxy-butyrate/acetoacetate ratio (mean $\pm$ SEM)

in 6 normal subjects during and after 30 minutes of

exercise with an oral alcohol dose (0.5 g/Kg body weight)

(●————●) and without alcohol (■--------■)
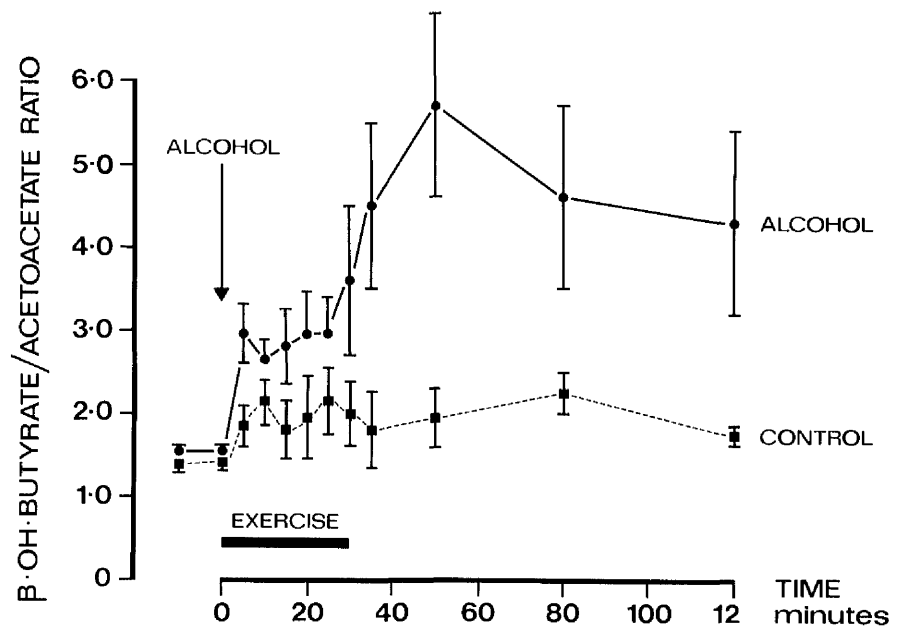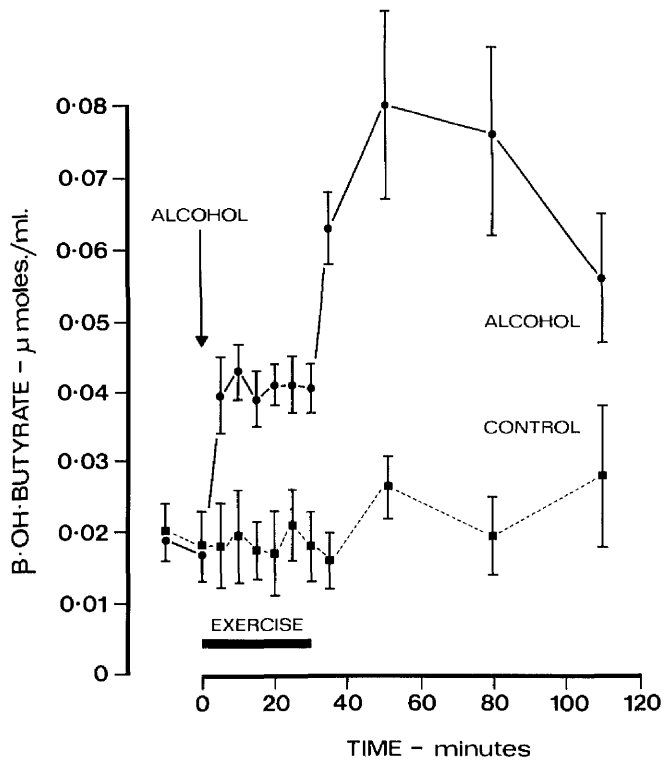
The ratio after alcohol remained significantly greater
(P < .01) than the levels without alcohol throughout the
post exercise period.

Blood glycerol (Fig. 2:4)

The blood glycerol level increased towards the end of
exercise and declined early in the post exercise period. A
similar trend occurred after alcohol had been taken, however,
with alcohol the glycerol level was significantly greater
than the corresponding control levels (samples at 15, 30,
35 and 40 minutes).

Plasma free fatty acids   (Fig. 2:4)

Without alcohol very little change occurred in the
FFA.   After alcohol, however, the FFA level increased towards
the end of exercise and in the initial post exercise period,
the samples taken at 25 minutes and all subsequent samples
having significantly greater (P < .05) FFA levels than the
corresponding control levels.

Blood glucose

Exercise did not produce any significant change in
glucose level.   Alcohol tended to produce lower levels of
glucose during and after exercise but the differences were not
significant.

Fig. 2 - 4

Blood glycerol (μmol/ml, mean $\pm$ SEM) and Plasma
free fatty acids (μ equiv/ml, mean $\pm$ SEM) in 6 normal
subjects during and after 30 minutes of exercise with an
oral alcohol dose (0.5 g/Kg body weight) (●———●)
and without alcohol (■————■)
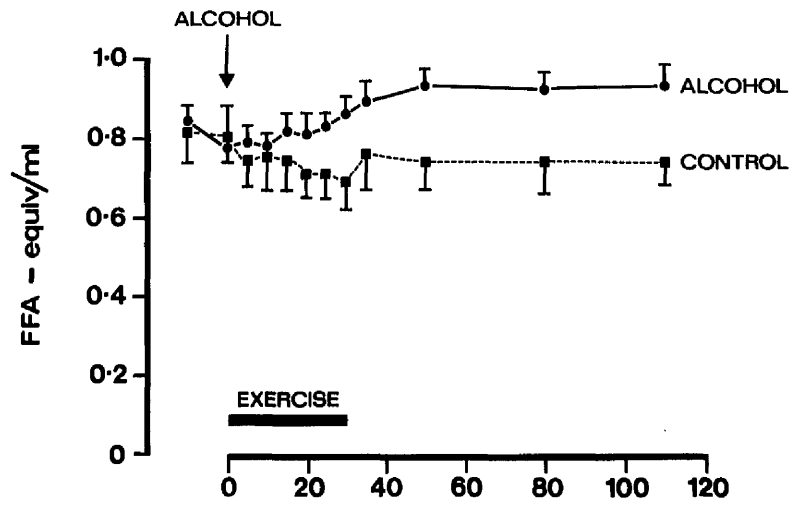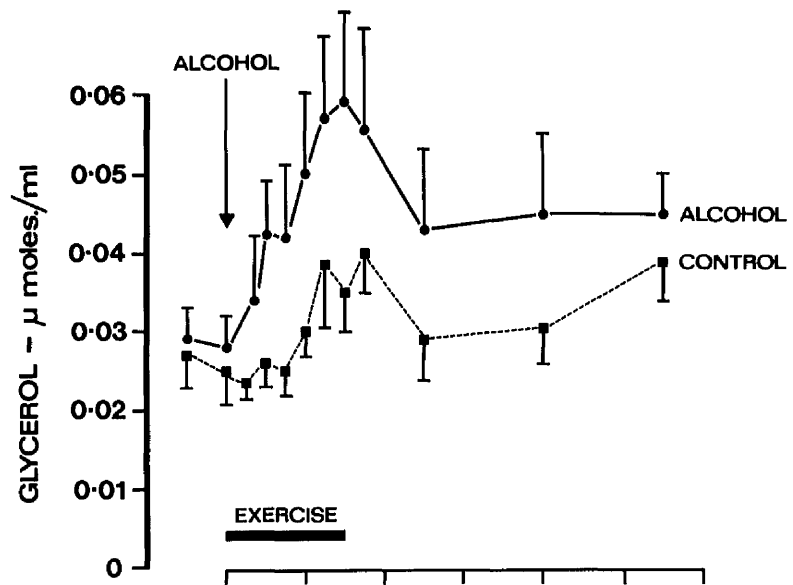
DISCUSSION

The difference between the pyruvate levels in the resting samples was small but significant. The difference is within the limits of the pyruvate assay and even if the difference was maintained in all the samples it is small relative to the change produced by alcohol and would not alter the significance of the lower pyruvate levels produced by alcohol.

The increase in lactic acid, pyruvic acid and lactate/pyruvate ratio during exercise has been well documented by many authors. De Coster et al (1969) have discussed the various patterns of evolution of lactic and pyruvate acids during and after exercise and my results for control subjects are consistent with their findings for normal subjects exercised at 600 Kg/min on a bicycle for 15 minutes. In my control subjects there was a tendency for the blood lactate level to fall towards the end of exercise and it has been demonstrated that this is a feature of prolonged exercise (Jones et al, 1965; Thomas et al, 1964; Astrand et al, 1963).

With alcohol, the lactate level during exercise was not increased above the control exercise level although the lactate pyruvate ratio was increased. After exercise it became evident that alcohol slowed the rate of removal of lactate from the blood. This evidence suggests therefore that alcohol does not lead to increased lactate production but decreases the conversion of lactate to pyruvate. These findings are in agreement with the results of Krebs et al (1969) who also

studied alcohol and exercise, and are consistent with the observation that alcohol inhibits gluconeogenesis from lactate.   We can conclude that the inhibition of hepatic gluconeogenesis by alcohol is due to the limiting effect of the low level of pyruvate which follows the decreased rate of conversion lactate to pyruvate.   The increase in blood lactate by alcohol at rest (Chapter 1) can also be interpreted as due to decreased conversion of lactate to pyruvate rather than increased lactate production.

With alcohol, the β-hydroxy-butyrate/acetoacetate ratio during and after exercise was greatly increased due principally to increased levels of β-hydroxy-butyrate.   The ratio was greater after exercise suggesting that the decreased mitochondrial oxidative capacity was less during than after exercise. This may be related to increased fat catabolism during exercise with alcohol which is consistent with the higher levels of FFA found with alcohol.   The rapid rise of β-hydroxy-butyrate after alcohol suggest that the 2 carbon acetate fragments produced in the metabolism of alcohol may be converted to ketones. However, Lundquist et al (1962) found that with labelled alcohol none of the radioactivity was associated with ketones.   The higher levels of ketones after exercise may be due to alcohol lowering the utilisation of ketones by the alteration of the redox state.

Moderate exercise of sufficient duration is normally associated with an increase in free fatty acids and glycerol (Basu et al 1960).   Normal, non-athletic subjects may also develop a post exercise ketosis (Johnson et al, 1969).   My

control subjects did not show any marked change in free

fatty acids or ketones and this is attributed to the relatively

low work load and duration of exercise.   It has been shown

repeatedly that alcohol can decrease the rate of fatty acid

oxidation by liver (Diluzio, 1968;   Leiber and Schmid, 1961;

Poggi and Diluzio, 1964) and this has been generally

explained in terms of the increased supply of reducing

equivalents originating in the cytoplasm and translocated

to the mitochondria when alcohol is oxidised.   Alcohol has

also been shown to increase fat mobilization from fat depots

in man and plasma FFA rises when the alcohol dose is sufficient

to induce intoxication (Mallov, 1961).   In addition, activation

of adipose tissue lipase in the epididymal fat pads leading

to increased fatty acid output occurs after acute administration

of ethanol to rats with an intact sympathetic nervous system

(Brodie et al, 1961).   The higher levels of glycerol and

free fatty acids reported in this chapter with alcohol and

exercise suggest that alcohol led to increased mobilization of

fat possibly by increasing the release of catecholamines during

exercise as catecholamines are known to increase during

exercise (Von Euler and Hellner, 1952;   Vendsalu, 1960;

Kolchen et al, 1971) and to stimulate lipolysis (Armstrong et al,

1961;   Havel and Goldfien, 1959;   Schotz and Page, 1959).

Such a possibility seems likely since the blood alcohol

levels were not sufficient to produce intoxication and

also since alcohol without exercise (Chapter 1) did not lead

to increased levels of glycerol and FFA.   The further

possibility exists that the apparent increase in FFA with alcohol and exercise was due to a decrease in the rate of fatty acid oxidation produced by alcohol. On the basis of the results reported in this chapter it was not possible to determine the relative importance of these possibilities in contributing to the increased levels of glycerol and FFA. The results suggest a number of future experiments in which it would be possible to differentiate between decreased oxidation and increased mobilization of fat.

The results reported in this chapter suggest that relatively small amounts of alcohol can produce major changes in metabolite concentrations and alter the pattern of fuel utilization during exercise.

SUMMARY

1. The effect of alcohol on the metabolic response to
exercise was studied in a group of five normal healthy
subjects by giving an oral dose of alcohol (0.5 g/kg
body weight) and performing 20 minutes of exercise on
a bicycle ergometer at 600 k p·m   Blood samples were
withdrawn at intervals during and after exercise.

2. Alcohol produced greater lactate/pyruvate and β-hydroxy-
butyrate/acetoacetate ratios ~~greater~~ than those normally
produced during exercise.

3. In agreement with other published findings alcohol
did not lead to increased blood lactate during exercise
but led to decreased removal of lactate from the blood
after exercise.

4. Alcohol led to increased mobilization of fat during
exercise an effect normally associated with greater
levels of blood alcohol.

CHAPTER 3

THE METABOLIC RESPONSE TO EXERCISE IN ATHLETIC SUBJECTS

INTRODUCTION

The normal metabolic changes associated with exercise have been considered in the previous chapter. Athletic subjects are known to have lower concentrations of lactic and pyruvic acids in the blood during exercise compared with unfit subjects. (Cobb and Johnson (1963); Saltin and Karlsson (1971). Johnson et al (1969) have demonstrated that the development of post exercise ketosis is reduced in athletes.

In view of the differences in the metabolic response to exercise between athletes and non athletes it was decided to study the effects of alcohol on the metabolic changes associated with exercise in athletes. It was considered that a relatively strenuous form of exercise would be required to produce metabolite changes sufficient to demonstrate any effects of alcohol.

METHODS

Subjects

The effect of alcohol on the metabolic response to exercise was further studied in a group of six trained male athletes aged 20 - 23 years. The subjects were relatively uniform with respect to height 174.0 ± 2.9 cm (mean $\pm$ SEM) and weight 68 ± 3.6 kg (mean $\pm$ SEM). The subjects were students of the Scottish School of Physical Education at Jordanhill College. None of them had taken alcohol on the day before the investigation and they were taking normal unrestricted diet.

Procedure

The subjects performed thirty minutes of exercise by running round a gymnasium on two occasions with and without an oral dose of alcohol (0.5 g/kg body wt.). To facilitate blood sampling the 30 minute exercise period consisted of three successive 10 minute periods of continuous running. The exercise was interrupted on the completion of each 10 minute exercise period and a blood sample taken from a catheter placed in an antecubital vein. The interval between the 10 minute exercise periods was no greater than 2 minutes. Blood samples were also taken at rest, before and after alcohol, and at 5, 15, 30, 60 and 90 minutes after exercise. The subjects commenced exercise 30 minutes after the oral dose of alcohol had been taken.

RESULTS

Blood lactate (Fig. 3:1)

Without alcohol, blood lactate increased during exercise reaching a maximum level in the sample taken 10 minutes after the first exercise period. The relatively high levels of lactate produced are consistent with the large amount of work done in performing the exercise.

Alcohol did not significantly alter the blood lactate levels produced during exercise. However, 15 minutes after exercise alcohol produced a significantly greater blood lactate level $(P < 0.05)$ and the level remained significantly greater $(P < .01)$ than the corresponding control levels in all subsequent samples taken.

It therefore appears that alcohol did not lead to an increased lactate level during exercise but that it was associated with a decreased removal of lactate from the blood. This is in agreement with the similar results described in chapter 2.

Blood pyruvate (Fig. 3 : 2)

Alcohol produced decreased pyruvate levels during exercise significantly lower $(P < .05)$ than the control values for samples taken at 10 and 20 minutes. The pyruvate level remained significantly lower than the control levels during the post exercise period for samples taken at 35 minutes $(P < .001)$ and 45 and 60 minutes $(P < .05)$. Samples taken at 90 and 120 minutes were not significantly different from the corresponding control levels.

Fig. 3.1 : Blood lactate (μ mol/ml, mean $\pm$ SEM) during

and after 30 minutes of exercise in six

athletic subjects with an oral dose of

alcohol (0.5g/Kg body weight(⊖————⊚)), and

without alcohol (▫————▫).
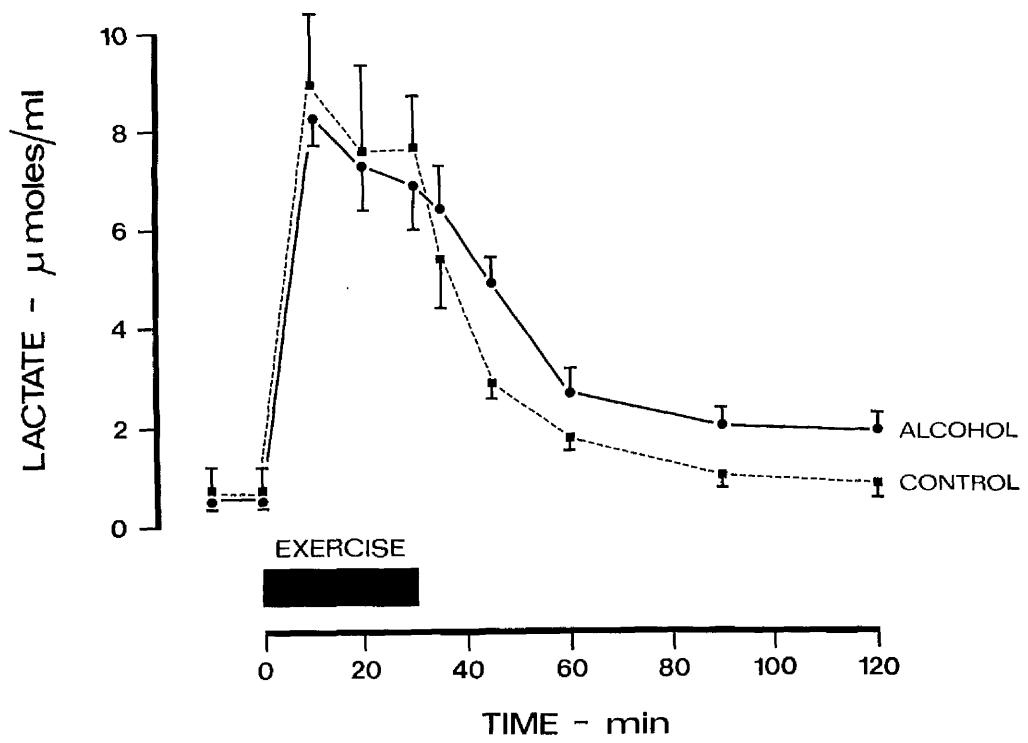
Lactate/pyruvate ratio (Table 3:1)

The lactate pyruvate ratio increased during exercise but recovered very quickly after exercise.

Alcohol produced greater lactate/pyruvate ratios during exercise.   After exercise, with alcohol, there was no recovery of the lactate/pyruvate ratio within the duration of the experiment all post exercise samples being significantly greater than the corresponding control samples ($P < .05$).

Blood acetoacetate

Without alcohol the acetoacetate level decreased during exercise and increased after exercise the level in samples taken at 90 and 120 minutes being significantly greater than the resting level ($P < .05$).

Blood β-hydroxy-butyrate (Fig. 3:2)

Exercise without alcohol produced β-hydroxy-butyrate levels significantly greater ($P < .05$) than the resting level (samples at 10 and 20 minutes). β-hydroxy-butyrate levels then decreased towards the end of exercise and in the initial post exercise period.   The level increased in the late post exercise period, samples taken at 60, 90 and 120 minutes being significantly greater ($P < .01$) than the resting level.

After alcohol the β- hydroxy-butyrate level increased above the resting level.   After exercise the level continued to rise initially samples taken at 40, 50 and 60 minutes being significantly greater ($P < .01$) than the corresponding control levels without alcohol.

Table 3:1 :   Lactate/pyruvate ratio during and after 30 min of exercise

in six athletic subjects with an oral dose of alcohol

(0.5g/Kg body weight) and without alcohol control.

LACTATE/PYRUVATE RATIO

Sample Time (min)		Control	Alcohol
Rest		14.4 ± 2.04	16 ± 1.20
			23.8 ± 1.16
Exercise	10	37.2 ± 6.4	40.4 ± 4.8
	20	27.4 ± 3.99	38.2 ± 4.9
	30	33.4 ± 5.6	34.2 ± 2.9
	35	16.8 ± 1.5	30.8 ± 1.69
	45	16.0 ± 2.0	41.8 ± 3.6
	60	13.8 ± 2.29	41 ± 3.1
	90	12.8 ± 3.34	35.2 ± 3.2
	120	15.0 ± 3.0	43.8 ± 7.2

Fig. 3.2 :   Blood pyruvate and β-hydroxy-butyrate (μmol/
ml, mean $\pm$ SEM) during and after 30 minutes of
exercise in six athletic subjects with an oral
dose of alcohol (0.5g/Kg body weight (⊙———⊚))
and without alcohol (▣————▣).
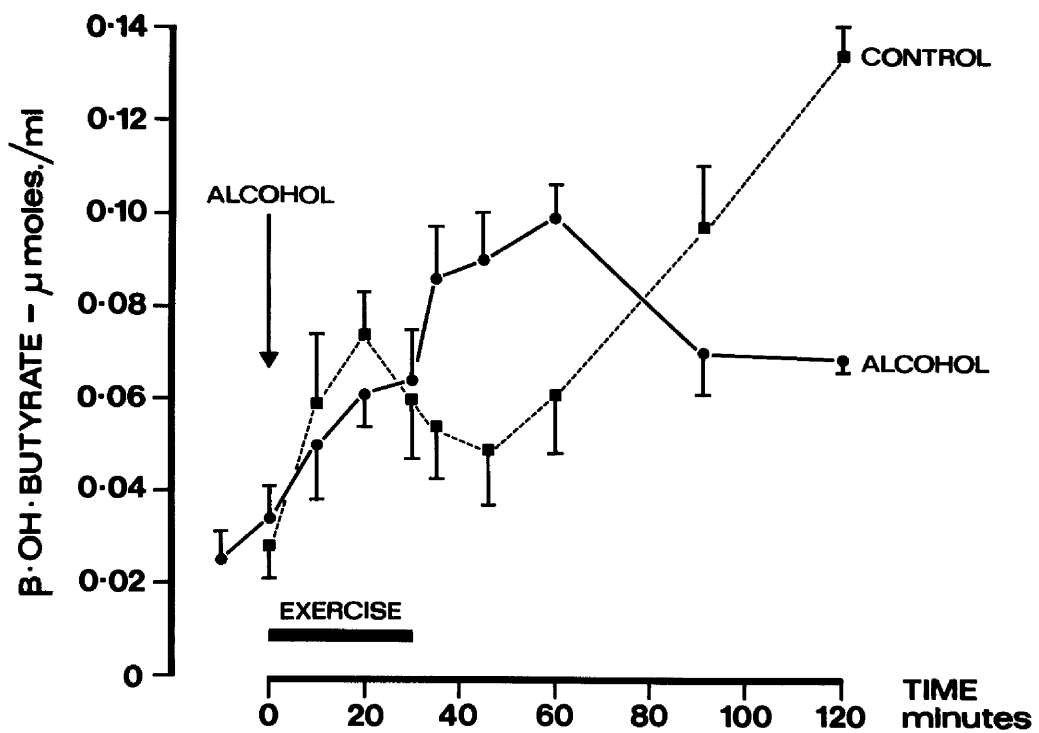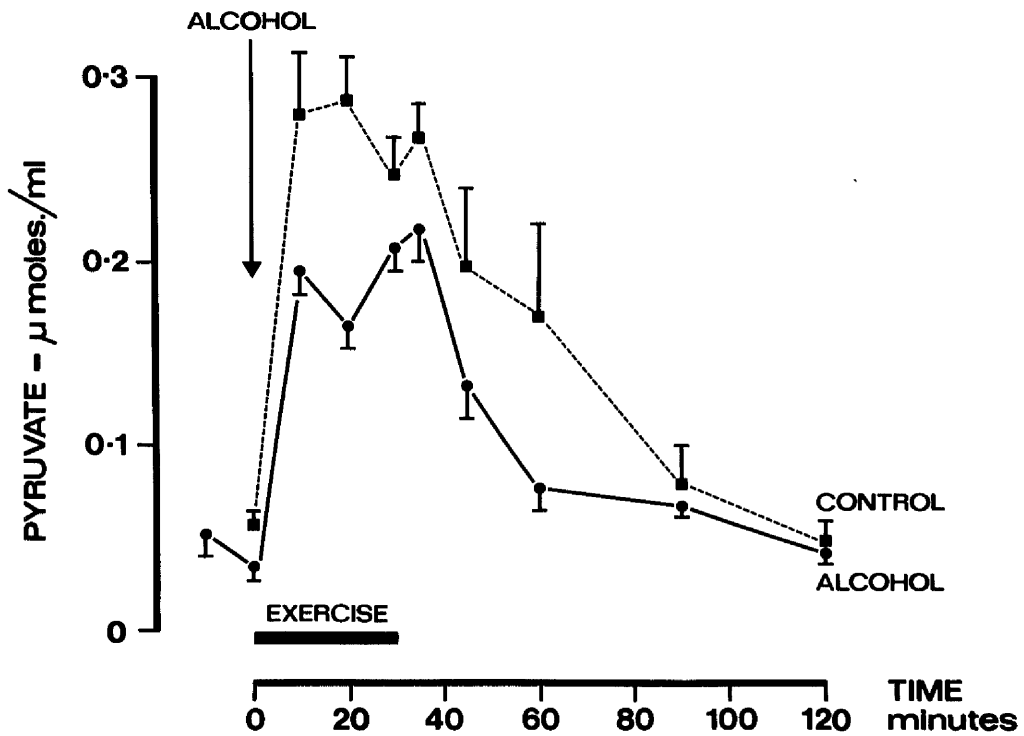
Blood glycerol (fig. 3:3)

Without alcohol, blood glycerol increased during exercise. The peak mean level was 0.126 moles at the end of exercise. Apart from the higher blood levels associated with the strenuous type of exercise the pattern of glycerol change was very similar to that of the control subjects described in the previous chapter.

With alcohol higher blood glycerol levels occurred during exercise. The levels in samples taken at 10, 20 and 30 minutes were significantly greater ($P < .01$) than the corresponding control samples.

Plasma free fatty acids

In the control experiment FFA fell initially during exercise then increased towards the end of exercise and remained increased in the first few samples taken after exercise.

With alcohol, the rise in FFA was greater during exercise and initially after exercise in samples taken at 30, 35, 45 and 60 minutes. The increase was significantly greater ($P < .05$) than the corresponding control levels.

Blood glucose (fig. 3:4)

In the control experiment without alcohol, there was very little change in blood glucose before and after exercise. With alcohol, there was a significant lowering of blood glucose ($P < .01$) in all samples after exercise (35, 45, 60, 90 and 120 minutes).

Fig. 3.3 Blood glycerol (μmol/ml, mean $\pm$ SEM) during
and after 30 minutes of exercise in six
athletic subjects with an oral dose of alcohol
(0.5g/Kg body weight (⊙———⊙)) and without
alcohol (▣----▣).
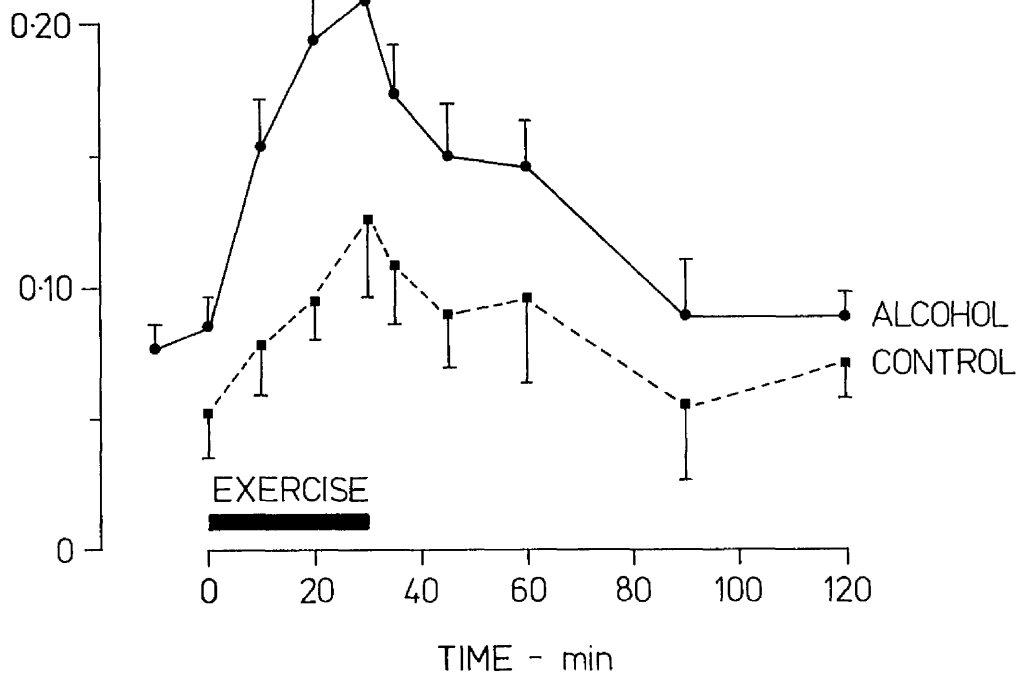
Fig. 3.4   Blood glucose (mg/100 ml, mean $\pm$ SEM) during
and after 30 minutes of exercise in six athletic
subjects with an oral dose of alcohol (0.5g/Kg
body weight (⊙————⊙)) and without alcohol
(⊡– – –⊡).
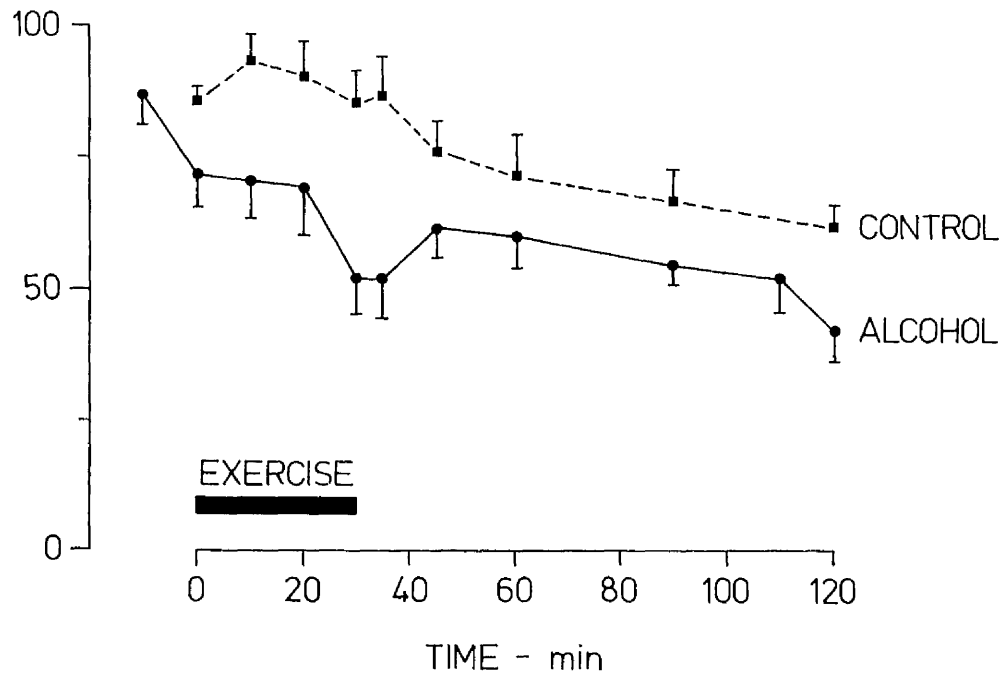
Plasma human growth hormone (HGH)   (Fig. 3:5)

Resting HGH concentrations were not significantly different with and without alcohol. They rose to a maximum at 20 minutes of exercise in the control study whereas with alcohol the maximum occurred at the end of exercise. Plasma HGH concentrations were higher with alcohol both during and after exercise but differences were significant only at the end of exercise and at 5 minutes after exercise.

Fig. 3:5   Plasma HGH (μmol/ml, mean $\pm$ SEM) in six healthy subjects
during and after exercise with an oral alcohol dose
(0.5g/Kg body weight) (●——●) and without alcohol (■——■).

PLASMA HGH
(μU/ml)

EXERCISE

ALCOHOL
CONTROL

TIME - min
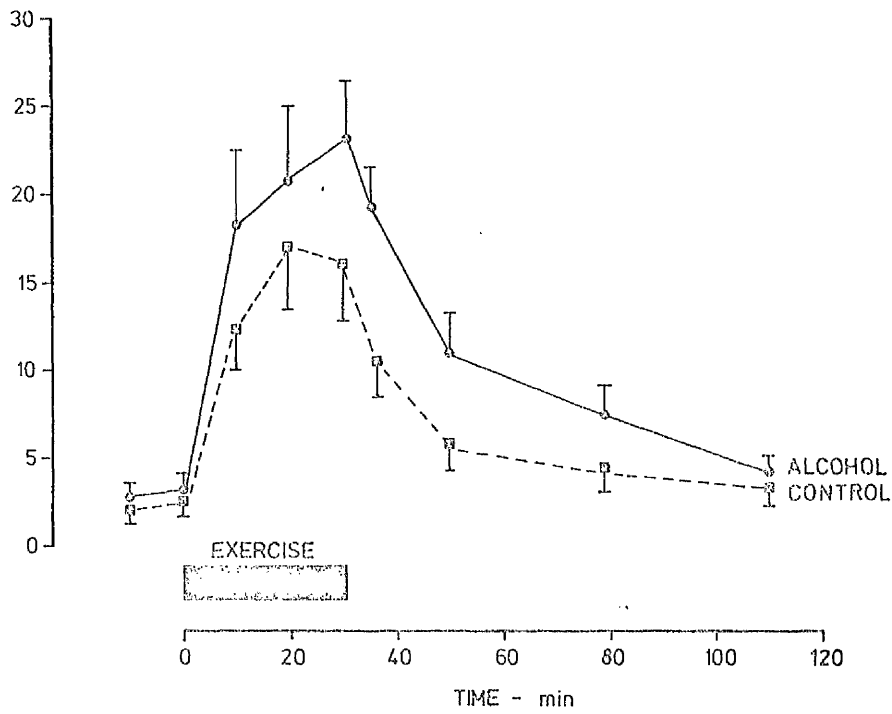
DISCUSSION

The relatively high levels of lactic and pyruvic acids in the blood of the athletic subjects described in this chapter are related to the relatively strenuous exercise that the subjects performed. The subjects exercised without alcohol, did in fact develop a small post exercise ketosis and this is also probably related to the strenuous type of exercise. Unfortunately, it was not possible to include a group of normal untrained subjects as a comparison with the athletes.

With alcohol, as in the previous chapter, lactate levels during exercise were not greater than the control levels. After exercise however, lactate levels remained elevated which confirms the observation in the previous chapter that alcohol decreased the rate of conversion of lactate to pyruvate. Although the athletes performed a more strenuous form of exercise than that described in the previous chapter, the lactate/pyruvate ratio changes were very similar. The failure of the lactate/pyruvate ratio to return to normal after alcohol reflects the increase in the NADH/NAD ratio which is probably the basic cause of the failure of lactate utilisation.

The increase in the level of FFA and glycerol, with alcohol, is consistent with the results reported in the previous chapter and suggests that alcohol produced increased mobilisation of fat. In addition, it appears that alcohol abolished the post exercise ketosis present at the end of exercise without alcohol and it may be that alcohol also increases the rate of oxidation of fat during exercise. However, this result requires further study.

As discussed in the previous chapter it was not possible
in this limited study to determine if the increased levels
of FFA produced by alcohol and exercise were due to decreased
oxidation of fatty acid or increased mobilisation of fat.

There was a significant lowering of blood glucose levels
with alcohol after exercise. It has been shown that alcohol
can produce hypoglycaemia after a fast of 48 hours which
produced some glycogen depletion (Field et al, 1963).
It is therefore suggested that when glycogen stores are
decreased during strenuous exercise alcohol induced
hypoglycaemia may result. The lowered blood glucose
levels reported here could also result when the energy
demand for glucose as a fuel is not matched by glycogenolysis
or gluconeogenesis from other metabolites. The low levels
of pyruvate associated with alcohol suggest that alcohol
will certainly reduce the rate of gluconeogenesis from
pyruvate. It is evident that strenuous prolonged exercise
taken in association with alcohol may lead to low levels
of blood glucose with the consequent dangers of fainting
and coma associated with hypoglycaemia. Low levels of
blood glucose could also produce lack of concentration
and effects on the central nervous system likely effect
performance.

The results of growth hormone changes suggest that the
release of growth hormone with exercise is greater following
the ingestion of alcohol by normal subjects. The means by
which alcohol produces such a change during exercise are
still unknown. Hypoglycaemia (Hunter et al., 1965), fall of

plasma FFA (Hartog et al., 1965) and a rise of blood lactate
(Sutton et al., 1969) have been reported as . possible
stimuli for producing an increase in plasma HGH concentration
during exercise. The last two possibilities appear to be
unlikely in this situation, because the changes of plasma
FFA and blood lactate during exercise were similar with and
without alcohol. The findings of a significant drop in
blood glucose and a rise in plasma HGH concentrations
during exercise with alcohol suggest the possibility that
hypoglycaemia might be a factor. There is also the
possibility that alcohol produced the greater elevation
of HGH by sensitizing the release mechanisms to such other
stimuli.

The secretion of HGH from the pituitary gland is
controlled by the hypothalamus through an intermediary
neurohormone system which is probably dependent on
catecholamines transmission (Forhman, 1972; Martin, 1973;
Merimee and Rabin, 1973). Gursey and Olson (1960) have
shown that in the rabbit the administration of ethanol is
followed by a rapid release of serotonin and nor-adrenaline
from brainstores. It is therefore possible that alcohol
interferes with HGH production by directly affecting the
aminergic activity of the hypothalamus. The reports of
Jenkins and Connolly (1968) and other workers (Bellet et al.,
1971); Merry and Marks, 1972) who found that in normal
individuals an amount of alcohol sufficient to produce mild
to moderate intoxication, stimulates pituitary and adrenal

activity as assessed by the release of cortisol and HGH. This and the present observation of a greater growth hormone response to exercise after alcohol by normal subjects are consistent with an acute stimulating effect of alcohol on pathways regulating the release of HGH.

The results reported in this chapter confirm and extend the results described in the previous chapter. The lowered blood glucose levels associated with alcohol and exercise suggest the danger of taking alcohol in association with strenuous exercise and merit further study. The increased release of growth hormone with alcohol suggests a number of future experiments under the same conditions but with different types of subjects.

SUMMARY

1. The effect of alcohol on the metabolic response to exercise
was further studied in a group of six trained male athletes
by giving an oral dose of alcohol (0.5g/Kg body wt.) and
performing exercise by running round a gymnasium for 30 min.
Blood samples were withdrawn at intervals during and after
exercise.

2. Relatively high concentrations of lactate and pyruvate were
produced consistent with the relatively strenuous type
of exercise performed.

3. In agreement with the results reported in the previous chapter
alcohol did not lead to greater lactate production but
decreased the rate of removal of lactate from the blood
after exercise.

4. Alcohol produced greater levels of ketones immediately after·
exercise but abolished the post exercise ketosis that
occurred towards the end of the post exercise period without
alcohol.

5. Alcohol was associated with greater levels of glycerol and
FFA after exercise consistent with the result reported in
the previous chapter that alcohol led to increased
mobilisation of fat during exercise.

6. Alcohol produced a significant lowering of blood glucose
during exercise an effect associated with strenuous exercise
not previously reported.

7. Greater HGH levels occurred during and after exercise with
alcohol.   This result is consistent with other published
findings that alcohol stimulates pituitary and adrenal
activity.

CHAPTER 4

THE EFFECT OF ALCOHOL ON GLUCOSE TOLERANCE

INTRODUCTION

Alcohol has been reported to produce an intolerance to glucose during glucose tolerance tests (Dornhorst and Ouyang, 1971; Dundee, 1972; Wapnick, 1972) but other authors who have carried out similar investigations have been unable to confirm this finding (Shanley et al, 1972; Philips and Sharfit, 1971). Shanley et al reported that alcohol produced a delay in the insulin response to the glucose tolerance test and this effect on the insulin response may be responsible for the reported decreased uptake of glucose. However, Dornhorst and Ouyang did not find a delayed insulin response and attribute the alcohol induced glucose intolerance to a general decreased peripheral uptake of glucose. The absence of the delayed insulin response in the work of Dornhurst and Ouyang may be due to the simultaneous administration of glucose and alcohol as Shanley et al performed the glucose tolerance test after sufficient time had been allowed for alcohol absorption. In addition, Metz et al, 1969, have reported that alcohol pretreatment led to an increased insulin response and increased glucose tolerance in the glucose tolerance test. It therefore appears that to some extent the different results reported by the various authors could be due to variations in the time allowed for alcohol absorption and in the drinking habits of the various groups of subjects.

As part of a general study of the metabolic effect of alcohol it was decided to study the effects of alcohol on glucose tolerance by giving glucose and alcohol simultaneously

and in a subsequent investigation by giving glucose after sufficient time had been allowed for alcohol absorption. The results of the investigation of alcohol and glucose tolerance in which alcohol and glucose were given simultaneously are given and discussed in this chapter.

METHOD

Subjects

The effect of alcohol on glucose tolerance was studied in six normal healthy subjects. The subjects were relatively uniform with respect to height, 171.7 ± 5.2 cm (mean $\pm$ SEM) and weight, 74.2 ± 5.2 Kg (mean $\pm$ SEM). The subjects had been fasting overnight prior to the experiment and had not taken alcohol on the day before the experiment.

Procedure

An oral glucose tolerance test (50 g) was performed on two occasions, with and without an oral dose of alcohol (0.5 g/Kg body weight). Alcohol and glucose were given simultaneously. Blood samples were taken from a catheter in an antecubital vein before alcohol, before glucose and at 30 minute intervals after glucose for $2\frac{1}{2}$ hours.

Blood lactate   (Fig. 4:1)

There was no significant difference between the pre-
glucose samples on the two occasions.   The glucose
tolerance test was associated with increased blood lactate
level but the difference was not significant.

With alcohol and glucose the blood lactate was
greater than the resting level in the sample taken at
60 minutes but the difference in this and subsequent samples
was not significant.

Blood pyruvate   (Fig. 4:1)

There was no significant difference between the pre-
glucose samples on the two occasions.

The glucose tolerance test was associated with increased
blood pyruvate levels but the increase was not significant.
With alcohol and glucose the blood pyruvate level was
significantly lower than the resting level $(P < .01)$ and
the corresponding levels without alcohol $(P < .05)$.

Blood β-hydroxy-butyrate   (Fig. 4:2)

There was no significant difference between the pre-
glucose samples on the two occasions.   The glucose tolerance
test was associated with a depression of β-hydroxy-butyrate
the levels in samples taken at 60, 90 and 120 minutes being
significantly lower than the resting level $(P < .05)$.

Alcohol produced a marked increase in β-hydroxy-
butyrate the levels being significantly greater than the
corresponding levels without alcohol $(P < .01)$.

Fig. 4 - 1.   Blood lactate and blood pyruvate

(μ mol/ml, mean $\pm$ SEM) in six normal

subjects before and after an oral glucose

tolerance test (50g) with and without

an oral dose of alcohol (0.5g/Kg body weight).

Fig. 4 - 2. Blood β-hydroxy-butyrate and

acetoacetate (μ mol/ml, mean $\pm$ SEM)

in six normal subjects before and after

an oral glucose tolerance test (50g)

with and without an oral dose of alcohol

(0.5g/Kg. body weight).
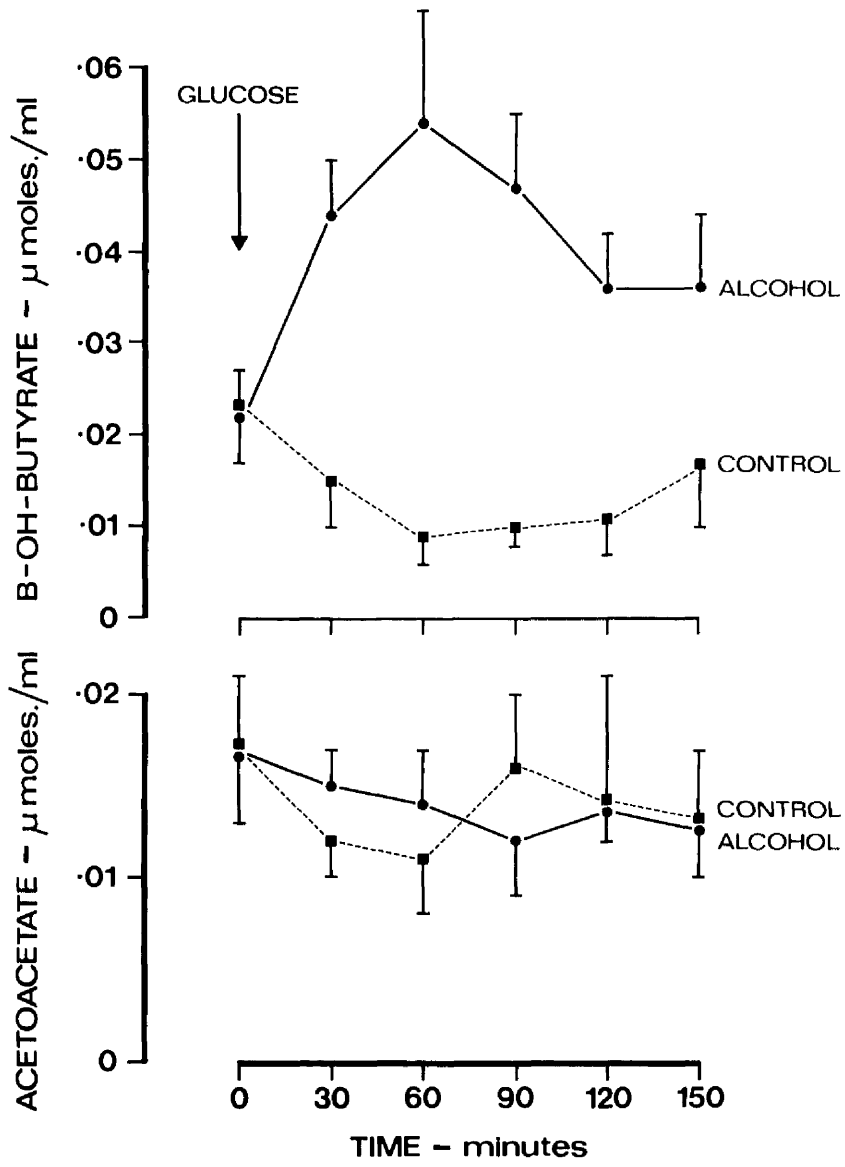
Blood acetoacetate (Fig. 4:2)

There was no significant difference between the pre-glucose samples on the two occasions. The glucose tolerance test was associated with a depression of the acetoacetate levels but the difference was not significant.

With alcohol and glucose the depression of acetoacetate was less marked and again was not significant.

Blood glycerol (Fig. 4:3)

There was no significant difference between the pre-glucose levels on the two occasions. The glucose tolerance test produced decreased blood glycerol levels significantly lower ($P < .05$) than the pre-glucose level in samples taken at 30, 60, 90 and 120 minutes.

With alcohol and glucose there was no decrease in blood glycerol levels all samples not being significantly different from the initial pre-alcohol and glucose sample.

Plasma free fatty acids (Fig. 4:3)

The levels of FFA before glucose were not significantly different on the two occasions. Oral glucose produced a depression in FFA levels, the levels of samples taken at 30, 60, 90 and 150 minutes being significantly lower than the pre-glucose level. At 150 minutes the FFA level was not significantly different from the pre-glucose level.

Alcohol delayed the depression of FFA the level not being significantly lower than the pre-glucose level until 60 minutes ($P < .01$) and all subsequent samples had FFA levels significantly lower than the pre-glucose level ($P < .01$).

Fig. 4 - 3.   Blood glycerol (μ mol/ml, mean $\pm$ SEM)

and plasma free fatty acids (in equiv./

ml, mean $\pm$ SEM) in six normal subjects

after an oral glucose tolerance test

(50g) with and without an oral dose of

alcohol (0.5g/Kg body weight).
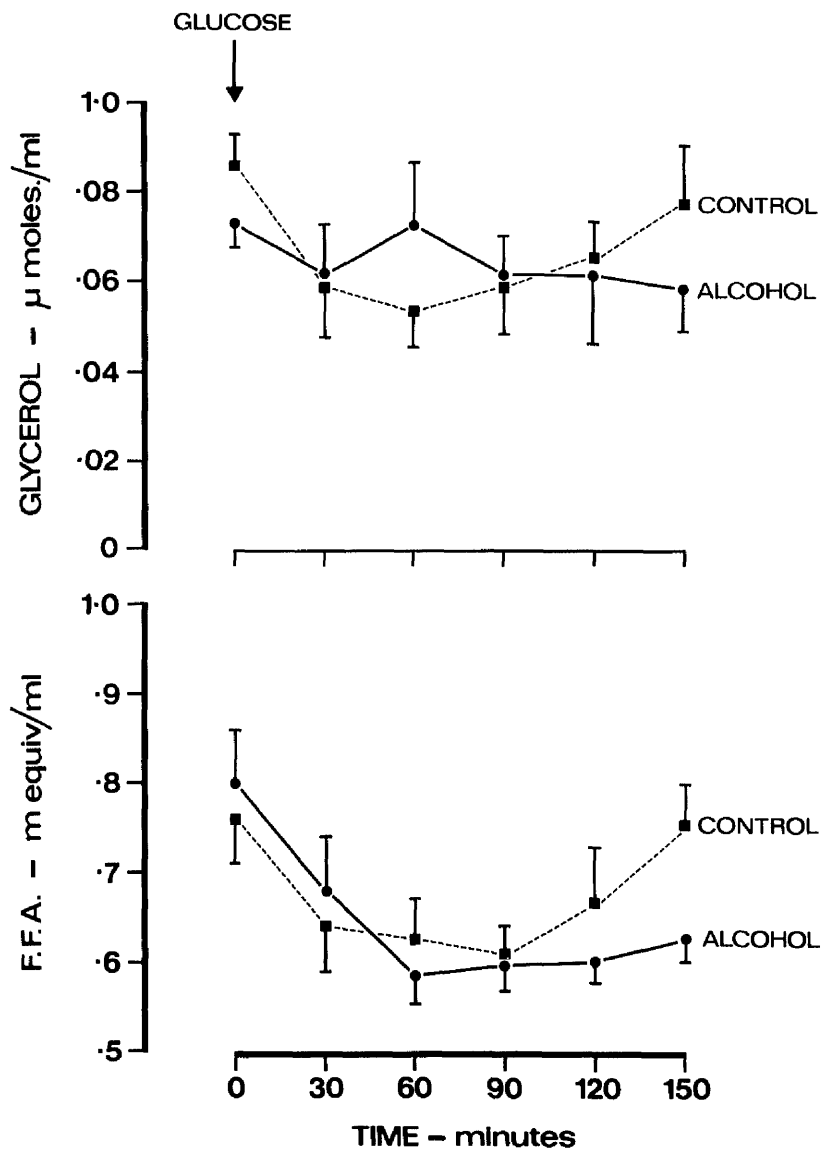
The FFA level of the sample taken at 150 minutes was the only level that was significantly less ($P < .05$) than the corresponding control sample without alcohol.

Blood glucose (Fig. 4:4)

There was no significant difference between the blood glucose levels before the glucose tolerance test on the two occasions.   There was no significant difference between the glucose tolerance curves on the two occasions. However, there is a suggestion that with alcohol the maximum blood glucose level was delayed.

Plasma insulin (Fig. 4:4)

There was no significance between the insulin levels of the pre-glucose levels on the two occasions.   It is evident from the figure that the maximum insulin level occurred at 60 minutes in the control test and at 90 minutes with alcohol.   With alcohol the insulin level in the sample at 90 minutes was significantly greater ($P < .05$) than the corresponding control level without alcohol.

Fig. 4 - 4. Blood glucose percentage change from

rest (mean $\pm$ SEM) and plasma insulin

(μ units/ml, mean $\pm$ SEM) in six

normal subjects after an oral glucose

tolerance test (50g) with and without
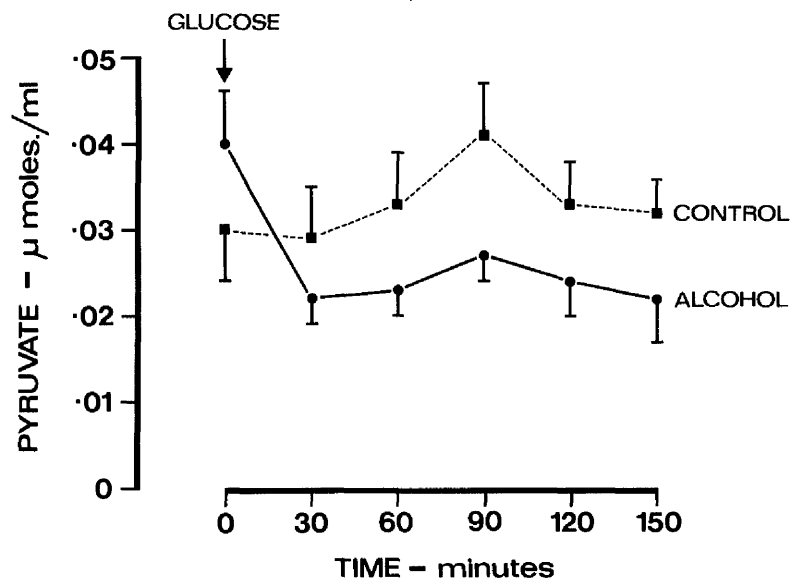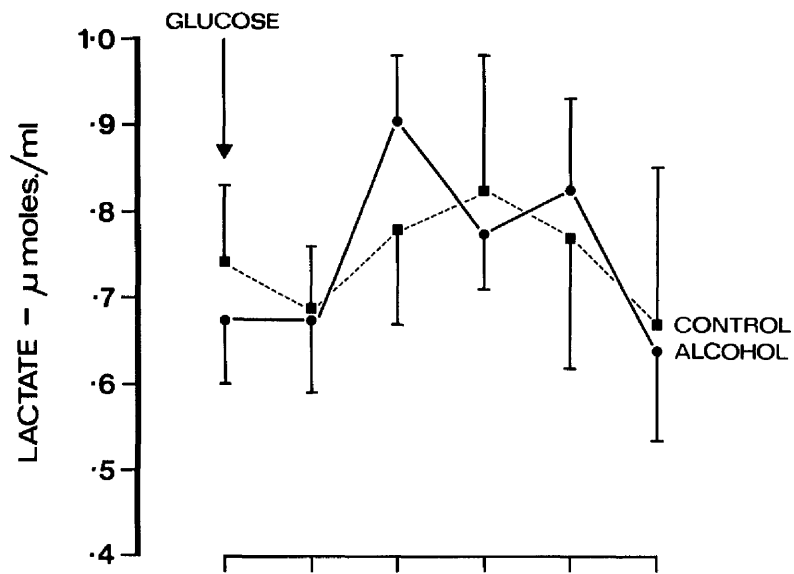
an oral dose of alcohol (0.5g/Kg

body weight).
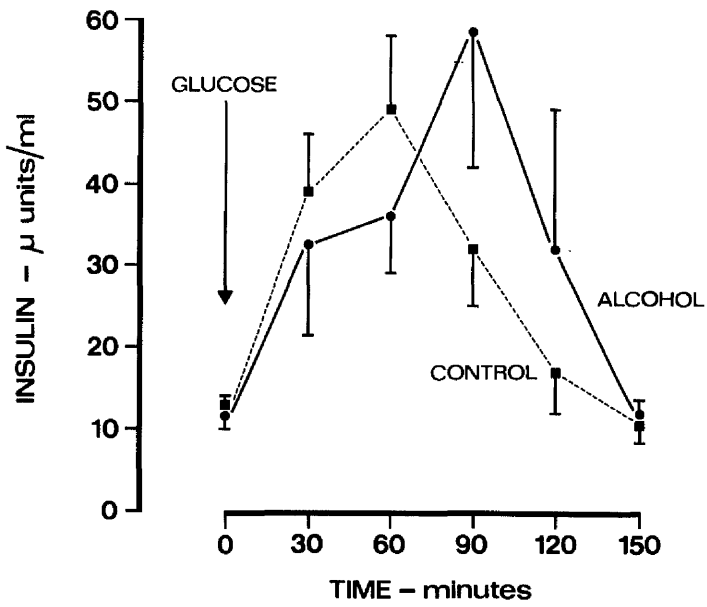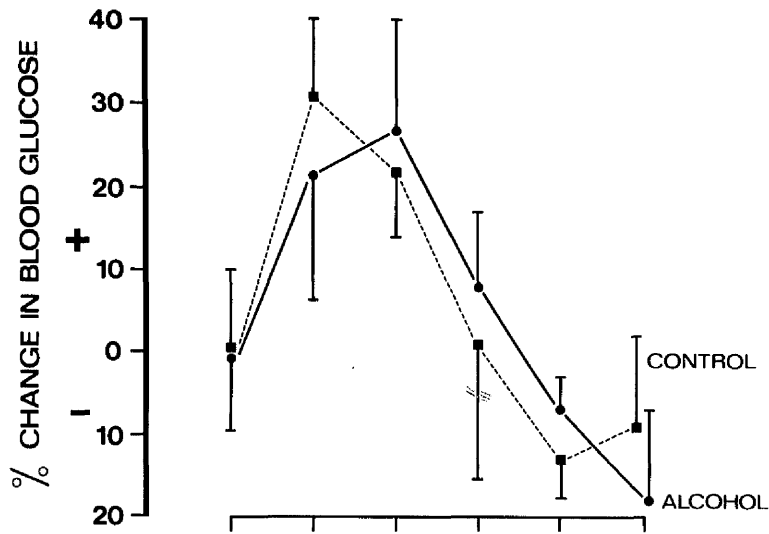
DISCUSSION

The control glucose tolerance test without alcohol
showed the normal pattern of change of glucose and other
metabolites following oral glucose. Glucose intolerance
is normally demonstrated when above normal glucose
concentrations are maintained in the blood for up to two
hours after a glucose tolerance test. The results reported
in this chapter suggest that although alcohol delayed the
appearance of a peak glucose concentration it did not produce
significant glucose intolerance. The demonstration of
alcohol induced glucose intolerance by Dornhorst and
Ouyang (1971) and Wapnick (1972) is particularly interesting
in that this effect was produced by relatively small
amounts of alcohol (0.5 g/Kg body weight) and (50 ml.
ethanol) respectively. It is known that high concentrations
of alcohol can produce glucose intolerance in alcoholics
(Philips and Sharfit, 1971), but the mechanism of the
diabetogenic effect of alcohol is unknown.

The ultimate fate of carbohydrate utilization is by
the Krebs cycle and since the metabolic pathway of alcohol
via acetaldehyde and acetoacetate is simpler than the
glycogen route followed by glucose it is possible that
alcohol is preferentially utilized by the tissues. However,
the delay in insulin release reported in this chapter and
by other authors (Wapnick, 1972; Philips and Sharfit, 1971)
probably depends on a specific action of alcohol on the
pancreas or its controlling hormones. It is known that
catecholamines can inhibit insulin release in man (Porte et al,
1966; Karama, 1966) and also that alcohol can release

catecholamines (Ogata and Mendelson, 1970). It is therefore possible that catecholamine release by alcohol may lead to inhibition or lowering of insulin release and this could be a basis for alcohol induced glucose tolerance under some conditions. However, alcohol levels associated with catecholamine release are much greater than the levels used in the investigation reported here and also the depression of FFA reported in Chapter 1 produced by the same dose of alcohol does not suggest that fat mobilization occurred as might be expected following catecholamine release. The depression of FFA and glycerol reported in this chapter is most likely due to a depression by glucose.

Wapnick (1972) and Phillips and Sharfit (1971) observed a delayed insulin release in investigations where alcohol was given and time was allowed for absorption before performing the glucose tolerance test. Dornhorst and Ouyang who administered glucose and alcohol simultaneously did not find a delay in insulin release. The results reported in this chapter when glucose and alcohol were given simultaneously suggests that a delay in insulin release can occur under these conditions and that it is not the length of time between giving alcohol and performing the glucose tolerance test that is the important factor.

The rather variable results reported by the various authors discussed here regarding the effects of alcohol on glucose tolerance suggest that the subjects chosen for study may differ in their susceptibility to alcohol induced glucose tolerance. The previous history of alcohol consumption by the subjects may be an important factor.

It was decided to repeat this investigation in a different group of subjects and to give glucose after sufficient time had been allowed for alcohol absorption. The results of this investigation are reported in the following chapter.

SUMMARY

1. The effect of alcohol on oral glucose tolerance was
    studied in six normal subjects by giving alcohol and
    glucose simultaneously and taking blood samples at
    intervals for $2\frac{1}{2}$ hours.

2. Alcohol produced a delay in the appearance of the peak
    glucose concentration but failed to produce any
    significant glucose tolerance.

3. Alcohol produced a delay in the insulin response to
    the glucose tolerance test a result not previously
    reported for the simultaneous administration of
    alcohol and glucose.

CHAPTER 5

THE EFFECT OF ALCOHOL ON GLUCOSE TOLERANCE IN ATHLETIC SUBJECTS

INTRODUCTION

In the previous investigation (Chapter 4) an alcohol induced glucose intolerance was not observed. However, there was a suggestion of a delayed peak in blood glucose and plasma insulin with alcohol.

It was considered that the failure to demonstrate any intolerance to glucose might have been due to the fact that insufficient time had been allowed for alcohol absorption and it was decided to repeat the investigation performing the glucose tolerance test 30 min. after alcohol administration. It was decided to study a group of athletically fit subjects since it has been suggested that some athletes may show a glucose intolerance during the glucose tolerance test (Davidson, 1966).

METHODS

Subjects

Five normal healthy, athletic subjects were studied. The subjects were relatively uniform with respect to height, 174.5 ± 2 cm (mean $\pm$ SEM) and weight 70 ± 3.5Kg (mean $\pm$ SEM). The subjects had fasted overnight and none of them had taken alcohol on the day before the investigation. The subjects were all students of the Scottish School of Physical Education and were undergoing regular athletic training for a variety of sports.

Procedure

An oral glucose tolerance test (50g) was performed on two occasions with and without an oral dose of alcohol (0.5g/Kg body weight). Alcohol was given 30 min. prior to the oral glucose tolerance test and blood samples were taken from a catheter placed in an antecubital vein. Samples were taken before alcohol, after alcohol but before glucose and at 30, 40, 50, 60, 90, 120 and 150 min. after glucose administration.

RESULTS

Blood lactate (Fig 5:1)

There was no significant difference between the pre-alcohol sample and the pre-glucose (control) samples. The control glucose tolerance test was associated with increased lactate levels but the increase was not significant.

Before glucose, alcohol produced a significant ($P < .05$) rise in blood lactate and after glucose, the blood lactate continued to rise the level being significantly higher ($P < .05$) than the corresponding control values at 30, 40, 50, 90 and 150 min.

Blood pyruvate (Fig.

There was no significant difference between the pyruvate levels of the pre-alcohol and the pre glucose (control) sample. The control glucose tolerance test was associated with increased mean pyruvate level 30-90 minutes but the increase was not significant.

Alcohol produced a significant decrease ($P < .01$) in the pyruvate level before glucose and after glucose the pyruvate level remained significantly lower ($P < .01$) than the pre-alcohol level. There was however a samll rise in the mean pyruvate level at 30 - 60 min. but the rise was not significant.

Blood β-hydroxy-butyrate (Fig. 5:2)

There was no significant difference between the pre-alcohol and the pre-glucose control sample. The control glucose tolerance test was associated with a depression of the β-hydroxy-butyrate level and samples at 50, 60, 90, 120 and 150 min. were significantly

Fig. 5.1:   Blood lactate and pyruvate (μmol/ml mean $\pm$ SEM)
in six athletic subjects during a glucose toler-
ance test (50g) with an oral dose of alcohol
(0.5g/Kg body weight) taken 30 minutes before
glucose (●————●) and without alcohol (■ — — —■).
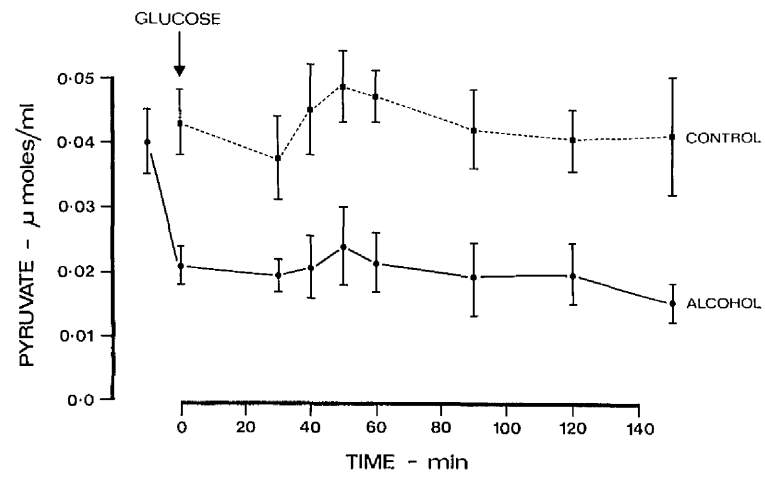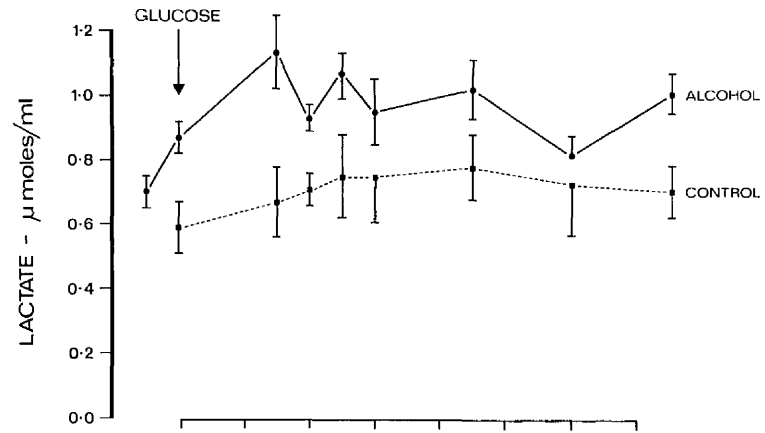
lower (P < .05) than the pre-glucose level.

With alcohol, the depression was reduced and although the samples at 60 and 90 min. were significantly lower than the pre-alcohol levels, they were significantly higher (P < .05) than the corresponding control values. It was a significant observation that at no time was the β-hydroxy-butyrate level after alcohol greater than the resting level.

Blood acetoacetate (Fig. 5:2)

There was no significant difference between the pre-alcohol and the pre-glucose (control) sample. The control glucose tolerance test appeared to produce a depression of the acetoacetate level but the difference was not significant.

Alcohol produced an immediate depression in acetoacetate, the post alcohol level being significantly lower (P < .05) than the pre-alcohol level. Thereafter, there was no no significant difference between the post alcohol level and the levels recorded in the following glucose tolerance test. The acetoacetate levels at 30, 40, 50 and 90 min. were significantly lower than the corresponding control values.

Blood glycerol (Fig. 5:3)

There was no significant difference between the pre-alcohol and the pre-glucose (control) level. Glucose produced a depression in blood glycerol the levels at 60 and 90 min. being significantly lower (P < .05) than the resting level.

Fig. 5.2: Blood acetoacetate and β-hydroxy-butyrate

(μ mol/ml, mean $\pm$ SEM) in six athletic subjects

during a glucose tolerance test (50g) with an

oral dose of alcohol (0.5g/Kg body weight)

taken 30 minutes before glucose (●————●)

and without alcohol (■– – – –■).
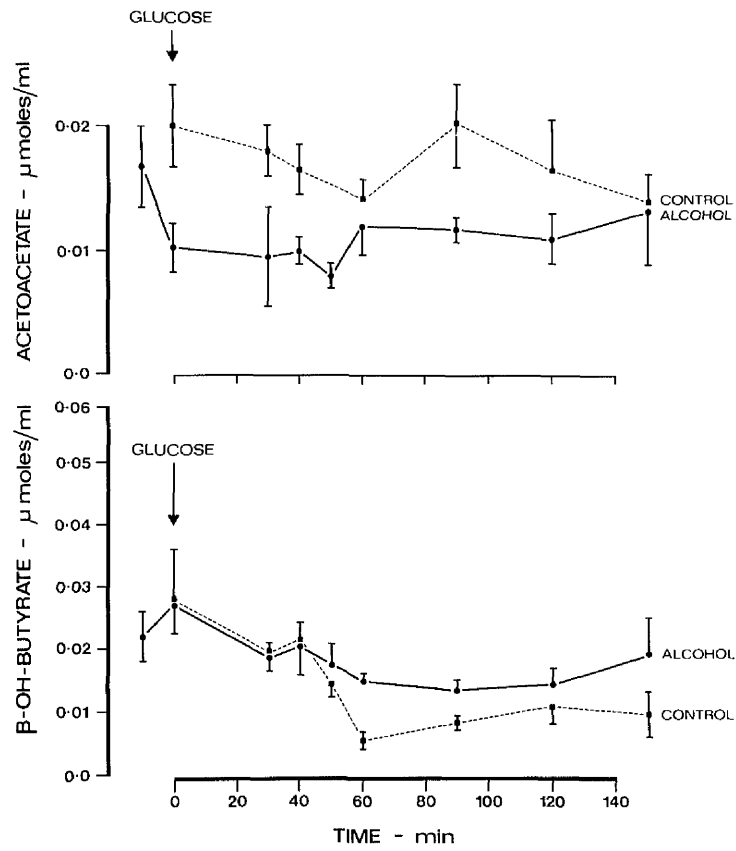
Fig. 5.3:   Blood glucose ($\%$, mean $\pm$ SEM) and blood glycerol

(μ mol/ml, mean $\pm$ SEM) in six athletic subjects

during a glucose tolerance test (50g) with an

oral dose of alcohol (0.5g/Kg body weight) taken

30 minutes before glucose (●———●) and without
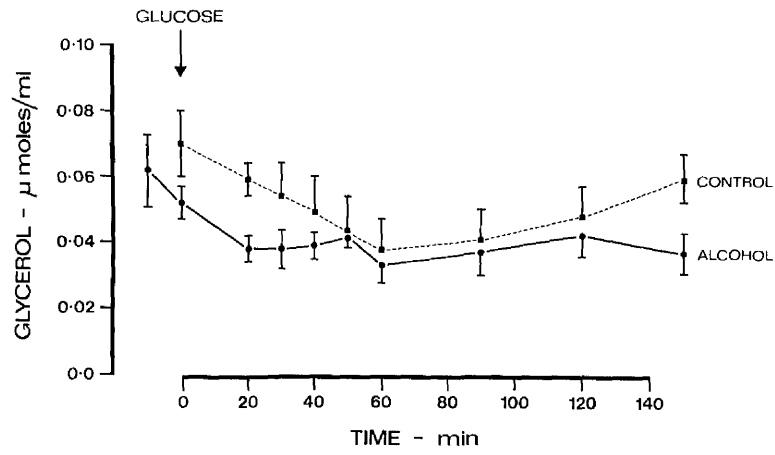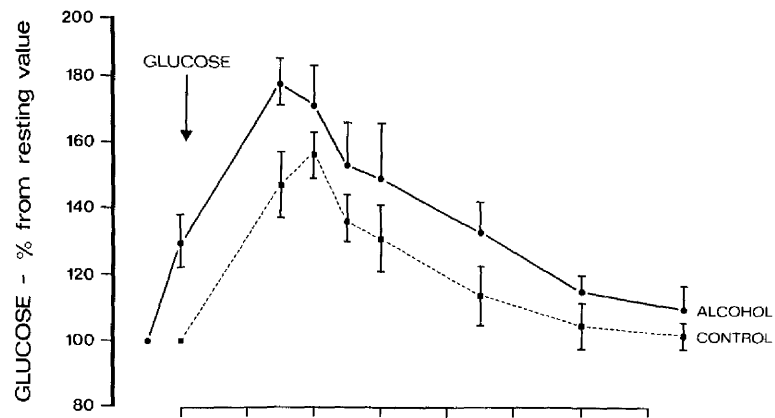
alcohol (■— — —■).

With alcohol, a greater depression in glycerol levels occurred in the levels at 20, 30 and 150 min. being significantly lower $(P < .05)$ than the corresponding samples without alcohol.

Blood glucose

There was no significant difference between the pre-alcohol and the pre-glucose (control) sample. Alcohol produced a significant rise $(P < .05)$ in blood glucose before the glucose tolerance test.

After glucose administration, all the glucose levels, with alcohol, were significantly greater than the corresponding control values. The mean blood glucose levels did not suggest any alcohol induced glucose intolerance but the individual levels of two subjects did suggest some intolerance, Table 5:1.

TABLE 5:1

BLOOD GLUCOSE mg/100 ml

CONTROL		ALCOHOL	
A	B	A	B
72	71	95	74
		111	136
122	105	159	150
119	120	206	149
120	93	182	151
111	106	190	149
107	84	162	109
89	87	114	103
83	76	101	100

DISCUSSION

Davidson (1966) has reported that a group of subjects undergoing physical training exhibited glucose intolerance during a glucose tolerance test. In the control experiment, none of the athletic subjects showed any signs of glucose intolerance and the levels of lactate, pyruvate and glycerol followed the normal pattern associated with the glucose tolerance test and described in the previous chapter. With alcohol, blood glucose appeared to increase immediately after alcohol an observation not previously reported and not found in the previous chapter with non-athletic subjects. This rise in blood glucose level may be an effect associated with fitness or may possibly be apparent only when sufficient time has been allowed for alcohol absorption. The failure to detect an increase in blood glucose after alcohol in chapter 1 suggests that the fitness of the subjects is the important factor. The impairment of glucose tolerance, in some subjects, reported here may be related to the fact that alcohol was given 30 min. before glucose. However, other authors who have allowed time for alcohol absorption (Kapnick and Jones, 1972; Phillips, 1971) have failed to demonstrate any impairment of glucose tolerance in normal subjects. It may be that the choice of subjects is very important in relation to fitness and other factors. Taken together, the athletic subjects did not demonstrate any impairment of glucose tolerance although individually, the curves of two subjects did suggest some intolerance. It is possible that a study of a larger group of athletes

or a group of more strenuously trained athletic subjects would establish how general the effect is in fit subjects.

With alcohol, there was no increase in the β-hydroxy-butyrate levels of the fit subjects as previously described for unfit subjects (Chapter 4). It is known that athletes do not develop a marked post exercise ketosis (Johnson, et al , 1969) and it may be that with a greater ability to utilise ketones β-hydroxy-butyrate does not increase in athletes after alcohol at rest.

The results reported in this chapter for unfit subjects suggest that fitness affects the metabolite changes normally associated with alcohol and in particular ketones are not produced during ethanol metabolism in fit subjects. The use of alcohol may therefore be valuable in further studies of the production and utilisation of ketones by athletic and non-athletic subjects. The results suggest that fitness may in some subjects be associated with the development of glucose intolerance by alcohol.

SUMMARY

1. The effect of alcohol on glucose tolerance was studied
   in five normal healthy athletes by giving an oral
   dose of alcohol (0.5 g/kg body weight) and after
   allowing 30 minutes for absorption performing an oral
   glucose tolerance test (50 g). Blood samples were
   withdrawn at intervals for $2\frac{1}{2}$ hours.

2. The control glucose tolerance without alcohol failed
   to demonstrate any glucose intolerance in the athletes.

3. Blood glucose increased immediately after alcohol
   (before glucose) a result not previously found in other
   studies of the effects of alcohol reported in this
   thesis. It was considered that this effect may be
   related to the fitness of the subjects.

4. Alcohol did not produce any significant glucose
   intolerance although the glucose concentrations of
   two subjects did suggest some intolerance.

5. The athletes did not show any increase in β-hydroxy-
   butyrate concentrations after alcohol as previously
   reported in other chapters for normal non-athletic
   subjects.

<u>CHAPTER 6</u>

THE EFFECT OF FRUCTOSE ON ALCOHOL INDUCED

CHANGES IN BLOOD METABOLITES.

INTRODUCTION

Many attempts have been made to find a means of increasing the rate of ethanol oxidation in man. Physical exercise, vitamin supplements, glucose, fructose, sucrose, galactose, thyroid hormones, caffeine, diet and starvation have all been tried (Pawan, 1972). Of these procedures only the administration of fructose was found to increase ethanol oxidation, and starvation and high fat diet to decrease it. Wallgreen and Barry 1970, have reviewed the published reports and concluded that fructose alone had a proven but very variable capacity to accelerate ethanol oxidation. Brown et al 1972, performed a controlled trial of the effect of fructose on alcohol metabolism by giving fructose intravenously and found a 25% increase in the rate of alcohol oxidation after fructose. This is similar to the increase in alcohol oxidation by fructose reported by Patel et al 1969.

Fructose is metabolised in the liver where it causes increased lactate formation, high energy phosphate depletion, increased uric acid and inhibition of protein synthesis (Woods and Alberti, 1972). Woods and Alberti pointed out the dangers of using fructose and alcohol mixtures as large levels of lactate are liable to be produced by fructose and also since alcohol inhibits lactate utilisation. It is pointed out that the use of fructose to increase alcohol oxidation is likely to produce lactic acidosis in alcoholic subjects with liver damage.

Although the ability of fructose to increase the rate of alcohol oxidation has been established there has as yet been no clear demonstration of how this effect is produced. It was decided to investigate the effect of fructose on alcohol induced metabolite changes and to determine to what extent fructose altered the NADH/NAD ratio in such a way as to promote alcohol oxidation. In view of the problems associated with fructose/alcohol mixtures an investigation was designed in which relatively small amounts of fructose and alcohol would be given orally.

METHODS

Subjects

Six normal healthy male subjects were studied. The subjects were relatively uniform with respect to height, 176.3 $\pm$ 2.9 cm (mean $\pm$ SEM) and weight 71.2 $\pm$ 5.2 Kg (mean $\pm$ SEM). The subjects had been fasting overnight, had not taken alcohol on the day before the investigation and were on normal unrestricted diet. The subjects remained at rest throughout the experiment.

Procedure

The subjects received an oral dose of alcohol (0.5 g/Kg body weight) on two occasions with an oral dose of fructose (50 g) and without fructose. The fructose was administered 30 minutes after the alcohol. Blood samples were taken from a catheter in an antecubital vein before alcohol and at 30 minute intervals during the investigation for $2\frac{1}{2}$ hours.

RESULTS

Blood lactate (Fig. 6 : 1)

There was no significant difference between the pre-alcohol samples on the two occasions. In the control experiment alcohol produced increased blood lactate concentrations significantly greater ($P < .05$) than the pre-alcohol level (samples taken at 30, 60 and 90 minutes).

With fructose and alcohol a greater and more prolonged rise in blood lactate occurred. Samples taken at 60, 90, 120 and 150 minutes had blood lactate concentrations significantly greater ($P < .01$) than the corresponding control samples without fructose.

Blood pyruvate (Fig. 6 : 1)

There was no significant difference between the pre-alcohol samples on the two occasions.

In the control experiment alcohol produced a depression in blood pyruvate, all samples taken after alcohol being significantly less ($P < .05$) than the pre-alcohol level.

Fructose did not appear to modify the alcohol induced depression of pyruvate, and there was no significant difference between the pyruvate concentrations after fructose and the corresponding control levels.

Blood β-hydroxy-butyrate (Fig. 6 : 2)

There was no significant difference between the pre-alcohol levels on the two occasions. In the control experiment alcohol produced a significant increase in β-hydroxy-butyrate, samples taken at 30, 60 and 90 minutes being significantly greater ($P < .01$) than the pre-alcohol level. With fructose the level of β-hydroxy-butyrate

Fig. 6.1:   Blood lactate and pyruvate (μmol/ml, mean

$\pm$ SEM) in six normal subjects after and oral

dose of fructose (50g) administered 30 minutes

after alcohol (●———●) and without fructose

(■———■).
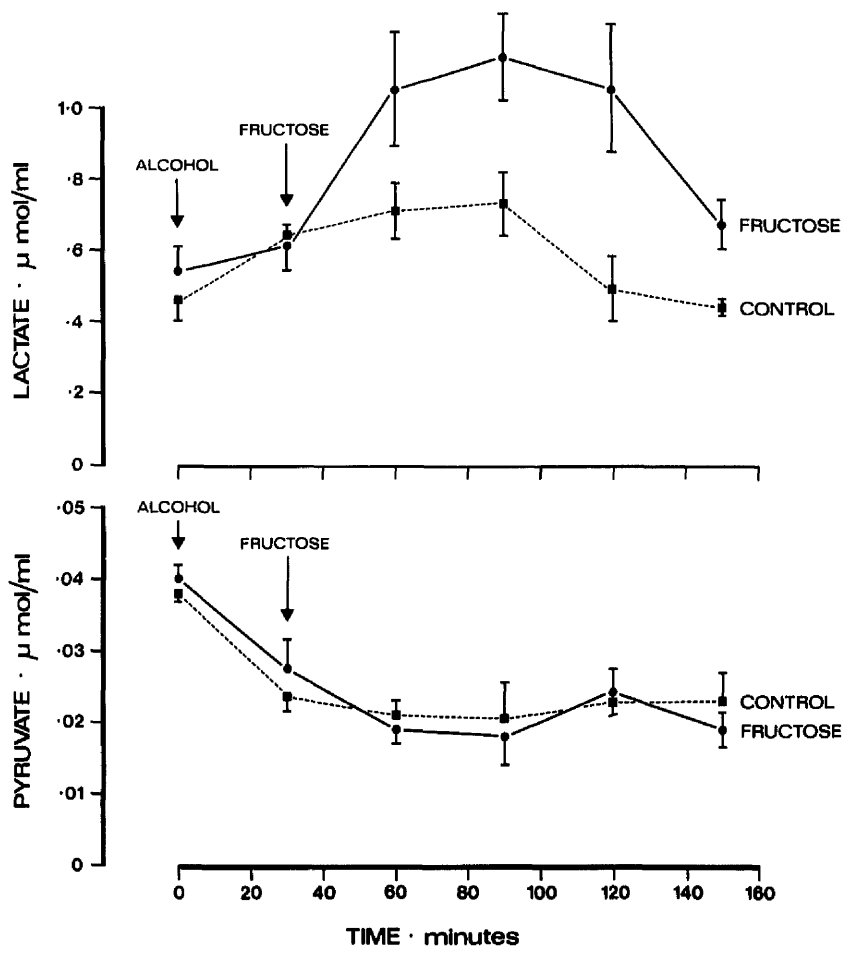
remained elevated longer than in the control experiment, samples taken at 120 and 150 minutes being significantly greater ($P < .01$) than the corresponding control levels. The sample taken at 60 minutes was however significantly smaller ($P < .05$) than the corresponding control level, this sample being the first sample taken after fructose.

Blood acetoacetate (Fig. 6 : 2)

There was no significant difference between the pre-alcohol levels on the two occasions.

In the control experiment alcohol produced a depression in acetoacetate which returned to the pre-alcohol level within the duration of the experiment. With fructose the acetoacetate remained low, samples taken at 90, 120 and 150 minutes being significantly less ($P < .01$) than the corresponding control values.

Lactate/pyruvate & β-hydroxy-butyrate/acetoacetate ratios

(Table 6 : 1)

Alcohol appeared to produce an increase in both the lactate/pyruvate and β-hydroxy-butyrate/acetoacetate ratios but within the duration of the experiment the ratios returned towards the pre-alcohol levels.

With alcohol and fructose the increase in both ratios was maintained for as long as sampling continued.

Fig. 6.2:   Blood β-hydroxy-butyrate and acetoacetate

(μmol/ml, mean $\pm$ SEM) in six normal subjects

after an oral dose of alcohol (0.5g/Kg body

weight) with an oral dose of fructose (50g)

administered 30 minutes after alcohol (●———●)

and without fructose (■———■).
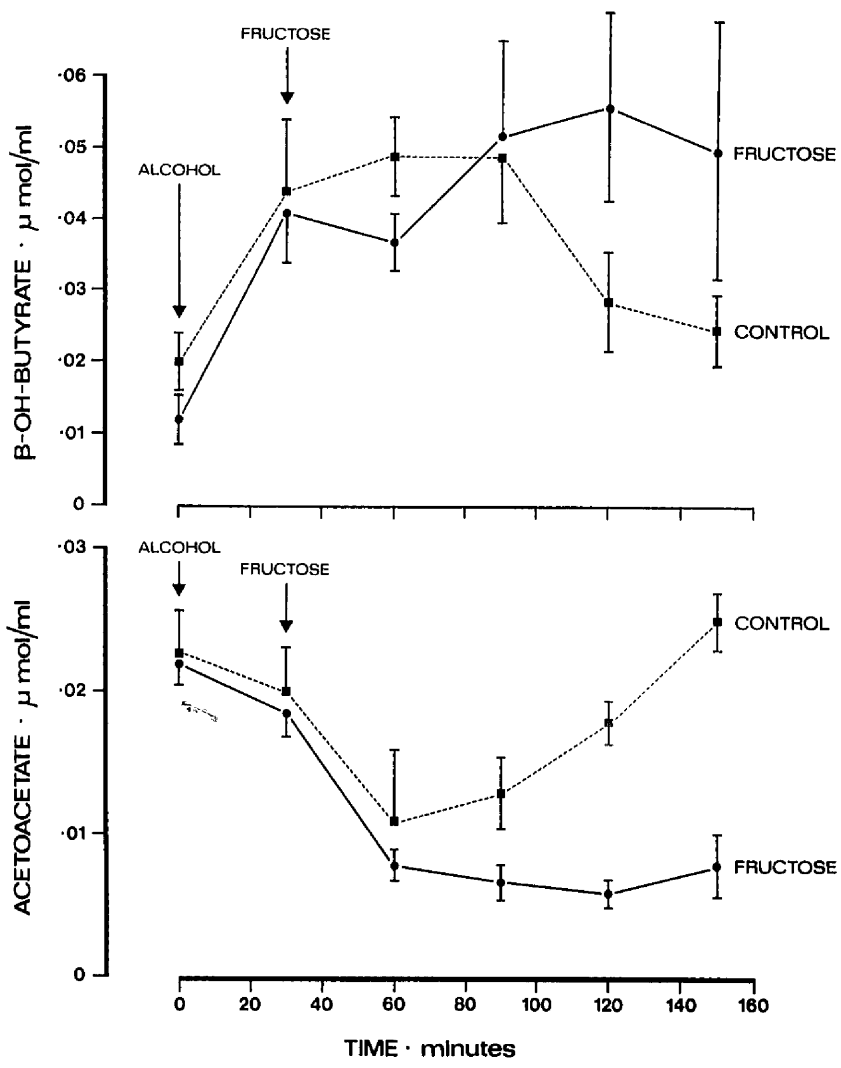
TABLE   6 : 1

LACTATE/PYRUVATE   RATIO

Sample time	0	30	60	90	120	150
Control	12.4	27.0	31.8	34.3	19.2	18.3
Fructose	13.0	22.2	52.0	55	44.2	36.0

β-HYDROXY-BUTYRATE/ACETOACETATE   RATIO

Sample time	0	30	60	90	120	150
Control	0.5	2.1	4.4	3.7	1.6	0.9
Fructose	0.5	2.2	4.4	6.0	8.7	6.0

Blood glycerol (Fig. 6 : 3)

There was no significant difference between the blood glycerol levels of pre-alcohol samples on the two occasions.

After alcohol, in the control experiment, there was no significant change in the blood glycerol level. Fructose produced an increase in blood glycerol the levels for samples taken at 60, 90 and 120 minutes being significantly greater ($P < .01$) than the corresponding control levels.

Fig. 6.3:   Blood glycerol (μmol/ml, mean $\pm$ SEM) in six

normal subjects after and oral dose of alcohol

(0.5g/Kg body weight) with an oral dose of

fructose (50g) administered 30 minutes after

alcohol (●———●) and without fructose (■———■).
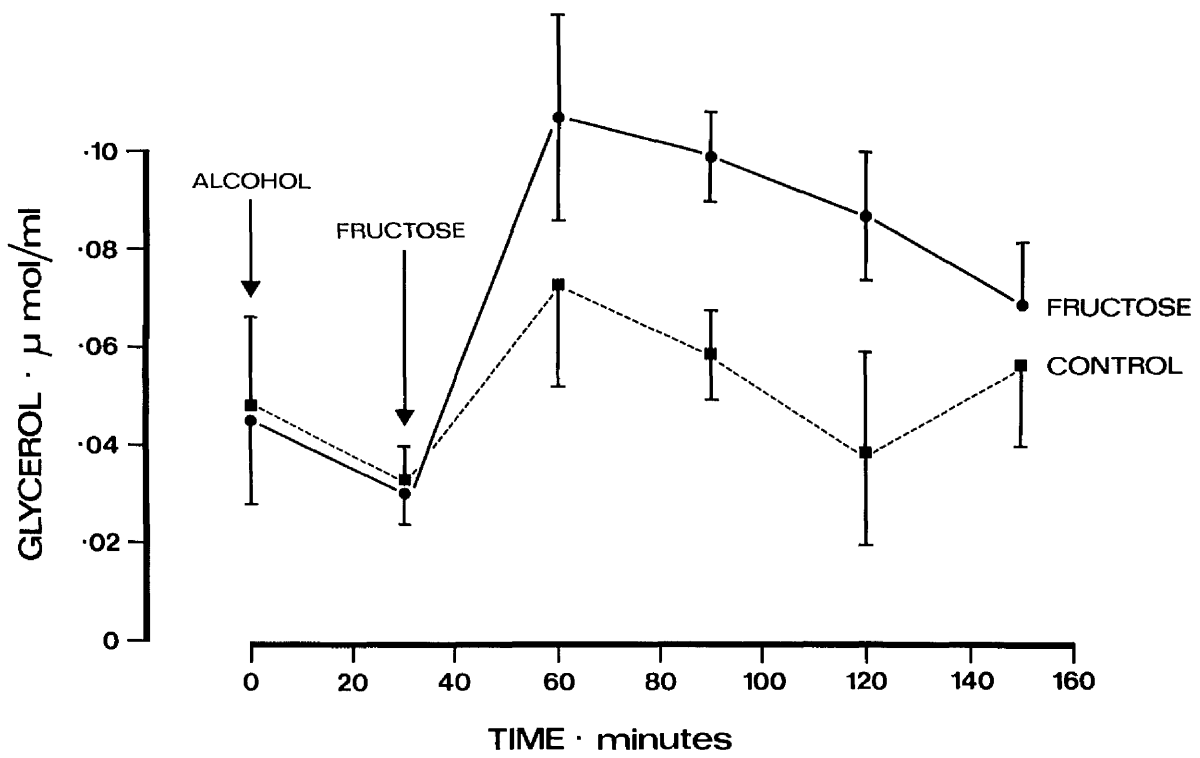
DISCUSSION

The metabolite changes in the control experiment
without fructose were very similar to the pattern of metabolite
changes induced by alcohol and described in Chapter 1.
Fructose led to increased levels of blood lactate which is
in agreement with previous reports.   (Berhstrom et al, 1968;
Sahebjami and Scalettar, 1971; Cook, 1970).   Sahebjami was
able to demonstrate that continuous infusion of fructose
could lead to high levels of lactate, lowering of plasma
bicarbonate and the development of lactic acidosis.   As
previously discussed (chapters 2 and 3) ethanol inhibits
gluconeogenesis from lactate and therefore administration of
both ethanol and fructose is liable to produce high levels of
lactic acid.   Of particular importance is the fact that
fructose given to increase the rate of alcohol breakdown is
most likely to be given to alcoholics many of whom suffer
from liver damage which is often associated with imparied
lactate uptake (Berry and Schuer, 1967; Berry, 1967;
Oliva 1970; Woods and Alberti, 1972).   Therefore the
administration of fructose to alcoholics with high alcohol
levels is accompanied with a serious risk of lactic acidosis.

Fructose led to an increase in the cytoplasmic (lactate/
pyruvate ratio) and mitochondrial (β-hydroxy-butyrate/
acetoacetate ratio) NADH/NAD ratio.   There was no suggestion
of fructose lowering the NADH/NAD ratio so as to promote an
increased rate of alcohol oxidation.

Theoretically, any NADH requiring system which can
oxidise the ADH-NADH complex directly would tend to enhance
the rate of the ADH reaction since the dissociation of this
complex appears to be the rate determining step (Theorell

and Chance, 1951), and the increased rate of alcohol oxidation by fructose might be explained on this basis. Fructose and glyceraldehyde have both been found to increase the rate of alcohol oxidation in liver slices (Thieden and Lundquist, 1967). Normally glyceraldehyde is oxidised to glycerate rather than being reduced to glycerol, but the oxidation pathway is blocked when alcohol and fructose are metabolised (Tygstrup et al, 1965). It is possible that acetaldehyde formed from alcohol will competitively appropriate the enzyme aldehyde dehydrogenase which would be required for glycerate formation from glyceraldehyde. In support of this explanation, glycerol production is greatly enhanced when liver slices are incubated with fructose and alcohol (Rawrat, 1970; Thieden and Lundquist, 1967). The result reported in this chapter of a significant increase in blood glycerol when alcohol and fructose were given supports this explanation. Presumably, NAD produced by the reduction of glyceraldehyde to glycerol would be immediately reduced to NADH by further alcohol oxidation. Unfortunately, the results reported in this chapter are unsatisfactory in that it was not possible to measure blood alcohol concentrations and to demonstrate any increase in alcohol oxidation produced by fructose.

SUMMARY

1. The effect of fructose on the metabolite changes
    produced by alcohol was studied in six normal healthy
    subjects by giving an oral dose of alcohol (0.5 g/Kg.
    body weight) and fructose (50 g) 30 minutes after
    alcohol administration.   Blood samples were taken
    at intervals for $2\frac{1}{2}$ hours.

2. Fructose produced greater concentrations of blood
    lactate consistent with the known effects of fructose.

3. Fructose was associated with an increase in the
    lactate/pyruvate and the β -hydroxy-butyrate/
    acetoacetate ratios.

4. Fructose led to increased glycerol levels consistent
    with the idea that fructose can increase the rate of
    alcohol oxidation following the reduction of
    glyceraldehyde, produced from fructose, to glycerol.

5. Due to the failure of the blood alcohol estimation it
    was not possible to demonstrate any increase in the
    rate of alcohol oxidation attributable to fructose.

CHAPTER 7

THE METABOLIC RESPONSE TO EXERCISE IN PATIENTS WITH CHRONIC ALCOHOLISM

INTRODUCTION

Chronic alcoholism is associated with a large number of metabolic disorders. Hypothalamic/pituitary/adrenal function is often disturbed (Merry and Marks, 1969 and Mendelson, 1971). Alcohol ingestion can give rise to a glucose intolerance (Domhorst and Ouyang, 1971) and under certain conditions to hypoglycaemia (Moyniham et al, 1967). The chronic ingestion of alcohol may also be associated with derrangement of liver function and the development of liver disease. The liver disease of the alcoholic may partly be due to poor nutrition but it is evident that other factors are involved as liver disease can often develop in alcoholics with adequate dietary intake (Klatskin, 1961). One of the important liver diseases of the alcoholic is Laennec's Cirrhosis which is apparently produced in part by the alteration of lipid metabolism by alcohol (Porta, Koch and Hartcroft, 1969).

It has already been reported (Chapter 3) that ethanol can lead to increased secretion of H.G.H. during exercise. Since hypothalamic and pituitary function is often disturbed in patients with chronic alcoholism, exercise studies in this situation may provide information about the role of the hypothalamus in regulating the release of growth hormone.

Due to the large number and variety of metabolic disorders associated with chronic alcoholism and the possible value of studying HGH release in this disease, it was decided to study the metabolic response to exercise in a group of patients suffering from this disease. It was hoped to determine the value of the exercise test in studying the effects of chronic alcohol by virtue of any differences between the metabolite changes of a group of alcoholics and a group of normal control subjects.

METHODS

Subjects

Six subjects who had been diagnosed as chronic alcoholics were studied 1 - 2 weeks after they had entered hospital for treatment. Prior to the investigation liver function tests were carried out on the alcoholics and the results were within normal limits. The alcoholics were relatively uniform with respect to height, 173 ± 3.0 cm (mean $\pm$ SEM) and weight 72.2 ± 3.2 Kg and none of them had taken alcohol since admission.

In addition, six normal control subjects were studied. The control subjects were relatively uniform with respect to height 176 ± 3.0 cm. (mean $\pm$ SEM) and weight 74.2 ± 3.6 Kg and had not taken alcohol on the day before the investigation.

Procedure

Both groups of subjects performed an exercise test, after an overnight fast, on a bicycle ergometer at 600 kpm for 20 min.. Blood samples were taken from a catheter placed in an antecubital vein at rest before exercise, at 5 min. intervals during exercise and at 15, 30, 60 and 90 min. after exercise.

Dr. W.R. Sulaiman who was interested in growth hormone release performed the analysis of HGH.

Blood lactate (Fig. 7.1)

Both the patients and the subjects showed increased
blood lactate levels during exercise and a rapid recovery
to within the resting level after exercise.   There was
no significant difference between the resting lactate levels
in the two groups.   At the end of exercise the blood lactate
of the alcoholics was significantly greater $(P < .01)$ than
the corresponding level for the control subjects.

Blood pyruvate (Fig 7:1)

There was no significant difference between resting
pyruvate levels of the two groups.   At the end of exercise
the pyruvate level of the alcoholics was significantly greater
$(P < .05)$ than the corresponding level of the control subjects.
During the post exercise period, the blood pyruvate of the
alcoholics remained significantly greater $(P < .01)$ than the
corresponding control levels.

Total ketones (Fig. 7:2)

There was no significant difference between the total
blood ketones of the alcoholics and controls at rest.
Both groups showed a similar fall in ketones during exercise.
After exercise the total ketones rose in both groups, however
the levels reached by the alcoholics were significantly
greater $(P < .001)$ than in the controls for samples taken at
90 and 120 mins.

Blood glycerol (Fig. 7:3)

There was no significant difference between the resting
glycerol levels in the two groups.   In all samples taken

during exercise the blood glycerol of the alcoholics was significantly greater ($P < .01$) than the corresponding levels for the control subjects. The only significant difference between the blood glycerol of the two groups occurred in the samples at 90 min. when the blood glycerol of the alcoholics was significantly greater ($P < .05$) than the corresponding control level.

Plasma free fatty acids (Fig. 7:3)

The pattern of change of FFA was very similar in both the alcoholics and the control subjects. The alcoholics tended to have higher FFA levels but the difference was not significant.

Blood glucose

There was no significant difference between the blood glucose of the alcoholics and the control subjects either during or after exercise.

Heart rate

Table 7:1

Mean heart rate beats/min.

	Alcoholics	Controls
End of exercise	140	136
5 min post exercise	102	96

The mean heart rate of the alcoholics was greater both at the end of exercise and five minutes after exercise but the difference was not significant.

Fig. 7.1:   Blood lactate and pyruvate $(\mu \text{mol/ml}$, mean $\pm$

SEM) during and after 20 minutes of exercise in

6 chronic alcoholics (●————●) and six normal
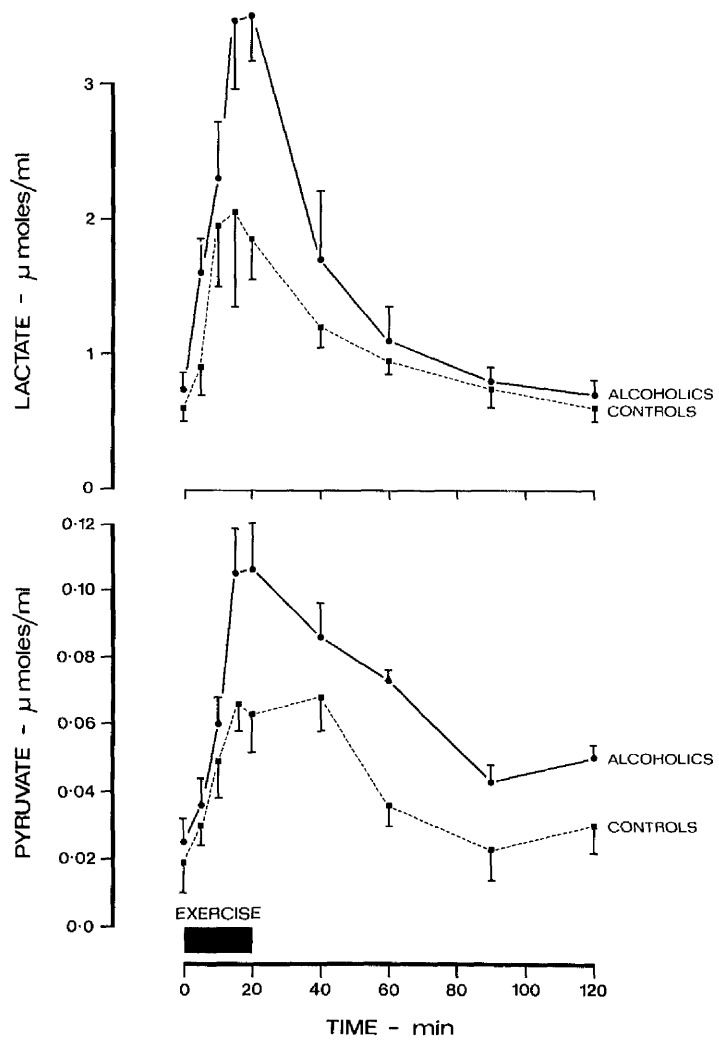
control subjects (■————■).

Fig. 7.2:   Total blood ketone - bodies (3-hydroxy-butyrate +

acetoacetate; μ mol/ml, mean $\pm$ SEM) during and

after exercise in six chronic alcoholics (o——o)

and six normal control subjects (□———□).
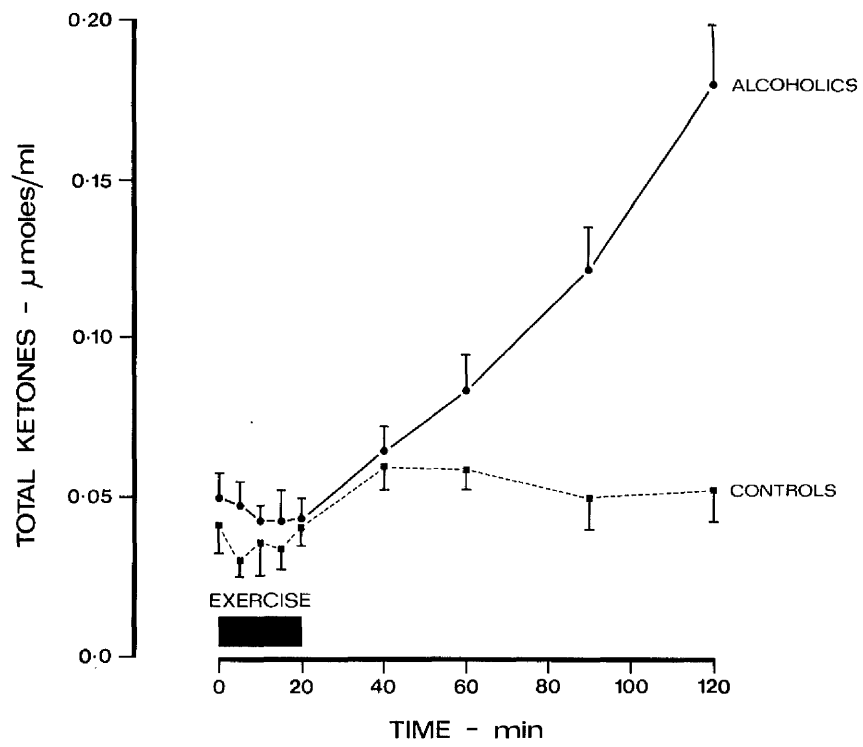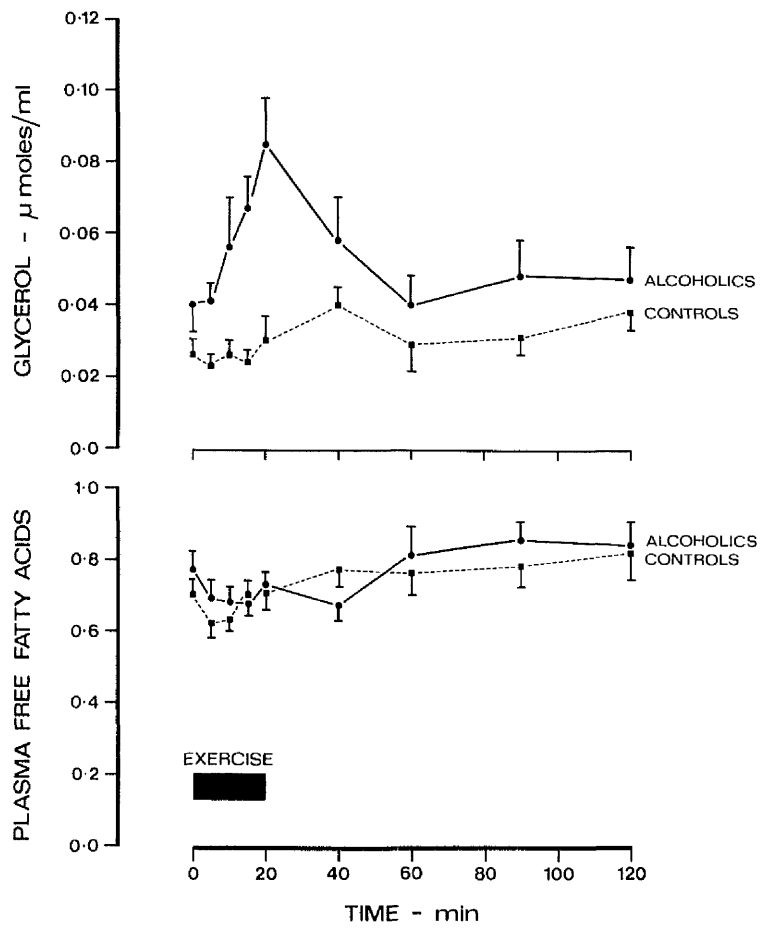
Fig. 7.3:   Blood glycerol (μmol/ml, mean $\pm$ SEM) and plasma

free fatty acids (m equi/ml, mean $\pm$ SEM) during

and after 20 minutes of exercise in six chronic

alcoholics (●———●) and six normal control

subjects (■———■).

Growth Hormone

Fig. 7:5 : Plasma HGH concentrations were similar at rest in the patients

and the controls. HGH concentrations rose in both groups. The

highest value was achieved at the end of exercise, but the

concentrations were 40% higher in the controls (10 µU/ml mean ±

1.5 µU SEM) than in the alcoholics (6µU/ml ± 0.8 µU SEM). In

patients and controls HGH concentrations fell in the period

following exercise to approximately pre-exercise values at

60 min after the end of exercise.

Acetoacetate Tolerance test

The alcoholic subjects appeared to develop a
significant post exercise ketosis which was absent with
.the control subjects.   Because the alcoholics had
exercised at the same rate as the controls and because
their heart rate was not very much greater it appeared that
post exercise ketosis was not due to a lower level of
fitness in the alcoholics.   An acetoacetate tolerance
test was therefore carried out to see if the alcoholics
showed a decreased utilization of ketones.

Method

An acetoacetate tolerance test was performed on the
same alcoholic subjects that had performed the exercise
test, and a group of control subjects both groups were
required to drink a solution of 0.4 M sodium acetoacetate,
blood samples being taken before and 40 mins. after
acetoacetate and then at 20 min. intervals a further
5 samples were collected.

Results Fig.7:4

Both groups of subjects showed similar concentrations
of total blood ketones, the only significant difference
being in the sample taken at 60 min. when the total ketones
of the alcoholic subjects was significantly greater $(P < .01)$
than the corresponding level for the control subjects.

Between 60 and 120 minutes, the decreasing levels
of acetoacetate are approximately linear and therefore
the appropriate linear regression equations were calculated
for both groups.   The values of R the regression coefficient
for each group were compared and found not to be significantly

different.   It appears that instead of showing a decreased
rate of ketone utilisation, the alcoholic, if anything,
showed an increased rate of ketone utilisation.   The
difference in the levels at 60 min. is most likely due
to different rates of absorption of ketones a problem
which is likely to arise in any experiment involving an
orally administered substance.   In this experiment the
alcoholics appeared to have the lower rate of absorption
which is in general agreement with the findings that
the alcoholic has impaired absorption (Mezey et al 1970).

Results of other metabolites, lactate, pyruvate,
glycerol and FFA showed very similar changes in both
groups and there were no significant differences between
the alcoholics and control subjects.   Lactate and pyruvate
showed little change whereas both FFA and glycerol were
depressed in both groups after the acetoacetate.

Fig. 7.4:   Total blood ketones (β -hydroxy-butyrate +

acetoacetate; μ mol/ml, mean $\pm$ SEM) during

and after oral administration of acetoacetate

(200 ml of 0.4 M) in six chronic alcoholics

(o———o) and six normal control subjects

(□———□).
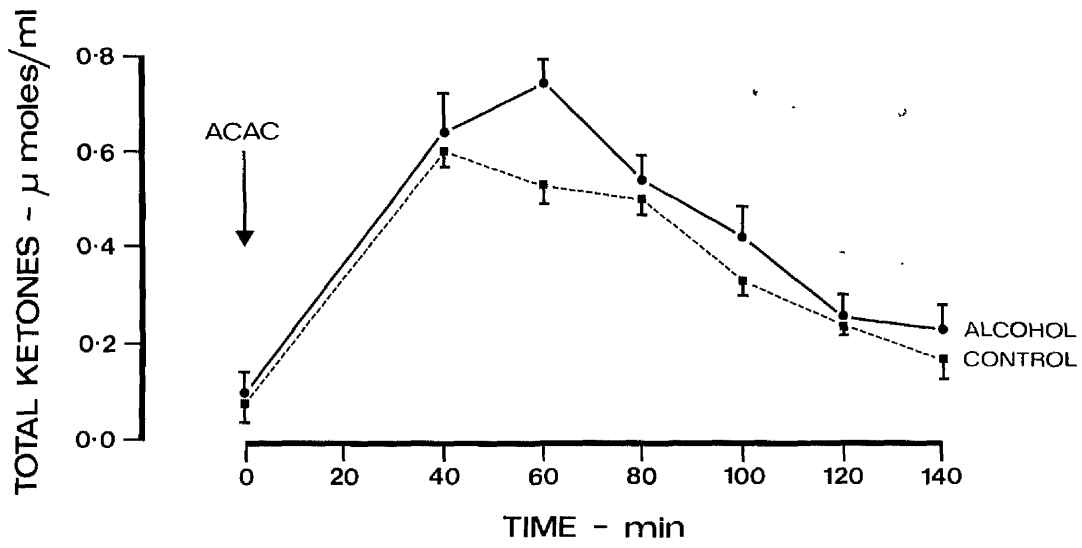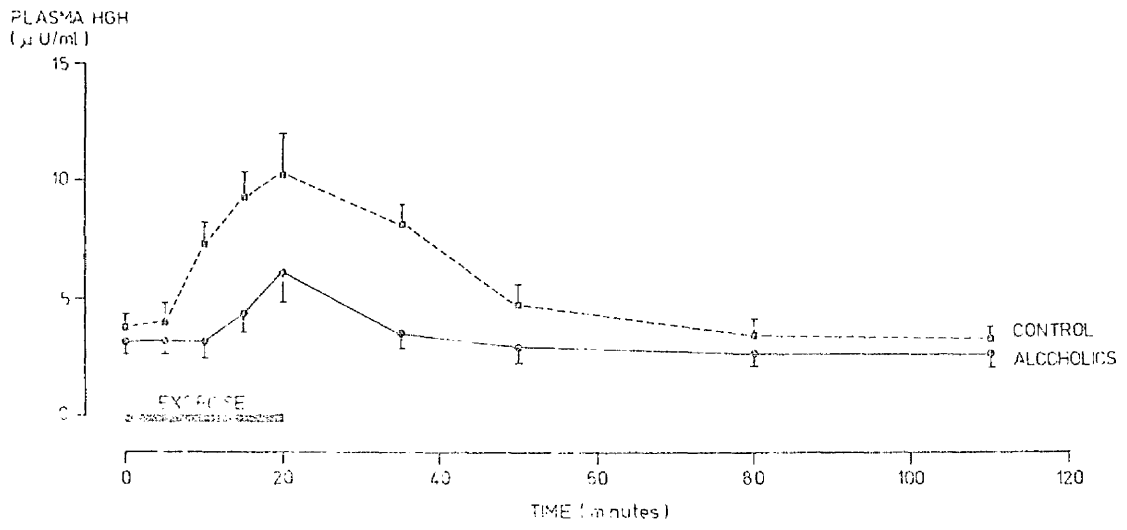
PLASMA HGH
(µ U/ml)

Fig. 7:5   Plasma HGH $(\mu U/ml$, mean $\pm$ SEM) during and after
20 minutes of exercise in five healthy control
subjects (■———■) and six patients with chronic
alcoholism (●———●).

DISCUSSION

The alcoholic subjects performed exactly the same exercise test as the controls and had similar heart rates but produced significantly greater lactate and pyruvate levels and also developed a post exercise ketosis. Johnson et al (1969) have shown that unfit subjects tend to develop higher levels of ketones after exercise than fit subjects. Therefore, in my experiments, it seems reasonable to conclude that the alcoholics were not as fit as the controls. It is possible that this apparent lower fitness was due to decreased oxidative capacity possibly produced as a result of chronic alcohol consumption.

Klinkerfuss (1967) reported that muscle biopsy of alcoholics revealed swollen pale muscle fibres. It has also been shown that chronic alcoholics often show abnormalities of the mitochondria (Lieber and Schmid, 1961). It therefore appears that chronic alcoholism could produce effects sufficient to lower the oxidative capacity and such a mechanism could explain the results described in this chapter.

It is a further possibility that the development of post exercise ketosis in the alcoholics was due to decreased utilisation of ketones. This possibility was tested by performing an acetoacetate tolerance test but the test failed to demonstrate any decreased tolerance to acetoacetate.

It therefore appears that exercise testing of alcoholics revealed some alteration of normal metabolic response to exercise. It is possible that lowered oxidative capacity can be produced by chronic alcohol ingestion but it is not

possible to draw definite conclusions from such a limited study.   The results suggest that a more detailed study of this subject would be of value.

Merry and Marks (1969) observed that plasma cortisol concentrations in patients with chronic alcoholism were paradoxically depressed after alcohol administration. They suggested that chronically self administered alcohol has a depressor effect on hypothalamic function.   This suggestion is also supported by the present findings of lower growth hormone release during exercise in chronic alcoholics.

SUMMARY

1. The metabolic response to exercise was studied in a
    group of six chronic alcoholics and six normal control
    subjects by performing 20 minutes of exercise at 600 k.p.m.
    on a bicycle ergometer. Blood samples were withdrawn
    at intervals during and after exercise.

2. Although both groups of subjects performed the same
    exercise test and had similar heart rates the concentrations
    of lactate, pyruvate, total ketones and blood glycerol
    were greater in the alcoholics than the controls.

3. To test if the increased ketone concentrations of the
    alcoholics was due to decreased utilisation an acetoacetate
    tolerance test (200 ml. 0.4 M acetoacetate, was performed
    but failed to show any differences between the alcoholics
    and the controls.

4. These results suggest that the alcoholics had a lower
    oxidative capacity which may be related to chronic
    consumption of alcohol.

5. The alcoholics had a lower release of growth hormone
    during exercise which may result from a depression of
    hypothalamic function by chronic alcohol consumption.

CHAPTER 8

THE METABOLIC RESPONSE TO EXERCISE IN PATIENTS WITH ISCHAEMIC HEART DISEASE

INTRODUCTION

The value of planned exercise programmes for patients
with ischaemic heart disease has become increasingly
recognised (Lancet, 1972; Redwood, Rosing and Epstein,
1972). In designing such programmes it is of value to
have a further understanding of their physiological and
metabolic rationale (Lange Anderson, Shephard, Denolin,
Varnauskas and Masironi, 1970; American Heart Association,
1972). Observations of the changes in metabolites in blood
as a result of exercise have concentrated upon long term
effects at rest or upon the exercise period. Major
differences have been observed between fit and unfit subjects,
for example, at rest, plasma triglycerides are lower in
athletes (Hurter, Swale, Feyman and Barnett, 1972) and during
exercise lactate and pyruvate concentrations are higher in
untrained subjects (Robinson and Harmon, 1941; Holmgren and
Ström, 1959; Cobb and Johnson, 1963; Juchems and Kumper,
1968; Saltin and Karlsson, 1971). We have observed that
differences also occur in the post-exercise period, thus
post-exercise ketosis is much more marked in unfit subjects
compared with fit individuals (Johnson, Walton, Krebs and
Williamson, 1969; Johnson and Walton, 1972; Jennett,
Johnson and Rennie, 1972).

In order to examine the metabolic changes occurring in
the blood of patients who have ischaemic heart disease we
have, therefore, studied the changes occurring both in
exercise and during recovery. We now report observations
indicating major differences in lactate and pyruvate
concentration in some patients after myocardial infarction
compared with others and with normal controls.

Subjects

Nineteen male patients aged 37-53 years (mean 48 years) were studied. All had been treated in the Coronary Care Unit, Victoria Infirmary, Glasgow, during the preceeding 6-18 months for definite myocardial infarction. Evidence consisted of history, serial ECG changes and/or significant enzyme changes. All had a Peel Index of less than 10 (Peel, Semple, Wang, Lancaster & Dall, 1962), and so were considered to have had infarcts of a mild to moderate severity. No significant complications had occurred in the period since discharge from hospital. Six male subjects were studied similarly as controls. They were aged 38-57 years (mean 44 years) and had no clinical or ECG evidence of heart disease.

All subjects were given a full verbal and written explanation before consenting to the procedure. Recent physical activity at work, recreation and sport was recorded and the appropriate New York Heart Association classification assessed (Schiller & Morris, 1971). No special rehabilitation or physical conditioning measures had been instituted and no medicines other than glyceryl trinitrate had been prescribed.

Procedure

A 12-lead ECG, blood pressure and heart rate were
recorded at rest and then a median antecubital vein was
cannulated. Two physicians, one of whom was a cardiologist,
were present during tests and facilities for cardiac
resuscitation were available. The subjects were fasted
for at least 7 hours prior to the test.

The exercise was performed on a treadmill (Collins
No. P-3900), and the patients were continuously monitored
by a single modified V5 lead (CB5) (Marriott & Fogg, 1970).
The Bruce multistage treadmill test was used (Macdonough &
Bruce, 1969). The only modification was that the patient
sat on a chair, placed temporarily on the treadmill platform
between the periods of exercise for one minute. A blood
sample could be withdrawn, and the single ECG lead and blood
pressure recorded. In the test the subject walked at a
steady rate for up to six three minute periods. At each
stage the speed and elevation were increased in a standard
fashion:

Stage	I	II	III	IV	V	VI
k.p.h.	2.75	4	5.5	6.75	8.0	8.75
Treadmill elevation (%)	10	12	14	16	18	20

The test was terminated in the patients either on
completion of Stage IV or on development of symptoms of angina,
claudication, extreme breathlessness, exhaustion, or on
appearance of cardiac insufficiency manifested by an inapp-
ropriately low systolic blood pressure response during the
exercise. The healthy control subjects carried out the
test until exhausted or intensely breathless. Circulatory
responses (blood pressure, heart rate) and ECG (single lead

CB5) were monitored, 1, 3 and 5 min after stopping the

exercise.   Blood samples (14 ml) were taken, 3, 5, 15,

30 and 45 min after the end of exercise.

ECG records were classified according to the following

grades of ischaemia (Mattingly , 1973):

1.   Early or mild ischaemia (+): Transient straigh-
tening of ST segment with minimal (less than 0.5 mm)
or no segment displacement or alternatively a similar
slight displacement of a previously existing
straightened ST segment.

2.   Moderately advanced ischaemia (++): 0.5-1.0 mm
displacement of a "ischaemic" or "near ischaemic"
type ST segment (slow upstroke of depressed segment)
in one or more leads.

3.   Advanced ischaemia (+++): 1.0-2.0 mm displacement
or an "ischaemic" ST segment.

4.   Far advanced ischaemia (+++): no examples in
this series.

Each blood sample was divided, 4 ml of blood being

deproteinized by addition to 5 ml perchloric acid (10% w/v)

in a pre-weighed tube for the determination of lactate and

pyruvate (Hohorst, Kreutz & Bücher, 1959), Williamson,

Mellanby & Krebs, 1962) and glycerol (Kreutz, 1962).   The

remainder of the blood sample (10 ml) was added to a

heparinised tube for the determination of plasma free fatty

acids (FFA) by a colorimetric method after chloroform

extraction (Itaya & Ui, 1965).   All specimens were stored

on ice prior to centrifugation.

Significance of difference between the groups (see below

for their descriptions) was tested using the Mann-Whitney U

non-parametric test for small samples (Mann & Whitney, 1947).

RESULTS

Blood lactate (figs. 8: 3a & 3b)

Blood lactate increased with increasing exercise stages in all subjects.. The most significant outcome of the study has been the demonstration that a proportion of patients who have recovered from a previous myocardial infarction have an unusual post-exercise metabolic pattern. Thus in some of the patients blood lactate concentrations continued to rise for several minutes after muscular work had ceased, instead of falling progressively from the end of exercise, as expected, and as it did in the control subjects. The patients as a whole showed a wide variation in lactate concentrations immediately after exercise, from those in whom they fell immediately to those in whom there was a continued rise. In an attempt to relate ischaemic ECG changes to changes in blood metabolites, the patients and subjects were divided into three groups as follows:-

Group 1. 5 patients. showing moderate or advanced ischaemic changes in the ECG.

Group 2. 14 patients showing early or mild ischaemic changes in the ECG.

Group 3. 6 control subjects with no clinical or ECG evidence of heart disease.

During the exercise, the blood lactate concentrations of coronary subjects Group 1 and 2 were significantly greater ($P < 0.05$ at Stage III) than the corresponding values for the control group. After exercise, the blood lactate concentrations of the control subjects fell rapidly and returned to normal resting concentrations within 30 min..

Fig. 8 - 3a & 3b   Blood lactate (μmol/ml, mean $\pm$ SEM)

(a) and as a percentage of the final

exercise value (b) (mean $\pm$ SEM) in

Group 1 (5 coronary patients) and

Group 2 (14 coronary patients) and

also in six control subjects (O-----O).
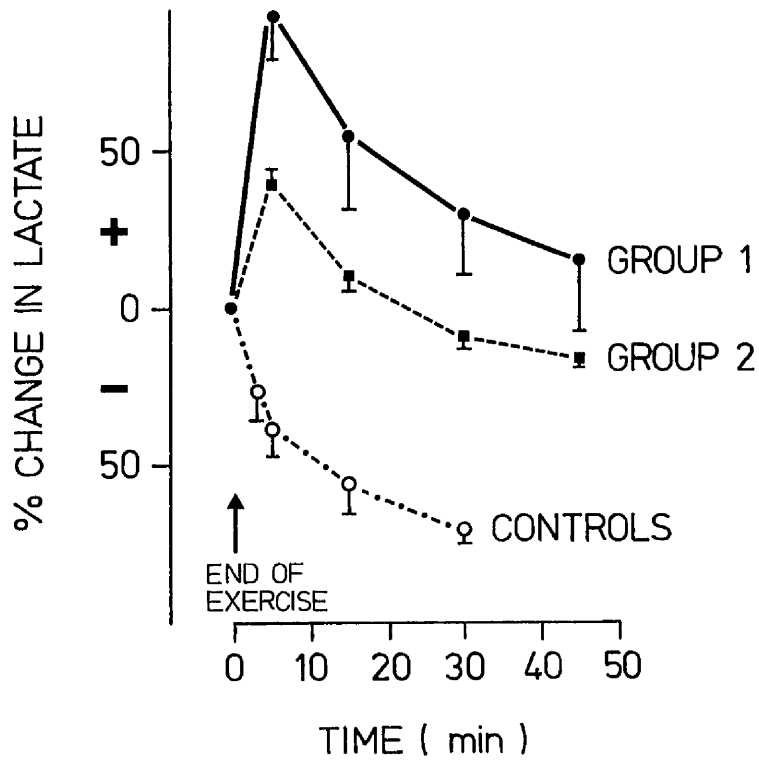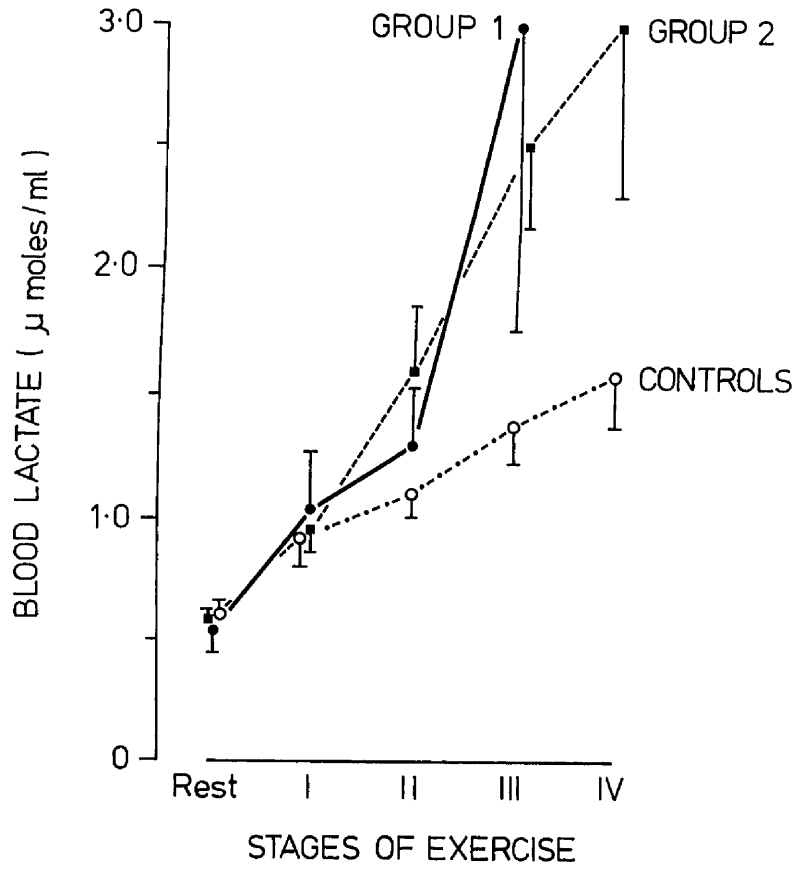
In Group 1 of the coronary subjects, lactates continued to rise after exercise, the maximum observed concentrations being at 5 min.. This was 100% greater than the lactate concentration at the end of exercise and was significantly greater ($P < 0.001$) than the corresponding control value. In Group 2 there was also a rise in lactate concentration 5 min. after exercise and the concentrations were significantly greater ($P < 0.01$) than the corresponding control values. In the Control subjects the blood lactate concentrations fell immediatly after exercise.

Heart rate (fig. 8:1)

There was some variation in the resting heart rates and although Groups 1 and 2 were not significantly different from each other they were significantly greater than the values of the controls at rest. Heart rates increased with increasing exercise stages in all subjects. At Stage II, the mean heart rates of Groups 1 and 2 coronary subjects were significantly higher than the corresponding mean heart rates of the controls ($P < 0.02$ and $P < 0.01$ respectively). It therefore appears that Group 1 subjects did not significantly increase their heart rates after Stage II.

Blood pressure (fig. 8:2)

There was no significant difference between the resting blood pressures of the coronary subjects and of the controls. During exercise, the blood pressures of Group 1 rose very little and were significantly lower than those of Group 2 at Stages I and II ($P < 0.05$ and $P < 0.01$) respectively. At Stage III the mean blood pressure of Group 1 was lower than the corresponding control value but the difference was not significant. At no stage were the blood pressures of Group 2 significantly different from the control values.

Fig. 8 - 1   Heart rate (beats/min, mean $\pm$ SEM) of Group

1 (5 coronary patients) and Group 2 (14

coronary patients) and also in six control

subjects (O--—·O) at rest and at the end of

each stage of exercise.
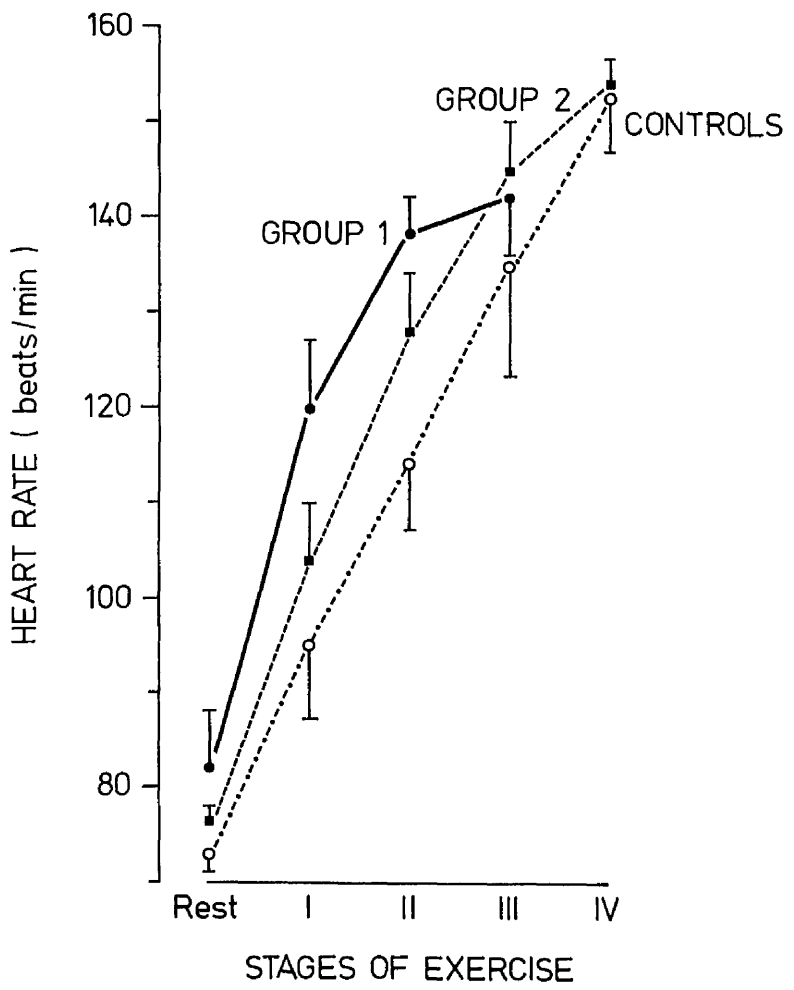
Fig. 8 - 2　Systolic blood pressure (mm Hg, mean $\pm$ SEM) of Group 1 (5 coronary patients) and Group 2 (14 coronary patients) and also in six control subjects (O——·O) at rest and at the end of each stage of exercise.
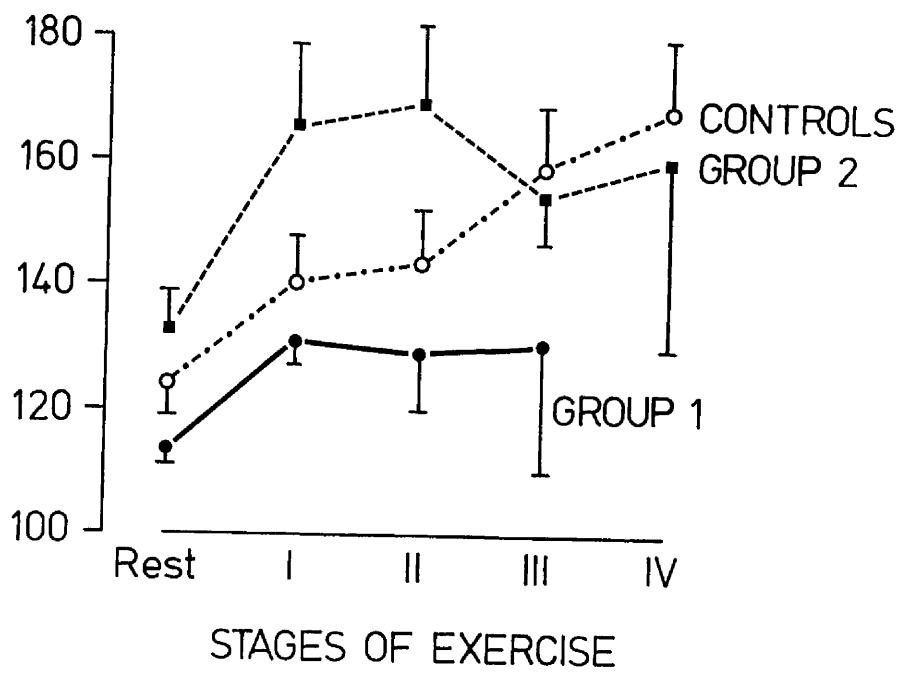
Blood pyruvate (figs. 8:3 & 8:4)

Blood pyruvate increased with increasing exercise stages in all subjects. After exercise, the blood pyruvate of control subjects fell and returned towards resting concentration. In Group 1 of the coronary subjects, pyruvate continued to rise after exercise and 5 min. later it was observed to be 100% greater than the value at the end of exercise, and the concentrations were significantly greater (P< 0.01) than the corresponding values for the controls. 30 min. after exercise, the pyruvate concentration of Group 1 coronary subjects was still 50% greater than that at the end of exercise. In Group 2 coronary subjects, blood pyruvate concentrations were also increased 5 min. after the end of exercise.

Lactate/pyruvate ratio (fig. 8:5)

The lactate/pyruvate ratio of the control subjects recovered rapidly after exercise. The ratios in the two groups of coronary subjects were all significantly greater (P< 0.05) than the corresponding control samples. In Group 1, the ratio in the initial post-exercise period was greater than the corresponding samples for Group 2 (5 and 15 min.) but the difference was not significant.

Blood glycerol (Fig. 8:6a)

Blood glycerol levels continued to rise for a few minutes after exercise in all subjects. There was no significant difference at any time between any of the groups of coronary subjects and the corresponding values for the controls.

Fig. 8 - 4   Blood pyruvate as a percentage of the final

exercise value (mean $\pm$ SEM) in Group 1

(5 coronary patients) and Group 2 (14

coronary patients) and also in six control
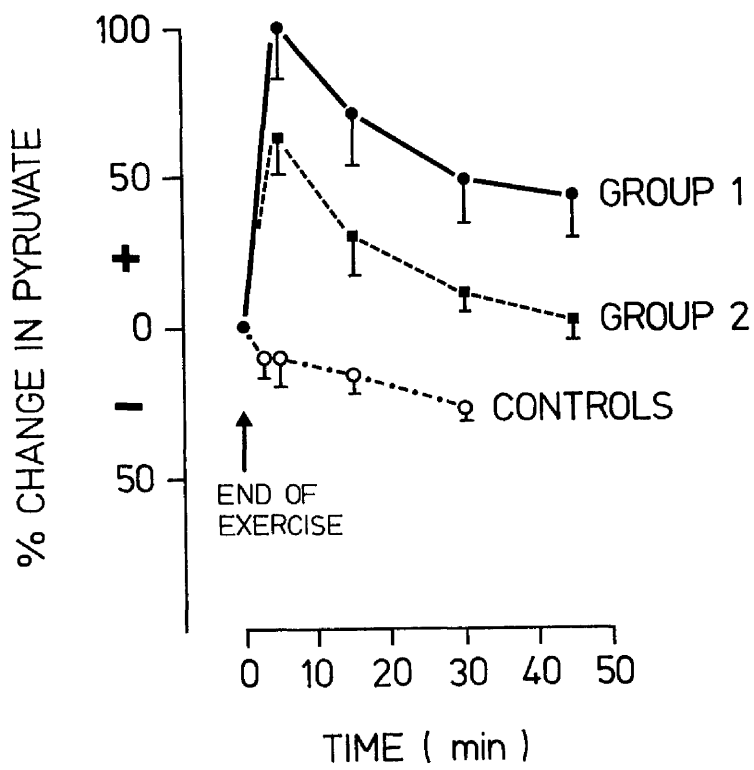
subjects (O—·—·—O).

Fig. 8-5 Lactate/pyruvate ratio (mean $\pm$ SEM) in

Group 1 (5 coronary patients) and

Group 2 (14 coronary patients) and also

in six control subjects (O—·—·—·O) after

exercise.
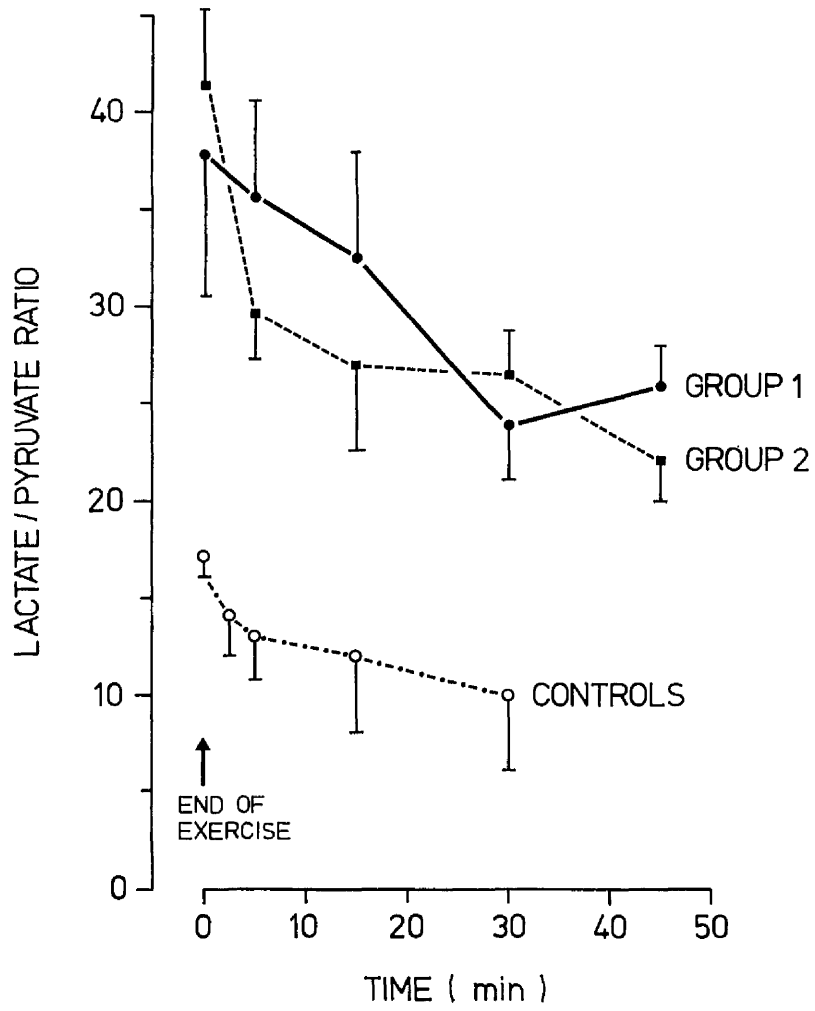
Plasma FFA (fig. 8:6b)

After exercise, plasma FFA rose in all subjects. There was, however, no significant difference throughout the investigation between the FFA concentrations of coronary subjects and the corresponding control values.

Fig. 8 – 6a and 6b   Concentrations of blood glycerol (a)

and plasma free fatty acids (b) as a

percentage of the final exercise value

(mean $\pm$ SEM) in Group 1 (5 coronary

patients) and Group 2 (14 coronary

patients) and also in six control

subjects (o– – – –o).
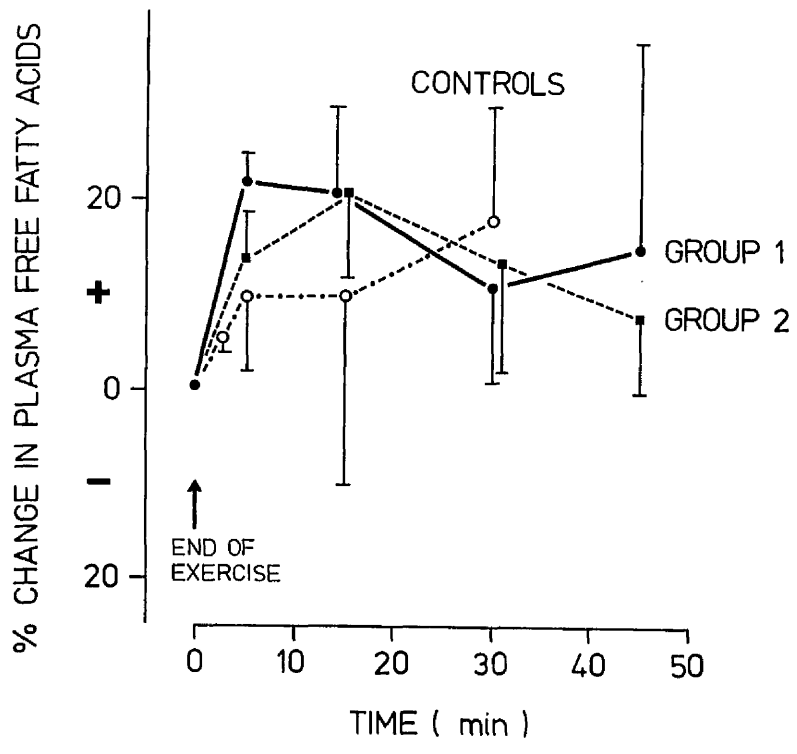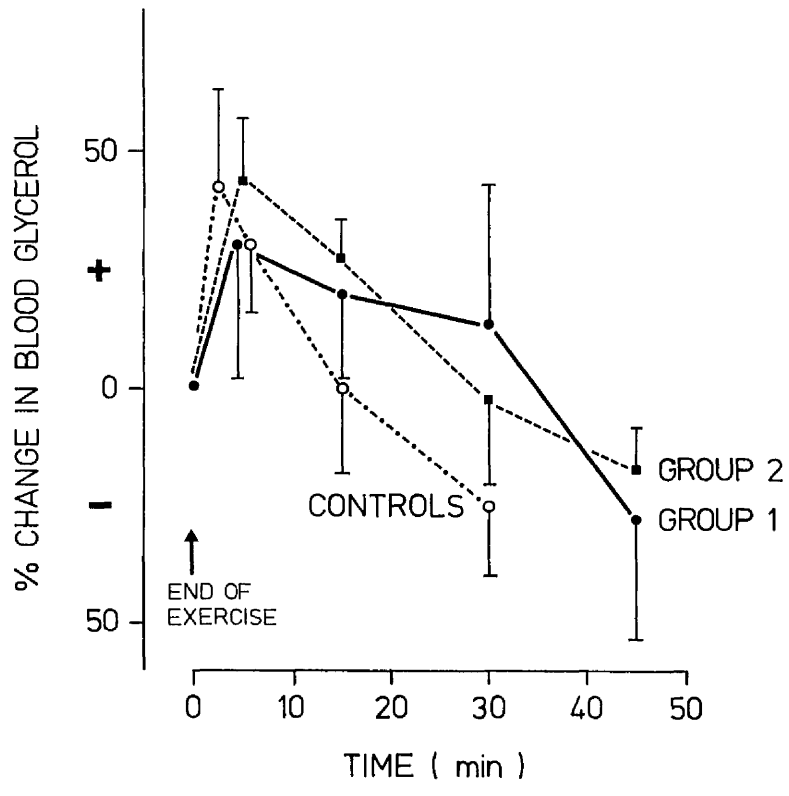
DISCUSSION

Our results would suggest that cardiac insufficiency
in ischaemic heart disease may be manifested by characteristic
metabolic as well as haemodynamic and ischaemic ECG responses
to exertion.   Patients in Group 1, selected from the whole
study group because of moderate or advanced ischaemia changes in
ECG were found to be considerably more physically disabled
than the rest as judged by changes in heart rate, systolic
blood pressure responses and ECG changes and had a pronounced
rise in blood lactate for at least $\frac{1}{2}$ hour after stopping
exercise.   The failure of Group 1 patients to increase
their heart rate with increasing work load beyond Stage II
may signify cardiac insufficiency.   Similarly, failure in
Group 1 of the systolic blood pressure to rise with
increasing level of exercise and its actual lowering in some
subjects when Stage III was reached, may signify poor force
of contractability of diseased left ventricular muscle.
Neither the resting or exercise ECGs were as clear cut, nor
was the New York Heart Association classification of limit-
ation of recent activity.   The control subjects showed the
greatest increases in heart rate in response to the exercise
test, implying that they exercised relatively harder than
the other subjects and were working nearer to their maximal
capacity (Åstrand, Cuddy, Saltin & Stenberg, 1964).

The greatest elevation of blood lactate during exercise
was observed in patients in Group 1.   This may be because
the proportion of maximal work they performed was relatively
higher than in the other subjects and levels of lactate are

known to be related to the degree of work intensity in
individual subjects (Wassermann, van Kessel & Burton,
1967). A further possibility is that the lower blood
lactates during exercise in the controls and Group 2
implies greater physical fitness in these subjects.
Subjects in good athletic training have lower concentrations
of lactate and pyruvate during exercise compared with
untrained subjects (Robinson & Harmon, 1941; Holmgren &
Ström, 1959; Cobb & Johnson, 1963; Juchems & Kumper, 1968;
Saltin & Karlsson, 1971). This biochemical concommitant
of athletic fitness might be secondary to the greater blood
flow (and hence supply of oxygen) found in the contracting
muscles of fitter individuals compared with sedentary or
poorly trained individuals (Elsner & Carlson, 1962). A
further explanation for the lower lactates in athletically
fit individuals is that there is a relative increase in
mitochondrial respiratory enzymes as a result of athletic
training (Holloszy, 1967) for blood lactate concentrations
rise during exercise because of anaerobic metabolism within
muscle cells, cytoplasmic glycolysis exceeding mitochondrial
oxidative capacity (Keul, Doll & Keppler, 1967). Such
observations refer to physical training and we do not know
if the minor differences in fitness in our patients with
ischaemic heart disease would produce changes in metabolites
of the extent we observed during this investigation.
Another possible explanation of the higher lactates during
exercise in Group 1 compared with the controls is that there
may have been a poorer arterial perfusion of the working
muscles of the Group 1 patients, related to their less marked

blood pressure response to the exercise.

After completion of the exercise in this group of patients there was a remarkable increase (average,+ 100%) in blood lactate and pyruvate concentrations, a phenomenon which we have not observed in other subjects. Another investigation of patients with coronary heart disease carrying out recreational activities (Sharrock & Nye, 1971) has shown that after playing table tennis they had a lactate concentration three minutes later which was above the level found just at the end of the exercise. The elevation was, however, only 6% and was an isolated observation as no other post-exercise observations were made. A rise in blood lactate after exercise can occur in normal subjects but only if the exercise is very severe: thus if the work is exhaustive an increase of up to 30% only has been observed and ascribed to continued diffusion of lactate into the circulation from skeletal muscle (de Coster, Denolin, Messin, Degre & Vondermoten, 1969) as the concentration of lactate is higher in working muscle than in venous blood (Sachs & Sachs, 1937).

There was no difference in the rate of disappearance after exercise of glycerol from the blood but plasma FFA were depressed in Group 1 compared with Group 2. The depression in Group 1 may have been related to their very much higher lactate concentrations as glucose and lactate may reduce FFA values towards normal (Gupta, Young, Jewitt, Hartog & Opie, 1969).

The continued rise in concentrations of lactate and pyruvate we have observed immediately after exercise in some of the patients could have been due to either a higher rate of production of lactate or to a lower rate of utilization. Increased production could have resulted from anaerobic activity either in skeletal muscle, the myocardium or the liver. Heart muscle normally metabolises lactate as a fuel (Keul & Doll, 1968) but lactate is released by the heart into the circulation in experimental myocardial ischaemia (Fisher, Heimbach, Ledingham, Marshall & Parratt, 1973). The greater lactate/pyruvate ratio in Group 1 after exercise also suggests that their higher blood lactate levels were related to increased anaerobic metabolism compared with the other groups (Huckabee, 1958). Our observations do not however indicate the source of the lactate.

Physical fitness of patients who have had an episode of myocardial infarction is frequently assessed by means of a graded exercise test such as the one we have carried out in this study. Our observations imply that, in addition to heart rate, blood pressure and ECG monitoring, objective evidence of the effect of the exercise may be obtained from metabolic observations, particularly during the post—exercise period. It is also possible that such biochemical studies may provide additional evidence of the value of physical conditioning programmes for patients with coronary heart disease.

SUMMARY

Nineteen patients who had recovered from acute myocardial infarction were studied in order to investigate the relationship between the severity of myocardial insufficiency developing during exercise and changes in metabolites in the blood during and after the exercise. They were compared with controls by means of a standard exercise test.

In 5 patients with moderate or advanced ischaemic ECG changes, the blood lactate and pyruvate concentrations continued to rise, up to a further 100%, after they stopped exercising. The other patients, including some who developed symptoms suggestive of myocardial ischaemia, and all the controls, failed to show this marked increase in blood concentrations after the exercise.

We conclude that the development of myocardial insufficiency during exercise is associated with major changes in metabolites in the peripheral blood after the exercise has ceased. Study of metabolites could be of practical value in the evaluation of the severity of myocardial disease and of the response to rehabilitation.

CHAPTER 9

THE LEVELS OF HVA AND 5-HIAA IN THE C.S.F. OF
PATIENTS WITH PARKINSON'S DISEASE TREATED WITH PIRIBEDIL

INTRODUCTION

Since brain biopsy is unethical unless performed incidentally to cerebral surgery, the nearest approach to the study of brain metabolism is by examining the c.s.f.. The measurement of amine metabolites in c.s.f. provides a useful method of assessing the turnover of biogenic brain amines.

Concentrations of dopamine and serotonin in the brain of patients with Parkinson's disease have been reported to be lower than in a control group (Hornykiewicz, 1962) and this has led to the suggestion that the metabolism of dopamine is important in this disease. Homovanillic acid (HVA) the main metabolite of dopamine and 5-hydroxy-3-indoleacetic acid (5-HIAA) the main metabolite of serotonin have also been found to be reduced in the c.s.f. of patients with Parkinson's disease (Guldberg et al 1967; Guldberg et al, 1969). Patients with Parkinson's disease have been treated with L-3-4-dihydroxyphenylalanine (levodopa) a dopamine precussor, and improvement has been observed in the akinetic symptoms of many patients. (Birkmayer and Hornykiewicz, 1961; Calne et al, 1969). The metabolites HVA and HIAA have been measured in the c.s.f. of patients undergoing levodopa therapy and it has been reported that levodopa gives rise to increased concentrations of HVA in the c.s.f. (Pullar et al, 1970). However, not all patients respond to levodopa therapy and also the dosage of levodopa required is often associated with side effects e.g. nausea.

Levodopa is now established as the drug of first choice in the treatment of Parkinsonism and is thought to work by increasing the concentration of dopamine in the corpus striatum and globus pallidus (Hornykiewicz, 1970).

Dopamine-receptor stimulators might be expected to have a similar effect upon dopamine receptors. The first dopamine-receptor stimulator to be tried in Parkinsonism was apomophine, given by frequent subcutaneous injection. Apomophine improved the main symptoms of severe Parkinsonism, but was of no clinical value because of its severe and unpleasant side-effects (Schwab et al 1951, Cotzias et al, 1970). A second drug, pyrimidyl-piperonyl-piperazine or piribedil, was investigated experimentally by Corrodi et al (1971), who suggested that it might be more useful than apomophine because of its longer action. It is principally a dopamine-receptor stimulator, though some potentiation of dopamine release may occur (Fuxe, 1973).

Early clinical experience with levodopa suggested that it was ineffective, an erroneous conclusion resulting from the fact that too small a dose had been used. As dopamine agonists are known to increase the turnover of dopamine, and hence to reduce the concentration of its metabolites in the cerebrospinal fluid (c.s.f.) (Corrodi, et al 1971), the c.s.f. of 9 patients with Parkinsonism had been examined for Homovanillic acid (HVA), and also 5-Hydroxyindoleacetic acid (5-HIAA), before and during treatment with piribedil in order to confirm that the drug was reaching the brain in suitable concentrations. Despite

a definite pharmacological action, careful investigation

of the clinical and physiological effects of the drug showed

no convincing therapeutic action.   These effects are

compared with the action of levodopa.

SUBJECTS

The patients were males (aged 47-71 years). Three
had post-encephalitic Parkinsonism and the remainder had
idiopathic Parkinson's disease of at least two years duration.
They had no evidence of cerebrovascular disease and their
symptoms were bilateral. Four of the patients had not
previously received levodopa. Three had been unable to
tolerate a therapeutic dose of levodopa because of nausea.
The remaining two patients had derived benefit from levodopa
and one of these took levodopa concurrently with piribedil;
five of the patients who were already taking anticholinergic
drugs continued on the same treatment in addition to piribedil.
The nature of the study was explained to each patient
beforehand and their permission obtained for the investigat-
ions.

METHODS

All patients were admitted to hospital for the duration
of the trial so that they were under continual clinical
observation. Each was assessed in detail both before
treatment and three to six weeks later, at least four days
after achieving their maximum dose of Piribedil (Trivastal,
Servier). The drug was given orally, starting with a dose
of 20 mg b.d. and increasing every 2 - 4 days in 40 mg steps
to a maximum of 200 mg per day. If side effects appeared,
the dose was reduced to the previous level for a further
four days after which an increase was again attempted.
Three patients tolerated 200 mg per day, three 160 mg per
day and three 120 mg per day. All side effects were recorded.

Assessments of rigidity, tremor and bradykinesia were made during the trial and the results are reported in full elsewhere.   (McLellan, Chalmers and Johnson, 1974).

Analysis of cerebrospinal fluid

10 ml samples of cerebrospinal fluid (c.s.f.) were obtained by lumbar puncture before and after treatment and stored at -20°C.   Homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were determined by the methods of Ashcroft, Crawford, Dow and Guldberg (1968) with the modifications of Pullar, Weddell, Ahmed and Gillingham (1970).   Control measurements were made on c.s.f. obtained from patients not suffering from cerbral disease.

RESULTS

Cerebrospinal fluid

There was a significant correlation $(P < 0.05)$ between the concentration of HVA and 5-HIAA in the c.s.f. of the patients with Parkinson's disease. There was no significant correlation between these metabolites in the control subjects (Fig. 9:1)

There was no significant difference between the HVA and 5-HIAA levels of the trial patients and controls (Fig .9:2) There was a significant decrease $(P < 0.005)$ in the HVA levels of trial patients before and after piribedil. There was also a significant decrease $(P < 0.001)$ in the 5-HIAA levels. These changes suggest decreased breakdown of cerbral dopamine and serotinin as a result of piribedil.

Fig. 9.1 : Correlation between concentrations (ng/ml c.s.f.)

. of HVA and 5-HIAA in lumbar c.s.f. in eight

patients with Parkinson's disease and eleven

normal control subjects.
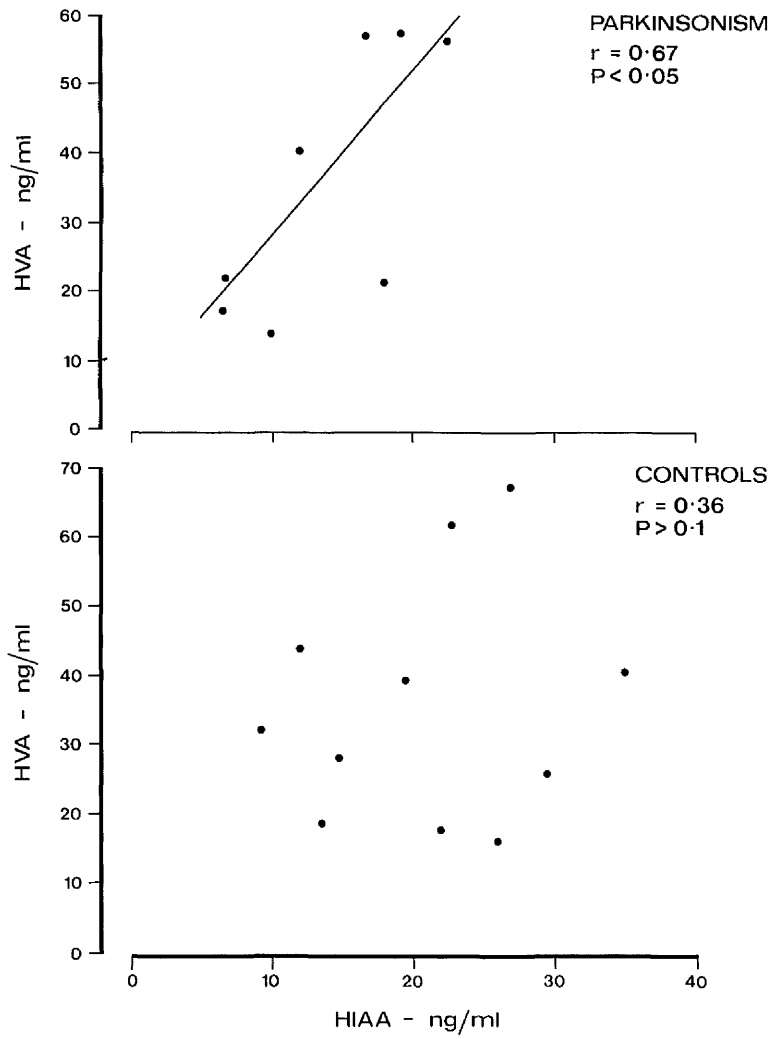
Fig. 9:2 HVA and 5-HIAA concentrations (ng/ml, mean $\pm$

SEM) in nine patients with Parkinsons desease

before and after treatment with Trivastal and

in eleven normal control subjects.

(a)

HVA

p < 0·005

CONTROLS

TRIVASTAL

BEFORE AFTER

(b)

5-HIAA
ng/ml

p < 0·001

CONTROLS

TRIVASTAL

BEFORE AFTER
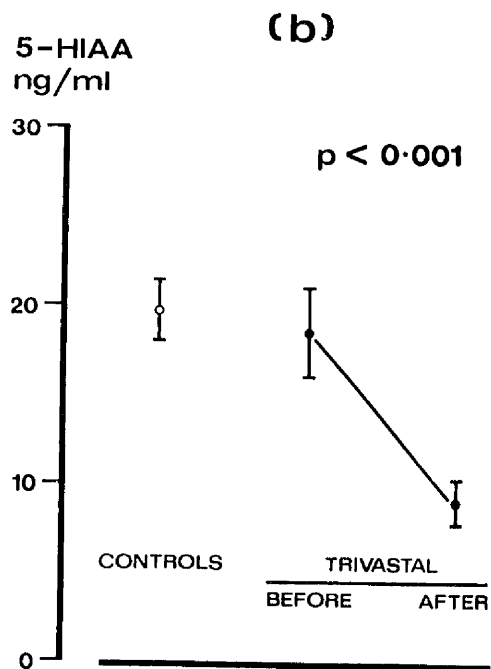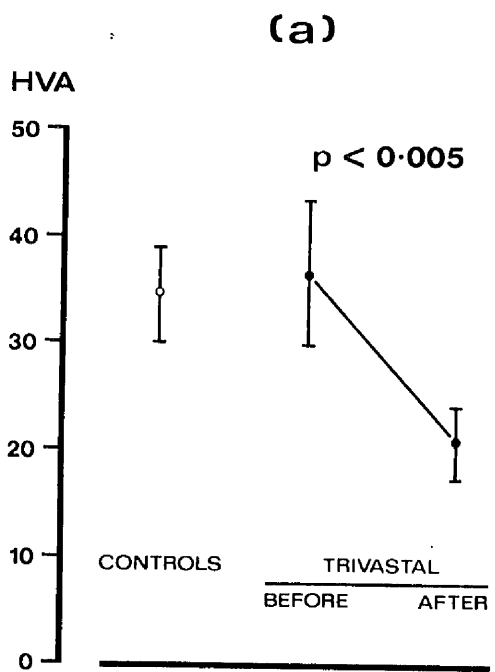
DISCUSSION

      Rigidity did not improve clinically during treatment
with piribedil and there was no change in the torques
induced by muscle vibration. This contrasts with the
effects of levodopa upon patients with rigidity (McLellan,
1973). The improvement in tremor in three patients was
not associated with shortening of the muscle silent period,
again contrasting with the response in patients whose tremor
improves with levodopa (McLellan, 1972). It is possible
that tremor may improve in some patients but these results
need to be interpreted with caution because resting tremor
is characteristically intermittent and of all the abnormal-
ities of Parkinsonism, tremor is known to respond best to
placebo. The improvement observed in the patients' speed of
walking was not accompanied by improvement in their manual
dexterity.

      The concentrations of HVA and HIAA in the c.s.f. of
the patients studied were not lower than control levels.
Untreated patients with Parkinsonism tend to have lower
than normal levels of HVA and HIAA in c.s.f. but normal
levels often occur. (Guldberg et al, 1969). Patients
with Parkinsonism whose c.s.f. HVA levels are normal
appear to respond poorly to levodopa (Godwin-Austen,
Kantamaneni and Curzon, 1971) so that by this criterion
the patients studied were a therapeutically unpromising
group.

      The correlation between HVA and HIAA concentrations
has been previously observed, (Guldberg,1967) in the
ventricular c.s.f. and Pullar et al (1970) in lumbar c.s.f.
and is not fully understood but may reflect the progressive

nature of the disease, the brain levels of dopamine
and 5 LT being reduced in step.

Studies upon rats have shown the piribedil stimulates
dopamine receptors (Corridi, Fuxe, and Ungerstedt, 1971).
The fall observied in c.s.f. HVA levels after treatment
with piribedil suggests a decreased turnover of dopamine
similar to that found in animals and this concept is
further supported by the development of chorea in the patient
whose HVA showed the greatest fall.   The observations on
c.s.f. suggest that the drug has a definite neuropharmacological
action but the clinical observations on this small number
of patients failed to demonstrate an associated clinical
benefit.

The contrast between the effectiveness of levodopa
and the ineffectiveness of piribedil is surprising.   In
a review of the action of piribedil Fuxe (1974) pointed
out that a severe fall-out of dopaminergic nerve terminals
could block the effect of piribedil;   further, concurrent
stimulation of serotonin receptors (which has not been
described in animals treated with piribedil) may interfere
with the effects of dopamine receptor stimulation.
Piribedil, in combination with levodopa or with anticholin-
ergic drugs, is ineffective, and the possibility remains
that activation of other receptors, in addition to dopamine
receptors, contributes to the therapeutic action of levodopa.

SUMMARY

1. Nine patients with Parkinsonism have been studied
    before and after treatment with Piribedil for three
    to six weeks.

2. Piribedil appeared to have a slight anti-Parkinsonism
    effect upon bradykinesia and possibly upon tremor
    but did not improve rigidity.   The chief toxic effects
    were drowsiness and confusion and two patients
    experienced nausea.

3. Changes in homovanillic acid in the cerebrospinal
    fluid suggest that the drug reduces the turnover of
    endogenous dopamine.   In spite of this definite
    neuropharmacological action, no clear-cut
    associated clinical benefit was demonstrated.

CHAPTER 10

THE LEVELS OF HVA AND 5-HIAA IN THE C.S.F. OF PATIENTS WITH HUNTINGTON'S CHOREA TREATED WITH TETRABENAZINE AND THIPROPAZATE

INTRODUCTION

Chorea is present in a wide range of disorders (Bruyn, 1973) but the commonest causes in current neurological practice are probably levopa therapy, cerebrovascular disease and Huntington's chorea. Levodopa induced chorea disappears when the dose of levodopa is reduced, or when tetrabenazine is added (Godwin-Austen, 1971). The other forms of chorea are said to respond to thiopropazate(Bruyn, 1962; Lyon, 1962; Brain, 1969; Simpson, 1971). A pilot study at the Institute of Neurological Sciences, Glasgow, has confirmed previous reports of the effectiveness of tetrabenazine, especially in Huntington's chorea and in chorea associated with cerebrovascular disease (McLellan, 1972). Since no controlled comparative trails of the various remedies have been reported, the drug of first choice has not been identified. A double-blind cross-over trial of thiopropazate (Dartalan) and tetrabenazine (Nitoman) which were the drugs most commonly used at this institute for the treatment of chorea has therefore been performed. Both drugs are thought to supress chorea by their action upon dopaminergic synapses in the basal ganglia. Thiopropazate is a phenothiazine. Phenothiazines block dopaminergic synapses, causing compensatory overactivity of dopaminergic neurons and increased concentrations of the dopamine metabolite homovanillic acid in the cerebrospinal fluid (c.s.f.) (Hornykiewicz, 1966).

Tetrabenazine, like reserpine, reduces the concentration of dopamine in the striatum, substantia nigra and pallidum. It was decided to study changes in c.s.f. as well as clinical effects to elucidate further the metabolic action of tetrabenazine and thiopropazate and to see whether observations on c.s.f. could be used to predict the effects of therapy. The results of this study are given and discussed in this chapter.

METHODS

Subjects

Ten patients were studies. The nature and purpose of the investigations were explained to the patients and their next of kin, and their consent was obtained. There were six females and three males with Huntington's chorea; two of the females were identical twins aged 59 years. The mean age of the patients with Huntington's chorea was 51 (range 36 - 60 years). The tenth patient was a man (aged 22 years) with chorea and athetosis as a result of cerebral palsy. All the patients were admitted to hospital for the trial.

Trial design

The maximum recommended daily dose of tetrabenazine is 200 mg (8 x 25 mg tablets) and of thiopropazate is 30 mg (6 x 5 mg tablets). Tetrabenazine tablets are yellow and compressed while thiopropazate tablets are white and coated. In order to test the formulation that is actually available for clinical use, a 'double dummy' technique was used. Each patient was admitted for 6 consecutive weeks, and this period was divided into three phases of two weeks each. During each phase, the patient took tablets gradually increasing the dose until the maximum dose was reached.

Procedure

A full clinical examination was performed and tests of manual dexterity were made before starting phase 1 and at the end of each phase of the trial. On admission

to hospital and at the end of each phase, a film was made of the patient in standardised postures and performing standard tasks to assess the degree of chorea.

At the end of each phase, c.s.f. (10 ml) was obtained by lumbar puncture and stored at -20° C. Homovanillic acid (H.V.A.) and 5-hydroxy-3-indolacetic acid (5-H.I.A.A.) were determined by the methods of Ashcroft, Crawford, Dow and Guldberg (1968); with the modifications of Pullar et al (1970).

RESULTS Table 10:1

The results of clinical assessment, cinematography and test of manual dexterity are reported in full elsewhere. (McLellan, Chalmers and Johnson, 1974).

H.V.A. concentrations were at the lower limit of control values and 5-HIAA concentrations were less than control values in the patients on placebo and did not alter significantly after thiopropazate, but there was a highly significant increase in H.V.A. during treatment with tetrabenazine ($P < 0.001$). Analysis of the c.s.f. findings in individual patients did not show a clear correlation with the clinical effects of treatment. The five patients who responded best to tetrabenazine had mean values similar to the group as a whole, at all stages of the trial. The c.s.f. could not, therefore, be used to predict the response to treatment, nor to monitor its effects. The patient in whom severe Parkinsonism developed had H.V.A. and 5-HIAA concentrations within the range for controls while on placebo. The identical twins had similar levels of H.V.A. and HIAA with placebo and also during treatment with tetrabenazine.

Table 10:1 : Concentrations (mean ± SEM) of HVA and 5-HIAA in

CSF of controls and patients with Huntington's Chorea*

| — | Controls | Huntington's chorea | | |
		Placebo	Thiopropazate	Tetrabenazine
HVA (ng./ml.)	34.0 (± 4.3)	28.5 (± 4.5)	26.5 (± 2.5) (N.S.)	54.5 (± 10.5) (P < 0.001)
5-HIAA (ng./ml.)	19.5 (± 2.2)	14.5 (± 2.0)	14.2 (± 1.2) (N.S.)	17.3 (± 2.8) (N.S.)

* The patient in whom severe parkinsonism developed was excluded.

N.S. = not significant.

DISCUSSION

Thiopropazate is recommended for the treatment of
chorea in current standard reference works (Brain, 1969;
Simpson, 1971) but the present observations suggest that
tetrabenazine is now the drug of first choice, at least for
Huntington's chorea.   Previous non-comparative studies have
shown that tetrabenazine also suppresses facial dyskinesia
(Pakkenberg, 1968) and choreiform and ballistic involuntary
movements of varied aetiology (McLellan, 1972;   Dalby, 1969;
Swash et al, 1972).   Drug induced dyskinesias are said
to respond both to thiopropazate (Carruthers, 1971;
Singer and Cheng, 1971) and to tetrabenazine (Brandrup,
1961;   Godwin-Austen, 1971) but no trial has been reported
comparing these drugs.

The biochemical background of Huntington's chorea
has been reviewed by Barbeau (1973), who provides evidence
for the role of dopamine in the pathophysiology of chorea.
Nevertheless, the concentration of dopamine and not
adrenaline in the lasal ganglia of patients with Huntington's
chorea is usually normal, and so is the urinary excretion
of H.V.A. and 5-H.I.A.A. (Bruyn, 1962).   In untreated
Huntington's chorea the c.s.f., H.V.A. concentration has
been reported variously as below normal (Curzon et al, 1972;
Chase, 1972), at the lower limit of normal (Bernheimer
et al, 1966;   Birkmayer, 1966;   Aquilonious and Sjostrom, 1971)
and as normal (Klawans, 1970).   Concentrations of 5-H.I.A.A.
in c.s.f. have been reported within a normal range (Curzon
et al, 1972;   Chase, 1973).   In the results reported in
this chapter 5-H.I.A.A. was below control values, a finding
also previously observed (Mattsson and Persson, 1973).

Possibly, the reduced levels simply reflect neuronal loss in the striatum. The rise in catecholamine metabolite levels in c.s.f. with tetrabenazine accords with the drug's known action as a depletor of neuronal stores of catecholamines (Hornykiewicz, 1966). Our observations of raised H.V.A. with tetrabenazine implies that there is increased metabolism and hence breakdown of dopamine. However, there appears to be no simple relation between the clinical effectiveness of tetrabenazine and its effects upon dopamine or serotonin turnover.

Using the doses recommended for clinical treatment, tetrabenazine appears to be better than thiopropazate in suppressing chorea in patients with Huntington's chorea. Suppression of chorea improved manual dexterity. The dose could be adjusted to provide satisfactory suppression without excessive side-effects, although side-effects sometimes occurred for the first time after several weeks of treatment. These observations indicate that tetrabenazine is the drug of first choice for the suppression of chorea in patients with Huntington's chorea.

SUMMARY

1. Nine patients with Huntington's chorea and one with
    chorea were admitted to a double-blind cross-over
    trial of tetrabenazine, thiopropazate and placebo.

2. Thiopropazate $(P < 0.01)$ and tetrabenazine $(P < 0.001)$
    both significantly controlled chorea.

3. The administration of tetrabenazine was accompanied
    by a pronounced rise $(P < 0.001)$ in the concentration
    of H.V.A. in c.s.f., suggesting increased cerebral
    dopamine metabolism.

4. The effect of thiopropazate was much smaller and
    was not statistically significant.

5. It is suggested that tetrabenazine is the drug of
    first choice for the suppression of chorea in
    patients with Huntington's chorea.

SUGGESTIONS FOR FURTHER RESEARCH

The most significant result found in the study of the effect of alcohol on the metabolic response to exercise was the apparent increased mobilisation of fat during exercise with alcohol.   It is possible that alcohol led to greater catecholamine release in response to exercise and it would be of considerable interest to study the effects of alcohol on catecholamine release at rest and during exercise in a group of athletes and non-athletes.

The fall in blood glucose observed during exercise with alcohol in a group of athletic subjects is also of considerable interest.   It would be of value to study this effect further and to consider the importance of the type of exercise performed and its duration, the amount of alcohol taken and the effect of a high carbohydrate diet designed to increase glycogen levels. In addition it would be of interest to consider changes in Insulin the main hormone regulating blood glucose levels.   The ability of alcohol to increase the release of human growth hormone suggests that further studies of the effects of alcohol may be of value in determining the factors that control growth hormone release.

The investigations into the effects of alcohol on glucose tolerance failed to demonstrate any marked glucose intolerance. It was possible, however, to demonstrate that alcohol delayed the insulin response to glucose and it may be that this delay is related to catecholamine release or an other effect of alcohol and it would therefore be of interest to investigate this effect further.   The investigations of the effects of fructose on alcohol metabolism were unsatisfactory in that it was not possible to measure blood alcohol in these investigation.   It would

therefore be of interest to investigate if the increased blood glycerol concentrations are associated with an increased rate of alcohol breakdown.

The exercise test is of considerable value in the study of the metabolism of subjects with a variety of diseases. The results of the study of patients with chronic alcoholism are interesting although it is not possible with such a limited study to draw any definite conclusions about the alcoholics. Although the results could be explained in terms of reduced fitness of the alcoholics further investigation is required.

It is not clear what factors are responsible for the increased blood lactate and pyruvate concentrations produced after exercise in coronary subjects and this effect requires further study. In addition, it would be of considerable value to study a group of coronary subjects before and after a period of training to assess the effects of the training on the response to exercise and in particular on the occurrence of increased lactate and pyruvate levels after exercise.

Since brain biopsy is unethical, the nearest approach to the biochemistry of the brain in man is the study of metabolites in c.s.f.. The studies repored in this thesis of brain amine metabolites in the c.s.f. of patients with neurological disease undergoing drug therapy were successful in providing an objective means of assessing the effect of the drugs on brain amine turnover. It will be possible to use such studies to determine the effects of a variety of drugs and it will also be possible to study brain amine metabolism in a number of degenerative neurological diseases. In addition, the measurement of brain amine metabolites provides a further means of studying the effect of alcohol on the central nervous system.

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PUBLICATIONS RESULTING FROM THIS WORK

The studies described in this thesis have led to the following publications:-

McLELLAN, D.L., CHALMERS, R.J. & JOHNSON, R.H. (1974)
A double-blind trial of Tetrabenazine, thiopropazate and placebo in patients with chorea.
The Lancet i; 104-107

McLELLAN, D.L., CHALMERS, R.J. & JOHNSON, R.H. (1975)
Clinical and Pharmacological evaluation of the effects of piribedil in patients with Parkinsonism.
Acta. Neurologica Scandinavica 51; 74-82