ETHANOL AND ACETALDEHYDE METABOLISM
IN SHEEP

A thesis presented in partial
fulfilment of the requirements
for the degree of

Doctor of Philosophy
in Biochemistry

at

Massey University,

New Zealand.

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1973

ABSTRACT

Ethanol and acetaldehyde metabolism in sheep has been studied in three different types of experiments: in purified enzyme systems, in liver homogenates and in the intact animals. Particular emphasis has been placed on the aldehyde oxidase enzyme from sheep liver, a molybdo-flavoprotein with a broad specificity which includes aldehydes, quinines and N^1-methyl nicotinamide. This thesis describes a method for preparing an enzyme solution in which sheep liver aldehyde oxidase constitutes 85% of the total protein present. Investigations of its physical and kinetic properties show that the sheep liver enzyme differs from the aldehyde oxidases previously prepared from pig and rabbit livers. In addition, an antibody to sheep liver aldehyde oxidase has been prepared from rabbit serum and has been shown to act as a specific, competitive inhibitor of the enzyme. This has been used to assess the contribution that aldehyde oxidase makes to acetaldehyde oxidation in sheep liver homogenates under several different conditions.

The effects of steroids on ethanol and acetaldehyde metabolism has been investigated, special interest being taken in the effects of progesterone. Progesterone stimulates sheep liver aldehyde oxidase activity _in vitro_ and inhibits sheep liver aldehyde dehydrogenase. Administration of progesterone to castrated sheep _in vivo_ increased the rates of ethanol and acetaldehyde oxidation, and aldehyde oxidase has been identified as a factor in decreasing acetaldehyde concentrations in the homogenates of livers from these animals during the metabolism of exogenous ethanol. Low endogenous ethanol concentrations in peripheral venous blood of sheep are positively correlated with high progesterone levels in sheep due to its experimental administration, and to pregnancy and the oestrus cycle.

Studies of the effects of disulphiram on ethanol and acetaldehyde metabolism have shown that the compound inhibits sheep liver aldehyde oxidase and aldehyde dehydrogenase enzymes _in vitro_, increases endogenous concentrations of acetaldehyde in peripheral venous blood, and causes acetaldehyde accumulation during ethanol metabolism _in vivo_. When diazepam is present together with disulphiram it provides protection from all but one of the effects shown by disulphiram alone. It does not alter the disulphiram inhibition of sheep liver aldehyde dehydrogenase. Amitryptyline is an inhibitor of both aldehyde oxidase and aldehyde dehydrogenase enzymes. It seems to increase the aldehyde

oxidase response to disulphiram, and its <u>in vivo</u> administration causes acetaldehyde accumulation in peripheral blood during and in the absence of metabolism of exogenous ethanol.

Investigations into the effects of ethanol on ethanol and acetaldehyde metabolism in sheep have shown that ethanol increases the activity of aldehyde oxidase <u>in vitro</u> and its chronic administration accelerates acetaldehyde oxidation <u>in vivo</u>. A supplementary study of the interrelationships between the relative concentrations of NADH and NAD^+, and ethanol and acetaldehyde metabolism shows that aldehyde oxidase participation in acetaldehyde oxidation is dependent on the NAD^+ concentrations, and that acetaldehyde oxidation can account for much of the NADH accumulation that occurs during ethanol metabolism <u>in vivo</u>.

Acetaldehyde oxidation during ethanol metabolism in sheep can be diverted through the aldehyde oxidase catalyzed pathway, avoiding dependence on the NAD^+-linked aldehyde dehydrogenase enzyme. The results in this thesis have shown that aldehyde oxidase can catalyze up to two-thirds of acetaldehyde oxidation in sheep liver when NAD^+ is limited, and that the pathway is dependent on the endocrine state and the pattern of ethanol consumption of the animal.

ACKNOWLEDGEMENTS

The author wishes to thank her supervisors, Prof.R.D.Batt and Dr.R.M.Greenway, for their help and advice throughout the course of this study.

In addition she expresses sincere appreciation of the help, advice and technical assistance rendered by other members of the staff of the Department of Chemistry and Biochemistry, Massey University; in particular, thanks are due to Miss L.Burson who willingly performed many duties outside her usual sphere of work.

The staff of the Animal Physiology Unit, especially Mr.L.Robertson, were generous in giving freely of their time and knowledge in experiments involving live animals.

The author also thanks Mrs.E.Self for typing the manuscript and remaining patient during inevitable delays.

Finally the patience and perseverance of Mr.R.E.Hendtlass must be recognised, for without these this thesis would not have been.

Upon the advice of Prof.R.D.Batt, this thesis is presented in the form of several papers to facilitate its later publication.

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a.m.	ante meridian
by vol.	by volume
CoQ	coenzyme Q
D	diffusion constant
$\triangle$	change in
DCI	2,6-dichlorophenol indophenol
DM	dry matter
E.C.No.	Enzyme Commission Number
EDTA	ethylenediamine-tetra-acetate
FAD	flavin adenine dinucleotide
g	gravity
HCl	hydrochloric acid
K	degrees Kelvin
K_i	inhibitor constant
K_m	Michaelis constant
LD_{50}	lethal dose for 50% of a population
M	molecular weight
MeB	methylene blue
mmoles	millimoles
mg%	$mg/100cm^3$
N	number of samples
NAD^+	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
$NADP^+$	nicotinamide adenine dinucleotide phosphate
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NE	not estimated
OD	optical density
p	relative density
$p<$	probability less than
pH_{max}	optimum pH
R	universal gas constant
R_f	distance travelled relative to the front
RNA	ribonucleic acid
s	sedimentation coefficient
S	Svedburgs
SD	standard deviation

SE_M	standard error of the mean
-S-S-	sulphydryl group
tert.	tertiary
T	absolute temperature
$\bar{\bar{v}}$	partial specific volume
vol.	volume
w/v	weight/volume
v/v	by volume
'zero'	taken at zero time