

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**THE EFFECT OF ETHANOL ON LIVER
GLYCOGEN
OF FED ANIMALS**

A thesis presented in partial fulfilment
of the requirements for the degree of
Master of Science
in
Biochemistry
at Massey University

BRIAN CHENG

1996

ABSTRACT

A hyperglycemic effect of ethanol has been reported in fed animals, but is poorly documented and in general, little work has been done on the effects of ethanol on carbohydrate metabolism in the fed state. This study is a further extension of research investigating the effect of ethanol on liver carbohydrate metabolism, as liver is a major source of glucose output in fed animals. It has been suggested that the hyperglycemic condition might be caused by an ethanol-stimulated liver glycogenolysis.

Because it was possible that the actions of ethanol were a direct effect on carbohydrate stores rather than an effect mediated by hormones, ethanol was tested on a simple unicellular organism *Ochromonas danica* where hormonal mechanisms are absent. It appears that ethanol does cause a reduction of *Ochromonas* carbohydrate stores in the absence of hormones. The major difficulty in using this organism was that its carbohydrate content could change according to the osmotic pressure in the external environment which therefore had to be strictly controlled.

Following the initial work using *Ochromonas danica*, further experiments were carried out using fed rats to assess the effects of ethanol on tissue glycogen stores. Sprague-Dawley rats were administered with an acute dose of ethanol (6g/kg). The effect of ethanol on the liver glycogen content of the animals was examined at 45, 90 and 180 minutes after the dose given. It appears that ethanol would lead to a significant decrease in liver glycogen content in both male and female rats at any given time. However, the decrease was not as much as that reported in the literature. Presumably this is due to the differences in ethanol administration, assays of liver glycogen and the strains of animals used in the experiment. The glycogen content in other tissues such as heart, kidney and muscle was also investigated but little difference was observed with ethanol treatment except in muscle, which showed some increase in glycogen content especially in the males. It is interesting that the free glucose concentrations in these tissues were not elevated as might have been expected if liver glycogen breakdown had occurred. Moreover, ATP levels were also observed to be unchanged.

The female rats were found to metabolise ethanol at a slower rate than males. The ethanol concentration in their extrahepatic tissues was similar to the calculated theoretical value for initial ethanol absorption. However in male rats, it is lower than the theoretical value. This indicates that the ethanol clearance curve for these tissues was not linear, and this implies that other factors such as delayed absorption or a first-pass effect (Lieber et al., 1994) might occur.

ACKNOWLEDGEMENTS

I would like to thank my supervisors, Dr. Kathryn Kitson and Dr. Malcolm Chick, for their invaluable help and advice in guiding me throughout the course of this work and in completion of my thesis. I am also grateful to Dr. John McIntosh for his permission in using the freezing point osmometer, Mr. Chris Burrows for technical assistance and to all the members of the team working on alcohol research in the Department of Biochemistry for continual advice and discussion of the project.

In particular I wish to thank Mr Pooranalingam Jeyarathan for his help in searching valuable references.

LIST OF CONTENTS

	Page
ABSTRACT	ii
ACKNOWLEDGEMENTS	iii
LIST OF CONTENTS	iv
LIST OF ABBREVIATIONS	v
LIST OF FIGURES	vi
LIST OF TABLES	vii
 Chapter 1 INTRODUCTION	 1
1.1 General	1
1.2 Ethanol Metabolism	2
1.2.1 Alcohol dehydrogenase system	2
1.2.2 Microsomal ethanol oxidising systems (MEOS)	4
1.2.3 Catalase system	4
1.2.4 Acetaldehyde dehydrogenase	5
1.2.5 NADH	7
1.2.5.1 Reoxidation of endogenous NADH	7
1.2.5.2 NADH from ethanol oxidation	7
1.3 Glycogen Metabolism	10
1.4 Interactions of Ethanol and Carbohydrate Metabolism	13
 Chapter 2 THE EFFECT OF ETHANOL ON THE RESERVE POLYSACCHARIDE OF <i>OCHROMONAS DANICA</i>	
2.1 Introduction	15
2.2 Materials	16
2.3 Methods	16
2.3.1 Culture media for the experiments	16
2.3.2 Maintenance and growing of <i>Ochromonas danica</i>	17
2.3.3 Growing <i>Ochromonas danica</i> for experiment	17
2.3.4 Measurement of dry cell mass	17
2.3.5 Measurement of chrysolaminarin content of cells	17
2.3.6 Demonstration that the material isolated from water extract was a poly β 1,3-glucoside	19
2.3.7 The effect of ethanol on the growth of <i>Ochromonas danica</i>	19
2.3.8 The effect of ethanol on chrysolaminarin catabolism	19

2.4	Results & Discussions	22
2.4.1	The growth of <i>Ochromonas danica</i>	22
2.4.2	The efficiency of the polysaccharide extraction procedure	22
2.4.3	The extracted polysaccharide from <i>Ochromonas</i> cells	24
2.4.3.1	The identity of the extracted polysaccharide	24
2.4.4	The effect of ethanol on chrysolaminarin catabolism	28
2.4.4.1	The effect of ethanol on the growth of <i>Ochromonas danica</i>	28
2.4.4.2	The maintenance of osmotic pressure in <i>Ochromonas</i> cells	28
2.4.4.3	Effect of ethanol on catabolism of chrysolaminarin by <i>Ochromonas danica</i>	30

Chapter 3 EFFECTS OF ETHANOL ON GLYCOGEN, GLUCOSE CONCENTRATION AND ATP FROM LIVER AND OTHER TISSUES

3.1	Introduction	35
3.2	Methods	35
3.2.1	Animals and materials	35
3.2.2	Experimental protocol	36
3.2.3	Metabolite assay methods	37
3.2.3.1	Enzymatic analysis of tissue glycogen content	37
3.2.3.2	Glucose assay	41
3.2.3.3	ATP assay	41
3.2.3.4	Ethanol assay	41
3.3	Results & Discussion	42
3.3.1	Glycogen recovery	42
3.3.1.1	Recovery from glycogen standards made up in perchloric acid	42
3.3.1.2	Recovery of glycogen from liver samples	44
3.3.2	Effects of ethanol on tissue glycogen content	46
3.3.2.1	Effect of ethanol on liver glycogen content	46
3.3.2.2	Muscle glycogen	58
3.3.2.3	Heart & Kidney glycogen	63
3.3.3	Effects of ethanol on other metabolites	67
3.3.3.1	Tissue glucose contents	67
3.3.3.2	Tissue ATP content	70
3.3.3.3	Ethanol concentrations in tissue of treated rats	74
Chapter 4	CONCLUSIONS AND FUTURE WORK	84

Appendixes	86
REFERENCES	94

ABBREVIATIONS

GENERAL

ATP	adenosine-5'-triphosphate
BSA	bovine serum albumin
cAMP	cyclic adenosine 3',5'-monophosphate
EDTA diNa	sodium ethylenediamine tetra acetic acid
FRS	free reducing sugar
H ⁺	hydrogen ion
H ₂ O	water
HCl	hydrochloric acid
KHCO ₃	potassium hydrogen carbonate
KClO ₄	potassium perchlorate
KOH	potassium hydroxide
MgCl ₂	magnesium chloride
NAD ⁺	nicotinamide adenine dinucleotide (oxidised form)
NADP	nicotinamide adenine dinucleotide phosphate (reduced form)
OAA	oxaloacetate
PCA	perchloric acid
PEG	polyethylene glycol
RS	reducing sugar
αKG	α-ketoglutarate

ENZYMES

ADH	alcohol dehydrogenase
AGS	amyloglucosidase
G6PD	glucose 6-phosphate dehydrogenase
GP	glycogen phosphorylase
GS	glycogen synthase
HK	hexokinase
MDH	malate dehydrogenase
PhK	phosphorylase kinase
PKA	protein kinase A
PP1G	protein phosphatase 1G

LIST OF FIGURES

Figure		PAGE
1	Scheme showing the malate-aspartate shuttle	8
2	The structure of glycogen	8
3	Reaction cascades for the hormonal control of glycogen metabolism	12
4	Arrangement for sampling <i>Ochromonas</i> cultures	18
5	Standard curve for chrysolaminarin assay	20
6	Standard curve for reducing sugar assayed by the method of Bernfeld (1955)	21
7	Effect of ethanol on growth of <i>Ochromonas danica</i>	29
8	The relationship between PEG concentration and osmolarity	31
9	Effect of ethanol on dry cell mass	33
10	Effect of ethanol on chrysolaminarin content	34
11	Summary of procedures in treatment of samples	40
12a	Liver glycogen content of male and female animals at given time after ethanol treatment (First experiment)	48
12b	Liver glycogen content of male and female animals at given time after ethanol treatment (Second experiment)	54
13a	Relationship between liver glycogen content and animal body weight	50
13b	Relationship between liver weight and animal body weight	51
14a	Muscle glycogen contents of male and female animals at given time after treatment (First experiment)	61
14b	Muscle glycogen contents of male and female animals at given time after treatment (Second experiment)	62

15a	Ethanol concentrations of different tissues in male rats (First experiment)	77
15b	Ethanol concentrations of different tissues in female rats (First experiment)	78
16a	Ethanol concentrations of different tissues in male rats (Second experiment)	79
16b	Ethanol concentrations of different tissues in female rats (Second experiment)	80