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.

BIOCHEMICAL AND HAEMATOLOGICAL ASPECTS OF ETHANOL METABOLISM IN HUMANS

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

Hon Wing KWOK 1983

ABSTRACT

Macrocytosis or raised mean cell volume (MCV) (which as measured by the Coulter S counter) is one of the results of alcohol abuse. There is a need to identify (chronic) alcoholics by laboratory tests. The obvious measurement of blood alcohol is not suitable as ethanol is so rapidly cleared from the body. It is usually undetectable 2 - 3 hours after drinking. To this the following battery of tests: MCV, fast haemoglobin, gamma glutamyl transferase and thiamine, have been examined.

These tests which were performed, on a population consisting of 115 random hospital patients, 14 patients attending diabetic clinic and 13 'normal' volunteers. For ethical reasons it was not possible to obtain samples from known alcoholics. Instead those samples which contained red cells above 92 fl of MCV were suspected of including alcoholics and correlated with other parameter which may be assumed to be elevated in alcoholics.

The results showed that there were 23 abnormal findings likely to be associated with heavy drinkings in 70 bloods selected for high MCV.

Aug 1 . 1

ACKNOWLEDGEMENTS

I wish to express my indebtedness to my supervisors, Prof. R.D. Batt, for his kindly approval of this project, Drs. John McIntosh and Ken Couchman for their cordial encouragement and superb guidance, and Drs. R. Greenway and M. Wilson for their advice during this work. I also wish to thank the members of the Alcohol Research Group at Massey University, Dr. K. Crow, Mr. T. Braggins and Mrs. K. Newland for their help in many ways during this project. I would also like to acknowledge my gratefulness to the staff of the Haematology Department of the Palmerston North Hospital who collected and provided the blood samples and blood parameters.

ABBREVIATIONS

General

Gly Hb glycosylated haemoglobin

Hb haemoglobin

Hb A adult haemoglobin

IEF isoelectric focussing

MCV mean cell volume (mean corpuscular volume)

PCV packed cell volume

RBC red blood cell

THF tetrahydrofolate

Chemicals, enzymes

ALAT alanine aminotransferase

ASAT aspartate aminotransferase

DMSO dimethyl sulphoxide

EDTA ethylenediaminetetraacetate (potassium salt)

GDH glycerolphosphate dehydrogenase

GGPNA gamma glutamyl-p-nitroanilide

GGTP gamma glutamyl transpeptidase (gamma glutamyl transferase)

HMFF hydroxymethylfurfural

NADH reduced nicotinamide adenine dinucleotide

TBA 2-thio barbituric acid

TCA trichloroacetic acid

TIM triose phosphate isomerase

TK transketolase

TPP thiamine pyrophosphate

Tris tris (hydroxymethyl) aminomethane

CONTENTS

				Page
Abstract				
Acknowledgements				
Abbreviations			iv	
List	List of contents			v
List	of figure	es		viii
			CHAPTER 1	
			INTRODUCTION	1
			CHAPTER 2	
		HAE	MOGLOBIN ADDUCTS	4
2-1.	Introduc	ction		4
2-2.	Glycosyl	lation		4
2-3.	Carbamy	lation		5
2-4.	Acetalde	ehyde addu	et	5
2-5.	Aspirin	adduct		5
2-6.	The dete	ermination	of haemoglobin adducts	6
2-7.	Methods			6
	2-7-1.	Column ch	romatography	6
		2-7-1-a.	Preparation of resin	6
		2-7-1-b.	Buffers	7
		2-7-1-c.	Preparation of haemolysate	7
		2-7-1-d.	Column chromatography of haemoglobin	7
		2-7-1-e.	Regeneration of resin	8
		2-7-1-f.	Measurement of haemoglobin	8
		2-7-1-g.	Elution of haemoglobin	8
		2-7-1-h.	Reproducibility	8
	2-7-2.	Isoelectr	ic focussing	12
		2-7-2-a.	Haemolysates	12
		2-7-2-b.	Preparation of gels	12
		2-7-2-c.	Electrophoresis	13
		2-7-2-d.	Measurement of pH gradient	13
	2-7-3.	Hydroxyme	thylfurfural assay	19
		2-7-3-a.	Production of hydroxymethylfurfural	
			from haemoglobin	19

		Page			
	2-7-3-b. Effect of antioxidants on HMFF				
	formation from haemoglobin	19			
	2-7-3-c. Precision of the method	22			
2-8.	Acetaldehyde adduct	23			
	2-8-1. Introduction	23			
2-9.	Conclusions	23			
	CHAPTER 3				
	BIOCHEMICAL AND HAEMATOLOGICAL MARKERS OF ALCOHOL ABUS	<u>SE</u> 26			
3-1.	Introduction	26			
3-2.	MCV	27			
3-3.	Determination of gamma glutamyl transpeptidase	31			
	3-3-1. Principle	31			
	3-3-2. Preparation of beef liver gamma glutamyl				
	transpeptidase (GGTP)	31			
	3-3-3. Assay procedure	31			
	3-3-4. The calculation of the enzyme activity	32			
	3-3-5. Precision of the method	32			
	3-3-6. Normal values	32			
3-4.	Thiamine deficiency	34			
	3-4-1. Introduction	34			
	3-4-2. Principle	34			
	3-4-3. Method	35			
	3-4-4. Calculation of the enzyme activity	35			
	3-4-5. Normal ranges	36			
	3-4-6. Results in the normal plasma	36			
	3-4-7. Conclusions	36			
	CHAPTER 4				
	RESULTS IN NORMAL VOLUNTEERS AND HOSPITAL PATIENTS	37			
4-1.	Source of blood samples	37			
	4-1-1. Normal volunteers	37			
	4-1-2. Hospital patients	37			
4-2.	Fast haemoglobin	39			
4-3.	Hydroxymethylfurfural	39			
4-4.	The relationship between fast haemoglobin and HMFF				
	results	39			
4-5.	Gamma glutamyl transferase				
4-6.	Thiamine deficiency	48			
4-7.	Combination of data				

	CHAPTER 5	Page
	DISCUSSION	52
	APPENDIX I	ā
Table 1		56
Table 2		57
Table 3		60
Table 4		62
	APPENDIX II	
Materials		63
	REFERENCES	64

LIST OF FIGURES

Figure	<u>Title</u>	Page
2-1-1.	Absorbance of cyanmethaemoglobin at different	
	wavelengths from 225-600 nm	9
2-1-2.	Absorbance of cyanmethaemoglobin at 415 nm	10
2-1-3.	The elution profile of fast haemoglobin from a	
	Biorex 70 column with pH 6.7 buffer	11
2-2-1.	The pH gradient on polyacrylamide gel purchased	
	from LKB and used in isoelectric focussing	15
2-2-2.	The pH gradient on polyacrylamide gel prepared	
	in the laboratory and used in isoelectric focussing	16
2-2-3.	Isoelectric focussing of human haemoglobins on	
	polyacrylamide gel purchased from LKB	17
2-2-4.	Isoelectric focussing on human haemoglobins on	
	polyacrylamide gel prepared in the laboratory	18
2-3-1.	Rate of production of HMFF from the normal	
	haemolysate pool and fructose standards	20
2-3-2.	The absorption of HMFF and HMFF + Hb at 443 nm	21
2-5-1.	Isoelectric focussing of haemoglobins to which	
	acetaldehyde had been added (LKB pH 5.5-8.5 gel)	25
3-1.	Frequency histogram for MCV	30
3-2.	The change in absorbance of p-nitroaniline due to	
	GGTP activity	33
4-1.	Histograms for MCV for the three groups of subjects	38
4-2.	Histograms for fast Hb for the three groups of subjects	40
4-3.	Histograms for HMFF for the three groups of subjects	41
4-4.	Correlation curve between HMFF and Fast Hb for the	
	diabetics.	42
4-5.	Correlation curve between HMFF and Fast Hb for	
	normal MCV group	43
4-6.	Correlation curve between HMFF and Fast Hb for	
	high MCV group	44
4-7.	Histograms of GGTP	47
4-8.	Histograms of transketolase activity	49
4-9.	Correlation between transketolase activity and the	
	effect of adding excess thiamine pyrophosphate for	
	the high MCV group bloods	50