

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.

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

Kenneth G. Couchman
1974

SUMMARY

Alcohol metabolism in humans has been studied by examining blood, urine and breath samples taken at frequent intervals for 3 hours after an alcohol load of 0.5ml/kg in a fasting condition.

A gas chromatographic method was developed for the simultaneous estimation of acetaldehyde, ethanol and acetone levels in blood and urine specimens and various column packings were investigated. Porapak Q was the most suitable material and the method finally adopted used the headspace gas phase over urine or perchlorate precipitated blood specimens to which had been added sodium sulphate to displace the volatile components from the aqueous phase. Protein precipitation was necessary in order to prevent the loss of acetaldehyde from the blood samples. A gas sampling valve was fitted to enable similar determinations in breath samples but was not used in this study.

Assays by enzymatic methods were developed for lactate, pyruvate, β -hydroxybutyrate, glucose and glycerol utilising the changes in concentration of NADH which was measured by fluorometry and the merits of converting NAD^+ to a fluorescent compound was examined. Twenty male and eight females volunteered for the study. Blood samples were obtained from an intravenous catheter, a procedure supervised by a physician. Blood alcohol levels were monitored by breath tests with an electrochemical device, (an Alcolimiter) for detecting ethanol.

There were considerable variations in the peak alcohol levels and in the time taken for equilibration in the body. Estimates of

the rate of metabolism of ethanol and of body content were in agreement with those published in the literature. Breath testing was found to be a satisfactory means of estimating blood alcohol concentration in the post-absorptive phase. The Alcolimiter gave readings that were on an average 10mg/100ml low, but this could be corrected by recalibration. Blood acetaldehyde levels rose to 0.1mg/100ml and occasionally to 0.2mg/100ml.

A fall in blood pyruvate level, which remained low throughout the test period, was seen to coincide with the increase in blood alcohol. There was a tendency for lactate to rise at the same time. This was not consistent, but the ratio of lactate to pyruvate increased 2 or 3-fold in most cases which reflected the change in cytoplasmic redox ratio.

Small increases were observed in blood glucose even though the alcoholic drink was free of sugar. There were increases in blood glycerol levels in all subjects and some of these were quite large. These findings were contrary to some reports which have appeared in the literature.

The excretion of electrolytes was examined in the urine but the results were difficult to interpret.

Alcohol concentrations in urine samples were measured and compared to the blood levels and the diuretic effect of alcohol was noted. These findings, together with those reported in the literature have been discussed together with their significance in interpreting disturbances of metabolism when alcohol is consumed.

More assays are thought to be required including those for blood acetate, blood triglycerides with free fatty acids and some hormones. It is considered that the use of labelled compounds could add a new dimension to the in vivo investigations on human volunteers.

ACKNOWLEDGMENTS

I am indebted to my supervisor, Professor R. D. Batt for his continuing interest in the study and help with the preparation of the manuscript for the thesis. I am also grateful to Dr L. Bieder for his medical supervision of the work with volunteers and assistance in formulating the protocol. My appreciation is due and acknowledged to Terry Braggins for technical assistance, Dr R. Greenway both for advice and the assays of electrolytes, Dr R. Brooks for help with estimations using atomic absorption spectroscopy; and to all the members of the team working on alcohol research in the department for continual advice and discussion of the project.

The thesis was typed and copied by Ms Marion Trevor to whom I am indebted for her patience and care at all stages of preparing the final manuscript.

LIST OF CONTENTS

| | | <u>Page</u> |
|-----------|---|-------------|
| Chapter 1 | <u>INTRODUCTION</u> | 1 |
| Chapter 2 | <u>THE ESTIMATION OF ACETALDEHYDE, ACETONE AND ETHANOL IN BREATH, URINE AND BLOOD SAMPLES</u> | 5 |
| 2.1 | DEVELOPMENT OF GAS CHROMATOGRAPHIC METHOD | |
| 2.1.1 | Introduction | 5 |
| 2.1.2 | Sample preparation | 6 |
| 2.1.3 | Gas chromatograph | 6 |
| 2.1.4 | Integration | 7 |
| 2.1.5 | Syringes | 9 |
| 2.1.6 | Distilled water | 9 |
| 2.1.7 | Internal standardisation | 11 |
| 2.1.8 | Standards | 12 |
| 2.1.9 | Determination of optimum operating conditions | 12 |
| 2.2 | COMPARISON OF COLUMN PACKINGS | |
| 2.2.1 | Porapak Q | 14 |
| 2.2.2 | Carbowax 1500 (5%) on Chromosorb W-DMCS | 20 |
| 2.2.3 | Carbowax 400 (10%) on Chromosorb W-DMCS | 25 |
| 2.2.4 | Carbowax 1540 (5%) on Chromosorb W-DMCS | 26 |
| 2.2.5 | Conclusions | 27 |
| 2.3 | APPLICATION TO BLOOD AND URINE SAMPLES | |
| 2.3.1 | Introduction | 28 |
| 2.3.2 | Effect of blood volume | 29 |
| 2.3.3 | Stability of blood sample | 31 |
| 2.3.4 | Precipitated blood samples | 33 |
| 2.3.5 | Headspace gas enrichment | 35 |
| 2.3.6 | Gas chromatograph operation | 36 |
| 2.3.7 | Standards | 37 |
| 2.3.8 | Urinary alcohol | 39 |
| 2.3.9 | Conclusions | 39 |
| 2.4 | BREATH TESTING BY GAS CHROMATOGRAPHY | 40 |
| 2.5 | BREATH TESTING BY ELECTRO-CHEMICAL ANALYSIS | 41 |

| | | <u>Page</u> |
|-----------|--|----------------------|
| Chapter 3 | <u>THE ESTIMATION OF NON VOLATILE CONSTITUENTS IN BODY FLUIDS</u> | |
| 3.1 | INTRODUCTION | 45 |
| 3.2 | REAGENTS | 45 |
| 3.3 | FLUOROMETRY | |
| 3.3.1 | Instrumentation | 46 |
| 3.3.2 | Measurement of NADH | 47 |
| 3.3.3 | Measurement of NAD^+ | 48 |
| 3.4 | LACTATE | 49 |
| 3.5 | HYDROXYBUTYRATE | 50 |
| 3.6 | PYRUVATE | 51 |
| 3.7 | PYRUVATE BY DIRECT MEASUREMENT OF NAD^+ | 54 |
| 3.8 | ACETOACETATE | 57 |
| 3.9 | GLYCEROL | 58 |
| 3.10 | GLUCOSE | 59 |
| 3.11 | CALCIUM AND MAGNESIUM | 59 |
| 3.12 | SODIUM, POTASSIUM AND CHLORIDES | 60 |
| Chapter 4 | <u>RESULTS</u> | |
| 4.1 | SUBJECTS | 61 |
| 4.2 | EFFECTS OF ALCOHOL | 61 |
| 4.3 | 1/ Absorption, 2/ Equilibration, and 3/ Elimination of alcohol | 63 65 65 |
| 4.4 | BODY WATER ESTIMATES | 67 |
| 4.5 | ACETALDEHYDE | 68 |
| 4.6 | ACETONE | 68 |
| 4.7 | 1/ Lactate 2/ Pyruvate, 3/ β -hydroxybutyrate and 4/ Redox ratios | 69 69 70 71 |
| 4.8 | GLUCOSE | 71 |
| 4.9 | GLYCEROL | 72 |
| 4.10 | URINE ALCOHOL LEVELS | 72 |
| 4.11 | DIURETIC EFFECT OF ALCOHOL | 73 |
| 4.12 | EXCRETION of Ca^{++} , Mg^{++} , Na^+ , K^+ and Cl^- . | 75 |
| 4.13 | BREATH VERSUS BLOOD ALCOHOL LEVELS | 78 |

| | <u>Page</u> |
|------------|--|
| Chapter 5 | <u>DISCUSSION</u> |
| 5.1 | EFFECTS OF ALCOHOL 80 |
| 5.2 | ABSORPTION, EQUILIBRATION AND ELIMINATION OF ALCOHOL AND THE ESTIMATION OF TOTAL BODY WATER 82 |
| 5.3 | ACETALDEHYDE 86 |
| 5.4 | KETOGENESIS 89 |
| 5.5 | LACTATE, PYRUVATE AND THE REDOX RATIO 91 |
| 5.6 | CARBOHYDRATE METABOLISM 93 |
| 5.7 | FAT METABOLISM 94 |
| 5.8 | DIURESIS 95 |
| 5.9 | THE ELIMINATION OF ELECTROLYTES AND MINERALS IN THE URINE 97 |
| 5.10 | BLOOD ALCOHOL ESTIMATES BY BREATH TESTING 98 |
| Chapter 6 | <u>CONCLUSIONS AND FUTURE WORK</u> |
| Appendix 1 | <u>A WORKBOOK OF PROCEDURES</u> |
| Appendix 2 | <u>TABLES OF RESULTS</u> |
| References | |

LIST OF FIGURES

| | | <u>Page</u> |
|------|--|-------------|
| 2.1 | A gas chromatogram of 0.5 μ l. water on unpacked stainless steel columns before and after treatment with phosphoric acid | 8 |
| 2.2 | A gas chromatogram illustrating integrator function | 10 |
| 2.3 | Van Deemter plots of acetaldehyde and ethanol on Porapak Q | 16 |
| 2.4 | Gas chromatogram, 1 μ l. acetaldehyde/ethanol on Porapak Q showing tailing at high temperatures | 17 |
| 2.5 | Calibration curves for ethanol and acetaldehyde on Porapak Q | 19 |
| 2.6 | Gas chromatogram of water and acetaldehyde on 5% Carbowax 1500 on Chromosorb | 22 |
| 2.7 | Gas chromatogram, headspace gas of acetaldehyde, ethyl methyl ketone and ethanol on 5% Carbowax on Chromosorb | 24 |
| 2.8 | Effect of blood volume on equilibration of headspace gas | 30 |
| 2.9 | Effect of incubation at 55°C. on the levels of acetaldehyde, ethyl methyl ketone and ethanol in the headspace gas | 32 |
| 2.10 | Calibration curves for acetaldehyde, acetone and ethanol. Headspace gas on Porapak Q | 38 |
| 2.11 | Variation of 'Alcolimiter' readings with temperature on a standard 100mg/100ml. simulated breath sample | 44 |
| 3.1 | Decay of fluorescence of NADH in the assay of pyruvate | 52 |
| 3.2 | Effect of NADH concentration on the pyruvate assay | 53 |
| 3.3 | Standard curve for pyruvate assay at various NADH concentrations | 55 |
| 4.1 | Graphs illustrating the variation of the rate of absorption and equilibration of alcohol | 62 |

LIST OF TABLES

| | <u>Page</u> |
|--|-------------|
| Table 4.1 The age, sex and drinking habits of the subjects with their interpretation of the effects of the alcohol dose | 41 |
| 4.2 Results of the application of the Widmark formulae | 66 |
| 4.3 The ratios, urine: blood alcohol levels during the absorptive and postabsorptive phase | 74 |
| 4.4 The average excretion in urine per 30 minutes of Cl^- , Na^+ , K^+ , Ca^{++} , and Mg | 76 |
| 4.5 Patterns of excretion of electrolytes and minerals | 77 |
| Appendix 2 Tables:- | |
| 1 Blood alcohol | |
| 2 Blood acetaldehyde | |
| 3 Blood acetone | |
| 4 Blood lactate | |
| 5 Blood pyruvate | |
| 6 Blood β -hydroxybutyrate | |
| 7 Redox ratios | |
| 8 Blood glucose | |
| 9 Blood glycerol | |
| 10 Urine alcohol | |
| 11 Urine volume | |
| 12 Urine specific gravity | |
| 13 Urine sodium | |
| 14 Urine chloride | |
| 15 Urine potassium | |
| 16 Urine calcium | |
| 17 Urine magnesium | |
| 18 Alcolimeter readings | |