$ALDH_2$ & ADH_2 Enzyme Variation in Asian Subjects:

A Study of Biochemical, Pharmacokinetic, Physiological and Psychomotor Variables and Ethanol Sensitivity after Low-dose Ethanol.

A thesis submitted in fulfillment of the requirements
for the degree of
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by
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Preface

The work presented for examination in this thesis was carried out under the supervision of Associate Professor Graham Starmer of the University of Sydney, and Dr John Whitfield of the Royal Prince Alfred Hospital, Sydney.

No portion of this work has been submitted by the candidate for the award of any other degree.

The guidelines set out by the British Journal of Pharmacology regarding SI units and abbreviations have been followed throughout this thesis.

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Here in particular I would like to specifically thank my Supervisors, Associate Professor G.A. Starmer and Dr J.B. Whitfield, to them I am forever indebted for their invaluable advice, guidance and friendship over these past years.

Also, I would like to express my gratitude to everyone at the Molecular Biology Laboratory at Rachel Forster Hospital (where much of my time was spent "PCRing").

Not to be forgotten, David Mascord must also be thanked for his advice on the statistical methods.

Keeping to the Asian (Chinese) aspect of this thesis, I would finally like to say...

Xie Xie (thank you) to all who have helped me by way of encouragement, support and/ or in more practical ways during the last five years, I am truly grateful.

! (expresses my feelings upon completion of this thesis!)

Abstract

The influence of $ALDH_2$ and ADH_2 genotype on the biochemical, physiological, psychomotor and subjective responses of Asian subjects to a challenge dose of ethanol were investigated.

One hundred and ten healthy male and female subjects of full or partial North East Asian descent (with ancestral origins in China, Japan, Korea and Vietnam), who were living in Australia at the time of testing, were genotyped for alcohol dehydrogenase (ADH_2) and aldehyde dehydrogenase ($ALDH_2$) enzymes using a combination of the polymerase chain reaction (PCR) with restriction enzyme digestion, allele specific oligonucleotide probing, or constant denaturant gel electrophoresis methods.

Volunteers were given a low oral dose (0.3 g kg^{-1}) of ethanol and were assessed subjectively for their degree of flush and for performance impairment using a battery of psychomotor tests (including divided attention, digit symbol coding, standing steadiness and critical flicker fusion frequency threshold). Self-report questionnaires were used to assess the subjects' perception of their intoxication and impairment. Blood ethanol concentrations (BECs) were monitored by breath analysis every fifteen minutes and blood samples were obtained from subjects before and at 15, 60 and 120 minutes after ethanol administration. Measurements were made of the blood or plasma levels of acetaldehyde, acetate, pyruvate, lactate and ethanol. The blood pressure (systolic and diastolic), heart rate and facial temperature were also recorded at regular intervals.

The effects of the ADH_2 genotype, $ALDH_2$ genotype, $ALDH_2$/ ADH_2 combination genotype, the degree of flush and gender on the psychomotor

performance, physiological, biochemical, pharmacokinetic and subjective responses of the subjects were explored to determine their influence on the response to ethanol.

When subjects were classified by **$ALDH_2$ genotype**, the BEC curve, acetaldehyde concentration, acetate concentration, facial temperature, heart rate, critical flicker fusion frequency threshold, digit symbol coding reaction time, standing steadiness and divided attention delay and excursion were all affected by whether the subject was $ALDH_2$ Homo11, Het or Homo22. The psychomotor performance of $ALDH_2$ Homo22 subjects was found to be more impaired in the divided attention delay, excursion and digit symbol coding reaction time tasks than in either Het or Homo11 subjects. The standing steadiness epoch time and critical flicker fusion frequency threshold were also most affected by ethanol in the $ALDH_2$ Homo22 group.

The effect of ethanol on the pyruvate concentration, heart rate, CFFF threshold and standing steadiness also differed significantly among subjects of different **ADH_2 genotype**. The ADH_2 Homozygote-22 (Homo22) subjects had a higher standing steadiness epoch time and critical flicker fusion frequency threshold than either Heterozygote (Het) or Homozygote-11 (Homo11) subjects.

The subjectively rated **degree of flush** was associated with differences in acetaldehyde concentration, acetate concentration, digit symbol coding reaction time, divided attention delay, facial temperature and heart rate measured. Psychomotor impairment in the divided attention delay task was greatest for the subjects who flushed with intermediate severity, although at two hours post-ethanol, the group which produced the most severe flushing was more severely affected. In the digit symbol coding reaction time, the subjects who produced the most severe flushing reactions were also most impaired.

The findings of this study suggest that BEC curve, acetaldehyde concentration, plasma ethanol level, digit symbol coding reaction time, divided attention delay and excursion measures, facial temperature and heart rate are all influenced by the **$ALDH_2$/ ADH_2 combination genotype**. Psychomotor performance was most impaired in subjects with the $ALDH_2$ Homo22/ ADH_2 Homo11, Het or Homo22 genotypes.

Based on the **gender** of the subject, pyruvate concentrations, facial temperature, systolic blood pressure, heart rate, standing steadiness, divided attention delay and reaction time were affected differently. Divided attention delay and reaction time were more affected after ethanol in female subjects than in the males. Female subjects were also steadier on the standing steadiness task, before but not after ethanol.

In terms of drinking details and consumption patterns of subjects tested, this study found that ADH_2 and $ALDH_2$ Homo22 subjects consumed the least amount of ethanol (<1 drink) per session when compared with the other groups. Similarly, female subjects and those who flushed most severely after ethanol also consumed the least amounts of ethanol. Subjects with the $ALDH_2$ Homo22/ ADH_2 Homo11, Het or Homo22 combination genotypes had the highest proportion of subjects who consumed less than one drink per session.

There have been no previous studies which have been specifically directed towards measurement of ethanol-induced psychomotor performance deficits (and to some extent, the biochemical and physiological responses) in Asian subjects, many of whom are much more affected by ethanol than their Caucasian counterparts and exhibit a flush reaction after very low doses.

The subjective effects which were reported in this study were interesting in that they were correlated with both genotype and the severity of the flush reaction. The perceived degree of intoxication was greatest in the $ALDH_2$ Homo22 and least in the Homo11 subjects.

There was a strong suggestion in these results that a severe ethanol reaction served to deter the subject from driving, and often from consuming ethanol in the future. This has important traffic-safety connotations, which probably extend to other potentially hazardous situations. The findings of this study are viewed as being important and could form the basis for specifically-directed educational campaigns at a time when ethanol consumption in many Asian countries is increasing rapidly.

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INTRODUCTION

Wide variations in the physiological, psychomotor and metabolic effects of ethanol appear to be the basis for the wide range of human responses to this well-known drug. Apart from the sociocultural differences found between individuals of different ethnic groups, genetic factors have now been shown to play an important role in the human response to ethanol as well.

Both alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) have been implicated in affecting the response to ethanol in people with genetic variations of these two enzymes. The inactive form of ALDH (ALDH2-2), which is found in many Asian people, has been associated with impaired acetaldehyde metabolism and with various accompanying adverse effects, such as nausea, dizziness and tachycardia etc. The ADH isozyme may also affect the rate of metabolism of ethanol because of differences in the *in vitro* enzyme activity of each isozyme.

However, although the effects of ethanol have been extensively investigated in the past, few studies have attempted to specifically measure the individual and racial differences in sensitivity to ethanol. These differences include physiological effects, such as the elevation of skin temperature and an increase in pulse and ventilation rates after ingestion of ethanol, as well as psychomotor impairment and subjective responses.

In general, Asians have traditionally consumed ethanol less frequently than their Caucasian counterparts. However, ethanol consumption in Asian countries has recently begun to increase and, coupled with the migration of Asian populations to Western countries, could conceivably encourage a 'Caucasian pattern' of drinking. Therefore, the potential for significant increases in ethanol-related health and social problems among Asians, both in their native and adopted lands, is obvious.

In addition to the expected health-related aspects of ethanol consumption in these populations, the effects of ethanol on psychomotor performance, of particular interest and importance in the drink-driving situation, need to be investigated because

information is clearly lacking. This information is vital because, as the level of consumption of ethanol in Asian countries has increased, so has motor vehicle ownership. Therefore, the results from this study would be expected to provide much needed advice and allow for more accurate predictive recommendations to deal with the related perceived problems that may arise.

A better understanding of the genetically-controlled factors which influence the biological handling of ethanol, and also the effects of 'social' amounts of ethanol in individuals of Asian descent, would be of great value in the prediction of the consequences of increased ethanol consumption by these populations. Therefore, this thesis presents the results of detailed studies which have examined the pharmacodynamics and pharmacokinetics of ethanol in subjects of Asian descent, who differ from Caucasian groups in ADH and ALDH polymorphisms which are known to affect ethanol metabolism. The results lead to a number of important conclusions with theoretical implications for an understanding of ethanol metabolism and more practical implications concerning the performance of complex tasks after the consumption of ethanol.

Since most of the studies on ethanol metabolism and its effects have been carried out on non-Asian subjects in the past, this thesis begins by summarizing information concerning the general pharmacokinetic, metabolic and psychomotor effects of ethanol in Caucasian subjects. This is followed by a brief historical review and a description of the current state of knowledge in this field including similarities and differences between Caucasian and Asian groups. Research pertaining specifically to Asian subjects and the ethanol metabolizing enzymes involved is also included. Finally, the main aims and objectives of this study, together with an outline of this thesis, are provided towards the end of this Chapter.

1.1. ETHANOL PHARMACOKINETICS

The rising part of the blood ethanol concentration (BEC) curve is the net result of the entry of ethanol into the body (mainly from the duodenum), the metabolism of part of the ethanol dose in the liver and the equilibration of ethanol into total body water. The uptake and equilibrium stages may contribute differently to the rising limb of the BEC curve. As the BEC reaches its maximum level there may be an overshoot, which has been ascribed to the BEC being briefly higher than the concentrations in other body water compartments (i.e. uptake into the blood is almost complete while equilibrium from the blood is still continuing). After the overshoot, there is a linear fall in the BEC, which results from a near-constant rate of ethanol metabolism as blood circulates through the liver. In the truly post-absorptive state, where uptake and equilibrium can be considered to be complete, the decrease in BEC with time closely approximates a linear relationship.

1.1.1. ETHANOL ABSORPTION

Ethanol is a small, neutral, water soluble molecule which requires no digestion and crosses biological membranes with ease. A large portion of an ethanol dose is absorbed passively, but rapidly, from the upper part of the small intestine and more slowly from the stomach. The rate of absorption is highly variable and is influenced by many factors, such as the concentration of ethanol in the beverage, the rate of consumption, the type of beverage, carbonation and the presence of food in the stomach (Goldberg, 1943; Dubowski, 1963).

The main determinant of the rate of absorption of ethanol is the presence of food in the stomach. Since there are no active transport processes for ethanol, an oral dose is mostly absorbed by passive diffusion from the stomach, small intestine and colon. Foods tend to retard ethanol absorption by prolonging the gastric emptying time and may also provide a physical barrier to passive absorption (Julkunen, DiPadova & Lieber, 1985). When there is food in the stomach, peak blood ethanol concentrations attained after a given dose of ethanol are lower and occur later compared with the fasting state (Millar, Hammersley & Finnigan, 1992). The type of food present in the stomach may also

influence ethanol absorption. Proteins and carbohydrates were found to slow down absorption, more so than fats (Haggard, Greenberg & Cohen, 1938; Agarwal & Goedde, 1990). The intravenous route of administration has been used, in experiments designed to investigate the effects of food on ethanol utilization, to avoid these absorptive interactions (Rogers, Smith, Starmer & Whitfield, 1987). This route also has been used to estimate the proportion of ethanol which is metabolized before reaching the circulation (Frezza, DiPadova, Pozzato, Terpin, Baraona & Lieber, 1990).

Beverages which contain ethanol in concentrations ranging from 15-30% (v/v) are absorbed most rapidly. Ethanol in whisky or brandy (~37.5% v/v) is absorbed more quickly than that in beer, even when the beverage is diluted to the same ethanol concentration (Haggard *et al.*, 1938). The rate of consumption also influences the rate of rise of the blood ethanol concentration. Provided that pylorospasm does not occur, rapid consumption of ethanol produces higher and earlier blood ethanol concentrations than when the same dose of ethanol is consumed more slowly (Dubowski, 1963). The volume of liquid, carbonation and the presence of ethanol congeners in the beverage can all affect the rate of ethanol absorption (Hopkins, 1966; Pohorecky & Brick, 1988).

A large number of drugs may also affect the rate of absorption of ethanol by increasing (e.g. hyoscine) or decreasing (e.g. metoclopramide) gastric emptying time.

The absorption of orally administered ethanol is usually complete 30-60 min after consumption in the fasted condition. About 45-60 min elapses from the conclusion of drinking to the point at which the peak BEC is attained. The peak BEC was found to increase as the dose of ethanol was increased, but the time to peak was not greatly affected by different doses (Jones, Jonsson & Neri, 1991).

1.1.2. ETHANOL METABOLISM

It should be mentioned that although ethanol is mainly metabolized in the liver, a small proportion of an oral dose never enters the systemic circulation but is metabolized by oxidation in the stomach (DiPadova, Worner, Julkunen & Lieber, 1987). Gastric oxidation of ethanol will be referred to in greater detail in a later section.

There are three pathways for ethanol metabolism in the liver hepatocyte: alcohol dehydrogenase (ADH), the microsomal ethanol oxidizing system (MEOS), and catalase.

Genetic, environmental, endocrine and other factors such as diet, drinking history and smoking can influence all three of these metabolic pathways (Vesell, Page & Passananti, 1971; Kopun & Propping, 1977; Pohorecky & Brick, 1988). MEOS is the major non-ADH pathway for ethanol oxidation and is NADPH-dependent and linked to the cytochrome P450 oxygenases. The catalase and MEOS systems for ethanol metabolism will not be discussed further because they do not contribute significantly to any of the observed effects at the BECs attained in this study.

There are several different ADH isozymes which differ in their gene sequence and molecular and catalytic properties (Crabb, Bosron & Li, 1987). Multiple classes of ADH isozymes, of which Class I in the liver is thought to be the most important for ethanol metabolism, exist. There are three genes producing ADH_1, ADH_2 and ADH_3 enzymes which can dimerise to homo- or hetero-dimers. In addition, there is known polymorphic variation for ADH_2 (1,2,3) and ADH_3 (1,2). The enzymes produced by ADH_1, ADH_2 and ADH_3 are also referred to as alpha, beta and gamma ADH in some literature.

Ethanol metabolism by ADH occurs predominantly in the cytosol of the liver cell. Here, ethanol is converted to acetaldehyde by the ADH enzymes:

$$\textbf{ETHANOL} + \textbf{NAD}^+ \xrightarrow{\textbf{ADH}} \textbf{Acetaldehyde} + \textbf{NADH} + \textbf{H}^+$$

Ethanol is oxidized to acetaldehyde *via* hydrogen transfer from the substrate to the co-factor, nicotinamide adenine dinucleotide (NAD^+), which is reduced to NADH. Oxidation of NADH by the mitochondrial electron-transport chain is the means by which electrons from the reduced electron carriers of intermediary metabolism are channeled to oxygen and protons to yield H_2O. This oxidation, which is extremely important to the

metabolic processes of all higher organisms, is coupled to ATP synthesis in a process known as oxidative phosphorylation. The reoxidation of NADH is dependent on the oxidative phosphorylation rate, which, during ethanol oxidation, is limited by the availability of ADP (Bernstein, Videla & Israel, 1973). In ethanol metabolism, the reducing equivalents of cytosolic NADH are transferred into mitochondria *via* shuttle mechanisms involving oxaloacetate and malate:

(1) **Cytoplasm:** **Inner Mitochondrial Membrane:** **Mitochondrial Matrix:**

OXALOACETATE + NADH + H^+ **MALATE + NAD^+**

→ NAD^+ + MALATE ⇨ **→ OXALOACETATE + NADH + H^+**

(Dicarboxylate carrier)

(2) **Mitochondria:** **Inner Mitochondrial Membrane:** **Cytoplasm:**

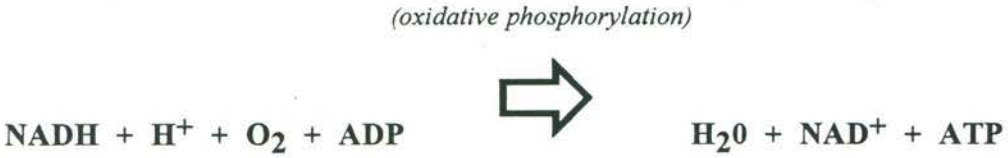
(Transported through the inner mitochondrial membrane)

OXALOACETATE ⇨ **OXALOACETATE**

(3) **Within the Mitochondrial Matrix:**

Reaction (1)...

...is coupled with oxidative phosphorylation:



Food or protein restriction has been shown to reduce ADH activity in rats by approximately fifty percent (Lindros & Hillbom, 1979). In contrast, the rate of ethanol metabolism can be increased acutely by carbohydrate (i.e. glucose and fructose) intake (Rogers *et al.*, 1987). The rate of ethanol metabolism in fasting subjects has been demonstrated to increase after glucose administration (Schmidt, Oehmichen & Pedal, 1987; Mascord, Smith, Starmer & Whitfield, 1988), although the mechanism of action has yet to be determined.

As mentioned earlier, there is also a first-pass effect during the absorption phase where gastric (ADH) metabolism of ethanol occurs. The effects of first-pass metabolism can be significant after low doses of ethanol, although there is a lesser effect in females (Frezza *et al.*, 1990). The first-pass effect is minimal in the fasting state (DiPadova *et al.*, 1987), possibly because of a shorter gastric emptying time and increased intestinal absorption, which may decrease the amount of ethanol which is available for metabolism in the stomach. More recently, it has been suggested that the mass of gastric mucosal

ADH available in the stomach is insufficient to play a major role in ethanol metabolism (Bye, Lacey, Gupta & Powell, 1966).

Acetaldehyde produced by ADH-catalyzed oxidation of ethanol is volatile, highly reactive and evanescent. There is normally rapid conversion of toxic acetaldehyde to the less harmful acetate, a process catalyzed by mitochondrial aldehyde dehydrogenase ($ALDH_2$). If any acetaldehyde escapes, it can be oxidized by the ALDH in the cytoplasm of the red blood cells and other peripheral tissues ($ALDH_1$). The acetate produced is finally converted to carbon dioxide and water, mainly in the extra-hepatic tissues.

1.1.3. ETHANOL ELIMINATION

The rate of elimination of ethanol is influenced by various factors including the activity of the ethanol-metabolizing enzymes in the liver. Enzymic activity encompasses both the concentration of enzyme (including the intrinsic activity of isozymes) and the metabolic and substrate conditions in the cells.

Ethanol elimination occurs mainly in the liver, begins before absorption has ended and proceeds at a practically constant rate once absorption is complete (Lundquist & Wolthers, 1958). At moderate BECs, the elimination rate is almost linear along the descending limb of the blood ethanol curve (Li, 1983). At low BECs, the rate of elimination of ethanol has been found to decrease in accordance with Michaelis-Menten saturation kinetics (Lundquist & Wolthers, 1958), although when BECs are high, ethanol is eliminated more rapidly (Salaspuro & Lieber, 1978).

The average rate at which the blood ethanol concentration curve declines in Caucasian subjects is about 15 mg dl^{-1} hr^{-1} (Harger & Forney, 1963) although this is highly variable among individuals. The total amount of ethanol which can be eliminated is about 6-9 g hr^{-1} in a normal, healthy subject.

Only two to ten percent of an ethanol dose escapes oxidation to be eliminated by the kidneys and lungs.

1.1.4. MALE VERSUS FEMALE RESPONSE TO ETHANOL

There are a number of differences between men and women in the pharmacokinetics and pharmacodynamics of ethanol, and also in the amounts which are habitually consumed. Females generally have a smaller volume of distribution per unit mass because of their higher body fat:body water ratio. Ethanol will not distribute into body fat and so the volume of distribution is smaller than in male subjects of the same body weight (Marshall, Kingstone, Boss & Morgan, 1983).

The rate of decrease of blood ethanol concentration was found to be similar for both sexes (Martin, Oakeshott, Gibson, Starmer, Perl & Wilks, 1985). Female subjects reached higher peak blood ethanol concentrations after a given dose of ethanol, and the time for complete elimination of ethanol was found to be shorter for the male subjects. These findings could be attributed to a greater gastric first-pass effect in men than in women (Frezza *et al.*, 1990).

A consensus has not been reached concerning the influence of the menstrual cycle on ethanol pharmacokinetics. Sutker, Allain, Brantley & Randall (1986) reported that females in the mid-luteal phase eliminated ethanol more rapidly than in the follicular and ovulatory phases. However, the peak blood ethanol levels attained after a given dose of ethanol did not differ as a function of menstrual cycle phase (Brick, Nathan, Westrick, Shapiro & Frankenstein, 1986). More recently, MacTavish (1992) reported that ethanol pharmacokinetics did not vary significantly over the menstrual cycle.

There is more consistent evidence to suggest that the behavioral effects of ethanol differ according to menstrual cycle stage. Ethanol-induced deficits in the psychomotor performance of women were found to be greater during the follicular phase of the menstrual cycle than at other times (Linnoila, Erwin, Ramm, Cleveland & Brendle, 1980). A reduction in reaction time on a digit symbol coding task from menses to pre-menses phases has been reported, together with a significant effect of menstrual cycle phase on critical flicker fusion frequency threshold (MacTavish, 1992).

Gender-based differences in speed-accuracy trade-off after ethanol have been found in the performance of subjects in tests of reaction time. Female subjects tended to

have a constant error rate, although this was at the expense of reduced speed, whilst the opposite was true for the male subjects (Martin *et al.*, 1985).

In addition, females given ethanol reported more negative subjective effects on mood, including feeling tired, when compared with males who reported being more relaxed, happy and alert after ethanol (Myrsten, Hollstedt & Holmberg, 1975).

The amount (frequency and quantity) of ethanol habitually consumed by men was found to be significantly greater than that of women (Weatherspoon, Danko & Johnson, 1994) although groups of heavy drinking women have been identified in Australian populations (Spragg, E. -personal communication, 1992). Waller & Blow (1995) reported that females are an increasing proportion of ethanol-involved drivers in both fatal and non-fatal crashes in the United States.

1.1.5. OTHER FACTORS

Many factors, other than those outlined above, have also been shown to influence the pharmacokinetics of ethanol in man. For example, the ethanol absorption rate decreases as the body temperature falls and as the level of exercise decreases (Agarwal & Goedde, 1990).

The age-related difference in ethanol pharmacokinetics which reflects, in part, changes in the total body water is also another factor that influences the effects of ethanol. Greenblatt, Sellers & Shader (1982) suggested that the increase in the proportion of adipose tissue which occurs with age might account for the age-related increase in sensitivity to ethanol. Tupler, Hege & Ellinwood (1995) found that elderly subjects exhibited significantly lower baseline performance levels than young subjects and achieved higher BECs with equivalent doses of ethanol. This was taken to indicate a need for caution in elderly people who engaged in neuromotor pursuits after drinking. This has not been a consistent finding, however, and Jones & Neri (1994) found no significant differences between the acute effects of ethanol in the sensory and motor functions of groups of men aged between 20 and 59 years.

1.2. EFFECTS OF ETHANOL IN GENERAL (CAUCASIAN) POPULATIONS

1.2.1. BREATH ETHANOL CONCENTRATION CURVES

A small fraction of the total amount of ethanol which is absorbed is expelled in expired air. Like all gases and volatile substances dissolved in the blood, ethanol distributes itself between pulmonary blood and alveolar air in the lungs. Given the ideal conditions for gas exchange at the alveolar membrane, a very large surface area, constant blood flow and a highly permeable diffusion surface, the ethanol concentration in alveolar air should reflect the pulmonary BEC (Jones, 1989). The value of the blood:breath ratio of ethanol at the alveolar-capillary membrane is not the same as the ratio deduced from analysis of blood and expired air. Various factors, such as the lower temperature of expired air, the haematocrit and the effects of oxalate and fluoride on the equilibration of ethanol between the blood sample and the head space, make the concept of a precise blood:breath ratio untenable (Jones, 1989). The use of a multiplier of 2300 permits an indirect estimate of the venous BEC from a breath measurement on a modern infra-red instrument.

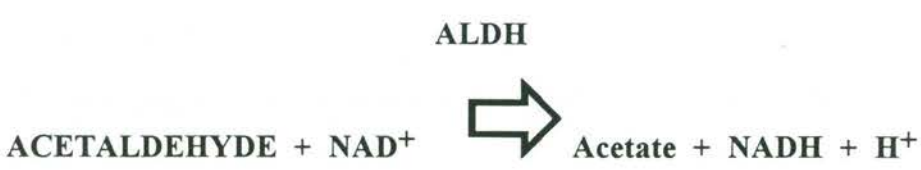
Breath analysis for ethanol, being both rapid and non-invasive, can be carried out at frequent intervals to determine the shape of the blood ethanol curve and hence allow calculations of the ethanol elimination rate, the apparent volume of distribution and the area under the curve, which is a measure of exposure to an ethanol dose.

1.2.2. EFFECTS ON ETHANOL METABOLITES

1.2.2.1. Blood Acetaldehyde

Acetaldehyde is a very potent, reactive and toxic first oxidative metabolite of ethanol. Because it is normally rapidly oxidized to acetate, acetaldehyde toxicity is

usually not manifested. The half-life of acetaldehyde is brief, being of the order of only 2.5-4 minutes (Ewing, Rouse & Pellizzari, 1974).



All the known pathways of ethanol oxidation in the liver result in the production of acetaldehyde. The pharmacological effects of acetaldehyde in the body are predominantly mediated *via* the release of vasoactive substances, including catecholamines (Eade, 1959). In many Asian populations, the release of these catecholamines causes the flushing and tachycardia which are manifest as the alcohol flush reaction (discussed further in section 1.5.).

Elevated acetaldehyde levels have been associated with ethanol aversion and there is a positive correlation between the elevated blood acetaldehyde levels which occur after ethanol ingestion and $ALDH_2$ deficiency in some Japanese people (Goedde & Agarwal, 1987).

ALDH inhibitors, such as disulfiram (Antabuse) and calcium carbimide are used in the aversion therapy of alcoholism. If patients who are receiving these drugs consume ethanol, the acetaldehyde which is produced accumulates instead of being rapidly metabolized, and causes extreme discomfort to the subject (Truitt & Walsh, 1971; Kitson, 1977). In a study using subjects who were receiving calcium cyanamide (a potent ALDH inhibitor) therapy, the elevated acetaldehyde levels which occurred after ethanol ingestion were found to increase heart rate and to decrease diastolic blood pressure (Peachey, Maglana, Robinson, Hemy & Brien, 1981). Catecholamine release, as a result of increased acetaldehyde levels, enhanced left ventricular function and caused peripheral vasodilation in flushing Asian subjects (Kupari, Lindros, Hillbom, Heikkila & Ylikahri, 1983).

The oxidation of acetaldehyde may be influenced by dietary manipulation. A low protein diet has been shown to decrease ALDH activity in rats (Lindros, Pekkanen & Koivula, 1977), although this did not increase blood acetaldehyde concentrations.

Some of the pharmacological effects of acetaldehyde are antagonistic to those of ethanol. For example, the catecholamine-releasing effect of acetaldehyde has the potential to counteract ethanol-induced sedation. Some drugs (e.g. penicillamine) can trap acetaldehyde and therefore decrease the levels in the circulation (Nagasawa, Goon, DeMaster & Alexander, 1977).

1.2.2.2. Plasma Acetate

Acetate is mainly formed in the liver from acetaldehyde and is oxidized further to carbon dioxide and water in the peripheral tissues. Endogenous acetate levels are normally low but after ethanol, the limited capacity of the liver to further oxidize acetate or to convert acetate to lipid causes plasma acetate levels to rise quickly to a plateau. Acetate concentrations have been found to increase up to twenty-fold after ethanol consumption (Lundquist, Tygstrup & Winkler, 1962; Israel, Orrego & Carmichael, 1994).

Adenosine, generated from the hydrolysis of adenosine monophosphate (AMP), has been implicated in mediating the effects of both ethanol and acetate. It has been suggested that the adenosine-acetate system modulates the motor effects of ethanol after social doses. After higher doses of ethanol, the importance of this system may be reduced because the production of acetate has been found to be maximal at low ethanol concentrations (Carmichael, Israel, Crawford, Minhas, Saldivia, Sandrin, Campisi & Orrego, 1991).

Acetate has been found to increase cardiac output, myocardial contractility and coronary blood flow (Liang & Lowenstein, 1978). However, acetate is much less toxic than acetaldehyde. After conversion to acetyl coenzyme A (acetyl CoA), which is one of

the major precursors of the Krebs Cycle, it becomes one of the more important metabolic intermediates.

1.2.2.3. Plasma Ethanol

The rate of increase of the plasma ethanol concentration is dependent on the route of administration. Much earlier peak BEC levels are attained when ethanol is given intravenously because the absorption step is eliminated (Rogers *et al.*, 1987). In general, ethanol is easily absorbed from all sites of administration and its distribution in the body is affected mainly by blood flow, vascularization and tissue water content (Greenblatt *et al.*, 1982).

The legal blood ethanol limit for drinking and driving in Australia (0.05 g per 100 ml) is equivalent to 10.9 mM ethanol.

1.2.2.4. Blood Lactate and Pyruvate - Derived Metabolic Products

The rate of production of lactate in all cells is determined by the rate of formation of pyruvate (with which the lactate is in equilibrium, because of the high activity of lactate dehydrogenase), and by the cytoplasmic redox potential, which influences the balance between lactate and pyruvate. The formation of pyruvate, in turn, is determined mainly by factors regulating glycolysis (Henry & Reed, 1974).

When ethanol is oxidized, the first step causes an increase of reducing equivalents (NADH) in the cytosol. This creates a redox potential shift which is reflected in a change in the lactate:pyruvate ratio. Therefore, the lactate:pyruvate ratio can act as an indicator of the redox status in the cytoplasm of liver cells. The pyruvate is converted to lactate *via* the liver in order to maintain a more constant NADH:NAD^+ ratio and to minimize changes in NADH concentration. Any excess lactate is released into the bloodstream and can be detected. An increase in the release of lactate into the circulation can cause metabolic acidosis, which can be further exacerbated, in severe cases, by hypoventilation resulting from ethanol-induced depression of the respiratory centre (Lieber, 1982).

The reoxidation of NADH is ultimately dependent on the rate of oxidative phosphorylation which, in turn, is limited by the availability of adenosine diphosphate (ADP); although shuttle mechanisms also play a part (Kuchel & Ralston, 1988).

Blood lactate concentration is normally about ten times that of pyruvate; increasing during ethanol metabolism. Lactate circulates in the blood at a concentration of about 1 mM and is removed from the circulation mainly *via* the liver. The liver then converts the lactate back into glucose or glycogen. Elevated levels of lactate are found in situations of lactic acidosis, exercise, and in some cases of liver disease. The reference range for pyruvate is less than 0.1 mM in normal healthy individuals (Gawehn, 1984; Noll, 1984; Drewes, 1974).

1.2.3. EFFECTS ON PHYSIOLOGICAL MEASURES

Ethanol ingestion causes a number of cardiovascular effects, including an increase in heart rate and peripheral vasodilation, although there is great variation among individuals. Blood pressure and pulse rates were found to be increased one hour after ethanol (0.75 g kg^{-1}) although by two hours, the blood pressure readings fell to below pre-ethanol values (Martin *et al.*, 1985).

Blood flow has been reported to increase in the skin of the face and extremities after ethanol consumption (Gillespie, 1967; Martin *et al.*, 1985) and an increase in the skin temperature results. The effect of these circulatory changes is an increase in heat loss and a subsequent fall in body temperature. Ethanol is thought to act on the thermo-regulatory mechanism and lower the hypothalamic set-point for temperature control but the fall in temperature is only slight due to the efficiency of the body's thermoregulatory mechanisms.

The vasodilator effects of ethanol are not exerted directly on the peripheral blood vessels but are mediated through the central nervous system (Morikawa, Matsusaka, Kuratsune, Tsukamoto & Mikisumi, 1968).

1.2.4. EFFECTS ON PERCEPTUAL, COGNITIVE AND PSYCHOMOTOR PERFORMANCE

The effects of ethanol on the central nervous system (CNS) are in many ways similar to those of the general anaesthetics, to which pharmacological class it belongs. An important difference however is, as explained above, that over 90% of the dose must be metabolized and since the metabolic capacity is limited, the duration of action of ethanol is rather protracted (Starmer, 1988).

Results from electrophysiological studies (Ritchie, 1985) have indicated that, like the general anaesthetics, the effects of ethanol are first manifested on the brain centres which are involved in the most highly integrated functions. The polysynaptic structures of certain cortical sites are particularly susceptible to disruption by ethanol (Himwich & Callison, 1972).

A fundamental property of the nervous system is the balance between excitation and inhibition. Ethanol appears to depress inhibitory functions somewhat selectively, with the net result that the cortex is released from central integrating control. Since the reticular formation has a key role in the control of sleep, wakefulness, attention and vigilance and, also regulates excitation and inhibition at the spinal level, the effects of ethanol are widespread. Thus, the analysis of sensory information, the control of intricate movement patterns, and memory, especially short-term memory, are all modified by ethanol.

Changes in CNS activity which follow the BEC are readily identifiable with the electroencephalograph (EEG). It was reported (Schwarz, Kielholz, Goldberg, Hobi, Gilsdorf, Hofsetter, Ladewig & Reggiani, 1980) that ethanol (0.8 g kg^{-1}) induced biphasic and possibly triphasic effects on both spontaneous background and stimulus-elicited activity. This consisted of stimulation in the absorption phase, depression in the elimination phase, and stimulation in the post-ethanol phase. A reduction of auditory-evoked responses was also found (Gross, Begleiter, Tobin & Kissin, 1966) after the ingestion of approximately 0.05 g kg^{-1} of ethanol and spinal motor neurones have been shown to be depressed (Megirian, Vasey & Posternak, 1958). Since peripheral nerves and muscle are much less sensitive to ethanol, it follows that the primary source of

disturbance in psychomotor performance is to be found in central mechanisms. These effects of ethanol can thus be conceptualized as involving alterations of the afferent input from the sense organs and/ or changes in the CNS, which confer a potential for disruption of the analysis of sensory information and the control of intricate movement patterns.

At the neurochemical level, ethanol has been found to have effects on membrane lipids and receptors and to disrupt the functions of neurotransmitters and second messengers, such as adenylate cyclase. The fluidity of biological membranes is well known to be decreased by high concentrations of ethanol which can, secondarily, influence the function of membrane proteins such as enzymes, receptors and ion channels. It is also likely that lower concentrations of ethanol may exert more subtle effects, such as the stimulation of phospholipid turnover. Ethanol has been reported to affect most neurotransmitter systems, which has led to a confusing picture. Current research interest centres on noradrenergic and GABA-ergic mechanisms (Johnston, G. - personal communication, 1987). It has been suggested that the GABA receptor-ionophore complex may represent a molecular substrate in the brain for the interactions between ethanol and many other drugs. The finding that acetaldehyde and biogenic amines are active at the opiate receptor sites has been suggested as a possible basis for ethanol dependency (Collins & Bigdell, 1975).

Ethanol causes changes in CNS function in a dose- and concentration-dependent manner (Moskowitz & Robinson, 1988). These changes have been exhaustively documented since the beginning of this century and include impairment of mental and cognitive ability, including judgment, and decreased perceptual and sensori-motor functioning (Jellinek & MacFarland, 1940; Wallgren & Barry, 1970).

It is generally accepted that significant deficits due to low doses of ethanol (producing BECs of about 0.05 g dl^{-1}) are readily identifiable and measurable in many tests of perceptual and motor skills related to those required in driving a motor vehicle. These deficits have been found to extend to simulated and actual driving impairment (Moskowitz & Robinson, 1988; Starmer, 1988).

As a general rule, the more demanding the task, in terms of the monitoring of sensory inputs, information processing, and the division of attention, the more likely is measurable impairment of performance to be encountered after small doses of ethanol. In practical traffic safety terms, this really means the detection of unforeseen hazards in time to correctly execute the appropriate emergency responses. There is probably no major conflict between the experimental findings on ethanol-induced performance deficits and the presumed role of ethanol in crash causation provided by epidemiological data.

A number of studies have demonstrated that impairment of performance persists for a considerable length of time after the complete disappearance of ethanol from the blood. These residual or 'hangover' effects have been described in laboratory measures of perception and psychomotor coordination (Kelly, Myrsten, Neri & Rydberg, 1970) and in driving simulator studies (Stening & Dureman, 1974). The residual effects of ethanol on closed-course driving performance were investigated in an experiment (Laurell & Tornros, 1982) where a car was driven at 50 km hr^{-1} around a cone-marked track. In addition, the subjects were required to react to an emergency signal and to carry out an avoidance manoeuvre. This task was considered to impose heavy demands on vigilance, reaction speed, and motor coordination. Performance measurements were made 8 hours after ethanol consumption had ceased, providing the BEC had declined to zero. The findings showed that all subjects hit more cones during the hangover period, with an average performance decrement of 20%. Also, the individual performance deficits did not correlate with subjective estimations of the severity of the hangover and were considered to be difficult to replicate (Lemon, Chesher, Fox, Greeley & Nabke, 1993).

Individual psychomotor functions are affected by ethanol to differing degrees. Generally speaking, cognitive functions have been found to be more resistant to the effects of ethanol than motor functions and, found to recover sooner (Goldberg, 1943).

Many variables have been shown to influence the effects of ethanol on psychomotor performance. In addition to the dose, the method of administration, age, previous experience with ethanol, practice on the task whilst intoxicated, stress, cigarette smoking, expectancy concerning an ethanol effect, food status and fatigue have all been

shown to influence the effects of ethanol on psychomotor performance (Moskowitz & Austin, 1983; Bennett, Cherek & Spiga, 1993; Fillmore, Mulvihill & Vogel-Sprott, 1994; Fillmore & Vogel-Sprott, 1995; Finnigan, Hammersley & Millar, 1995). Reduction of ethanol-induced impairment of psychomotor performance by the prior ingestion of food has been reported (Millar *et al.*, 1992). Both acute and chronic tolerance have been found to develop to the effects of ethanol (Franks, Starmer & Teo, 1977), reducing the effects of the drug over time.

Vision, especially dynamic visual acuity (Wallgren & Barry, 1970), glare resistance (Hogman, Bergman, Borg, Eriksson, Goldberg, Jones, Linde & Tengroth, 1977) and possibly colour perception (Rizzo, 1955) are all impaired by ethanol. The adverse effects of ethanol on eye movements include nystagmus (Aschan, Bergstedt & Goldberg, 1957) and a dose-dependent reduction in the efficiency of both saccadic (Wilkinson & Kime, 1974; Bittencourt, Wade, Smith & Richens, 1981) and smooth pursuit eye movements (Ali, Marshall & Richens, 1984). On the other hand, audition is relatively resistant to ethanol, except at high BECs (Eisenmenger, Schorn & Gilg, 1984). Ethanol reduces the sensitivity to odours and tastes (Goldberg, 1949) and, at high BECs, obtunds the sense of touch and raises the pain threshold and tolerance levels (Altounyan & Starmer, 1959).

Ethanol reduces vigilance (Moskowitz, Daily & Henderson, 1979), interferes with information-processing (Tharp, Rundell, Lester & Williams, 1974) and impairs memory - in particular short-term memory (Hutchinson, 1964; Ryback, 1971)- which influences the storage of information and its subsequent retrieval (Parker, Birnbaum & Noble, 1976). The effects of ethanol on mood and emotions are well-documented but are heavily influenced by expectancy (Rix, 1977) and were considered to be outside the scope of this review. Attention has therefore been placed on a discussion of the known effects of ethanol on the psychomotor parameters which have been monitored in this study.

1.2.4.1. Reaction Time

It has been reported by many groups that the effects of ethanol on reaction speed depend not only on the dose and the BEC attained but also on the demands and complexity of the task and the circumstances under which recordings are made. The more complex the circumstances, in terms of correct stimulus recognition and task-sharing, the more likely are significant ethanol-induced deficits to be encountered at low BECs (Jellinek & McFarland, 1940; Wallgren & Barry, 1970; Moskowitz & Robinson, 1988).

Reviews of the early literature (Carlson, Kleitman, Muehlenberger, McLean, Gulliksen & Carlson, 1934; Jellinek & McFarland, 1940; Wallgren and Barry, 1970) have all concluded that although it is well-known that simple reaction time can be increased by ethanol, the effect is only slight and a dose of at least 0.5 g kg^{-1} (to give a BEC of about 0.07 g dl^{-1}) is needed to produce a significant effect, and BECs of 0.15 to 0.20 g dl^{-1} must be attained before consistently measurable performance decrements are seen. Nevertheless, the effect of ethanol on simple reaction time is real and exhibits dose-dependency. With very low doses of ethanol, a slightly decreased simple reaction time has occasionally been reported (Forbes, 1947), usually in association with a greatly increased response variability, which is a feature of most ethanol effects (Starmer, 1988).

The questions of the nature and the intensity of the stimulus have also been examined. It has usually been found that ethanol has a greater effect on the response to auditory signals in comparison with visual signals (Dettling, 1956; Martin, LeBreton & Roche, 1957; Moreau, 1957; Chardon, Boiteau & Bogaert, 1959) and this difference also appears to apply in more complex situations (Joyce, Edgecombe, Kennard, Weatherall & Woods, 1959; Carpenter, 1959; Talland, 1966). It has been suggested (Carpenter, 1959) that under the influence of ethanol, the difference between the reaction times to simple auditory and visual stimuli is likely to be reduced. Evidence for an ethanol-induced decrease in differential sensitivity to two visual stimuli of different intensities has also been obtained (Lyon, Tong, Leigh & Clare, 1975). It was found that the more intense stimulus evoked a shorter reaction time, but that ethanol had a greater detrimental effect.

After an ethanol dose of 0.9 g kg^{-1}, there was no difference in reaction time to the two stimuli. An interesting finding (Lyon *et al.*, 1975) is that smokers, whether smoking or not, do not show the expected effects of ethanol on reaction time. The implications of these findings are of methodological importance.

Performance in choice reaction time tasks is well known to be more susceptible to disruption by ethanol (Carlson *et al.*, 1934) and significant decrements have been found after moderate (Lolli, Nencini & Misiti, 1964; Franks, Hensley, Hensley, Starmer & Teo, 1975) or even low (0.5 ml kg^{-1}) doses of ethanol (Warrington, Ankier & Turner, 1984). Dose dependency for this effect has been confirmed (Linnoila, 1973) but it is important to note that significant impairment does not always occur after low ethanol doses. For example, Bahnsen & Vedel-Peterson (1934) found that 30 min after a 0.5 g kg^{-1} dose of ethanol, the mean complex reaction time was significantly ($p<0.05$) shorter than that of a zero treatment group.

The possibility of error arises in choice reaction time tests and both error (Patay, van der Dreissche & Cormier, 1956; Moreau, 1957) and premature response rates have been found to increase after ethanol consumption (Cass & Frederick, 1961). The distribution of scores around the mean has often been found to be increased after ethanol, with the appearance of a much larger number of very long reaction times (Boyd, Morken & Hodge, 1962).

Choice reaction time tests represent relatively easy problems of discrimination and when greater demands of speed and/ or complexity are imposed on the subject, the effects of ethanol become progressively easier to demonstrate (Zirkle, King, McAtee & van Dyke, 1959). At a mean BEC of 0.05 g dl^{-1}, where performance in a choice reaction time was only slightly affected, the ability to compare certain characteristics of simultaneously presented stimuli was significantly reduced (Gruner, 1955).

When the demands of the task and the ethanol dose have been increased simultaneously, profound disruption of performance has been noted. For example, when a letter-canceling test was carried out at the same time as a complex reaction time task, ethanol dose: 1.0-1.2 g kg^{-1} (mean BEC=0.11 g dl^{-1}), reaction time increased more than

two-fold (Gruner, 1959; Rafaelsen, Bech & Rafaelsen, 1973; Rafaelsen, Bech, Christiansen, Christup, Nyboe & Rafaelsen, 1973). This indicates the importance of vigilance and division of attention in the assessment of ethanol effects, especially those which are related to driving. In divided attention situations, reaction times are invariably increased by ethanol (Rafaelsen *et al.*, 1973; Sugarman, Cozad & Zavala, 1973; Attwood, 1978).

Attwood (1978) investigated the effects of the functional separation of brake and turn signals at low BECs of 0, 0.02, 0.05, and 0.08 g dl^{-1}. Response errors and the latencies between the onset and the completion of the response were recorded. Performance was found to have deteriorated significantly at a BEC of 0.05 g dl^{-1} when both signals were combined, but only at 0.08 g dl^{-1} when they were separated.

Increased stimulus-response uncertainty after ethanol consumption at BECs of about 0.1 g dl^{-1} has been reported in subjects required to state the location of a spot projected on a screen (Robinson & Peebles, 1974). Ethanol-associated increases in localization time became greater as the central processing demands increased, particularly when the stimulus-response associations were novel. Thus, ethanol was considered to retard the selection of a response rather than stimulus recognition *per se*. Performance deficits under ethanol (0.25 and 0.5 g kg^{-1}) were found to depend on the interaction between task difficulty and compatibility, with the latter having a greater effect in more difficult tasks (Sutton & Burns, 1971).

A number of reports have indicated that impairment of reaction time by ethanol is not an obligatory relationship and can be overcome by the manipulation of factors such as motivation. Finger extension and flexion reaction times to visual and auditory signals (with performance feedback) were measured (Obitz, Rhodes & Creel, 1977) before and after ethanol (BECs: females -0.03 and 0.06 g dl^{-1}; males -0.02 and 0.04 g dl^{-1}). Reaction times were shorter for the auditory signals and the flexion and extension responses were approximately equal. Ethanol impaired the responses of the female subjects, but not of the male subjects. This finding was attributed to the competitive attitude of the males to the feedback and was taken to suggest that the effect of ethanol was not necessarily on

reaction time *per se*, but rather on factors which interact with it. After ethanol (mean BEC=0.09 g dl^{-1}), reaction time was adversely affected in a low, but not in a high, motivation condition (Heacock & Wikle, 1974). It was suggested that an increase in reaction time occurred as a result of inattention which could be overcome by positive reinforcement.

However, factors other than motivation may also operate. Impairment of reaction time was found in both a placebo group and a group treated with a moderate dose of ethanol (Huntly & Centybear, 1973). Since impairment in distance judgment was found in the ethanol-treated subjects, it was suggested that impairment of reaction time might be explicable as a misperception of when to react. An attempt was also made (Robinson & Peebles, 1974) to determine whether ethanol-induced increases in the reaction time to extrafoveal stimulation became greater as the stimulus eccentricity increased. The influence of a concurrent high-priority foveal task was also investigated. Measurements were made at BECs of 0.01, 0.05 and 0.10 g dl^{-1} and, although an increase in reaction time was found, it was independent of target eccentricity, which is contrary to the findings of Hamilton & Copeman (1970). Reaction times in a key-pressing task were tested at mean BECs of 0, 0.011, 0.037 and 0.055 g dl^{-1} (Shillito, King & Cameron, 1974). The task load was systematically varied and there was some evidence that the accuracy of performance was impaired at BECs of 0.037 and 0.055 g dl^{-1}, however at 0.011 g dl^{-1}, there was a slight facilitation. It was suggested that tasks where the main criterion is accuracy of performance are more likely to be sensitive to ethanol than those which require information processing. It was also noted that subjects used different strategies to maintain performance and that there was often a willingness to trade off accuracy against speed.

It has proved possible (Jennings, Wood & Lawrence, 1976) to distinguish reaction time effects resulting from performance efficiency from those involving speed-accuracy changes. Ethanol (0, 0.33, 0.67, 1.00, and 1.33 ml of 97% ethanol kg^{-1}) progressively reduced performance efficiency by decreasing the rate of increase of accuracy per unit time. Changes in speed-accuracy criteria were combined with a

decrease in efficiency at the higher ethanol doses. This suggested that if mean reaction time is considered without respect to accuracy, ethanol produces no discernible decrement in performance. When accuracy is considered, however, subjects may maintain a roughly constant reaction time performance by sacrificing accuracy. A gender difference in speed-accuracy trade-off has also been reported in reaction speed performance under ethanol (0.75 g kg^{-1}). Female subjects tended to keep their error rate constant at the expense of reduced speed and the reverse was true in the male subjects (Jennings *et al.*, 1976; Martin *et al.*, 1985).

Thus, ethanol appears to have a small effect on simple reaction time which is progressively increased when choice and information-processing components are added. In methodological terms, failure to record accuracy will result in an inability to detect speed-accuracy trade-off and, in consequence, to detect a decrement in performance.

1.2.4.2. Digit Symbol Coding (DSC)

Digit symbol coding is a test of perceptual motor speed which involves sustained attention on a highly repetitive task. It is a measure of the time with which subjects take to respond to a complex visual stimulus and involves both decision making and reaction to a stimulus. It should be treated as a complex reaction time task.

Digit symbol coding is well-known to be very sensitive to ethanol, with performance deficits occurring at BECs as low as 0.03 g dl^{-1} (Moskowitz & Robinson, 1988), that is, the reaction times increase after ethanol consumption.

1.2.4.3. Tracking

Most of the early studies (Jellinek & McFarland, 1940) found that small doses of ethanol (0.3-0.4 g kg^{-1}) had no statistically significant effects on tracking performance, but inter-subject variability was usually more prominent. The average performance deficit increased progressively as the BEC rose from 0.06 to 0.18 g dl^{-1} (Newman, Fletcher & Abramson, 1942; Lambercier & Martin du Pan, 1946) although some subjects showed no impairment of tracking performance at a BEC of 0.1 g dl^{-1}. Nevertheless, tracking tasks

have generally been considered to be ethanol-sensitive. In their review, Wallgren and Barry (1970) noted that there was general agreement that ethanol degrades tracking performance, although simple compensatory tracking tasks, which are directly comparable to steering a motor vehicle, have often not exhibited a performance deficit under ethanol.

Moskowitz (1973) drew attention to the fact that performance in tracking tasks has not always been found to be reduced after ethanol when this has been the sole task. Although compensatory visual-manual tracking performance was found to be progressively degraded (Reid & Ibraham, 1975) with increasing BEC, there was only a 15% decrement in tracking performance at a BEC of 0.100 g dl^{-1} if there was no requirement for either speed or accuracy (Crancer, Dille, Delay, Wallace & Haykin, 1969). At a mean BEC of 0.100 g dl^{-1}, compensatory tracking performance was not impaired unless an intermittent subsidiary task was added (Chiles & Jennings, 1970). The nature of the subsidiary task may be very important in determining the response to ethanol. Subjects who attained a peak BEC of 0.08 g dl^{-1} exhibited a greatly increased reaction time to one stimulus (meter level change), but not to another (warning light) during a continuous tracking task (Pearson, 1968).

The introduction of additional stressors has also been found to increase the sensitivity of the tracking tasks to ethanol impairment. It has been demonstrated that although no impairment was produced by ethanol in the stationary condition (mean BEC=0.074 g dl^{-1}), impairment was unmasked when the subjects were subjected to 48 s cycles of angular acceleration (Collins, Schroeder, Gilson & Guedry, 1971). It was suggested that although there may be little or no impairment in a static situation, performance may be seriously degraded during motion. This has an obvious relevance to the driving situation.

Pursuit tracking, probably because it involves monitoring two or more sources of information (Moskowitz, 1973), appears to be more sensitive to ethanol than compensatory tracking, and performance deficits have usually been found at BECs within the range 0.05-0.09 g dl^{-1}. A significant deficit in tracking performance was found at

mean BECs of 0.05 to 0.06 g dl^{-1} (Mortimer, 1963; Hughes & Forney, 1964). The relationship between ethanol dose (to produce peak BECs of 0, 0.025, 0.050, 0.075 and 0.100 g dl^{-1}) and pursuit-tracking performance deficit was explored and the response was shown to be BEC-dependent (Evans, Martz, Rodda, Kiplinger & Forney, 1974). Performance was further degraded when a sub-task (responding to lights) was included.

A dose-response relationship for ethanol-induced impairment on a combined tracking task has also been found, with a reduction in performance at BECs as low as 0.03 g dl^{-1} (Loomis & West, 1958). Where division of attention between a pursuit tracking and a signal detection test was required, a differential effect was found for ethanol (von Wright & Mikkonen, 1970). Signal detection was impaired by both 0.4 and 0.8 g kg^{-1} doses of ethanol, however, reduced pursuit tracking ability only occurred after the higher dose. In another study (Hamilton & Copeman, 1970) a similar combination of tests was used and it was found that the ethanol dose had to be increased to 0.63 g kg^{-1} before significant impairment occurred. Again, the introduction of noise as an additional stressor, resulted in the appearance of a performance deficit after a much lower dose (0.21 g kg^{-1}) of ethanol. Subjects with a mean BEC of 0.06 g dl^{-1} showed performance impairment in a combined pursuit tracking and cue-recognition task (Binder, 1971).

A critical tracking task, where the controlled element is unstable and the instability increases with time has been described (Klein & Jex, 1980). Following ethanol dosage, there was a reduction in the level at which the subject ceased to be able to track. At a BEC of 0.1 g dl^{-1}, 90% of subjects had impaired performance. Analysis of error responses in a critical tracking task revealed statistically significant impairment of performance at BECs as low as 0.05 g dl^{-1} and the effective time delay was also adversely affected (Dott & McKelvey, 1977). Highly significant ($p<0.001$) impairment was found in this task after low (0.54 g kg^{-1}) and moderate (0.75 g kg^{-1}) doses of ethanol (Peacock, 1991).

Not all studies have demonstrated ethanol-induced impairment of tracking ability however. Using a pursuit step-tracking apparatus (Gibbs, 1966), it was found that ethanol (peak BEC=0.10 g dl^{-1}) gave impairment on the improbable, but not on the

probable, steps. This finding could not be confirmed by others at a lower (approximately 0.05 g dl^{-1}) BEC (Landauer, Milner & Patman, 1969; Linnoila *et al.*, 1980).

The effects of small doses of ethanol (to produce BECs of 0.002, 0.024 and 0.057 g dl^{-1}) on the rate of decision making in a modified tracking task have been studied (Verhaegen, van Keer & Gambert, 1975). Two parallel lines were generated in a computer display to represent a road, and a cursor, operated by a steering wheel, was under the control of the subject and had to be kept between the two lines. The "road' curved in an unpredictable way and variation in task difficulty was also incorporated in the design, with a random 15 s of each minute being considered 'critical'. The number of times 'off road' and dwell time 'off road' were recorded for both critical and non-critical periods. From the findings it could be seen that there was a strong tendency for longer dwell times 'off road' to occur in the high ethanol condition, although there was no difference in the number of errors. Further analysis indicated that during the critical periods, both the number and duration of errors were significantly greater after ethanol consumption. The effect of the low ethanol dose did not reach statistical significance, except for the number of errors in the non-critical periods, which may reflect reduced vigilance.

The influence of task proficiency on ethanol-induced tracking performance impairment (0.88 ml of 96.4% ethanol kg^{-1}) was investigated and was found to be negligible, with sizeable deficits occurring in all groups (Bierness & Vogel-Sprott, 1982). Further studies, using both higher and lower ethanol doses, would appear to be warranted before a proficiency effect can be entirely dismissed.

1.2.4.4. Division of Attention

Divided attention has been repeatedly shown to be sensitive to the effects of ethanol. In a letter-cancellation task performed concurrently whilst responding to lights in the periphery, it was found that moderate doses of ethanol caused both an increase in reaction time to the peripheral flashes and a decrease in the accuracy of the cancellation task (Gruner, 1959; Gruner, Ludwig & Domer, 1964). Ethanol has also been shown to

have differential effects on auditory vigilance in a task where division of attention was required (Moskowitz *et al.*, 1979). After an ethanol dose of 0.5 g kg^{-1} (BEC=0.07 g dl^{-1}), there was no impairment in the vigilance condition, although it became highly significant when attention was divided. Dose-response studies indicated that only slight vigilance deficits occurred at a BEC of 0.1 g dl^{-1}, but deficits in the divided attention condition appeared at BECs as low as 0.015 g dl^{-1}.

It was suggested (Moskowitz & Austin, 1983) that one of the reasons-in-common for ethanol-induced deficits in the division of attention and in skills performance may be a decrease in the rate of information processing. The degree of inconsistency in the findings obtained to date may relate to differences in task demands and the various strategies adopted by subjects to deal with them. Under the influence of ethanol, there is a general tendency to shift the focus of main attention to the central region and also to increase attention to one sub-task at the exclusion of others. These suggestions have recently been reinforced by Koelega (1995). In real life, the reward/ penalty aspects in a particular situation would be included and would represent a learned modification of behaviour.

1.2.4.5. Standing Steadiness

One of the most noticeable effects of ethanol intoxication is that of a staggering gait (ataxia). Therefore, it is not surprising that attempts have been made to assess the extent of ethanol intoxication in terms of body sway. One of the most sensitive tests for detecting ethanol-induced impairment remains the Romberg test, in which the subject is instructed to stand as steady as possible with the eyes closed, so that the kinesthetic and vestibular senses provide the only sensory information. Body sway is then observed or measured (Wallgren & Barry, 1970).

The most prominent body sway movements have been found to occur in a common transverse axis of the ankle joints. Thus, the greatest sway occurs in the antero-posterior plane (Eklund & Lofstedt, 1970). Subjects have been found to sway both from the head as well as the hips (Edwards, 1942). Thomas & Whitney

(1959) showed that the trunk also rotates relative to the limbs. Eklund & Lofstedt (1970) used signals from a force transducer mounted beneath a platform to detect the pressure changes in the antero-posterior plane and found that they showed irregular, low frequency oscillations mostly below one Hertz. These oscillations were due to changes in the common centre of gravity on the area of support. In addition, faster oscillations of low amplitude reflected dynamic contractions of the leg muscles serving equilibrium regulation (Murray *et al.*, 1967). The CNS structures which regulate posture and equilibrium have been shown to have important reflexes in the basal ganglia and to receive information from a number of afferent (sensory) systems, of which somatic proprioceptors appear to be the most important (Martin, 1967). Ethanol effects on standing steadiness are believed to be a consequence of the impairment of these vestibulospinal reflexes.

Standing steadiness is thus predominantly an indicator of cerebellar function (Linnoila, Stapleton, Lister, Guthrie & Eckhardt, 1986) and remains a popular test for field sobriety because of its low threshold for ethanol impairment. It is also a non-learnable task which measures 'state' rather than 'trait' changes in subjects (Tiplady, 1988).

There have been many attempts to measure body sway ranging from rather crude observation methods to the use of relatively sophisticated electronic apparatus. In addition, there have been many modifications of the Romberg test, such as having the eyes open, eyes closed, placing one foot in front of the other, or raising one foot above the ground to increase the magnitude of body sway (Wallgren & Barry, 1970). The threshold for impairment appears to be at a BEC of about 0.03 g dl^{-1} (Hebbelinck, 1963). A number of modified statiometry techniques have identified that age-related differences occur in the response to ethanol, with younger subjects being affected to a greater extent (Wilson, Barboriak & Kass, 1970). A gender difference in response has also been reported (Martin *et al.*, 1985), in that the increase in body sway following ethanol ingestion (0.75 g kg^{-1}) was much more marked in young females than young males (mean age=23.1 years).

1.2.4.6. Critical Flicker Fusion Frequency (CFFF)

Intermittent light is perceived as flicker. If the frequency of flicker is increased, the flicker sensation becomes gradually less distinct until the separate flicker sensations fuse (fusion threshold). Alternatively, if the flickering is supra-threshold and is gradually decreased, the point at which flicker is perceived is known as the frequency threshold (Curran, 1990). Measurement of the critical flicker fusion frequency threshold (the average of the fusion and flicker thresholds) is now a widely used technique in psycho-pharmacology (Kranda, 1982).

A simple model of information-processing and the relationship to CFFF threshold has been advanced by Hindmarch (1982). CFFF threshold is seen as a measure of the integrative capacity of the CNS or the ability of the CNS to process discrete portions of information. The CFFF threshold can be influenced by a number of intrinsic (subject) and extrinsic (instrument and environmental) variables which have been extensively reviewed (Curran, 1990). If these variables are not controlled, it may be impossible to determine whether an observed change in CFFF threshold is drug-induced and/ or is the result of some other factor. Apart from extrinsic variables relating to the test apparatus, most extrinsic variables can be adequately controlled by choosing an appropriate light intensity, luminance of the surround, perceived brightness, area of stimulus, environmental illumination, stimulus colour, light-dark ratio, dark adaptation, wave form, starting point (ascending versus descending), continuous and discontinuous and duration of exposure (Curran, 1990).

The continuous method of limits is a quick and easy method for determining the CFFF threshold. The use of modern equipment with microchip-controlled flicker (such as the Leeds Psychomotor Tester, as used in these experiments) obviates the necessity to control the extrinsic variable. The intrinsic variables are, of course, controlled by standardized experimental procedures (see *Methods* section).

The CFFF task thus measures the level of mental alertness and cognitive potential of the subject. It is a simple, reliable, highly sensitive and non-learnable test which can be used to assess psychomotor performance impairment. Like body sway, CFFF is a non-

learnable task which measures changes of 'state' rather than 'trait' in subjects (Tiplady, 1988).

A number of drugs which are known to cause clinical sedation (including ethanol - Goldberg, 1943; 1949) are well-known to produce a reduction in CFFF threshold scores, and those causing clinical arousal produce an elevation of the CFFF threshold (Smith & Misiak, 1976; Hindmarch, 1982). Ethanol, which has many of the attributes of a general anaesthetic, has consistently been found to decrease the CFFF threshold value (Rosketh & Lorentzen, 1954; Yap, 1990).

Anxiety has also been found to affect the CFFF threshold value. Subjects who are highly anxious have been found to have lower CFFF threshold values than low-anxiety subjects (Hindmarch, 1979).

1.2.4.7. The Driving Situation

Ethanol-induced impairment found in most of the psychomotor performance tests described above indicates a potential for driving impairment. Although the studies described in this thesis do not specifically address the question of ethanol-induced deficits in driving performance, the relevance is obvious and deserves comment. Moreover, the subjects in this study were routinely asked whether they would be prepared to drive a motor vehicle (in their present condition) and their responses were recorded and analyzed.

It is well known that the consumption of ethanol can cause increased aggression and risk-taking behaviour at a time when perceptual and coordinative abilities are also reduced (Starmer, 1988). People who drink and drive are thus much more likely to commit traffic violations and to be involved in vehicle crashes as a result.

Driving a motor vehicle is a complex multi-functional task involving many aspects of psychomotor performance, such as visual search and recognition, vigilance, information-processing under variable demand, decision-making and risk-taking. Driving is also an overlearned task, where practice has obviated the need for conscious recall and where critical high demands are very infrequent. Impairment of driving has been defined,

in a general sense, as the failure to exert the expected degree of prudence or control to ensure safe operation of the vehicle under the traffic conditions pertaining at the time.

Only small doses of ethanol are required to adversely affect the ability of a driver to react quickly, correctly and in a coordinated manner to an unexpected emergency (Starmer, 1988). It should be noted, however, that such alterations of behaviour are not specific to ethanol and can also be caused by distraction, emotional lability, aggression, fatigue, physical or mental illness and many other factors, including age or prescription drug use, which can have complex interactions (Starmer, 1988). The medical and social costs of transport accidents to the community are immense, $6.6 billion in Australia in 1988 alone, and road accidents are the major ($6.1 billion) contributing factor (Bureau of Transport Communications, 1992). It is revealing to note that more years of life during the working age span were lost due to road accidents than through all forms of heart disease (Federal Office of Road Safety, 1991).

The legal BEC limit permitted for drinking and driving (excluding novice and bus and truck drivers) in Australia is 0.05 dl^{-1}, at which level the risk of crash involvement is approximately doubled (Borkenstein, Crowther, Schumate, Ziel & Zylman, 1974; McLean, Holubowycz & Sandow, 1980).

At lower BEC levels, the adverse effects of ethanol are more difficult to demonstrate unequivocally. However, in general, as has been discussed in relation to discrete psychomotor measures, as the complexity of the situation increases, the adverse effects of ethanol tend to become more prominent (Asknes, 1954; Starmer, 1988).

1.2.5. SUBJECTIVE RESPONSES

There is a considerable body of literature which deals with the effects of ethanol on mood but there are very few findings which can be directly applied to ethanol-induced impairment of driving or driving-related skills (Starmer, 1988). Changes in mood would however, intuitively be expected to exert an influence on psychomotor performance. Even small doses of ethanol produce responses which can be identified by the use of

appropriate visual analogue scales. Caucasian subjects are usually relaxed, happy, alert and confident after drinking small amounts of ethanol. However, it has also been reported (Warrington *et al.*, 1984) that a small dose of ethanol (0.39 g kg^{-1}), which produced a peak BEC of 0.03 g dl^{-1}, significantly increased drowsiness, reduced 'clear-headedness' and also tended to reduce feelings of aggression. Importantly, such effects might also be expected to have reduced motivation to perform optimally in the tests. These findings, which were obtained in Caucasian subjects, are in direct contrast to those in many subjects of Asian extraction who report various symptoms of discomfort and malaise after drinking only small amounts of ethanol. These symptoms include flushing, dizziness and a pounding in the head, which are rarely, if ever, reported by Caucasian drinkers (Ewing *et al.*, 1974).

1.3. RACIAL DIVERSITY

There is great diversity among different ethnic groups in their attitudes towards ethanol and in their patterns of ethanol consumption. Alcoholic beverages are totally prohibited in some Islamic countries whilst being extensively 'promoted' in countries like Australia. Traditional values usually determine approved drinking patterns within the mainstream of a given society and these can vary from regarding ethanol as having medicinal properties and only to be consumed on certain special occasions, to the position of being a 'social lubricant' which is to be consumed as often as possible.

Rapid social changes, consequent upon economic and industrial success, may also be associated with the increasing use of ethanol by a greater proportion of the younger generation, especially young males, in countries which have not been traditionally regarded as high ethanol consumers. In Taiwan, an increasing incidence of ethanol abuse has been reported in the 18-24 year age group (Yu & Liu, 1986). Consumption of ethanol in China has increased by over 20% during the last ten years. A decade ago, the Chinese market was ranked thirteenth in the world; today it is the second largest with almost ten percent of the world market. While *per capita* consumption of ethanol is still only a fraction of that in more mature markets, consumption in China is expected to continue to rise, given the growing affluence of the 1.2 billion people who, increasingly, regard beer as a staple rather than a luxury item (Baker, 1994).

Genetic factors, as mentioned earlier, are now believed to play an important part in influencing the considerable inter-individual variability found with respect to ethanol sensitivity and in the metabolism of ethanol. This may be explained in part due to the enzymes which catalyze ethanol and acetaldehyde metabolism exhibiting a genetically-determined heterogeneity (isozymes and enzyme polymorphisms) which leads to the large variety of individually different enzyme phenotypes (Harada, Agarwal & Goedde, 1980; Mizoi, Tatsuno, Adachi, Kogame, Fukunaga, Fujiwara, Hishida & Ijiri, 1983; Goedde &

Agarwal, 1989). Genetic variation in alcohol and aldehyde dehydrogenases is discussed in further detail later.

There now follows a brief historical perspective of the more important developments which have occurred as a result of studies on the effects of ethanol on Asian populations, followed by a more detailed description of the effects of ethanol on the majority of Asians -namely, the alcohol flush reaction itself.

1.4. HISTORICAL PERSPECTIVE AND MORE RECENT DEVELOPMENTS

" Red cheeks, grey beard;
This is me after drinking wine... "

-Bai Juyi

As early as the Tang Dynasty (618-907 AD) Bai Juyi, a well-known Chinese poet, had already included in at least one of his numerous poems, the characteristic symptoms of the alcohol flush reaction. Other references to 'red or rosy cheeks and drowsiness after consuming wine' abound in early Chinese classical literature, some dated even before the Tang Period. Hence, it can be seen by evidence of these early records, that the Asian reaction to ethanol has been popularly described by many, and as these poets have been known to painstakingly record various aspects of everyday life in their pieces, it can be inferred that, even then, the majority of people would be aware of the symptoms of the alcohol flush reaction.

Since then, much information has been obtained from the first descriptions of the alcohol flush reaction, including the discovery and subsequent studies on ADH and ALDH and the role of acetaldehyde, but many questions remain unanswered. This section of the thesis presents a chronological account of research progress in this field up to the present day.

1946-62 Perhaps the first relevant observation recorded was that of LaBarre (1946) who commented on the low incidence of alcoholism in Chinese villagers in the 1940s and speculated on the existence of differences in ethanol sensitivity among different populations. Almost twenty years later, when it was known that differences in reactions to drugs occurred in different racial groups, it was suggested that perhaps ethanol sensitivity might be similarly influenced (Kalow, 1962).

1972-73 The first published report on the ethanol flushing reaction was that of Wolff (1972) who found a greater ethanol sensitivity in Asian subjects than in Caucasians. This applied to adults as well as to full-term infants. The newborn infants exhibited a similar pattern of response to ethanol as the adults, which included an increased pulse pressure. This led to the conclusion that racial differences in ethanol sensitivity could not be explained in terms of cultural or dietary factors. Further studies by Wolff (1973) established that ethnicity was the main determinant of the ethanol flushing reaction. The incidence and intensity of flushing was found to be significantly greater in the Asian groups than in the Caucasian group. Moreover, the increase in pulse pressure and skin temperature was similar in half-Mongoloids as in those of 'pure' extraction. Hence, being born and raised in a Western society, and consuming entirely Western type diets did not affect the way subjects of Asian extraction responded to ethanol. It was concluded that the prevalence of the flushing response would inhibit the Asian groups from drinking as long as their social structure remained intact. However, if the social cohesion of a culture was broken down, a greater susceptibility towards ethanol intoxication might cause problems of alcoholism, as argued in the case of the native American Indians.

1974 Ewing *et al.* (1974) also measured the blood ethanol levels which were attained after ethanol administration to establish whether differences in ethanol absorption or metabolic rate could have influenced Wolff's findings. However, although no evidence for absorptive or metabolic differences were found, Ewing *et al.* (1974) confirmed that after ethanol, the Asian subjects' heart rates increased to a greater extent than Caucasian controls. Also, the blood pressure of the Asian subjects decreased to lower levels and there was significantly more skin flush in Asian than in Caucasian subjects. A similarity between the flushing response of some of the Asian subjects and the disulfiram reaction was also noted and the possible involvement of acetaldehyde was suggested.

1975-76 In 1975, Stamatoyannopoulos, Chen & Fukui postulated that an 'atypical' alcohol dehydrogenase (now more specifically known as ADH2-2) might be the source of the ethanol sensitivity responses which were found in subjects of Asian descent. The atypical ADH, found in about 90% of Asians, caused an unusually rapid acetaldehyde formation after ethanol. The following year, racial differences were demonstrated in the peak BEC which was attained after a given dose of ethanol per unit body weight (Reed, Kalant & Gibbins, 1976). There were also differences detected in acetaldehyde levels across different racial groups after ethanol.

1977-79 In 1977, two forms of aldehyde dehydrogenase -$ALDH_1$ and $ALDH_2$, were found in human liver tissue (Greenfield & Pietruszko, 1977). It should be noted here that although the nomenclature of ALDHs has been somewhat confusing in the past, the cytoplasmic form is now commonly known as $ALDH_1$ and the mitochondrial form as $ALDH_2$. Approximately fifty percent of a group of Asians were found by Harada, Agarwal & Goedde (1978) to have no mitochondrial $ALDH_2$ activity in their livers. The presence of elevated levels of acetaldehyde in Asian subjects who exhibited the flushing response was confirmed by Mizoi, Ijiri, Tatsuno, Kijima, Fujiwara, Adachi & Hishida (1979).

The fact that the ethanol sensitivity response occurred too infrequently to be adequately explained by an atypical ADH alone was also addressed by Mizoi *et al.* (1979). More than half of their Japanese subjects were found to lack $ALDH_2$, in contrast to the finding that all Caucasian subjects had both $ALDH_1$ and $ALDH_2$ (Goedde, Harada & Agarwal, 1979). This led to the suggestion that Asian individuals might experience ethanol sensitivity because they could not metabolize acetaldehyde quickly enough, because of a lack of the $ALDH_2$ isozyme, as opposed to the proposition that they produced more acetaldehyde after a given ethanol dose because they had an atypical ADH with a higher activity than had been previously supposed.

1982-83 Further studies found that apart from the Japanese, some Chinese, Vietnamese, Thai and South American Indians also lacked $ALDH_2$, although in differing frequencies. In 1982, all the members of a group of Japanese alcoholics were found to possess both $ALDH_1$ and $ALDH_2$ isozymes. This finding, which is in sharp contrast to the high incidence of a lack of $ALDH_2$ in the Japanese population at large, then led many researchers to consider that a lack of $ALDH_2$ might play a role in protecting the individual concerned against excessive drinking and alcoholism (Harada, Agarwal, Goedde, Tagaki & Ishikawa, 1982). Goedde, Agarwal, Harada, Meier-Tackmann, Du, Bienzle, Kroeger & Hussein (1983) also drew attention to a possible correlation between $ALDH_2$ deficiency and ethanol sensitivity in Asian subjects.

1984-87 Elevated breath acetaldehyde levels were correlated with increased body sway, nystagmus and facial flushing (Truitt, Rowe & Mehl, 1984). Body sway was found to be markedly elevated in Asian subjects, even after low doses of ethanol. Acetaldehyde has traditionally been considered to be difficult to measure in biological fluids. However, improved methods have now been developed for measuring acetaldehyde levels in blood using high performance liquid chromatography and sensitive fluorescence detectors (Peterson & Polizzi, 1987; Ung-Chhun & Collins, 1987).

Park, Huang, Nagoshi, Yuen, Johnson, Ching & Bowman (1984) differentiated between fast (one drink or less to evoke flushing) and slow (two drinks or more to flush) flushing, and suggested that fast flushing appeared to be a distinct phenomenon and might have a substantial influence on ethanol use. The possibility that fast flushing might be an early warning system in certain individuals was also raised. Earlier research findings on the relationship between $ALDH_2$ deficiency and the flushing response, and between $ALDH_2$ deficiency and moderate drinking practices were confirmed by Ohmori, Koyama, Chen, Yeh, Reyes & Yamashita (1986).

Wilkin & Fortner (1985) reported that individuals who flush after an oral ethanol dose were more likely to have cutaneous erythema after topical ethanol application. By using the ethanol patch test (considered by some to be a cutaneous model

of flushing), individuals deficient in $ALDH_2$ could be detected. The mechanism of action is believed to involve vasodilation due to the accumulation of acetaldehyde in the skin because both ADH and ALDH occur in cultured skin fibroblasts (Goedde *et al.*, 1979).

Synthetic oligonucleotide probes, encoding a portion of the subunit of ADH, have been used to facilitate molecular studies on human gene expression (Duester, Hatfiield, Buhler, Hempel, Jornvall & Smith, 1984). A full length cDNA coding for human ADH was cloned by Ikuta, Fujiyoshi, Kurachi & Yoshida in 1985. The cDNA for $ALDH_2$ has also been cloned and sequenced. Molecular abnormality of inactive $ALDH_2$ was found to be due to the substitution of Glu by Lys (G to A transition in the gene) at the fourteenth position from the COOH-terminal (Yoshida, Ikawa, Hsu & Tani, 1985). The molecular and structural analysis of the ADH and ALDH genes has continued since then (Duester, Smith, Bilanchone & Hatfield, 1986; Ikuta, Szeto & Yoshida, 1986; Jornvall, 1987).

1988 In 1988 Xu, Carr, Bosron, Li & Edenberg described the first direct determination of all known polymorphisms at both the ADH_2 and ADH_3 loci, using white blood cell DNA. The development of this method meant that liver biopsies were now not required to determine ADH phenotype. Instead, subjects were easily genotyped by amplifying their DNA using the polymerase chain reaction, combined with restriction enzyme analysis detection methods or with the use of appropriate allele-specific oligonucleotide probes (ASOPs).

1989 Crabb, Edenberg, Bosron & Li (1989) correlated $ALDH_2$ genotype with the phenotype of their subjects and concluded that the ALDH2-2 allele, which encoded the abnormal subunit, was dominant. Therefore, the ALDH2-2 protein was able to inactivate the ALDH2-1 allele in some, as yet undefined, manner.

1991 When non-alcoholic and alcoholic Taiwanese individuals were compared, the number of deficient $ALDH_2$ subjects was significantly higher in the non-alcoholic

group than in the alcoholic one (Chen, Hwu, Yeh, Morimoto & Otsuki, 1991), a finding which is in accord with that of Harada *et al.* (1982).

Acetaldehyde metabolism in people with the different $ALDH_2$ genotypes was studied by Enomoto, Takase, Yasuhara & Takada (1991). Their findings indicated that the metabolism of acetaldehyde in ALDH2-2 homozygote (Homo22) subjects was severely impaired, in contrast to the milder impairment found in heterozygote subjects.

1992 The presence of the deficient $ALDH_2$ enzyme was found to exert a strong inhibitory effect on ethanol consumption -Japanese subjects with the deficient form of $ALDH_2$ were found to drink significantly less than those with the active form (Higuchi, Muramatsu, Shigemori, Saito, Kono, Dufour & Harford, 1992). It was also reported (Wall, Thomasson, Schuckit & Ehlers, 1992) that flushers experienced more intense (though not necessarily negative) reactions to ethanol than non-flushers, even though their blood ethanol concentrations were essentially the same.

1993 The prevalence of alleles ADH2-2 and ADH3-1, as well as ALDH2-2 were found to be lower in alcoholics than in control groups. The presence of ALDH2-2 was also associated with the most intense flushing and a slower ethanol metabolism (Thomasson, Crabb, Edenberg & Li, 1993).

1994 More recently, cortisol levels (which increase in response to stress) after ethanol were reported to be higher in subjects with ALDH2-2 alleles than in ALDH2-1 homozygote subjects, although the blood ethanol concentrations were similar (Wall, Nemeroff, Ritchie & Ehlers, 1994). This finding is consistent with the belief that individuals with the ALDH2-2 allele experience more intense reactions to ethanol than others.

Thus, it can be seen that the alcohol flush reaction has been known to scientists for a considerable time. Early studies concentrated on the physiological variables and physical observations, although with the introduction of modern techniques in molecular biology, the genetic component became overridingly important. The ability to accurately detect and quantify acetaldehyde in body fluids has also played a very important role in the study of the alcohol flush reaction (Peterson & Polizzi, 1987; Ung-Chhun & Collins, 1987).

1.5. THE EFFECTS OF ETHANOL IN ASIAN POPULATIONS

Throughout this thesis the term 'Asians' will imply those people of Chinese, Japanese or Korean ancestry; in this case residing in Australia at the time of testing.

1.5.1. THE ALCOHOL FLUSH REACTION (AFR)

Differences in ethanol sensitivity among Asians refer primarily to the facial flushing response exhibited by certain individuals after ethanol consumption, although other symptoms may include dizziness, nausea and tachycardia. Severe changes in blood flow and cardiac effects associated with the AFR have been reported widely in Asians who have received only small doses of ethanol.

The AFR produces symptoms not unlike the effects of disulfiram. Disulfiram or Antabuse is a drug which is used to deter people from drinking because of the unpleasant side-effects which occur when ethanol is consumed whilst on disulfiram therapy. The mode of action of disulfiram is to inhibit the ALDH enzyme from degrading acetaldehyde, which then accumulates as ethanol is metabolized. Because acetaldehyde is toxic, various unpleasant adverse effects are produced, hopefully discouraging further ethanol use.

The actual flushing reaction in the AFR is usually rapid and intense, starting between two to ten minutes after ethanol consumption, reaching a peak at 30-40 min, and finally subsiding by 60-90 min (Wolff, 1973). In some individuals, ethanol ingestion can have other adverse effects associated with the flushing reaction which include stimulation of respiration, decreased blood pressure and nausea (Mizoi, Hishida, Ijiri, Maruyama, Asakura, Kijima, Okada & Adachi, 1980).

The symptoms of ethanol-induced flushing correlate with ALDH deficiency and high blood acetaldehyde levels. Subjects with ALDH deficiency have also been found to possess the atypical ADH2-2, but the contribution of this enzyme to the higher-than-normal blood acetaldehyde levels in flushing subjects remains unknown, although atypical ADH occurs in both flushing and non-flushing individuals. If ADH_2 type is important, it

may act by affecting the rate of ethanol metabolism and therefore the rate of acetaldehyde generation. Likewise, if ADH_2 type alters the risk of ethanol dependence, as has been recently shown in Japanese subjects (Higuchi, 1995), then it would be likely that it would be due to its role in producing or modulating the AFR. That is, ADH_2 type affects the rate of ethanol conversion to acetaldehyde and hence the steady-state acetaldehyde concentration. In a recent study, almost 86% of facial flushers were found to have the inactive form of $ALDH_2$ (Higuchi *et al.*, 1992). There is also evidence that flushers may experience a more intense response to ethanol than non-flushers (Wall *et al.*, 1992).

A combination of H_1 and H_2 histamine antagonists has been used to suppress flushing in subjects. The mechanism for this effect has not been established, although a combined central and peripheral antagonism by the histamine antagonist has been proposed (Miller, Goodwin, Jones, Gabrielli, Pardo, Anand & Hall, 1988).

1.5.2. ACETALDEHYDE - A TOXIC METABOLITE

It is acetaldehyde and *not* ethanol itself which causes most of the severe symptoms associated with the AFR (Goedde, Agarwal, Harada & Meir-Tackman, 1982). Primary ethanols must be metabolized to aldehydes before vasoactivity occurs (Wilken & Fortner, 1985). In addition, acetaldehyde exhibits stronger sympathomimetic actions than ethanol and causes the release of catecholamines from the adrenal medulla and sympathetic nerve endings. An increase in catecholamines causes an increase in heart rate and peripheral vasodilation with accompanying haemodynamic effects. Noradrenaline release would be expected to cause vasoconstriction, however, and if vasodilation occurs, it is likely to be due to adrenaline release from the adrenal medulla and/ or some other mechanism.

In contrast to non-flushers, acetaldehyde levels were found to be significantly higher in people who experienced facial flushing and tachycardia after ethanol (Harada *et al.*, 1981; Mizoi *et al.*, 1983). Although high acetaldehyde levels are associated with the AFR, substances such as histamine and bradykinin may actually mediate the response (Thomasson *et al.*, 1993).

Truitt *et al.* (1984) found similar breath ethanol levels in Asian (lacking $ALDH_2$) and Caucasian subjects although the breath acetaldehyde levels were two to seven times higher in the Asians than the Caucasians.

1.5.3. OTHER DIFFERENCES BETWEEN CAUCASIAN AND ASIAN POPULATIONS

Cultural factors are generally believed to play a major role in the differences in social drinking patterns found between different racial groups. Women have traditionally been expected to consume less ethanol than men and, in this instance, biological factors may also affect the social drinking pattern.

Racial differences also appear to exist in the pharmacokinetics of ethanol metabolism. Asian subjects have been found to absorb ethanol more quickly than their Caucasian counterparts (Zeiner, Paredes & Musicant, 1977). Since it is known that Asians have longer intestines than Caucasians (Hanna, 1978), the faster ethanol absorption has been attributed to a greater surface area being available for absorption (Hanna, 1978). However, this may or may not be the case for orally administered ethanol, given that the drug is primarily absorbed from the upper part of the small intestine and hence length would appear not to be a major contributing factor.

Ethanol elimination rates, calculated from the slope of the pseudolinear portion of the BEC curve in Chinese men and women have been determined (Thomasson *et al.*, 1993). ALDH2-2 homozygote and heterozygote subjects exhibited lower rates of ethanol elimination, while ALDH2-1 homozygote subjects with ADH2-2 or ADH2-1/ 2 genotype demonstrated no differences in ethanol elimination rate.

V_{max} of ADH2-2 (β2) *in vitro* is approximately forty times greater (Yin, Bosron, Magnes & Li, 1984) than ADH2-1 (β1), however the β2 subunit makes up only a fraction of Class I ADHs present in the liver, and cytochrome P4502E1 also influences the ethanol elimination rate (although in our low-dose study, only the ADH enzyme is of relevance). The ADH enzyme is subject to product inhibition by NADH and acetaldehyde; further details relating to the ADH enzyme will be discussed more specifically in the next section.

1.6. ENZYMES INVOLVED IN ETHANOL METABOLISM

Differences in ethanol sensitivity among individuals have been related to two ethanol metabolizing enzymes, ADH and ALDH. These two enzymes are responsible for the degradation of ethanol and, because they have various polymorphic and isozymic forms, it has been suggested that the combination of the two enzymes which occur in a given individual may play a part in determining his or her sensitivity to ethanol.

To some extent, variation in ethanol metabolizing enzymes would be expected to cause variation in ethanol metabolism. A reduction in activity of $ALDH_2$ leads to a build-up of acetaldehyde after ethanol use and this is believed to be the major factor in the alcohol flush reaction. However, genetic variation in ADHs (with markedly different *in vitro* properties) does not seem to produce proportional differences in ethanol metabolism between racial groups with differing ADH_2 gene frequencies.

1.6.1. ALCOHOL DEHYDROGENASE (ADH)

There are a number of molecular forms of this enzyme. ADH enzymes can be divided into various classes based on their different electrophoretic properties and substrate specificities. However, ethanol is primarily metabolized in the liver by Class I ADH. There is at least 85% base sequence homology within a class and about 70% between classes (Ehrig, Bosron & Li, 1990).

Within human Class I, there is genetic polymorphism. All three Class I genes are situated on the long arm of Chromosome 4. Being closely related by having 94% homology in nucleotide sequence, the ADH isozymes are dimers of peptide chains with molecular weights of 40,000. The combination of α, β and/ or γ polypeptide chains coded by three separate gene loci (ADH_1, ADH_2, ADH_3) associate to form homo- and hetero-dimers which are physiologically active. Association across various classes has not been observed. Allelic polymorphisms are found at two gene loci, the ADH_2 locus coding for $\beta 1$, $\beta 2$ and $\beta 3$ chains, and the ADH_3 locus which codes for $\gamma 1$ and $\gamma 2$. Heterodimers behave as a mixture of the parent homodimers; hence their subunits seem to function independently of each other (Bosron & Li, 1987).

Locus ADH_1, coding for α subunits is thought to be active in early foetal life whilst after birth, ADH_2 and ADH_3 progressively become more active (Smith, Hopkinson & Harris, 1971).

Polymorphism occurs at both the ADH_2 and ADH_3 loci and racial differences appear to exist in the gene frequencies of the polymorphic forms of ADH (Table1).

For ADH_2, a combination of the three different subunits (1,2,3) leads to six possible dimeric forms ADH2 -1,1; 1,2; 1,3; 2,2; 2,3; and 3,3 (Smith, Hopkinson & Harris, 1973).

	ADH2-1 (β1)	**ADH2-2** (β2)	**ADH2-3** (β3)	**ADH3-1** (γ1)	**ADH3-2** (γ2)
White American	> 95%	< 5%	< 5%	50%	50%
White European	90%	10%	< 5%	60%	40%
Oriental	35%	65%	< 5%	95%	5%

Table 1: Frequency of ADH alleles in different populations. (Bosron, Lumeng & Li, 1988)

Several polymorphisms occur at the gene locus which codes for the beta subunits including 'normal' (ADH2-1 or β1) and 'atypical' (ADH2-2 or β2) types. The β2 enzyme found in the majority of the Oriental population has been found to predominate, with over 85% of subjects testing positive (Fukui & Wakasugi, 1972).

The amino acid sequences of both β1 and β2 differ only in a single base change (Fig.1), at position 47, out of 374 residues (i.e. β1 arginine CGC cf β2 histidine CAC).

A low K_m for ethanol (Table2) means that the β2 enzyme is easily saturated at low concentrations of ethanol easily reached in social drinking however, the V_{max} is approximately forty times higher than β1 (Yin *et al.*, 1984).

The activity of ADH2-2 *in vitro* is considerably elevated, therefore, it might be expected that individuals with this genotype would eliminate ethanol much faster than those with the other variant. However, no significant difference in the rate of ethanol degradation has been reported between 'normal' and 'atypical' subjects. It has been suggested (Stamatopoulos *et al.*, 1975) that individuals with the atypical ADH gene will have increased acetaldehyde levels after ethanol consumption, although this proposal is not well-supported.

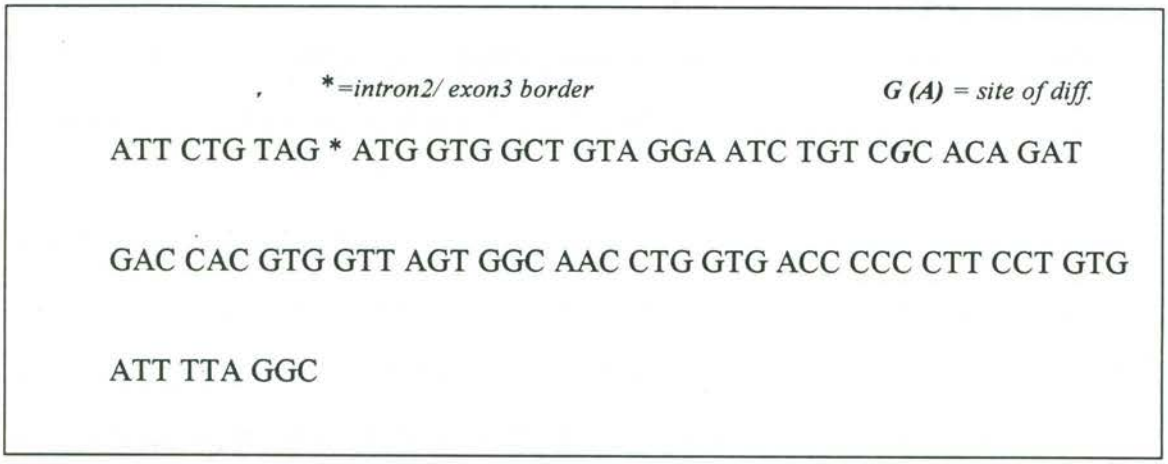


Fig.1: Partial sequence of the ADH_2 gene showing the site of difference.

ADH_2 Isozyme	**K_m ethanol (mM)**	**V_{max} (min^{-1})**
β1β1	0.05	9
β2β2	0.9	400
β3β3	34	300

Table 2: K_m and V_{max} values for ADH_2 isozymes.
(Bosron & Li, 1987)

The ADH2-3 (β3) allele seems to be found mainly in the Black American and African populations, having a frequency of less than five percent in the Chinese and Japanese populations which were tested (Bosron *et al.*, 1988).

Polymorphism also occurs at the ADH_3 locus and racial differences have also been noted. In Caucasian populations which were tested, a sixty percent frequency of ADH3-1 has been estimated (Harada *et al.*, 1978) while for Asian populations, frequencies above ninety percent have been found (Harada, Misawa, Agarwal & Goedde, 1980). Conversely, ADH3-2 was mainly found in Caucasian subjects and was mostly absent in Asian subjects (Harada *et al.*, 1978; 1980).

1.6.2. ALDEHYDE DEHYDROGENASE (ALDH)

Four groups of ALDH isozymes have been identified Class I, Class II, Class III and Class IV. These are coded by four independent gene loci $ALDH_1$, $ALDH_2$, $ALDH_3$ and $ALDH_4$ respectively. Isozymes within each group are different from other groups in terms of their electrophoretic and kinetic properties, affinity for various aldehydes, as well as in tissue distribution (Goedde, Agarwal & Harada, 1979).

Genetic variation/ polymorphism has been found to occur in $ALDH_1$, $ALDH_2$ and also in $ALDH_3$ isozymes (Santisteban, Povey, West, Parrington & Hopkinson, 1985). The structural genes for the two major isozymes of ALDH, $ALDH_1$ (cytoplasmic ALDH) and $ALDH_2$ (mitochondrial ALDH), have been assigned to Chromosomes 9 and 12 respectively.

A faster migrating isozyme with a low K_m (3 μM) for acetaldehyde ($ALDH_2$) has been found in liver mitochondria, and a slower migrating enzyme with a high K_m (30 μM) for acetaldehyde ($ALDH_1$) in the cytosol (Greenfield & Pietruszko, 1977). The mitochondrial $ALDH_2$ isozyme is responsible for the oxidation of acetaldehyde generated by ADH during ethanol metabolism. Individuals lacking activity of this isozyme have high concentrations of acetaldehyde during drinking. Virtually all Caucasians have the two

major isozymes $ALDH_1$ and $ALDH_2$ in their livers, while about fifty percent of Asians are 'atypical' in that they have only $ALDH_1$ and are deficient in $ALDH_2$ (Goedde *et al.*, 1979). An enzymatically inactive but immunologically cross-reactive material (CRM) was identified in atypical Japanese livers by Yoshida, Wang & Dave (1983). The CRM is thought to be a defective protein resulting from a mutation of the $ALDH_2$ locus.

Basically, two $ALDH_2$ phenotypes can be described (namely, flushers and non-flushers) for the three genotypes which have been identified (namely, ALDH2-1 homozygotes, $ALDH_2$ heterozygotes and ALDH2-2 homozygotes). Of the two phenotypes, a normal (ALDH2-1) and deficient (ALDH2-2) type, the deficient phenotype is inherited as an autosomal dominant trait, with a characteristic slow acetaldehyde metabolism, and such an individual would typically attain high blood and breath acetaldehyde levels -between two to seven times higher than normal concentrations -after ethanol ingestion (Truitt *et al.*, 1984).

$ALDH_2$ is a homotetramer and even one inactive ALDH2-2 subunit causes reduced enzyme activity. Some residual activity is expected in heterozygous subjects because approximately 6% (0.5^4) of the tetramers will contain no inactive subunits. Hence, although the AFR is inherited as a dominant condition, the homozygous ALDH2-2 subjects are likely to be more severely affected after ethanol.

The molecular and genetic basis of the $ALDH_2$ deficiency has been determined. The enzyme is composed of four identical protein subunits, each with five hundred amino acid residues (Jornvall, Hempel, von Bahr-Lindstrom, Hoog & Vallee, 1987). The active and inactive forms of the $ALDH_2$ enzyme differ due to a point mutation causing the enzymes to differ in their amino acid composition at one point, position 487 (Fig.2), with a Glu to Lys exchange in the inactive variant (Hsu, Tani, Fujiyoshi, Kurachi & Yoshida, 1985).

(Amino-terminus)

position 487

Gly Leu Gln Ala Tyr Thr ***Glu*** Val Lys Thr

Glu (Lys) = *site of difference*

Fig.2: Partial amino acid sequence of the $ALDH_2$ gene with the site of difference shown.

The other major ALDH isozyme, $ALDH_1$, can be detected in all cell types, including erythrocytes, and was found to be missing in a Japanese liver studied by Yoshida *et al.* (1983). This cytosolic liver ALDH was also found to be reduced in people with alcoholic liver disease and, abnormalities of $ALDH_1$ activity have been associated with ethanol reactions in a small number of Caucasians (Jenkins & Peters, 1983).

1.7. ISSUES RAISED BY THE RESEARCH TO DATE

Recent advances in molecular biology, including the use of the polymerase chain reaction (PCR) for faster and more practical genotyping, has enabled further studies to be conducted in this field (see *Methods* section). The advent of PCR, requiring only small amounts of isolated DNA samples, and of the use of DNA probes for ADH and ALDH genotyping permits the easier study of genetic variants in living subjects.

Although there has been considerable research into the molecular biology of the ADH and ALDH enzymes, especially as a result of recent developments in technology, there is little information concerning the psychomotor, physiological and biochemical effects of ethanol on people with different ADH and ALDH enzymes. Much of the research to date has been focused primarily on subjective responses to ethanol through the use of questionnaires which have been administered to various groups of people. While such an approach provides interesting information, it is limited by the subjective nature of the responses.

Psychomotor performance studies after ethanol consumption would be expected to yield valuable information concerning reaction time, coordinative ability, cognitive function and other variables which are crucial in operating machinery, driving a motor vehicle or working in a similar dangerous environment. Such psychomotor studies have been almost exclusively carried out in Caucasian subjects and none appear to have been specifically directed towards Asian subjects. This is important because there is good evidence that small amounts of ethanol can produce severe impairment in some, although not all, Asians. It was considered especially important to determine whether the psychomotor effects of a low dose of ethanol in Asian subjects differed according to whether or not they experienced the AFR, or according to $ALDH_2$ or ADH_2 genotype.

Although some early studies have measured changes in blood pressure and some other physiological variables after ethanol, the findings have seldom been related to genotype. The present state of knowledge is that, after ethanol, flushers have a higher

blood pressure and a more elevated heart rate than non-flushers but differences, if any, among the various flushing groups have yet to be properly defined.

The acetaldehyde concentration attained by Asians after ethanol has received considerable attention although various problems have been encountered with the assay procedures which have rendered many of the earliest findings rather suspect. There is no detailed information concerning other ethanol metabolites in Asians however. This information is important because if faster ethanol metabolism occurs giving rise to increased acetaldehyde concentrations, the lactate:pyruvate ratios should also increase. Whether or not the genetic make-up of the subject significantly affects other ethanol metabolite levels has not yet been established.

Thus, although the effects of ethanol in Caucasian subjects has been extensively studied, other racial groups have largely been neglected. Where Asian subjects have been used, the subgroups were usually combined in a single category ('Asians'). The AFR, which occurs to any significant extent only in Asian populations, has received some attention but the findings have been largely of a subjective or specialized nature, involving measurement of acetaldehyde concentrations, genetic codes and genotyping techniques. The effects of a low dose of ethanol on the psychomotor performance and on various physiological and biochemical measures (except acetaldehyde) of Asian subjects with different ADH and ALDH isozymes has yet to be systematically investigated and documented.

Many questions still remain to be answered. For example, should Asian people who are susceptible to the AFR be forewarned of the possible consequences of low-dose ethanol consumption and driving a motor vehicle? Many Asian countries have recently reported a higher incidence of ethanol consumption and ethanol abuse. If such trends are viewed together with increasing affluence and motor vehicle ownership, is this predictive of disproportionately greater traffic violation and crash rates in Asian countries, with their attendant morbidity and mortality?

This thesis is primarily concerned with a detailed examination of the effects of a modest dose of ethanol on a number of physiological, psychomotor performance and mood parameters of young male and female Asian subjects. The findings have also been related to the pharmacokinetics of ethanol and a number of biochemical parameters associated with the main ethanol metabolic pathway. Subjects were grouped according to their genetic ADH and ALDH status, and various other groupings including gender and degree of flush observed.

1.8. AIMS AND OBJECTIVES OF THIS STUDY

Subjects were classified into different groups based on genotype (ADH, ALDH & ALDH/ ADH combination), degree of flush and gender to test for differences in the effects of ethanol on:

- ⇨ Psychomotor performance;
- ⇨ Physiological variables;
- ⇨ Biochemical variables;
- ⇨ Ethanol pharmacokinetics and;
- ⇨ Subjective responses.
- ⇨ Ethanol consumption and drinking habits were also examined in each of the five groups.

Specifically, it was intended to:

- ➔ compare any differences among the three ADH_2 groups and to determine whether responses can be graded according to allele type (Homo11, Het or Homo22);
- ➔ compare any differences among the three $ALDH_2$ groups and to determine whether responses can be graded according to allele type (Homo11, Het or Homo22);
- ➔ compare any differences among the combinations of the $ALDH_2$ and ADH_2 enzymes and to see whether responses can be graded according to these combinations;
- ➔ compare any differences among the subjectively rated 'Degree of Flush' groups and to determine whether responses can be graded according to this rating;

- ➔ compare any differences which might exist between male and female subjects;
- ➔ determine which groups are more susceptible and more performance-impaired after ethanol consumption and;
- ➔ establish whether any of the differences can be attributed to diet, drug interactions or other socio-cultural differences.

1.9. OUTLINE OF THE THESIS

This thesis is divided into six major chapters including the current Chapter, which provides a broad overview of the research field and topic to be investigated, a historical perspective, a discussion of the issues raised by the research to date and a statement of the aims and objectives of this study. The other chapters are:

Chapter 2. Methods;

Chapter 3. Results;

Chapter 4. General Discussion & Conclusions;

Chapter 5. Appendices and;

Chapter 6. References.

Chapter 2 provides details of the various methods used in the study including information on the background, limitations and modifications made to the experimental methods which were adopted.

Sub-divisions contained within the Chapter include information on subject recruitment and screening, subject information, ethanol dosage, the procedure utilized, breath analysis, methodologies (for the molecular biology, biochemical, physiological, psychomotor & subjective aspects) and methods of statistical analysis.

Chapter 3 reports on the results obtained. In order to simplify the results, the information is arranged based on the following sub-divisions:

3.1. General results, including subject backgrounds, drinking histories, flushing details and excluded data etc;

3.2. Observations in flushers after ethanol consumption;

3.3. Results by ADH_2 genotype;

3.4. Results by $ALDH_2$ genotype;

3.5. Results by $ALDH_2$/ ADH_2 combination genotype;

3.6. Results by degree of flush;

3.7. Results by gender;

3.8. Summary of results.

Each section (3.3-3.7) is further subdivided into the following sections:

1. Group breakdown;

2. Drinking details;

3. BEC curves;

4. Biochemical results;

5. Physiological results;

6. Psychomotor results;

7. Subjective response results.

Chapter 4 presents a general discussion of the topic including the results obtained in these studies. Results from these studies are compared with those of previous studies whenever applicable. A conclusion is provided at the end of the section, summarizing the findings of these studies and possible practical implications of these findings.

Chapter 5 contains the Appendices, with further details on the methods used, together with samples of the questionnaires and other forms used in the study.

Chapter 6 provides a list of references in alphabetical order.

Chapter 2.

SUBJECTS, MATERIALS & METHODS

2.1. SUBJECT RECRUITMENT AND SCREENING

Subjects of Asian descent, but not necessarily of full Asian ancestry, were recruited by advertisement placed at a number of University campuses in the Sydney, Australia area. Persons interested in participating in the study were requested to telephone the Psychopharmacology Research Unit for further details and information.

Upon contact and subsequent confirmation of an interest to take part in the study, the subject was given an agreed appointment time and was provided with further information regarding the study.

The information sent to subjects included a map (with information on how to get to the Research Unit), a questionnaire (see Appendix A) and written instructions about how to prepare for the Study; such as to fast, and not to smoke cigarettes or drink ethanol for at least eight hours before the testing session.

Subsequently, to obtain a better representation of flushers compared to non-flushers in the group studied, it became necessary to recruit more flushing subjects. Hence, in a second group of subjects tested, screening for 'flushers' consisted of asking people to describe "what happens after you drink a *small* amount of alcohol". If the response was appropriate, subjects were then asked to describe their flushing pattern in more detail, their drinking habits and, whether their parents, siblings and other relatives flushed after ethanol. Appropriate subjects were then given appointment times and further details were sent out as with the first group of recruits.

2.2. ETHICS APPROVAL AND SUBJECT INFORMATION

The protocol adopted was approved by the Ethics Review Committee of the Central Sydney Area Health Service, and all subjects gave written informed consent at the beginning of the experiment.

Volunteers had to be at least 18 years old and prepared to sign a consent form stating that they understood the nature of the study and that they would not be permitted to leave the Research Unit and drive a motor vehicle if their breath ethanol reading exceeded 0.04 g dl^{-1} at the end of the test session (see Appendix B).

2.3. ETHANOL ADMINISTRATION

The ethanol dose (0.3 g kg^{-1}) was consumed at a constant rate over ten minutes.

Drinks were served chilled and presented as vodka (37% ethanol by volume) which was mixed with twice the volume of orange juice. The drink was divided into two equal portions to facilitate an even pattern of drinking over the ten minute period.

A correction factor of 0.87 was used in calculating doses for female subjects to allow for the lower volume of distribution of ethanol in females and thus, attain similar target blood ethanol concentrations in both male and female subjects.

2.4. PROCEDURE

The ethanol challenge testing was carried out at the Psychopharmacology Research Unit of the University of Sydney.

Only one subject at a time could be tested effectively due to the large number of variables which had to be recorded and the need to adhere closely to the planned time schedule.

On arrival at the Research Unit all subjects were breath tested for ethanol. If any ethanol was detected, the test session was canceled and the subject was asked to attend on another occasion.

If no ethanol was detected in the screening test, the subject was weighed so that the dose of ethanol to be given could be calculated (in grams per kilogram). The completed consent form and questionnaire forms were also collected at this point, as were the health (Appendix C) and meal diaries (Appendix D).

To facilitate completion of the questionnaire and to increase the consistency of the responses, the number of possible answers to the questions on drinking frequency, place, time, reasons and type of beverage preferred, were limited to a maximum of seven choices. The more general questions remaining in the questionnaire were left open for the subject to answer as he/ she wished. The languages spoken at home were used as an indication of how much of the ancestral culture had been retained in the subject's family. If the subject spoke English at home, then it was considered that the family would be more likely to be Westernized and thus to hold more Western attitudes than one which spoke an Asian language.

Meal diaries were also collected, primarily as a confirmation of fasting but also, so that the effects of the last meal could be considered if unexpected variations were encountered. The health diary recorded any drugs or herbal remedies which had been taken around the time of the experiment.

Although it was necessary to adhere closely to a strict time schedule, the testing situation was kept as informal as possible and the atmosphere was relaxed and friendly. Subjects were encouraged to participate enthusiastically and to perform as well as possible in the psychomotor tasks.

Experimental testing consisted of breath analysis, psychomotor and subjective tests, physiological measurements (heart rate, blood pressure and temperature) and venous blood sampling for biochemical determinations (a brief schedule of the flow of testing is provided later).

An initial 'run through' and practice period enabled subjects to attain a plateau level of performance and to be familiar with the computerized tasks and overall experimental procedure before the pre-ethanol control (t_0) measurements were made.

After the pre-ethanol control measurements had been obtained for the psychomotor performance tasks, pre-ethanol control measurements were also obtained for the skin (facial) temperature, subjective questionnaire, systolic and diastolic blood pressure, heart rate and the breath ethanol concentration. Blood samples were also taken from the subject at this point.

Ethanol (0.3 g kg^{-1}), in the form of two glasses of vodka and orange juice, was then given to the subject to be consumed evenly, under close supervision, over a ten minute period. During the subsequent three and a half hour testing session:

- ☑ skin temperature was measured at 5 minute intervals;
- ☑ blood pressure and heart rate were measured at 15 minute intervals;
- ☑ breath ethanol readings were obtained at 15 minute intervals;

☑ blood samples were taken for biochemical determinations at pre (t_0), 15 (t_{15}), 60 (t_{60}) and 120 (t_{120}) minutes after ethanol;

☑ psychomotor and subjective tests were also conducted at pre (t_0), 15 (t_{15}), 60 (t_{60}) and 120 (t_{120}) minutes after ethanol consumption.

The sequence of testing in each session is shown in the following schedule:

① Practice run-through and familiarization with tests.

Control measurements (t_0) taken.

Meal and health diaries filled in.

② Consumption of ethanol (0.3 g kg^{-1}).

③ Throughout the entire duration of the test session:

Skin temperature measurements were taken every 5 min and;

Breath analysis, blood pressure and heart rate readings taken every 15 min.

④ One cycle:

* Subjective questionnaire
* Skin temperature measurement (every 5 min)

⇩

* Blood pressure/ heart rate measurements (every 15 min)
* Breath analysis (every 15 min)

⇩

* Blood collection

⇩

* Psychomotor testing: 1. Standing steadiness test
2. Computerized test battery
-Tracking and Digit Symbol Coding tasks
3. CFFF task

↓

* Breath analysis and Blood pressure/ heart rate measurements.

⑤ Repeat step ④ at 15, 60 and 120 min after ethanol consumption.

The ethanol pharmacokinetic parameters, acetaldehyde levels, subjective assessments, flushing response, biochemical, physiological as well as psychomotor effects of ethanol were then compared among the various genotype(s) and flush groups.

2.5. ASSESSMENT OF DEGREE OF FLUSH

A subjective assessment of the degree of flush exhibited in subjects after consumption of ethanol was recorded by the researcher, who was unaware of the genotype results at that time.

Degree of flushing was initially separated into eight categories, ranging on a scale from 0-6, based on observations of where and to what extent flushing occurred. The determination of the degree of flush was based on the following criteria:

Rate:			
	'NF'	0	Non-flushers
	'FF'	1	Glow (faint)
		2	Distinct glow (cheeks faintly)
		3	Cheeks flushed
		4	Obvious facial flush (neck faintly)
	'LF'	5	Obvious facial, ears and chest flush
		5.5	As in 5 but with some flushing of the limbs
		6	'Lobster Flusher' (body/ face flushed)

The eight categories were later aggregated, for better statistical analysis, into three larger and more generalized groups: 'NF' for Non-Flushers, 'FF' for Facial Flushers (subjects rating between 1-4), and 'LF' for Lobster Flushers (those rating 5, 5.5 or 6).

2.6. BREATH ETHANOL CONCENTRATION ANALYSIS

Breath analysis has often been employed to measure blood ethanol concentrations (BECs) in man, especially if rapid and non-invasive sampling is required. Measurement of absorption at the infrared 3.4 nanometre wavelength was used in this study (Alcomat, Siemens, Karlsruhe, Germany).

The Alcomat operates by taking an initial zero reading from the surrounding air and comparing it with a breath sample taken from the subject (the subject must blow consistently into the mouth-piece continuously for at least 3 seconds for a successful breath sample). Since ethanol absorbs infrared radiation at 3.4 nm, the proportion of radiation actually reaching the detector in the Alcomat is decreased depending on the amount of ethanol present. The initial zero reading is then taken into account before the resulting BEC result is displayed on the LED display. The Alcomat instrument produces a readout in g dl^{-1} of blood using a blood:breath factor of 2100:1 -that is, 1 ml of blood contains the same amount of ethanol as 2100 ml of deep lung air.

In general, from the blood:breath regression curves, the breath ethanol concentrations obtained are lower than values obtained from blood samples. The correlation coefficients between BECs determined from breath and directly from venous blood have generally been found to exceed 0.956 (Dubowski, 1963). Using the Alcomat, the correlation coefficients between blood and breath readings obtained in two field studies (n=466 & 94) were reported to be 0.977 and 0.998 respectively (Slemeyer, 1985).

Although the Alcomat has a slope detector which can indicate the presence of mouth ethanol it was considered prudent that for the first BEC measurement, taken only five minutes after drinking had finished, for the subjects to rinse their mouths out with tepid water in order to avoid the possibility of readings from falsely elevated residual mouth ethanol.

2.6.1. ETHANOL ELIMINATION RATE

Ethanol elimination is a measure of ethanol metabolism. The post-absorption/ distribution pseudolinear part of the BEC curve was initially used to estimate the ethanol elimination rate. Data points used in the calculations were taken between 30 and 105 minutes after drinking; by this time all subjects had passed their peak BEC. A linear regression was then performed on the six post-peak data points to determine the ethanol elimination rate.

However, due to the low dosage of ethanol given and the resultant low blood ethanol concentrations which were attained, it was found that some subjects had reached zero BEC by the 105 min time-point. In such cases, it was difficult to identify a linear portion of the BEC/ time curve and to estimate the rate of ethanol metabolism in the conventional manner.

Instead, the rate of ethanol metabolism was estimated for each subject by averaging the observed decreases in breath ethanol (converted to equivalent blood values) between 30 and 90, 45 and 105, and 60 and 120 minutes after ethanol. This procedure gave the greatest weight to values between 60 and 90 minutes and reduced the influence of early readings (when absorption and distribution might have been incomplete in some subjects) and of the later readings (when some subjects had very low values and a diminished rate of fall).

Estimation of the pharmacokinetic parameters C_0, K_m and V_{max} from the breath ethanol results was performed by fitting the data to the integrated form of the Michaelis-Menten equation:

$$(C_0 - C) + (K_m \cdot \ln(C_0/ C)) = V_{max} \cdot t$$

where C is the observed concentration at time t; C_0 is the estimated concentration at time zero assuming instantaneous distribution; V_{max} is the rate of conversion of

substrate (ethanol) to product at infinite substrate concentration; and K_m is the substrate concentration giving half maximal activity.

2.6.2. VOLUME OF DISTRIBUTION

The volume of distribution (V_D) relates to the amount of ethanol in the body to the concentration of ethanol in the blood. It reflects the apparent space available for the drug in both the general circulation and in the tissues of distribution.

The apparent V_D, in a linear curve fit model, is calculated by extrapolation of the blood ethanol concentration curve back to the y-axis; however, for reasons explained above, the V_D was estimated from the C_0 value. The apparent V_D is equivalent to the total water content in the body (excluding dense bone, tooth enamel and body fat) together with the effects from gastric metabolism. In young, healthy subjects, body water has been determined to be equal to 61% of the body weight in males, and 55% in females (Delwaide & Crenier, 1973).

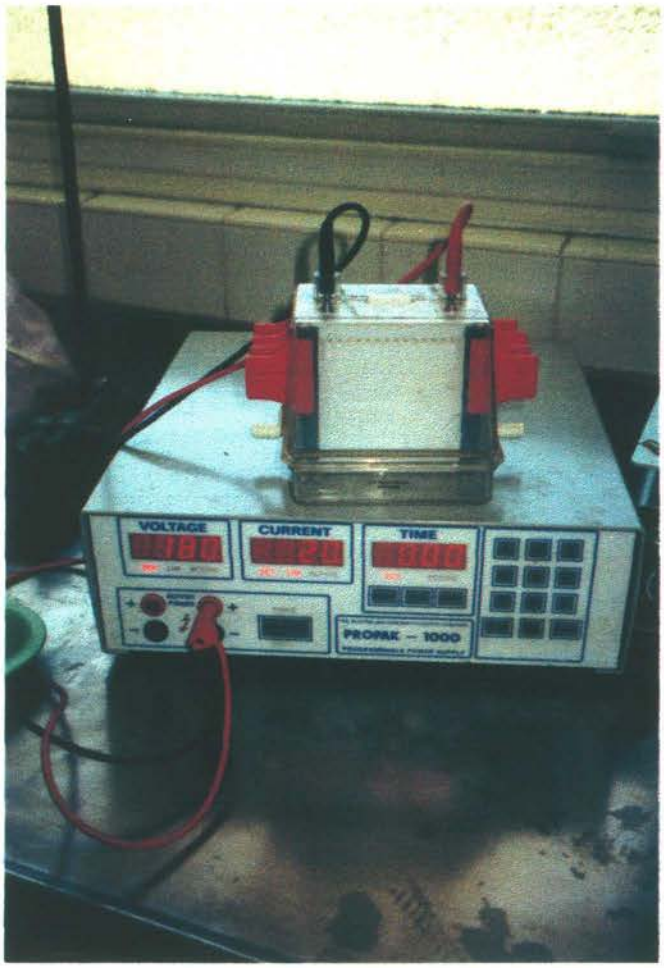
2.6.3. AREA UNDER THE CURVE

The area under the curve (AUC) is significant as a measure of the systemic availability (bioavailability) of the ethanol, indicating the degree of exposure of the peripheral tissues to the drug (i.e. the potential toxicity of the ethanol). A linear trapezoidal method was used to calculate the area under the BEC curve from time zero until the time of the last BEC reading.

2.7. MOLECULAR BIOLOGY METHODOLOGY

The advent of the polymerase chain reaction (PCR) technology has facilitated the study of genetic variants. It is now possible to screen individuals using isolated DNA samples rather than needing to rely on the more difficult methods of the past, including liver biopsies for ADH typing or time-consuming hair root samples for ALDH screening. The amplification permitted by the PCR process generates enough DNA to enable characterization by many methods, including restriction digestion analysis and hybridization with allele specific oligonucleotide probes (ASOP).

(a)



(b)

Fig.3: Photograph of equipment required for the PCR amplification technique.

(a) PCR instrument

Special 20 μL tips were loaded with the DNA, PCR buffer, dNTPs and lastly, the Taq Polymerase. The PCR amplification process took less than two hours to complete (see Appendices G and H).

(b) Gel electrophoresis equipment

After the PCR has ended, a portion of the product is loaded onto a polyacrylamide gel and electrophoresed for up to one hour. The gel is then stained with ethidium bromide and the result can be visualized under UV light.

PCR is a modern technique which amplifies specific samples of DNA (flanked by two different primers) by enzymatic synthesis. The procedure involves repeated cycles of heat *denaturation*, causing the unraveling of DNA so that it becomes single-stranded; *annealing*, when the two oligonucleotide primers hybridize specifically to their complementary sequences on the piece of DNA and primer *extension*, where new complementary strands of DNA are formed by the incorporation of deoxynucleotides and DNA polymerase. By repeated cycles of denaturation, annealing and extension, the new strands become templates for the DNA primers, which are present in excess, and an exponential amplification of the DNA bounded by the primers occurs, the number of target DNA copies approximately doubling at each cycle (Mullis, Faloona, Scharf, Saiki, Horn & Erlich, 1986; Schochetman, Ou & Jones, 1989).

The amplification process provides sensitivity, while the specific size of the amplified product, from the migration distance in an electrophoresed polyacrylamide gel, provides specificity. By staining the polyacrylamide gel with ethidium bromide, the PCR products, with distinct bands characteristic of the particular DNA to be studied, can be visualized under ultraviolet transillumination.

2.7.1. PREPARATION OF GENOMIC DNA FOR PCR

The white blood cell (WBC) layer obtained from centrifuged heparin blood tubes were proteinase-K treated in order to extract the DNA required for PCR amplification. The proteinase-K digests nuclei and whole cells to release DNA in a readily accessible form for the polymerases in the PCR reaction. The procedure used was as follows:

a) Eppendorf tubes containing the buffy coat layer obtained from subject's whole blood were centrifuged for ten minutes (3000 rpm).

The upper layer of WBCs was then separated from the blood and transferred to fresh tubes.

b) Magnesium chloride (1 mM) was then added and the tube mixed and centrifuged for twenty seconds at 13,000 G to break open and wash away the remaining red blood cells.

c) The red coloured supernatant was then pipetted out and the previous step repeated many times until a clean white pellet was obtained.

d) The pellet was then resuspended in 500 µl of 1xPCR buffer (50 mM Tris-HCl, 1.5 mM $MgCl_2$, 20 mM ammonium sulfate).

e) 5 µl proteinase-K (10 mg ml^{-1}) was then added and the whole tube incubated at 55°C for one hour.

f) At the end of the incubation period the proteinase-K enzyme was inactivated by heating the tube to 95°C for ten minutes.

g) The tube were then stored at -20°C until required for PCR.

2.7.2. ALCOHOL DEHYDROGENASE GENOTYPING METHOD (APPENDIX G)

A number of different methods for distinguishing between the different ADH alleles were investigated.

Some of the methods initially used included genotyping by use of allele specific oligonucleotide probes (ASOP) directed at single base pair differences among the different alleles and also, the utilization of photobiotin labelled nucleic acid probes in conjunction with dot-blot hybridization methods (the oligonucleotide probe hybridization procedure eliminated the preferential melting of A-T to G-C base pairs, permitting the stringency of hybridization to be controllable based on the probe length). However, these methods were very labour- and time-intensive so therefore it was decided to try other more convenient techniques.

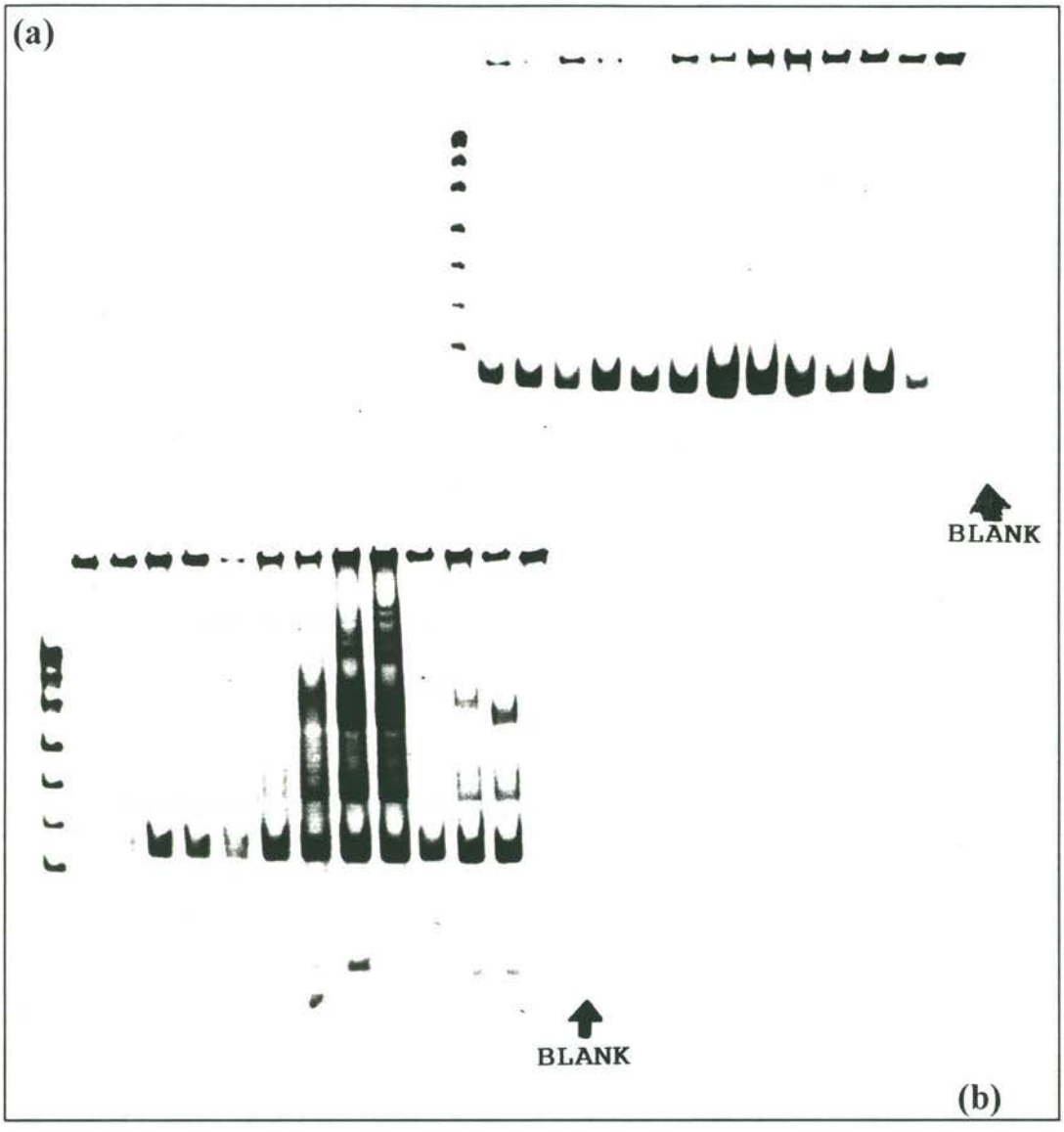


Fig.4: PCR amplification & confirmation gel electrophoresis results.
(a) ADH PCR; **(b) ALDH PCR.**

After the PCR reaction, a portion of the amplified product is 'run' on a confirmation gel. This provides information as to whether the amplification was successful or not, whether contaminants were present, and whether the appropriate DNA sections were amplified successfully. The rest of the PCR product can then be used in conjunction with other detection methods including ASOPs, restriction enzyme digests etc.

The use of fluorescent primers for amplification with PCR and subsequent gel electrophoresis was also investigated, making the numerous and time-consuming washes and hybridizations, required with the dot-blot methods, unnecessary. However, problems with the primers and the ease of contamination resulted in the method being less efficient than was initially hoped and it was decided to find a better method.

M45.5 (sense) Primer:

5' ATT CTG TAG ATG GTG GCT GT 3'

β2C (anti-sense) Primer:

5' GCC TAA AAT CAC AGG AAG G 3'

or

He46 (anti-sense) Primer: (Xu *et al.*, 1988)

5' GAA GGG GGG TCA CCA GGT TGC 3'

Fig.5: Primer sequences used for ADH_2 genotyping.

Eventually, after much experimentation, it was decided that the most reliable method to use in this study was through the use of MaeIII restriction enzyme digestion (Xu *et al.*, 1988) followed by DNA fragment size analysis using polyacrylamide gel electrophoresis and ethidium bromide staining. The amplification process involves the use of two primers acting at opposite ends of the DNA strand.

Binding to the template DNA, the primers are then extended and amplified by the PCR process described earlier. After this, a portion of the product is electrophoresed on a polyacrylamide gel to confirm that amplification has occurred successfully (Fig.4). Due to possession of a restriction site unique to β2, the MaeIII

enzyme is then used to distinguish between β2 and β1 subjects -cutting the β2 DNA into two smaller sized fragments, or not cutting at all if β2 is absent (Fig.6).

A flow diagram of the actual procedure used in genotyping subjects for ADH_2 is shown below:

Whole Blood
⇩
White Blood Cells
⇩
Extracted DNA
⇩
PCR Amplification
⇩
Confirmation Electrophoresis Gel
⇩
MaeIII Restriction Enzyme Digestion
⇩
Electrophoresis Gel
⇩
Detection (Staining with ethidium bromide)

One of the main problems encountered with the genotyping includes contamination of the PCR amplified product. Since PCR can amplify up very small amounts of DNA, contamination could occur even in the presence of only tiny amounts of unwanted target DNA sequences, causing false positives to be detected. Products not digested properly by the MaeIII enzyme and so yielding larger than actual sized bands were also another problem encountered with the restriction enzyme method. However, both these problems could be easily detected with control blanks and the MaeIII internal control.

T GTCAC ACA GAT... GC AAC CTG GTGAG C CCC CTTC
internal control

__ = MaeIII cutting sites

CAC => site cut => β2
CGC => site not cut => β1,3*

Fig.6: MaeIII cutting positions.

* As explained in an earlier section, β3 was assumed to be absent in the subjects tested in this study because the likelihood of its presence in Asian subjects is not great.

The MaeIII internal control is a small sized band of 13 base pairs in length. After complete digestion of the amplified product, the DNA to be examined should be cut in at least one position (i.e. at the internal control site). The other site of digestion would be in the area of interest, yielding different sized bands as a result. In this manner, the proper digestion of the amplified product could be confirmed by the presence of the smaller (13bp) sized band.

BP	Uncut	β1	β2	Het
76	--			
63		--		--
35			--	--
33			--	--
13		--	--	--
(13 = Int.control => should cut)				

Fig.7: Results of ADH_2 genotyping.

2.7.3. ALDEHYDE DEHYDROGENASE GENOTYPING METHOD (APPENDIX H)

Isoelectric focusing of hair root extracts has been employed in the past in determining ALDH enzyme activity and thus phenotype (Agarwal, Harada & Goedde, 1981). Using the molecular genetic techniques described above for ADH_2 genotyping, DNA was extracted from the white blood cells and amplification of the segment of the gene containing the $ALDH_2$ mutation site was performed utilizing PCR in combination with the appropriate primers. The amplified product was then run on a polyacrylamide gel for confirmation of amplification (Fig.4).

Following successful amplification, various approaches were used in order to distinguish between the various ALDH allele types.

18mer Primer:

5' AAT TAC AGG GTC AAC TGC 3'

Exon12 clamped Primer:

5' CGC CCG CCG CGC CCC GCG CCC GTC CCG CCC CCG CCC CCG

CCA CAC TCA CAG TTT TCA 3'
(Exon12 match)

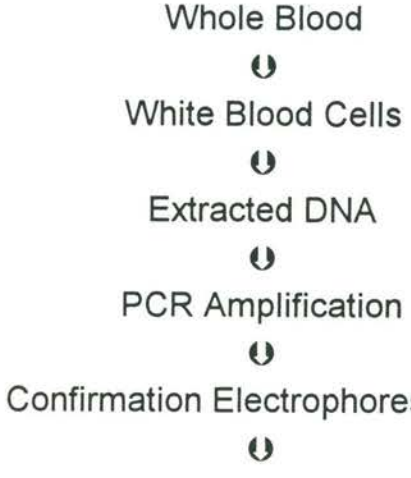
Fig.8: Primer sequences used for ALDH genotyping.

One of the methods used was through the use of radioactive probes that were labelled for the ALDH2-2 allele combined with dot blot hybridization. By hybridizing the amplified PCR products, transferred onto nitrocellulose membranes with radioactively labelled probes, products containing the ALDH2-2 allele hybridized with their complementary radioactive probes. Numerous stringent and non-stringent washes were then carried out on the membranes. The membranes, together with

radioactive probes selectively attached to the appropriate DNA for which it was probing, were then left overnight placed under a sheet of autoradiograph film. Results were inferred from the film on the following day. This method turned out to be time-consuming and labour-intensive therefore another method was investigated.

The method employed in this study, involved the use of 48% Constant Denaturant Gel Electrophoresis (CDGE) at 60°C on the amplified product (Zybenko, Nightingale & Whitfield, 1995). This method was based on the fact that sequence changes in a piece of DNA being tested would produce changes in the melting characteristics of that particular DNA strand. The different genotypes, varying by only one basepair, would migrate through the gel at different rates depending on whether they were heterozygote or homozygote. To increase sensitivity and to ensure that all changes could be detected, an additional very stable section of DNA with a higher melting temperature (G-C Clamp) was attached to one of the primers used in the PCR reaction, and hence onto the DNA of interest. With this method, the temperature at which the gels were run was extremely crucial, a 1°C change being equivalent to a 3% (approximately) change in denaturant solution concentration.

A flow diagram of the actual procedure used in genotyping subjects for $ALDH_2$ is shown below:



Constant Denaturant Gel Electrophoresis (CDGE)

↓

Detection (Staining with ethidium bromide)

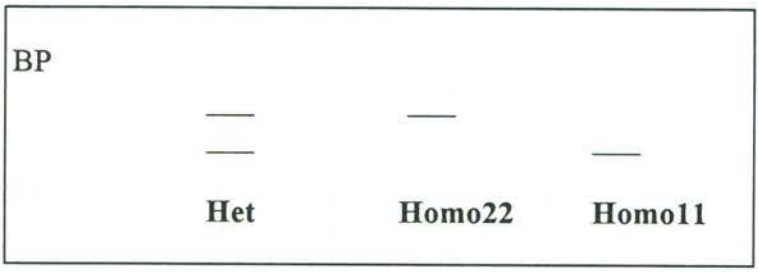


Fig.9: Results of ALDH genotyping: Bands visualized after ethidium bromide staining.

Problems encountered with genotyping for ALDH encompassed those associated with primer quality, contamination, specificity and the actual electrophoretic system itself. Temperature fluctuations caused by the surrounding room temperature when running the gels would occasionally occur, affecting the results obtained. It was found that the gel components used in the mixture needed to be freshly made; otherwise bands could not be distinguished clearly and easily.

2.8. BIOCHEMICAL METHODOLOGY

Venipuncture (Travenol Lab., Miniset 0.81x19mm) was used to withdraw blood from a forearm vein before ethanol consumption and at 15, 60 and 120 minutes afterwards. All assays were performed in duplicate, except the acetaldehyde assay which was carried out in triplicate.

After blood withdrawal, the blood samples were separated and treated as follows:

250 µl Blood	+	Acetaldehyde Tubes (triplicate)
10 ml Blood	+	Heparin Tube (acetate & ethanol assays)
5 ml Blood	+	5% Perchloric Acid Tube (lactate & pyruvate assays)

Tubes for the acetaldehyde assay were treated as described in the following sections. The other tubes were centrifuged for twenty minutes at 3000 rpm at the end of the session to obtain the plasma/ supernatant. The plasma layer was then transferred to fresh vials for storage or transport to another laboratory for analysis. Additionally, with the heparin tubes, the white blood cells on top of the plasma layer were removed from the centrifuged tube and transferred to autoclaved Eppendorf tubes for genotyping.

2.8.1. MEASUREMENT OF ACETALDEHYDE CONCENTRATIONS

High performance liquid chromatography (HPLC) and modern headspace gas chromatography methods to measure acetaldehyde levels in blood have approximately equivalent sensitivity (Fukunaga, Sillanaukee & Eriksson, 1993). However, it remains technically difficult to measure acetaldehyde concentrations accurately in samples, and the difficulties experimenters have been faced with when attempting to measure these acetaldehyde levels have been widely documented (Eriksson, 1980; Lindros, 1983; Fukunaga, Kogame, Adachi, Ueno & Mizoi, 1986).

Artifactual production of acetaldehyde was, and still is, a major problem and corrections must be made in calculations (Fukunaga *et al.*, 1993). Rapid disappearance, caused by enzymatic oxidation and/ or binding to other blood constituents, is also another important problem associated with blood acetaldehyde determination. The acetaldehyde in the blood, either endogenous or exogenous in origin, has been suggested to exist in 'free', 'loosely bound' or 'more firmly bound' forms (Okada & Mizoi, 1982; Lucas, Menez, Berthou, Pennec & Floch, 1986; Fukunaga *et al.*, 1993). The bound acetaldehyde originally existing *in vivo* may be released during the analytical treatment of blood samples and it is also believed that ethanol can be converted to acetaldehyde *in vitro*, and that this reaction might be catalyzed by blood proteins (Baraona, Behrens, Ma, Hernandez-Munoz, Uppal & Lieber, 1991; Fukunaga *et al.*, 1993). Therefore it can be seen that many important points must be considered when acetaldehyde determinations are made.

The assay used was based on the reaction of acetaldehyde with ammonium ion and 1,3-cyclohexanedione to form a fluorescent derivative (Ung-Chhun & Collins, 1987). This step was carried out immediately after the blood was obtained from the subject. The acetaldehyde tubes into which the fresh blood was transferred contained well mixed amounts of 50 µl of 0.5 M thiourea, 10 µl of 2 M sodium azide, 800 µl of 200 mM 1,3-cyclohexanedione (Sigma Chemical Co.) in 2 M ammonium chloride, and 10 µl of 0.6 M propionaldehyde (internal standard). These tubes were prepared up to two days before the testing session and then stored in a refrigerator until required. At the end of the testing session, all acetaldehyde + blood tubes were heated to 60^{o}C for one hour, then cooled in ice before being centrifuged for twenty minutes at 3500 rpm. The supernatant was then run on the HPLC.

The derivatised acetaldehyde product was then (within 24 hours of blood withdrawal) separated out on a C18 column (Waters Associates, 25 x 0.4cm) by reversed phase high performance liquid chromatography (HPLC) and quantitated fluorometrically (Shimadzu RF-535). The excitation filter was set at 375 nm and the

emission filter at 455 nm. A filtered acetonitrile/ water mixture (25:75 v/v) was used as the mobile phase. The total run time was less than seven minutes on the HPLC system.

This method was found to be sensitive, the detection limit for blood acetaldehyde levels was 0.1 μM, and could be used for small samples (250 μl). The precision of each measurement was 11.28 μM (mean=64.71). The assay was linear over the range of standards used, with a good linear relationship (r^2=0.99) being found between height of peak and amount of acetaldehyde measured. All values obtained were corrected for artifactual generation of acetaldehyde or pre-existing protein-bound acetaldehyde, by subtracting t_0 values (blank correction) from the value measured at the different times.

2.8.2. MEASUREMENT OF ACETATE CONCENTRATIONS

The method used to measure acetate levels in plasma involved the use of UV enzymic acetate assay kits (Boehringer Mannheim GMBH) and the following reactions:

* AcetylCoA Synthetase (ACS)
* Citrate Synthetase (CS)
* Malate Dehydrogenase (MDH)

Acetate was converted to acetyl-CoA in the first reaction which was catalysed by acetylCoA synthetase. The acetyl-CoA produced was then combined with oxaloacetate to form citrate in the presence of citrate synthetase (reaction 2). The oxaloacetate required for the second reaction was formed in a separate reaction (3) in the presence of malate dehydrogenase. This third reaction also causes the NAD^+ to be reduced to NADH. The acetate determination is based on this formation of NADH which is quantitated by an increase in absorbance spectrophotometrically (Shimadzu UV-260) at 340 nm, and the acetate concentration calculated from the standards (0.5, 1, 2 & 10 mM) used (Beutler, 1984). The precision of each measurement was calculated to be 0.029 mM (mean=0.861).

2.8.3. MEASUREMENT OF PLASMA ETHANOL CONCENTRATIONS

Ethanol levels in plasma were quantified by capillary column (25m x 0.25mm BP-20) gas chromatography (GC) using a flame ionization detector (Hewlett Packard 5890 Series) with n-propanol as the internal standard.

Gas chromatography is the most specific method for estimation of plasma ethanol concentrations available to date. This method enabled the measurement of ethanol levels to be determined from relatively small (200 µl) samples of plasma. The limit of detection of ethanol was found to be 1 mM, the assay was also linear up to 200 mM ethanol, and the method used has been shown to be sensitive, accurate and

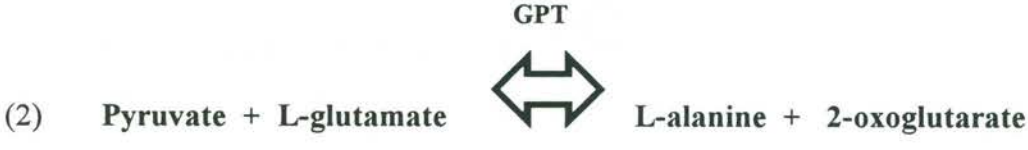
reproducible (RPAH Standard Methods SD15/2/90). The precision of each measurement was 0.623 mM (mean=19.62).

The run time for this assay was approximately five minutes. The mobile phase (nitrogen gas) was set at 35 kPa, air was set at 150 kPa and the fuel (hydrogen gas) was set at 250 kPa. The oven temperature was kept at 40°C.

2.8.4. MEASUREMENT OF LACTATE CONCENTRATIONS

The blood samples collected for both lactate and pyruvate analysis were deproteinized with an equal volume of 0.6 M perchloric acid immediately after withdrawal to prevent any further reactions occurring.

Lactate levels in plasma were measured using the following reactions:



* Glutamate-pyruvate Transaminase (GPT)
* L-Lactate Dehydrogenase (L-LDH)

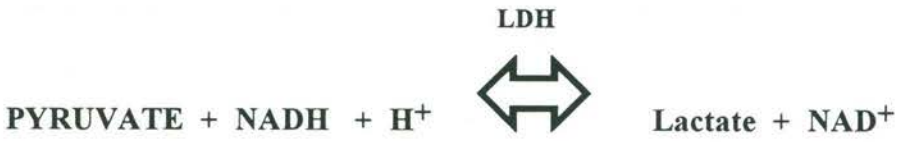
In the method used to measure lactate concentration, the lactate was oxidized to pyruvate in the first reaction. The second reaction converted the pyruvate to alanine, trapping the pyruvate and forcing the first reaction to completion. The

absorbance change at 340 nm (Shimadzu UV-260) was proportional to the lactate concentration in the sample. The precision of each measurement was calculated to be 0.046 mM (mean=0.587).

2.8.5. MEASUREMENT OF PYRUVATE CONCENTRATIONS

When ethanol is oxidized, the first step causes an excess of reducing equivalents (NADH) in the cytosol. This creates a shift in the redox potential of the cytosol, which is expressed as a change in the lactate:pyruvate ratio. Pyruvate is in equilibrium with lactate in the liver, and both lactate and pyruvate are believed to equilibrate between the liver cells and the blood. Therefore, the blood lactate:pyruvate ratio reflects changes in the NADH:NAD^+ ratio in the hepatocyte cytosol. Any excess lactate escapes into the bloodstream and can be detected.

Plasma pyruvate concentrations were also obtained by enzymatic means, based on the following reaction:



* Lactate Dehydrogenase (LDH)

The pyruvate concentration in blood was determined spectrophotometrically by measuring the change in optical density (at 340 nm) during the conversion of pyruvate to lactate. The resulting change in optical density was then compared with controls containing known amounts of pyruvate. The precision of the pyruvate concentration measurements was calculated to be 0.038 mM (mean=0.956).

2.9. PHYSIOLOGICAL METHODOLOGY

Subjects were seated comfortably in lounge chairs and allowed to relax before and during the measurement of physiological variables.

2.9.1. MEASUREMENT OF HEART RATE AND BLOOD PRESSURE

The systolic and diastolic blood pressure and heart rate were measured simultaneously from the antecubital artery using a digital sphygmomanometer (Yamasu YSE-320).

2.9.2. MEASUREMENT OF SKIN TEMPERATURE

Skin temperature was measured *via* a reusable skin temperature probe (YSI-401 series) which was attached to the subject's left cheek for the duration of the testing session.

The probe was connected to a digital pocket temperature monitor (Zentemp 2000) which the subject carried throughout the test, and from which readings were taken every five minutes.

The accuracy has been measured as $\pm 0.1^{o}C$ over the operating range of the thermometer (Zencor).

2.10. PSYCHOMOTOR METHODOLOGY

Psychomotor performance ability varies greatly among subjects according to many different factors. Under the influence of ethanol, this variation in performance ability has been found to increase.

Four major aspects of performance were measured in our test battery, these were co-ordination, steadiness, cognitive time and reaction time.

All the psychomotor tests incorporated in this study were chosen to monitor both the cognitive and motor effects of ethanol; the standing steadiness and CFFF tasks being highly sensitive and non-learnable.

The test battery (Fig.10) used in this study utilized an Apple IIe computer with a monochrome monitor, a response board with keys numbered zero to nine, a four button response pad, and a potentiometer connected to a steering wheel which was used for the divided attention tracking task.

A separate piece of equipment (Leeds Psychomotor Tester) was used for the critical flicker fusion frequency threshold task.

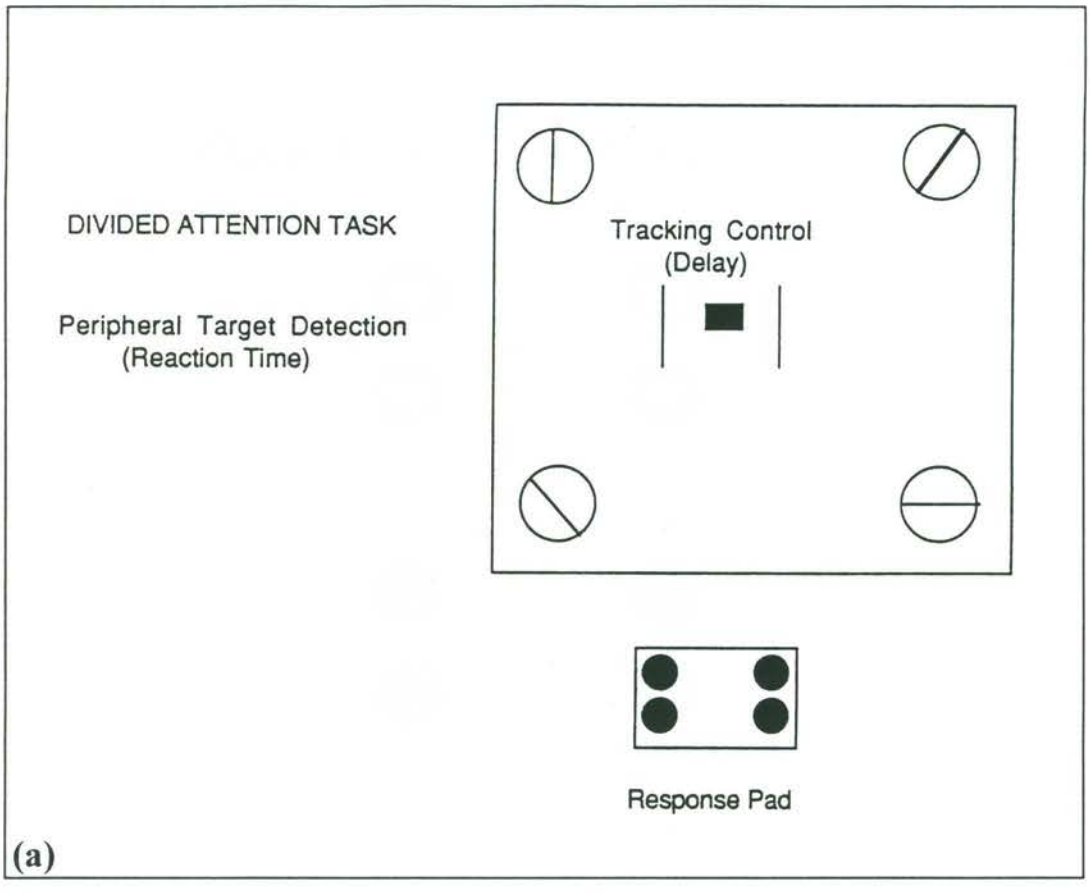
During testing, the subject was seated in a darkened room and headphones, emitting white noise, were worn by the subject to decrease the effect of distractions.

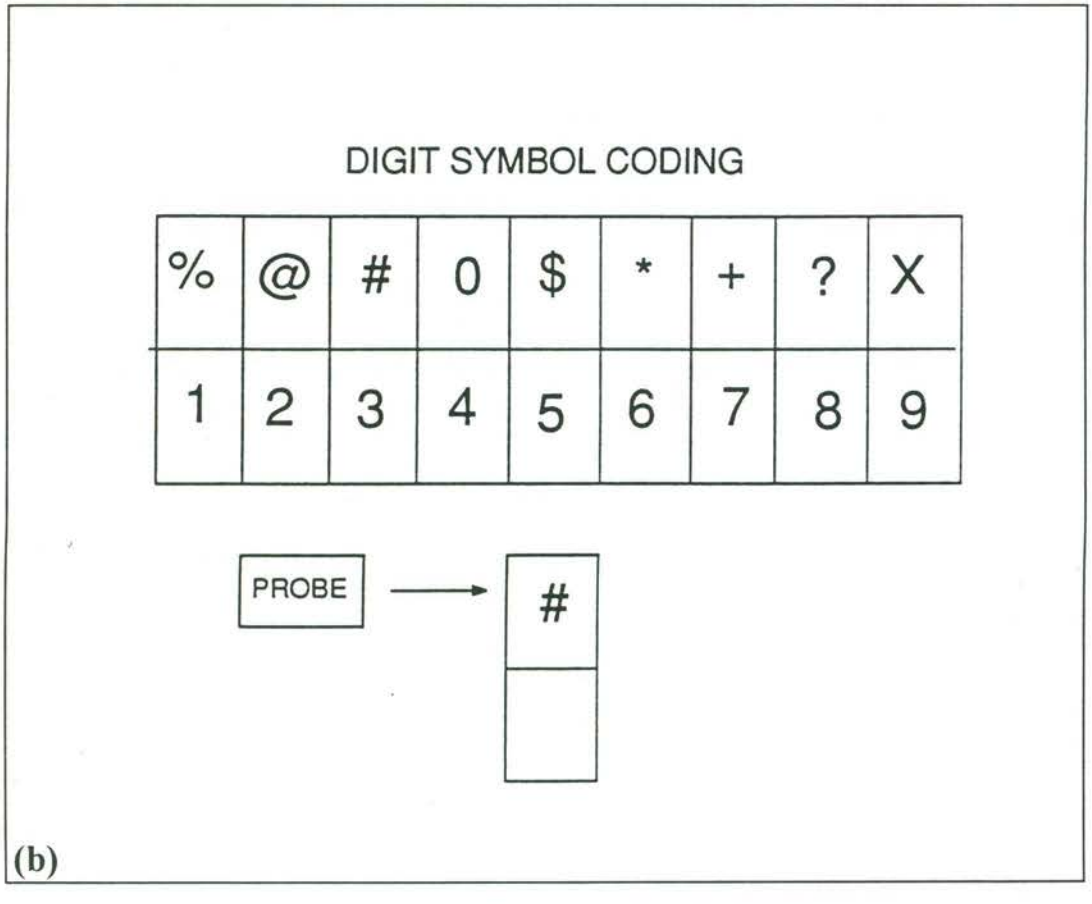
The psychomotor performance tests were explained in English or Chinese (depending on the subject's requirements) using a standard description to ensure consistency. Subjects were given as much time as they required to familiarize themselves with the various computerized tasks before commencement of actual testing.

Fig.10: Photograph of the test battery utilized.

2.10.1. DIVIDED ATTENTION TASK

In the divided attention task (Fig.11a), both the central and peripheral task were presented on one screen simultaneously. The central component was a pursuit tracking task -two vertical lines moving irregularly backwards and forwards across the screen, the subject being required to keep a small rectangular cursor within the two lines. The program monitored the subject's performance, and adjusted the speed of the moving display according to the number of errors the subject made in each five second interval. If no errors were made then the speed would be increased. The final ten speed readings were then averaged to give an indication of the subject's performance. The mean delay time taken to react, recorded at each five second interval, was used to assess ethanol induced impairment.


DIVIDED ATTENTION TASK
Peripheral Target Detection
(Reaction Time)
Tracking Control
(Delay)
Response Pad
(a)


DIGIT SYMBOL CODING
% @ # 0 $ * + ? X
1 2 3 4 5 6 7 8 9
PROBE
#
(b)

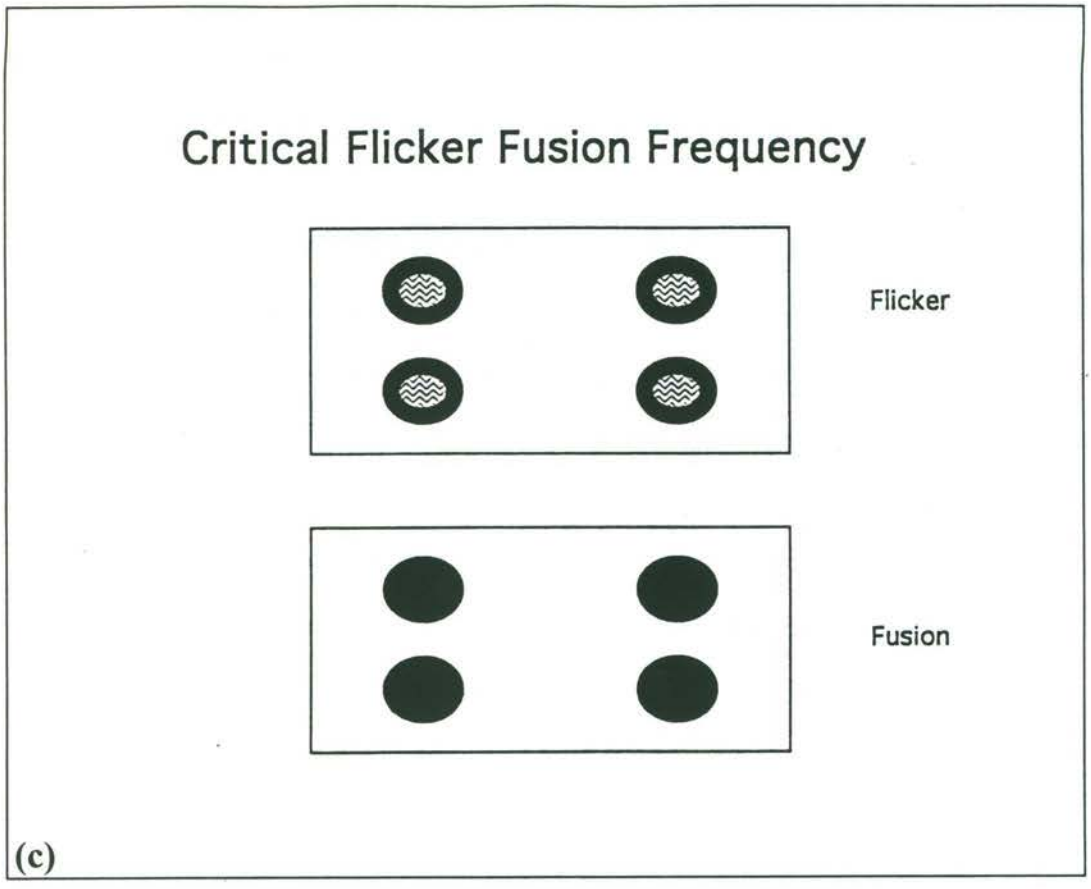


Fig.11: Schematic diagrams of **(a)** the Divided Attention test setup; **(b)** the Digit Symbol Coding task and **(c)** the Critical Flicker Fusion task.

The peripheral component was a visual discrimination task. On the screen at each corner a circle was displayed. Within the circle a line orientated in one of four directions (vertical, horizontal, oblique right or oblique left) was seen. The oblique right target was the designated target to which the subject was requested to respond with an appropriate button press. Each button press was recorded, and the reaction time for hits was recorded as was the number of errors, misses and false alarms.

2.10.2. DIGIT SYMBOL CODING (DSC)

This was a test of perceptual motor speed which incorporated sustained attention on a repetitive task.

The test involved the recognition of geometric figures from a table of nine different symbols and numerals (Fig.11b). Upon presentation of a randomized probe symbol, the subject was asked to key in as quickly as possible, the correct corresponding numeral. The mean correct reaction times and accuracy were recorded.

2.10.3. STANDING STEADINESS

A well known effect of ethanol intoxication is decreased balance, causing the subject to become ataxic. The severity of ataxia is dependent on the amount of ethanol consumed and the tolerance of the subject.

Body sway, in the forward and backward directions, was measured by asking the subject to stand on a platform beneath which a displacement transducer was mounted (Fig.12). The subject was instructed to stand as still as possible without talking or moving. Any shift in the subject's centre of gravity, of which sway is a function, created an electrical impulse which was amplified, integrated (Grass Integrator, model P10B) and recorded on a Grass polygraph (Quincy, Mass., USA) to give an overall measure of body sway (frequency and amplitude). The time(s) to accumulate a given amount of sway was recorded; the longer the time, the steadier the subject. Measurements were conducted with the eyes open.

2.10.4. CRITICAL FLICKER FUSION FREQUENCY (CFFF)

CFFF has been used as a means of measuring the ability of a subject to distinguish discrete sensory data and hence is used as a measure of cognitive ability and mental alertness.

In this study, the subject was required to discriminate flicker (20-70 Hz) in a set of four light-emitting diodes held in foveal fixation. The subjects were required to respond by button press, to the light emitting diodes (LEDs) beginning to flicker, and in a separate measurement, to respond when flickering disappeared (Fig.11c). Individual thresholds were determined by the psychophysical method of limits on three ascending and three descending scales.

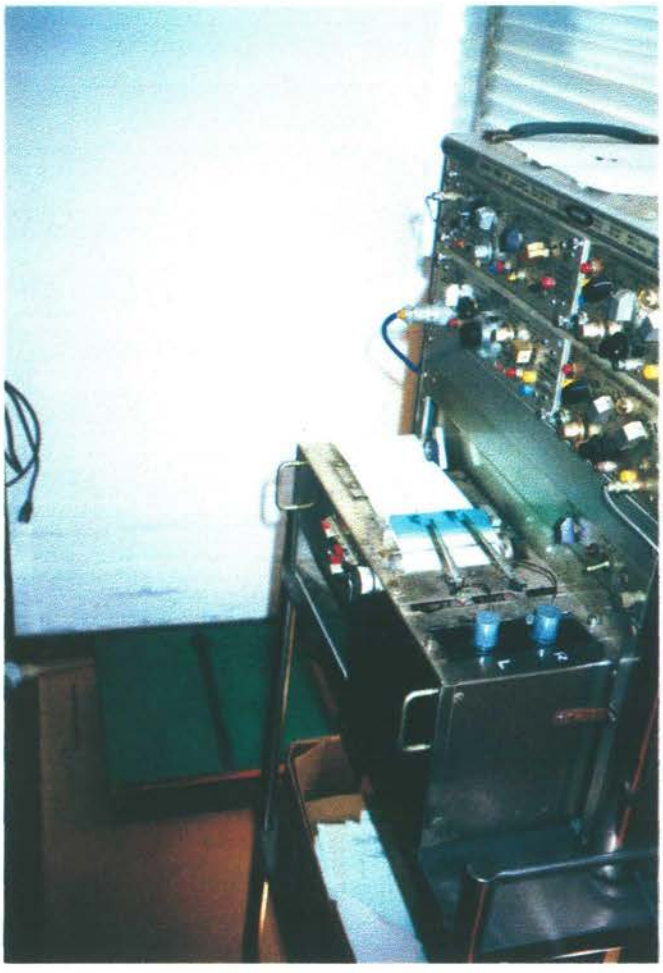


Fig.12: Photograph of Standing Steadiness equipment used.

2.11. SUBJECTIVE MEASURES OF INTOXICATION

Visual analogue scales using end-point adjective descriptions of a mood state were used to assess various aspects of ethanol intoxication.

The questions were presented as a series of questions in a questionnaire (see Appendix E):

1. Do you feel 0 (*alert*) ...9 (*drowsy*)

2. Do you feel 0 (*well coordinated*) ...9 (*clumsy*)

3. Is your level of concentration 0 (*low*) ...9 (*high*)

4. How intoxicated do you feel 0 (*not at all*) ...9 (*extremely*)

5. Do you feel 0 (*attentive*) ...9 (*dreamy*)

The subjects were required to complete the above questions by marking on a horizontal scale, of 0 to 9, how they felt at that particular time.

Additionally, Yes/ No questions were also asked in the subjective questionnaire. These questions were more specific in that they enquired if the subject experienced a headache, did he/ she feel uneasy or sick in the stomach, and also if he/ she felt any hangover effects. If subjects answered in the affirmative to any of these questions, they were then asked to provide further details.

2.12. STATISTICAL METHODS

The data analyses used in this study concentrated on testing the effects of genotype and flushing status on the pharmacokinetic, biochemical, physiological, psychomotor and subjective effects of ethanol. The factors and the dependent variables have been analysed individually for simplicity's sake and the results are presented in this manner.

Performance on the psychomotor test battery, biochemical, physiological and pharmacokinetic variables were analyzed using an ANOVA repeated measures analysis of variance.

The within-subjects factor was time (t_0, t_{15}, t_{60}, t_{120} for the blood or plasma measurements, and at t_5, t_{15}, t_{30}, t_{45}, t_{60}, t_{75}, t_{90}, t_{105}, t_{120}, t_{135} and t_{150} for breath ethanol readings).

The between-subjects factors were group: {Homo11, Het, Homo22} for ADH_2 and $ALDH_2$ genotypes; {GroupA, GroupB, GroupC, GroupD, GroupE} for $ALDH_2$/ ADH_2 combined genotypes; {males, females} for gender and {NF, FF, LF} for degree of flush.

Post-hoc Newman Kuels tests adjusted for multiple comparisons were performed on the means at the individual times.

Tests for correlation between rate of ethanol metabolism and metabolite concentrations were carried out using a rate which was calculated as described earlier, and the mean acetaldehyde, acetate or lactate: pyruvate ratio values over the t_{15}, t_{60} and t_{120} samples.

Statistical tests were performed using BMDP-Dynamic (BMDP Statistical Software Inc., Los Angeles CA), programs 2V, 6D, 7D, 3S and 3R; or StatXact

Turbo (Cytel Software Corporation, Cambridge MA), for Fisher's exact test on contingency tables.

A minimum significance level of $p<0.05$ was chosen to indicate statistical significance when analyzing the data.

Chapter 3.

RESULTS

3.1. SUBJECT DEMOGRAPHICS

All 110 subjects who were tested lived in Sydney, Australia, at the time of the study. Although most subjects were Australian citizens, some were overseas students who were studying in Australia at the time.

Males made up 61% of the total number of subjects who were tested. The average body weight of all subjects was 62.8 ± 9.3 (SD) kg. The female subjects were lighter 56 ± 8.1 (SD) kg than the males 67.3 ± 7.2 (SD) kg and the difference was statistically significant ($p<0.005$).

The ages of the subjects ranged from 18-46 years, with the average age being 23.2 ± 5.5 (SD) years. The mean age of the two sexes did not differ significantly ($p>0.05$); male subjects were 23.7 ± 5.6 (SD) years old and the females were 22.6 ± 5.1 (SD) years old.

The subjects all reported that they were in good health, and that they had not consumed food, smoked cigarettes or drank ethanol within eight hours before the testing session. The reported time from the last meal (either breakfast or dinner) until the time of testing was 13.2 ± 3.7 (SD) hours.

3.1.1. BACKGROUND OF SUBJECTS

Most of the subjects tested could trace their ancestral origins to China (74%). People of Korean or Japanese descent made up 7% of all subjects tested, whilst Vietnamese, mixed Asian/ Asian and mixed Asian/ Caucasian subjects made up 5%, 4% and 4% respectively. The remaining subjects came from a number of other countries in the Asian region including Burma, Cambodia, Taiwan, Hong Kong, Singapore, Malaysia and Indonesia (Fig.13).

It should be mentioned here that despite these group distinctions, many of the subjects (apart from the ones who were born in China) were of Chinese origin; this

could be inferred from their surnames and by subsequent questioning into the subject's family history and background.

45% of subjects tested reported growing up in a Westernized country (Australia, USA or Canada). 28% of subjects grew up in Singapore, Malaysia or Indonesia; 9% in China; 8% in Hong Kong or Taiwan; and the rest in other countries including Vietnam, Korea, Japan and various islands in the South Pacific (Fig.14).

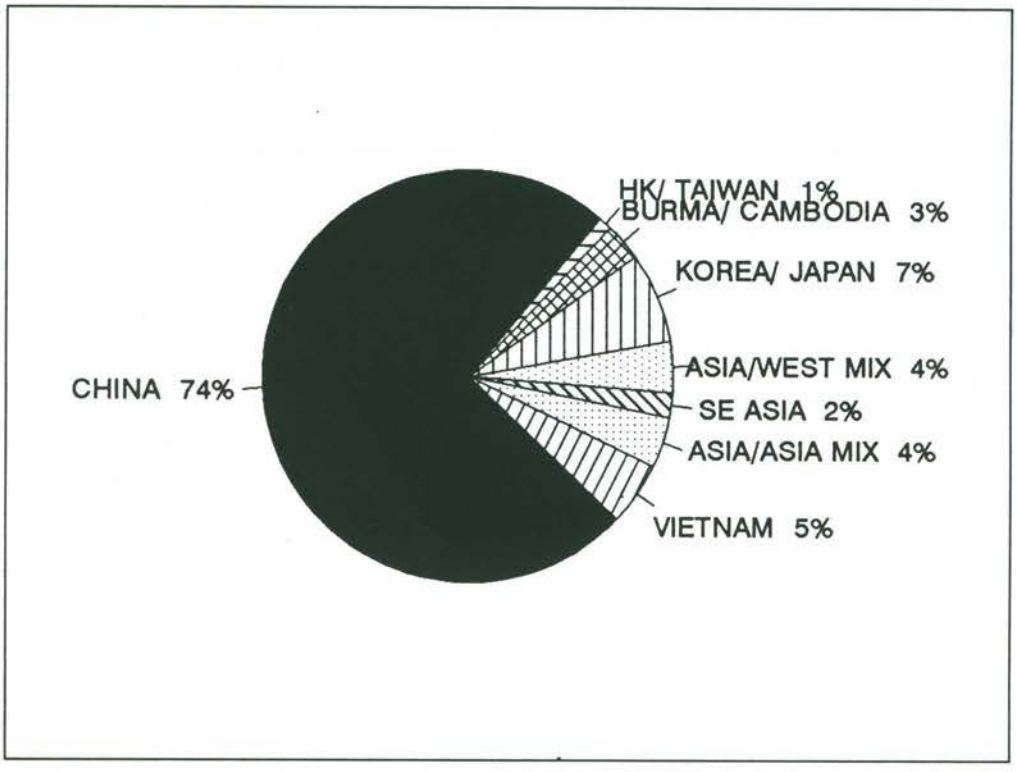


Fig.13: Country of origin of family of subjects tested.

It should be noted that these data only indicate the country in which the subject grew up and cannot be taken to indicate whether full assimilation into the prevailing culture, attitudes and behaviour of the 'host' country had been attained by the individual subject, or whether the traditional culture of the country of origin had been retained.

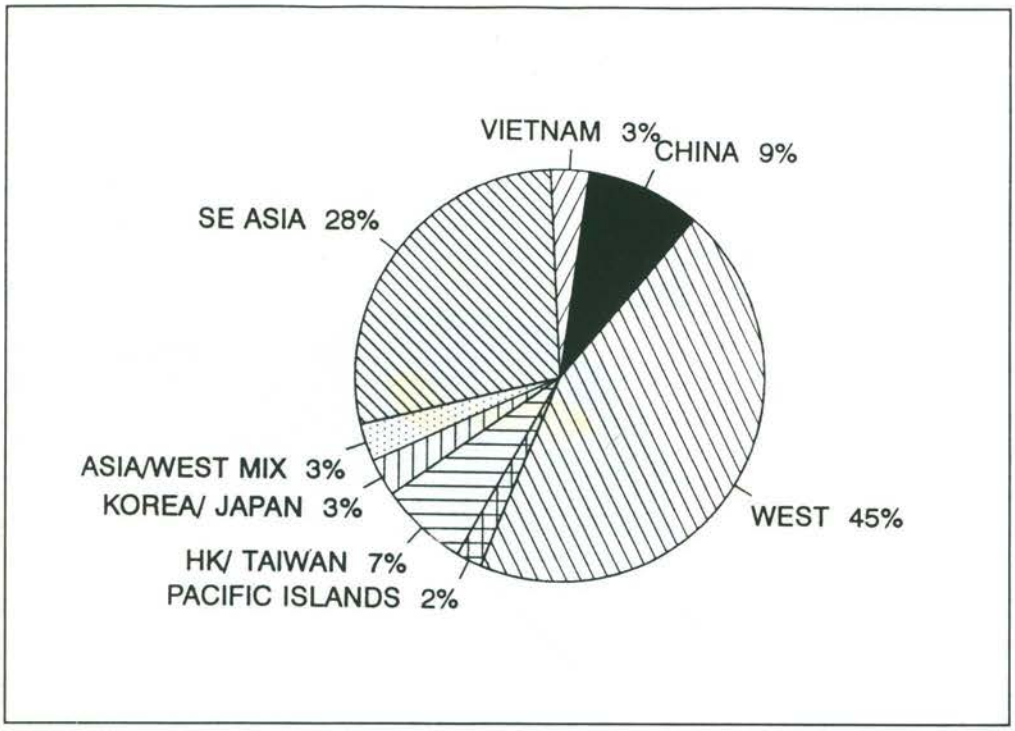


Fig.14: Country where subjects were brought up.

3.1.2. DRINKING DETAILS

Most (78%) of the subjects who were tested said that they considered themselves to be flushers who experienced a flush response after drinking ethanol. The remaining 22% of subjects did not flush after drinking.

Non-flushers Of the non-flushing group, 87% reported that they did not drink on a regular basis. Just over half of these subjects drank less than 1-2 times a month or not at all (52%), 35% drank 1-2 times a month, and 13% reported drinking 1-2 times a week (Fig.15a).

The usual amount of ethanol consumed per session by the non-flushers was two or more standard drinks (57%). However, 33% of this group reported that they

usually only drank one drink per session, and 10% drank even less than this amount (Fig.15b).

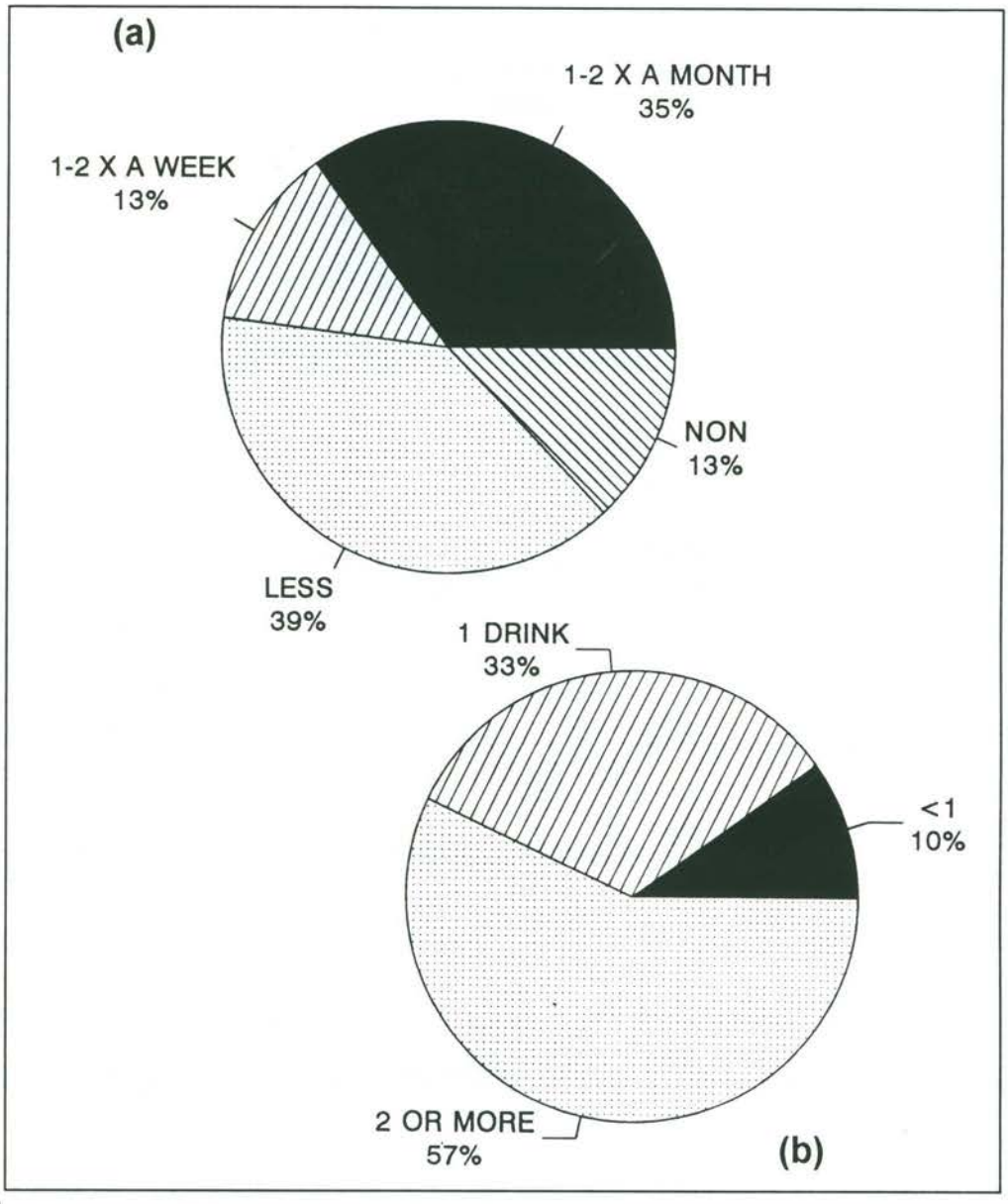


Fig.15: Ethanol intake **(a)** frequency and **(b)** quantity for non-flushing subjects.

The most popular beverage consumed by the non-flushers was beer (50%) followed by wine (25%) and spirits (20%) -Fig.16.

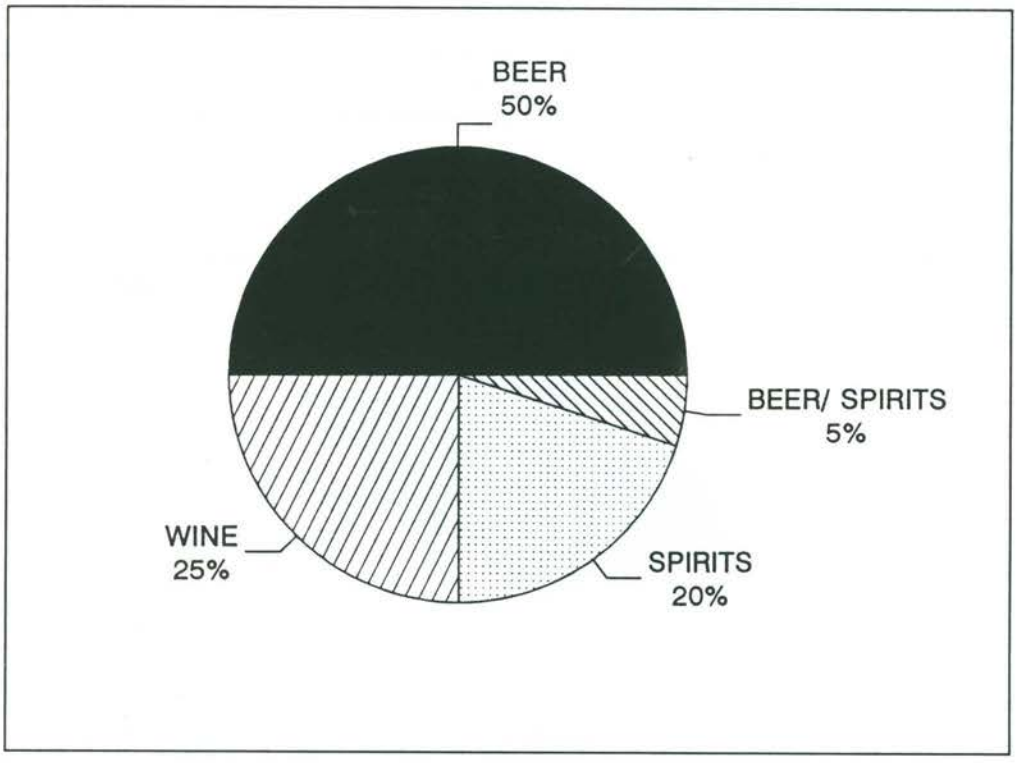


Fig.16: Choice of beverage preferred by non-flushers.

Flushers Subjects who reported flushing after ethanol were also mostly occasional drinkers (83%). Only 17% of flushers reported drinking on a regular basis.

Flushers who did not normally drink at all made up 5% of the flushing subjects; 38% of this group reported drinking less than 1-2 times a month, 35% drank 1-2 times a month, 21% reported drinking 1-2 times a week, and only 1% of subjects drank up to 3-4 times a week (Fig.17a).

The quantity of ethanol reported to be consumed by flushing subjects was one 'standard' drink (containing 10 g of ethanol) per session (44%). Only 14% of the flushing subjects recorded that they usually drank less than one standard drink per session, while 42% drank two or more drinks (Fig.17b). It should be noted that some subjects might have reported consuming one drink even if they usually did not finish all of it, perhaps explaining the low incidence of <1 drink subjects in this flushing group.

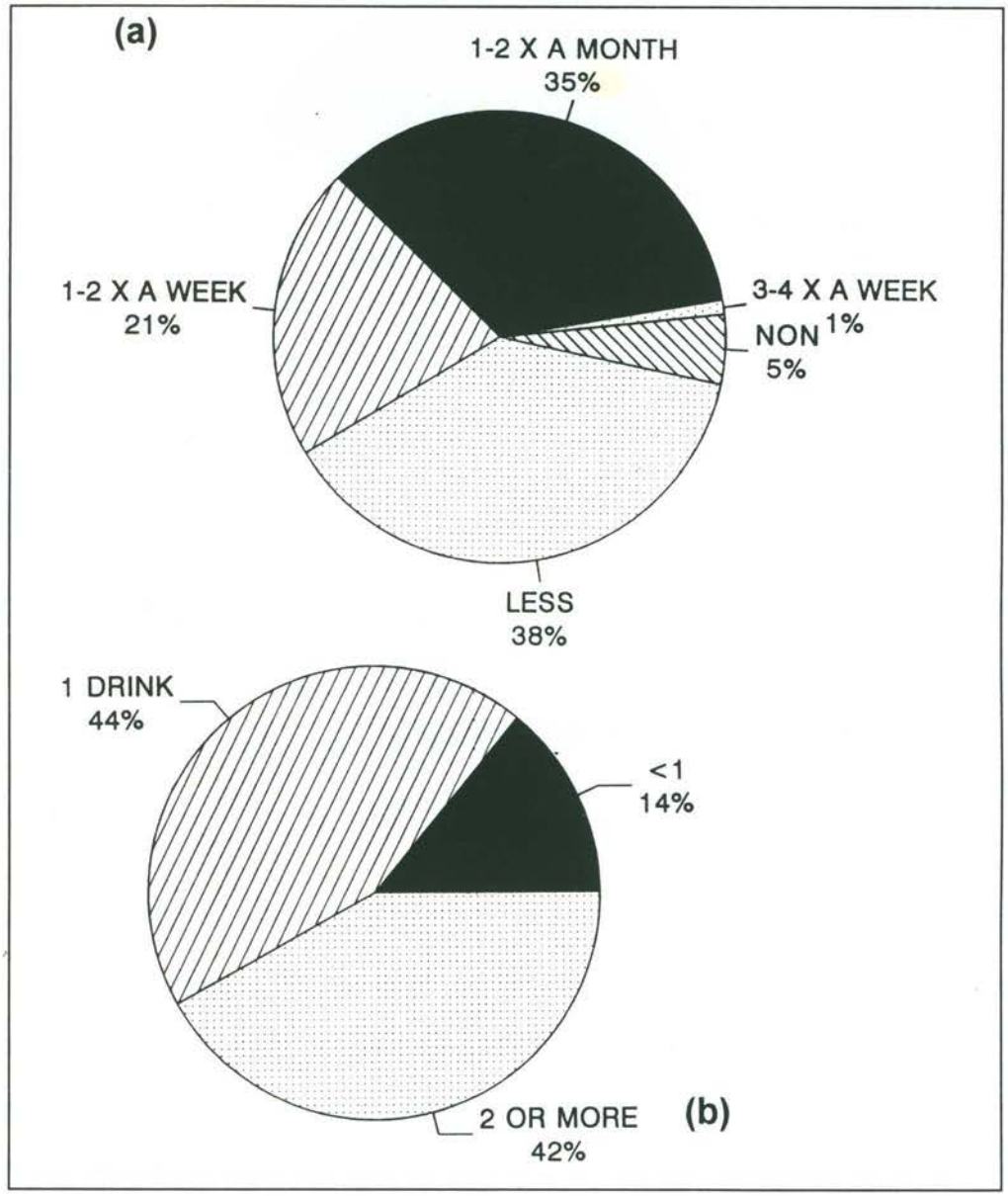


Fig.17: Ethanol consumption **(a)** frequency and **(b)** quantity flushers.

For Flushers, the beverage of choice was beer (39%), followed by spirits - including traditional wines (26%) and table wine (14%); the remaining subjects consumed combinations of beer and spirits (6%), beer and wine (5%) and spirits and wine (10%). The other subjects did not drink regularly enough to be able to specify which drink they consumed most often (Fig.18).

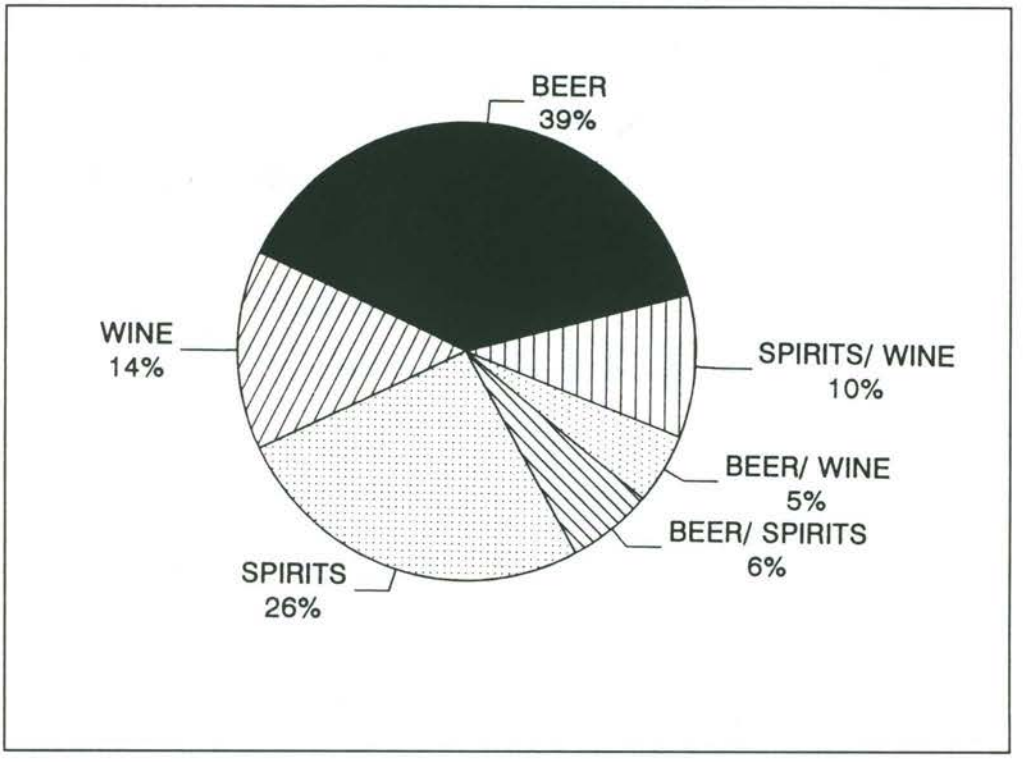


Fig.18: Choice of beverage preferred by flushers.

3.1.3. FLUSHING DETAILS

From analysis of the responses to the questionnaire given to subjects before the testing session, 79% of subjects reported that they usually experienced facial flushing after consumption of ethanol. 3% of subjects said they did not know if they flushed or not, and the remainder reported that they did not 'go red' after drinking. This self-reported flushing experience of subjects was found to parallel the observed flushing behaviour of subjects after ethanol challenge (79%); confirming accurate

reportage and honest co-operation in the study. The 3% of subjects who did not know whether they flushed or not after ethanol *did not* flush after ethanol challenge.

Fig.19 shows the breakdown of subjects according to the degree of flush as defined below:

Rating:	**'NF'**	0	Non-flushers
	'FF'	1	Glow (faint)
		2	Distinct glow (cheeks faintly)
		3	Cheeks flushed
		4	Obvious facial flush (neck faintly)
	'LF'	5	Obvious facial, ears and chest flush
		5.5	As in 5 but with some flushing of the limbs
		6	'Lobster Flusher' (body/ face flushed)

Non-flushers comprised 22% of the subjects who were tested, the remaining 78% of subjects flushed to varying degrees, with 41% of the subjects being rated in the LF group -the group with the worst flushing experiences.

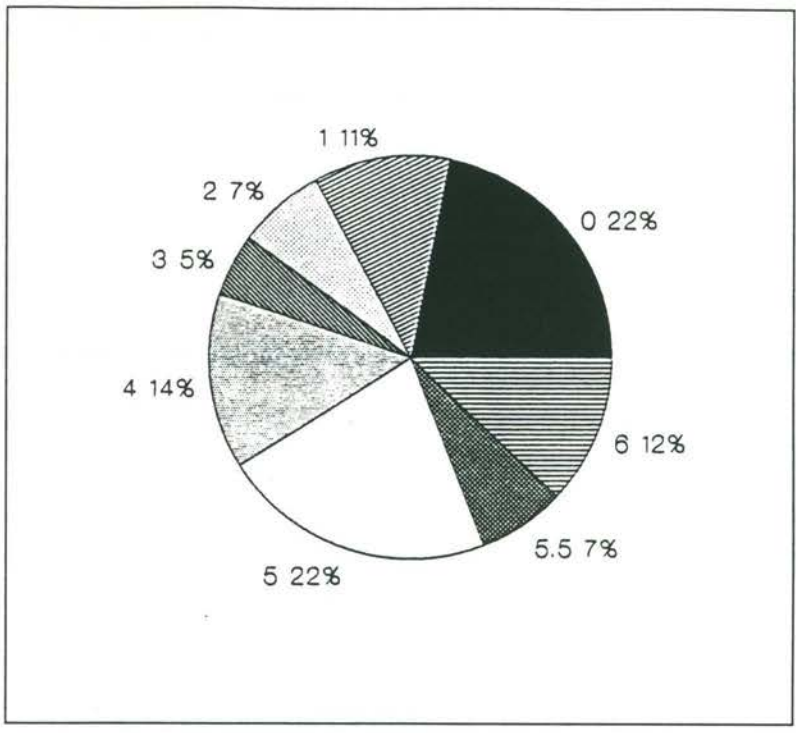


Fig.19: Breakdown of subjects according to degree of flush.

3.1.4. EXCLUDED DATA

The results from three of the subjects were not included in the analysis because, after ethanol, they felt too ill or were otherwise disinclined to complete the test session.

In addition, one of the subjects was excluded from the study because of his reported (in the health diary) use of a hypnotic drug the week before the test session.

3.1.5. SUBJECT MOTIVATION

The subjects said that they were motivated to participate in the study primarily out of curiosity concerning the alcohol flush reaction (AFR). Most of the subjects had personally experienced or knew of friends who had experienced the AFR and they wanted to find out more about this reaction and why it occurred. Many people also wanted to find out, in a test situation, how they would react to the consumption of

ethanol. Although subjects had a wide range of ability and educational achievement, the majority were university students who appeared to be especially interested in the outcome of the study.

In this Chapter, the findings obtained from the present study are organized into several sections:

3.2. Observations recorded

3.3. Results by ADH_2 genotype

3.4. Results by $ALDH_2$ genotype

3.5. Results by Degree of Flush

3.6. Results by ADH_2/ $ALDH_2$ genotype combined

3.7. Results by Gender

3.8. Additional results

At the end of this Results section, a summary of all significant findings is shown in table format.

3.2. OBSERVATIONS

Subjective assessment of the degree and extent of the flushing which occurred after ethanol challenge allowed the development of a rating scale (section 3.1.3.). The scale initially used was based on a rating of 0-6, with 0 indicating non-flushing subjects, and 6 indicating the worst flushers (later referred to as 'Lobster Flushers').

Closer inspection of the subjects' flushing patterns indicated that three major groups of flushers could be distinguished -

1. Non-flushers (NF)
2. Facial Flushers (FF)
3. 'Lobster' Flushers (LF)

The NF group did not exhibit any signs or symptoms of the AFR and some subjects even expected to receive more ethanol during the testing session; stating that they could only taste the orange juice!

The FF group flushed only on the face, ears, neck and sometimes the upper torso. The flushing usually occurred in only one of the above areas, although a less intense pattern of flushing in several areas was occasionally seen. The extent of flushing ranged from a simple 'glow' in some people, to a much more obvious facial reddening in others. Some people also reported feeling a little drowsy during the last hour of testing, although they felt normal again upon completion of the test session.

People in the LF group became flushed 'all-over' after ethanol. Areas which reddened included the palms, upper arms, lower arms, legs, chest, as well as the (more usual) ears, face and neck. Various other adverse effects were experienced by the people in this group, including tachycardia, headaches or pounding sensations in the head, nausea, dizziness and an overall generalized discomfort. The pounding sensations in the head which were reported by LF subjects were often localized in

specific areas, usually in the forehead initially and later spreading to the temporal and lateral areas and, eventually even to the occipital region. The sequence of most of the major symptoms was remarkably similar in all LF subjects.

A brief description of LF subject flushing follows (Fig.20-21):

About 5-10 minutes after consuming ethanol, LF subjects became red in the face (this reaction was rapid compared with that of FF subjects). At first, the redness was blotchy and uneven but this quickly spread to the whole face. The subjects also reported feeling *very* hot and many became uncomfortable (FFs usually reported feeling only slightly warm). Respiration was affected in some people, although not markedly. LF subjects usually appeared to be quite inebriated at this stage. The heart rate also increased rapidly.

About an hour after ethanol, the LF subjects reported feeling considerably less affected than at thirty minutes; the flushing had also started to subside. Frequently, however, the subjects would suddenly report feeling nauseous (the severity varying depending on the subject) and would begin to experience rigor and feel very cold. The LF subjects had usually stopped flushing at this point and their skin colour had either returned to normal or they became pallid. By this time, the BECs of many of the subjects had fallen to zero. Some LF subjects also reported visual disturbances just before they started to feel faint or, in some cases, to experience syncope.

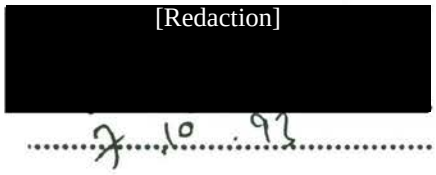
By about two and a half hours after the ethanol challenge, subjects no longer felt nauseated and the sudden chills had usually subsided. The LF subjects then reported a strong, uncontrollable urge to sleep (and frequently did so).

Thirty minutes later (when most LF subjects awoke) the subjects reported feeling very refreshed and much better than before. They left the Research Unit feeling happy and none-the-worse for their experience (although most have said that they would never drink ethanol ever again).

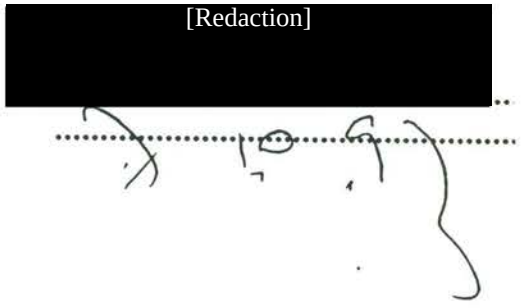
Another interesting observation which was noted in the subjects tested, particularly in those who flushed, involved their handwriting. Because subjects had to fill in their own subjective questionnaire forms by hand and to add additional comments, it was noted that the handwriting of many of the flushing subjects progressively deteriorated from about fifteen minutes after ethanol consumption. The handwriting became increasingly spread-out and larger; each letter was less well-formed, less clear and less controlled over time. This effect was apparent up to 120 minutes after ethanol. These handwriting changes were not seen in non-flushing subjects.

Examples of the changes in handwriting, seen after ethanol, are shown below:

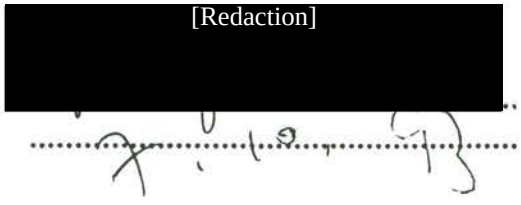
t_0



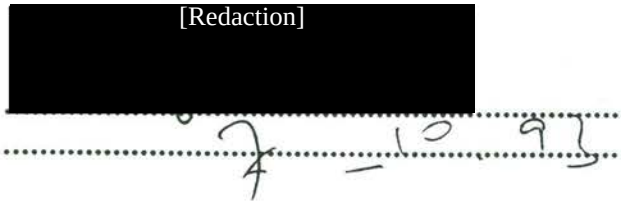
t_{15}



t_{60}



t_{120}



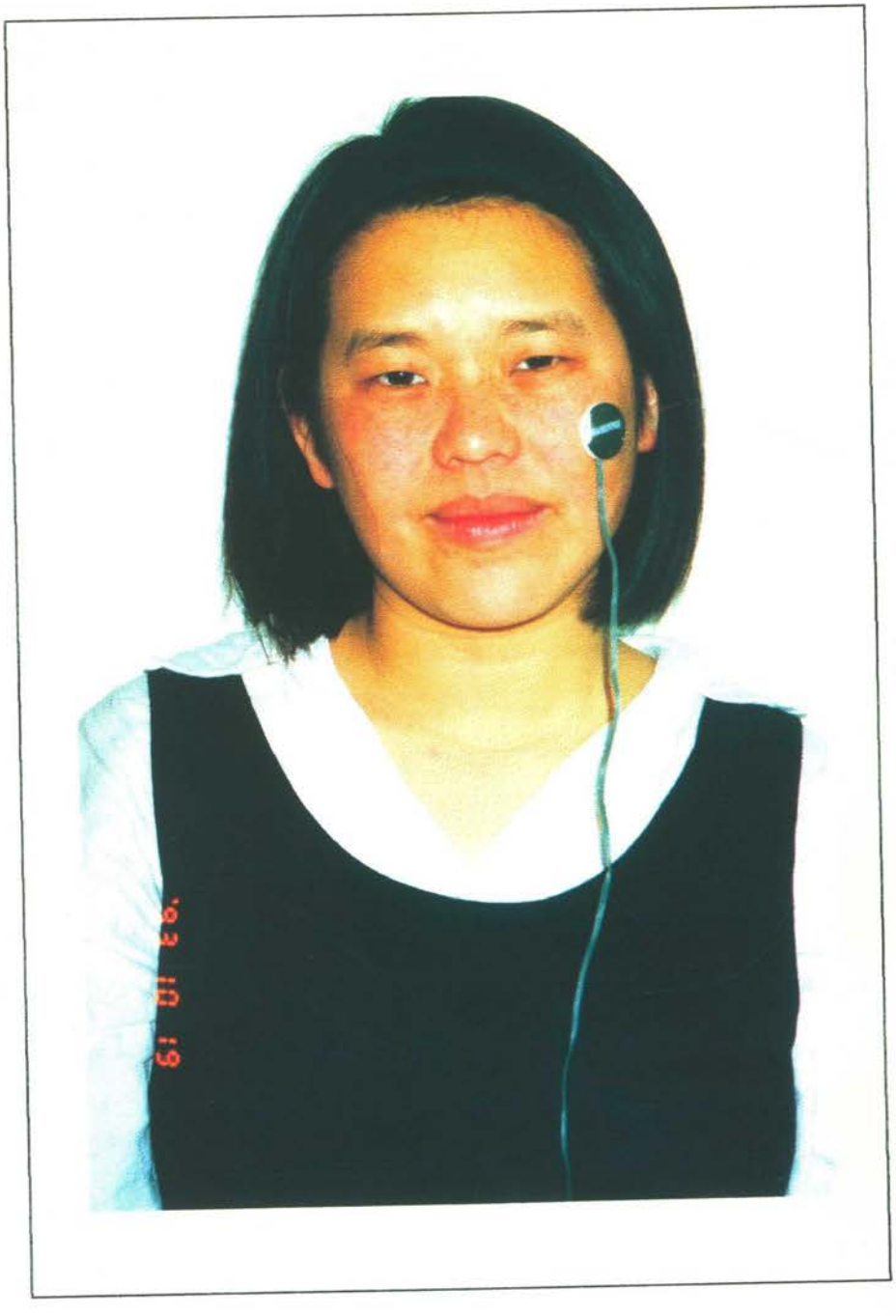


Fig.20: Photograph *before* ethanol administration in one of the LF subjects. *(With written permission from the subject)*

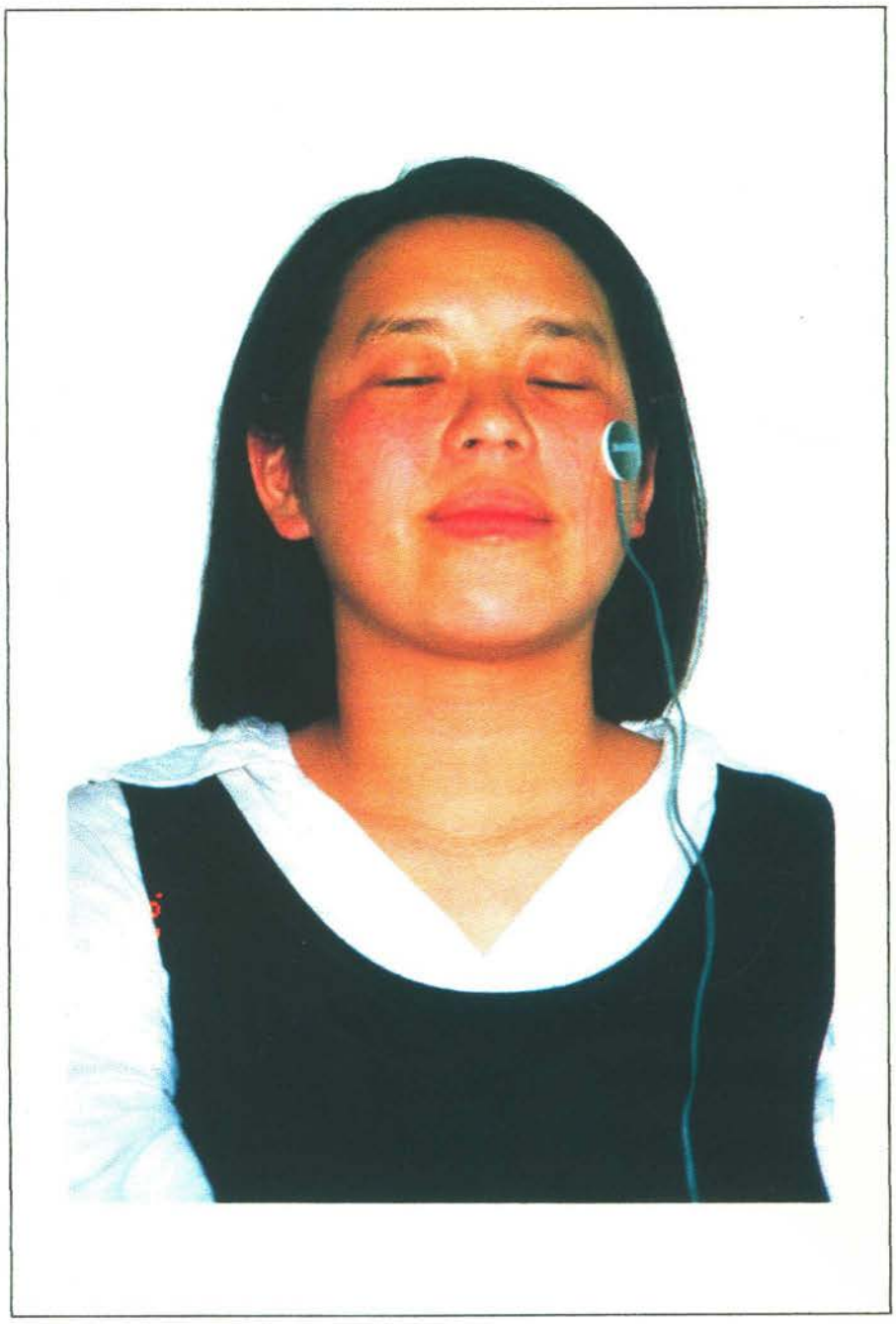


Fig.21: Photograph *after* ethanol administration in one of the LF subjects. *(With written permission from the subject)*

Note the puffy cheeks and eyelids compared with the previous photograph, the subject felt very drowsy, hot and lethargic at this point in time.

3.3. SIGNIFICANCE OF FINDINGS

Significant differences over time were found after ethanol ingestion for **all** (pharmacokinetic, biochemical, physiological, psychomotor performance and subjective measures of intoxication) variables measured. This finding is indicative of the sensitivity of the tests chosen, even to the relatively low dose of ethanol used in the study. Further confirming that our choice of tests used in this study to measure the levels of intoxication was appropriate.

A summary of all non-significant results for each grouping is given at the end of each section.

3.4. RESULTS BY ADH_2 GENOTYPING

3.4.1. BREAKDOWN OF SUBJECTS BY ADH_2

Heterozygote subjects made up 58% of all subjects in the ADH_2 genotype group. Homo22 subjects made up 10% of the total, with the rest (32%) being of the Homo11 genotype (Fig.22).

The gene frequency for the ADH2-1 allele was calculated to be 61% while that for the ADH2-2 allele was 39%.

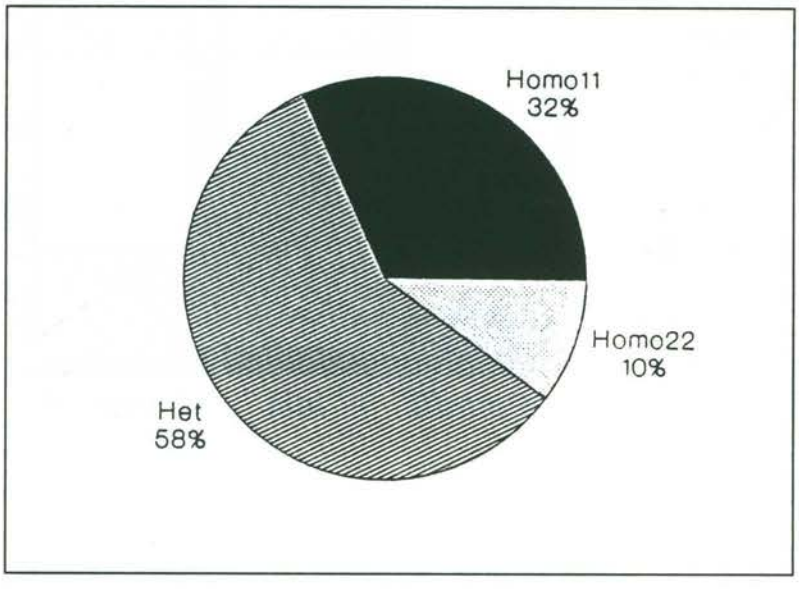


Fig.22: Breakdown of subjects based on ADH_2 enzyme type.

Subjects in the ADH_2 Homo11 group comprised a larger proportion of the total recruitment than the Homo22 group. However, this might have been influenced by selection bias consequent to an aversion of some potential subjects to drinking ethanol, and hence a reluctance to participate in the study.

3.4.2. DRINKING DETAILS

36% of ADH_2 Homo22 subjects reported that they habitually consumed ethanol less than 1-2 times a month or not at all. 55% of this group reported drinking 1-2 times a month, and a further 9% drank, on average, 1-2 times a week. From Fig.23, it can be seen clearly that Homo22 group had the highest proportion of subjects who reported that they never consumed ethanol.

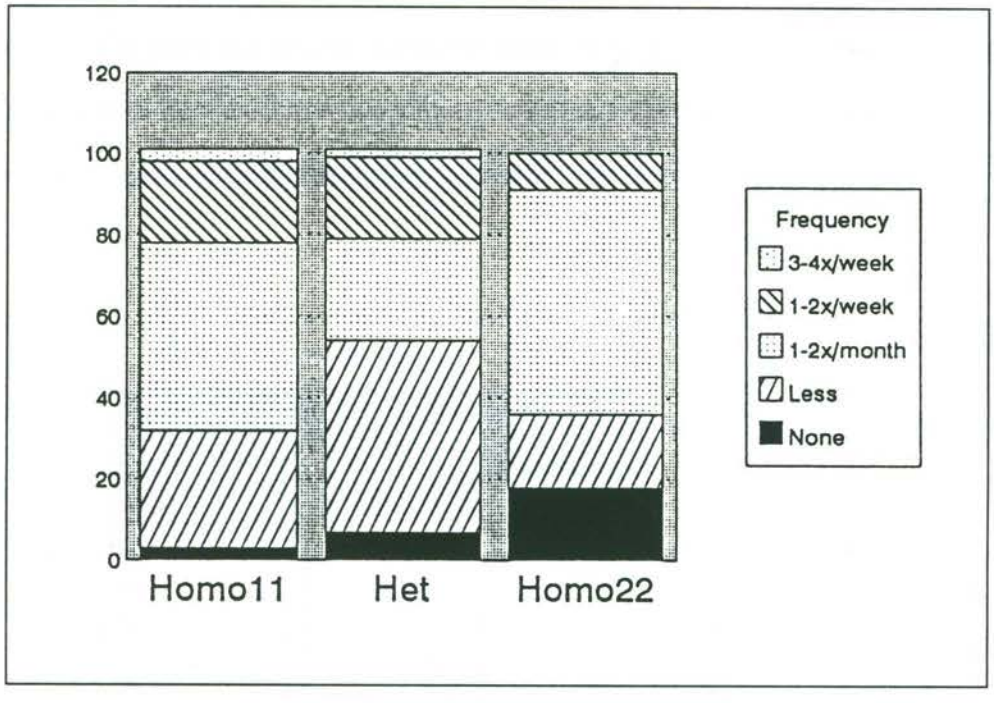


Fig.23: Drinking habits based on ADH_2 genotype: How often subjects consume ethanol.

Similarly, Homo11 subjects predominantly consumed ethanol 1-2 times a month (46%), with another 20% of this group consuming ethanol 1-2 times a week. 3% of subjects also reported drinking more frequently (3-4 times a week). Non-drinkers and those who consumed ethanol less than 1-2 times per month comprised the remainder (32%) of the subjects in this group.

Over half (54%) of the Het group were non-drinkers or drank less than 1-2 times a month. A quarter of the subjects reported drinking 1-2 times a month, 20% drank 1-2 times a week, and a further 2% reported consuming ethanol 3-4 times a week. However, these findings did not reach levels of significance in non-parametric tests ($p>0.05$).

Beer and wine were clearly the most popular beverages consumed by all three groups. Spirits were the next favoured beverage, but the reasons for such 'popularity' might be due to the fact that many subjects reported that they only drank ethanol on festive occasions (when higher strength ethanol is normally consumed).

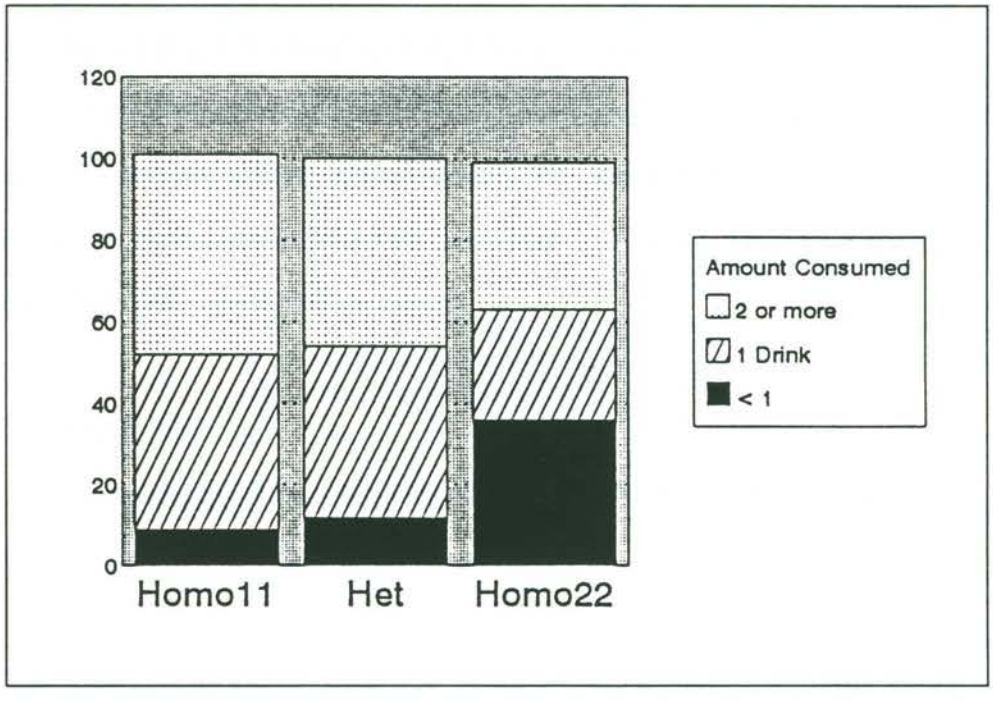


Fig.24: Quantity of ethanol consumed per session -by ADH_2 type.

49% of the ADH_2 Homo11 subjects reported that they usually drank two or more drinks per session, 43% of this group also reported that they usually drank one drink, whilst only 8% drank less than this amount (Fig.24).

The Homo22 group had the highest percentage of subjects reporting consumption of less than one drink per session. In general, this group consumed less ethanol than the other two groups (also with the lowest proportion of subjects drinking two or more drinks of all three groups). It should be remembered, however, that the ADH_2 Homo22 findings might also have been influenced by selection bias, in any case, these findings did not reach levels of significance with non-parametric tests ($p>0.05$).

3.4.3. BREATH ETHANOL CONCENTRATION CURVES

Group differences in the ethanol pharmacokinetics (Fig.25), reported below, were not statistically significant for the ADH_2 genotype but the results obtained are included for reference purposes and because the absence of differences is also worthy of comment.

3.4.3.1. PEAK BEC

The mean peak BECs were found to be 0.036, 0.033 and 0.031 g dl^{-1} for Homo11, Het and Homo22 subjects respectively.

3.4.3.2. TIME TO PEAK

The mean time taken to peak BEC was 15 min for all three groups.

3.4.3.3. ELIMINATION RATE

The mean ethanol elimination rates were calculated by linear regression and found to be 0.0164 ± 0.001 (SEM), 0.0148 ± 0.001 (SEM) and 0.0178 ± 0.001 (SEM) g dl^{-1} hr^{-1} for Homo11, Het and Homo22 groups respectively.

3.4.3.4. VOLUME OF DISTRIBUTION

The mean volumes of distribution were calculated to be 51.85, 58.87 and 51.05 litres for the Homo11, Het and Homo22 groups respectively.

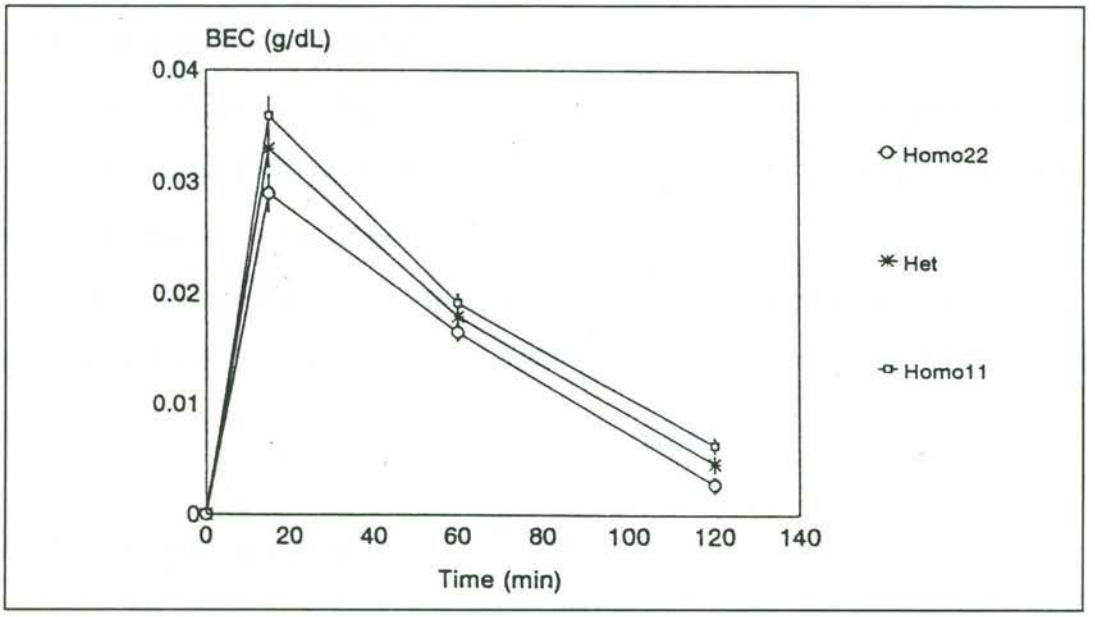


Fig.25: Breath ethanol concentration (g dl^{-1}) by ADH_2 genotype.

(N.B. Only four data points, corresponding to the time-points for blood ethanol measurements, are shown for clarity -see later section for full graph)

3.4.3.5. AREA UNDER THE CURVE

The mean areas under the three curves were calculated to be 0.15 ± 0.018 (SEM) g dl^{-1} min for the Homo11 group, 0.13 ± 0.012 (SEM) g dl^{-1} min for the Het group and 0.12 ± 0.027 (SEM) g dl^{-1} min for the Homo22 group.

3.4.4. BIOCHEMICAL VARIABLES

No significant effects of ADH_2 genotype were found on any of the biochemical variables (acetaldehyde, acetate, lactate and plasma ethanol concentrations), except for pyruvate levels.

3.4.4.1. BLOOD ACETALDEHYDE

ADH_2 Het subjects had the highest blood acetaldehyde concentrations over all time-points tested, followed by Homo11 and Homo22 subjects respectively, but the group differences were not significant.

The acetaldehyde concentrations measured after ethanol remained elevated above pre-ethanol levels for the duration of the test session (see section 3.4.8. for data tables).

3.4.4.2. PLASMA ACETATE

ADH_2 Homo11 subjects had the highest acetate levels, followed by Homo22 and Het subjects who had very similar values. Again, the group differences failed to reach statistical significance.

Acetate levels remained elevated for up to 120 min after ethanol consumption (see section 3.4.8. for data tables).

3.4.4.3. PLASMA ETHANOL

The ADH_2 Homo11 subjects attained the highest plasma ethanol concentrations after ethanol ingestion, this was followed by Het subjects and then by the Homo22 subjects. The group differences did not reach statistical significance, however.

Plasma ethanol concentrations began to decrease after reaching a peak at t_{15}, although by t_{120}, the mean ethanol concentrations had not yet declined to zero (see section 3.4.8. for data tables).

3.4.4.4. BLOOD LACTATE

No statistically significant group differences were found in the lactate concentrations attained. ADH_2 Homo22 subjects had the highest levels of lactate, reaching a peak 15 min after ethanol consumption, and then falling to pre-ethanol values.

The lactate concentrations of Het subjects rose to a peak 15 min after ethanol and then plateaued for the duration of the session.

In Homo11 subjects, the plasma lactate concentrations increased over time with the highest levels being found at t_{120} (see section 3.4.8. for data tables).

3.4.4.5. BLOOD PYRUVATE

A significant difference ($F_{2,101}=3.72$, $p<0.05$) was found among the three ADH_2 groups, both before and after ethanol, with the Homo22 subjects attaining the highest pyruvate concentrations over the entire testing session (Fig.26).

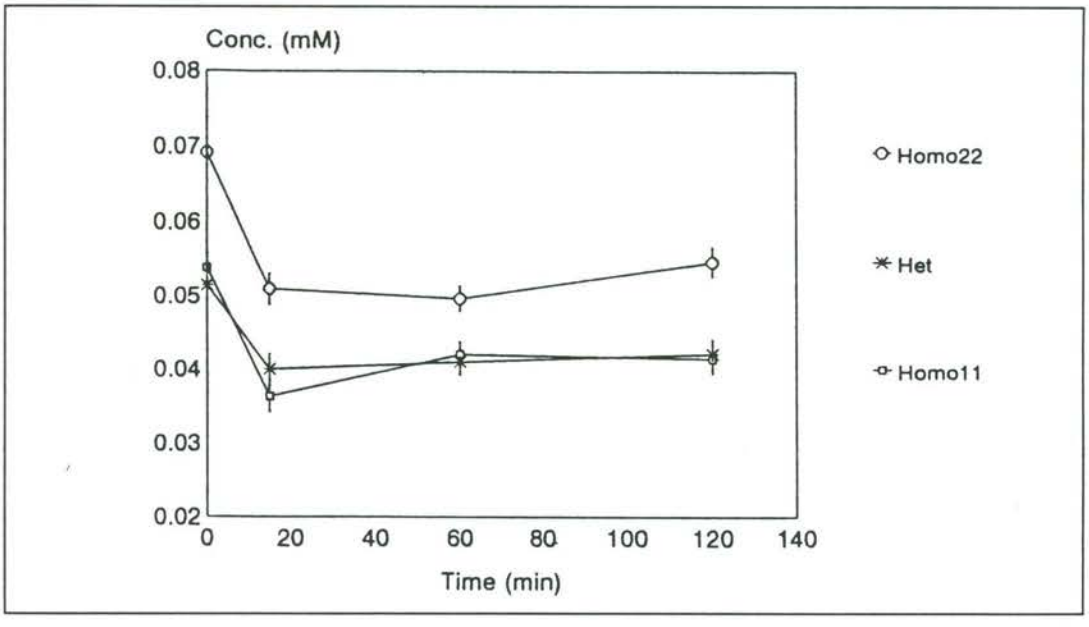


Fig.26: Pyruvate concentrations (mM) by ADH_2 genotype.

Pyruvate concentrations decreased after ethanol, reaching their lowest level at t_{15}, followed by a subsequent small rise.

3.4.4.6. BLOOD LACTATE:PYRUVATE RATIO

Differences in the lactate:pyruvate ratio by ADH_2 genotype did not reach levels of statistical significance (see section 3.4.8. for data tables).

3.4.5. PHYSIOLOGICAL VARIABLES

Subjects with the atypical ADH_2 (Homo22) genotype exhibited a significantly higher heart rate after ethanol than the other two ADH_2 groups. Systolic and diastolic blood pressure and facial temperature were not found to be significantly different among the groups.

3.4.5.1. HEART RATE

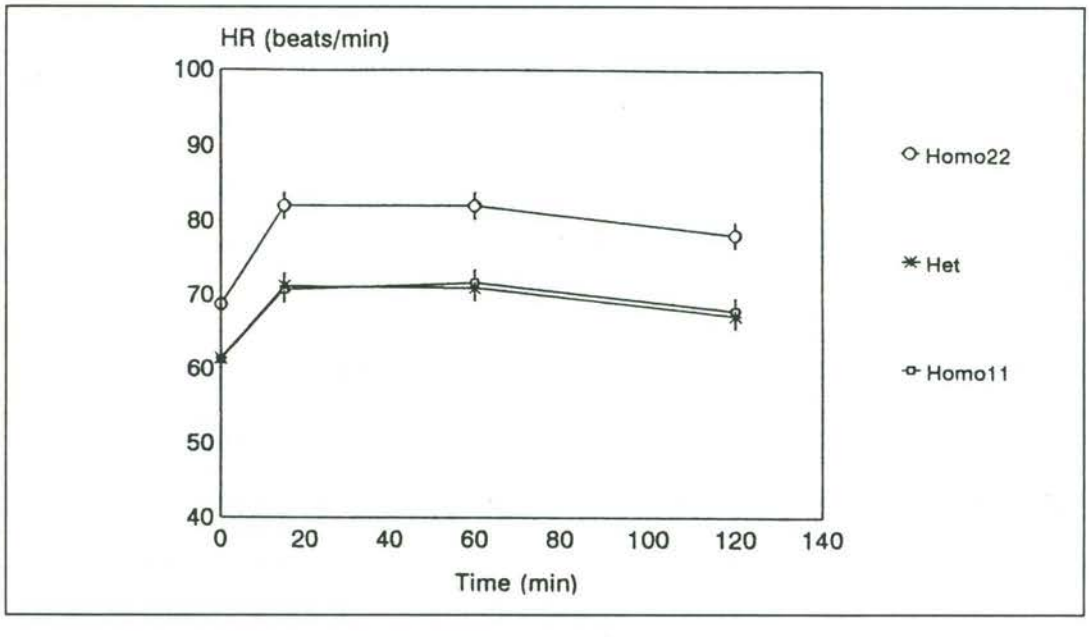


Fig.27: Heart rate by ADH_2 genotype.

Highly significant differences were found among the three ADH_2 groups for heart rate ($F_{2,102}$=5.89, $p<0.005$), with Homo22 subjects attaining the highest heart rate measurements overall (Fig.27).

Het and Homo11 subjects had very similar heart rate values, both lower than Homo22 subjects, although Het subjects had a peak effect at t_{15}, whereas that of Homo11 subjects peaked at t_{60}.

3.4.5.2. BLOOD PRESSURE

ADH_2 Homo22 subjects had the highest systolic blood pressures across all time-points. After ethanol, the systolic blood pressure increased to a peak value at t_{15} and then decreased to below pre-ethanol values. Het and Homo11 subjects did not differ greatly from each other in this respect and, in contrast to Homo22 subjects, systolic blood pressure decreased after ethanol.

The diastolic blood pressure changes were similar in both the Het and Homo11 subjects, decreasing after ethanol consumption to the lowest value at t_{60} and then increasing again at the time of the last measurement. Homo22 subjects differed somewhat in this response, with a fall in blood pressure immediately after ethanol followed by a rise at t_{60} and a second and greater fall at t_{120}.

The differences among the groups for both the systolic and diastolic blood pressure were not statistically significant (see section 3.4.8. for data tables).

3.4.5.3. FACIAL TEMPERATURE

ADH_2 Homo22 subjects attained the highest facial temperatures overall, with a peak response at t_{60}. Homo11 and Het subjects had similar temperatures up to t_{15} but Het subjects experienced a fall in temperature at t_{60}, whereas Homo11 subjects exhibited a rise. The differences among the groups did not reach statistical significance (see section 3.4.8. for data tables).

3.4.6. PSYCHOMOTOR VARIABLES

Standing steadiness was significantly different after ethanol and there was a strong trend towards significance in the CFFF task.

No significant differences were noted in the digit symbol coding or divided attention tasks.

3.4.6.1. DIVIDED ATTENTION

Delay Divided attention delay was the highest in the Het group, followed by the Homo11 and Homo22 groups respectively. In general, after ethanol, delay time was increased. The worst effects were experienced at t_{60} for Homo22 subjects, between t_{15} and t_{60} for Het subjects, and at t_{120} for Homo11 subjects. The differences among the groups did not reach statistical significance (see section 3.4.8. for data tables).

Excursion Divided attention excursion was affected in a similar way in both the Het and Homo11 groups. A sharp rise in excursion occurred in Het and Homo11 subjects after ethanol, but, this was followed by a fall at t_{60}, and a small rise at t_{120}. The Homo22 group, in contrast, behaved differently in that the initial rise in excursion value measured immediately after ethanol was not very great and the excursion decreased to below pre-ethanol values thereafter. The differences among the groups did not reach statistical significance (see section 3.4.8. for data tables).

Reaction Time Divided attention peripheral reaction time was similar among the three groups. After ethanol consumption, the reaction time increased, returning to pre-ethanol values by t_{60}. Homo22 subjects exhibited a second fall in reaction time, to below pre-ethanol values, at t_{120}. The differences among the groups were not of statistical significance (see section 3.4.8. for data tables).

3.4.6.2. DIGIT SYMBOL CODING

ADH_2 Het and Homo11 subjects had similar responses in DSC reaction time after ethanol, that is, a rise occurred at t_{15}, followed by a gradual fall.

Homo22 subjects demonstrated a much greater increase in DSC reaction time immediately after ethanol, resulting in the longest reaction times overall, however after 60 minutes, instead of continuing to fall, DSC reaction time increased at t_{120} (see section 3.4.8. for data tables).

3.4.6.3. STANDING STEADINESS

Statistically significant group differences among the three ADH_2 groups were found for standing steadiness ($F_{2,102}=4.70$, $p<0.05$). These effects occurred both before *and* after ethanol, indicating that the differences among the groups were not simply ethanol-related (Fig.28).

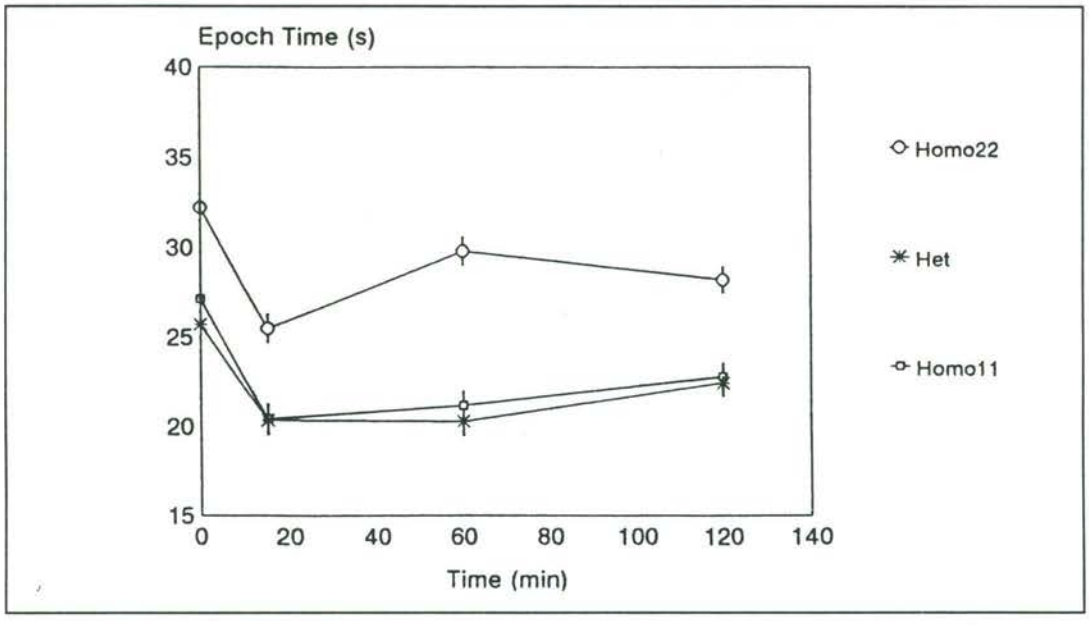


Fig.28: Standing steadiness by ADH_2 genotype.

As seen by the higher values overall, the ADH_2 Homo22 subjects were the steadiest of all subjects tested. Behaving in a similar manner to the Het and Homo11

subjects, the only difference in the Homo22 response occurred at t_{60}, where standing steadiness increased compared to the other two groups, this was followed by a decrease at t_{120}.

As with most of the other variables, the Het and Homo11 subjects behaved in a similar manner, recording a fall in steadiness after ethanol, followed by a slow recovery.

3.4.6.4. CRITICAL FLICKER FUSION FREQUENCY

A strong trend towards significance among the ADH_2 groups was found in the critical flicker fusion frequency variables after ethanol ($F_{2,101}=2.93$, $p=0.058$). It should be noted, however, that again these differences were apparent both before *and* after ethanol (indicating that the differences detected were not simply caused by ethanol) -Fig.29.

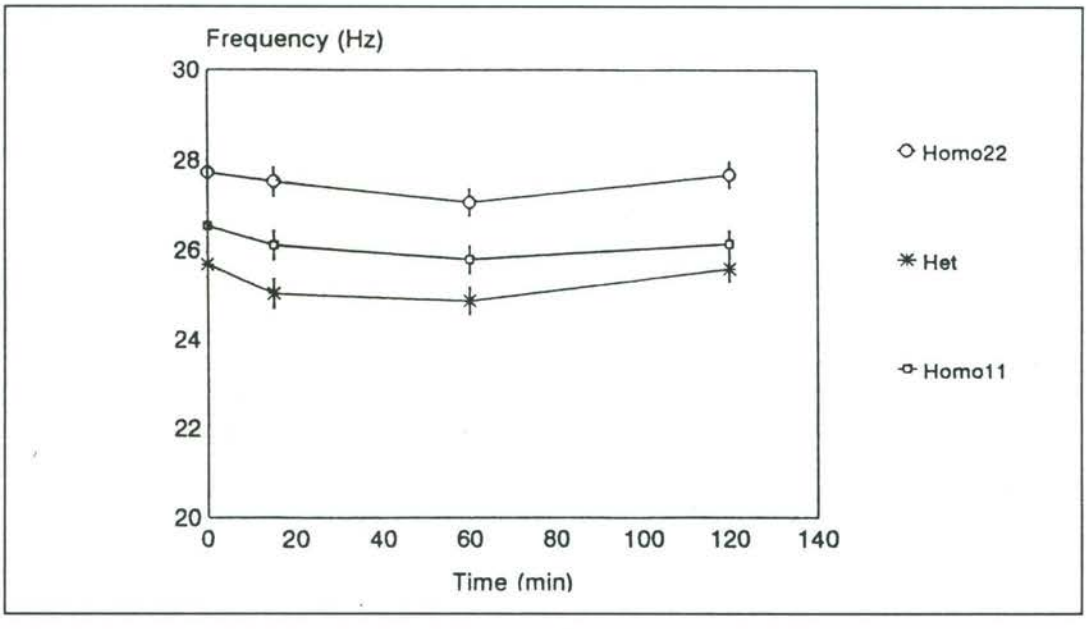


Fig.29: Critical flicker fusion frequency threshold by ADH_2 genotype.

ADH_2 Homo22 subjects had the highest CFFF values overall, followed by Homo11 subjects, with the Het subjects having the lowest CFFF threshold values of the three groups.

After ethanol, the general trend of response was similar in all three groups, CFFF values decreased to their lowest level at t_{60}, after which there was an increase to pre-ethanol levels.

3.4.7. SUBJECTIVE MEASURES OF INTOXICATION

When subjects were asked if their stomach felt uneasy or if they felt sick (Q9), the time by group interaction was found to be significant ($F_{4,206}=2.64$, $p<0.05$) over the duration of the testing session, after ethanol was consumed. The 'yes' response predominated at 120 min after ethanol for the Homo22 group, with the other two groups mainly answering 'no' (Fig.30).

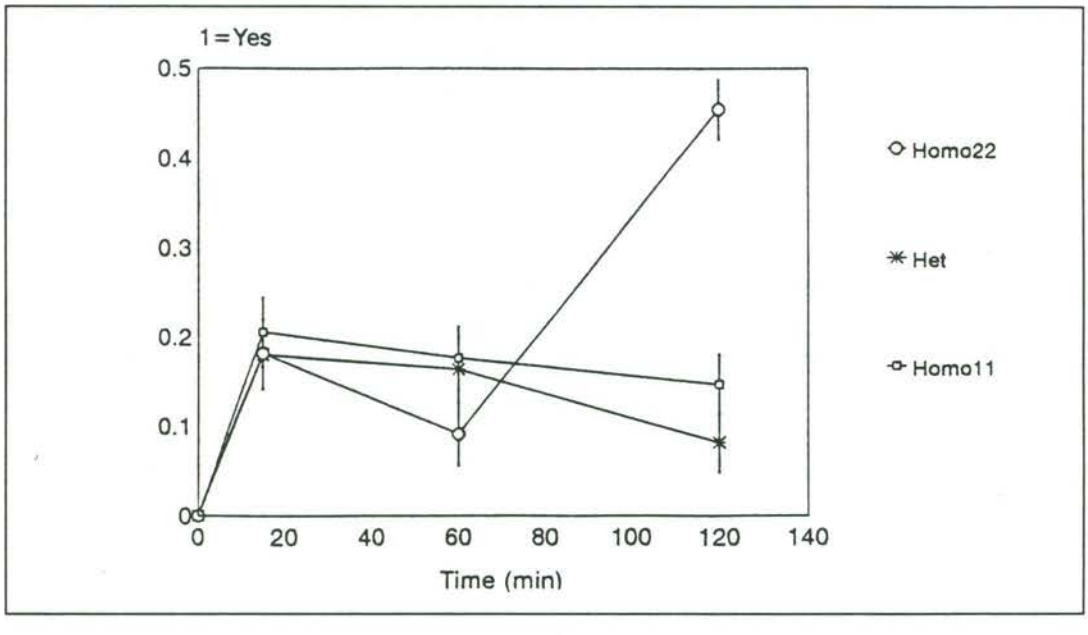


Fig.30: 'Does your stomach feel uneasy or sick?' subjective response results.

After ethanol, there was a significant difference ($F_{2,103}=3.85$, $p<0.05$) among the three groups when asked if they experienced any hangover-like effects at that particular moment (Q10). Homo22 subjects reported increasingly more hangover-like effects as time progressed, even up to 120 min after ethanol (Fig.31).

There were no significant differences among the three ADH_2 groups when they were asked whether they would drive a car or not, or whether they would accept another alcoholic drink if they were drinking in a social situation. Neither were there any differences in alertness, co-ordination, level of concentration, feelings of intoxication, attentiveness and presence of headache among the three groups tested.

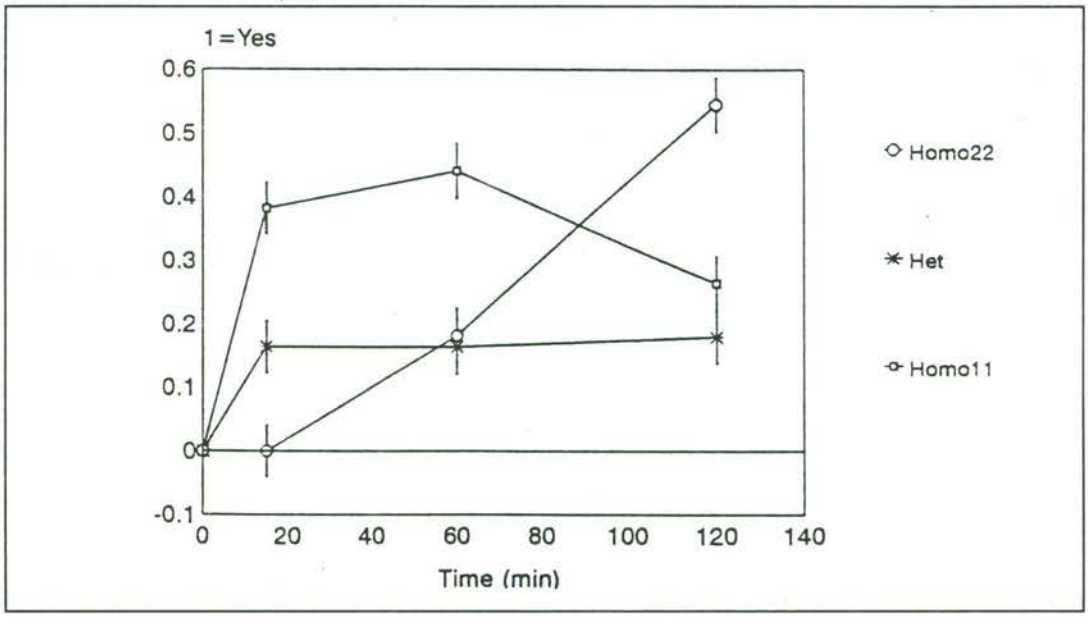


Fig.31: 'Any hangover-like effects?' subjective response results.

3.4.8. SUMMARY OF OTHER RESULTS BY ADH_2 GENOTYPE (NON-SIGNIFICANT)

The results for each variable below are listed in the following order for each time-point (t_0, t_{15}, t_{60}, t_{120}):

Homo11 mean ± SD; n=42

Het mean ± SD; n=54

Homo22 mean ± SD; n=11

VARIABLE	t_0	t_{15}	t_{60}	t_{120}
Acetaldehyde	32.2 ± 28.9	47.6 ± 41.0	43.3 ± 30.9	44.8 ± 32.1
(μM)	42.9 ± 44.6	61.2 ± 58.9	59.1 ± 59.6	60.5 ± 60.3
	27.5 ± 26.4	42.0 ± 29.3	39.6 ± 27.9	38.6 ± 32.8
Acetate (mM)	-0.01 ± 0.10	0.42 ± 0.26	0.40 ± 0.26	0.38 ± 0.26
	-0.02 ± 0.14	0.30 ± 0.75	0.35 ± 0.32	0.33 ± 0.20
	-0.10 ± 0.27	0.32 ± 0.20	0.36 ± 0.24	0.33 ± 0.17
Ethanol (mM)	0 ± 0	9.2 ± 2.9	8.6 ± 1.6	5.8 ± 1.5
	0 ± 0	8.8 ± 2.5	8.4 ± 1.6	5.2 ± 1.4
	0 ± 0	8.1 ± 2.5	7.8 ± 1.1	4.4 ± 0.8
Lactate (mM)	1.4 ± 1.0	1.7 ± 0.9	1.8 ± 1.0	1.8 ± 1.1
	1.3 ± 1.2	1.7 ± 1.0	1.7 ± 0.9	1.6 ± 0.9
	1.6 ± 0.9	1.9 ± 0.9	1.9 ± 1.0	1.7 ± 0.8
Lac:Pyr Ratio	31.7 ± 41.9	64.1 ± 107.1	46.4 ± 28.2	69.3 ± 129
	25.2 ± 14.0	46.5 ± 23.1	47.8 ± 38.2	45.5 ± 41.2
	21.4 ± 11.2	38.4 ± 14.8	37.7 ± 15.5	34.0 ± 13.1
Temperature	32.2 ± 1.3	33.7 ± 1.1	33.8 ± 1.0	33.2 ± 1.1
(°C)	32.3 ± 1.4	33.6 ± 1.5	33.5 ± 1.3	33.0 ± 1.3
	32.2 ± 1.8	34.1 ± 1.6	34.2 ± 1.0	33.6 ± 1.4

Blood Pressure	109.6 ± 12.6	109.0 ± 14.3	105.2 ± 14.0	106.2 ± 13.9
(Sys) (mmHg)	111.8 ± 12.0	108.0 ± 13.0	105.4 ± 10.9	104.7 ± 12.9
	116.9 ± 16.3	118.6 ± 15.9	112.0 ± 15.4	109.0 ± 14.4
Blood Pressure	73.1 ± 11.2	69.4 ± 10.0	66.8 ± 10.8	69.6 ± 9.4
(Dia) (mmHg)	71.2 ± 10.1	68.4 ± 11.3	66.3 ± 10.1	67.7 ± 9.3
	76.0 ± 10.6	67.5 ± 11.7	68.9 ± 12.5	65.4 ± 10.7
Digit Symbol	1.6 ± 0.3	1.7 ± 0.3	1.6 ± 0.3	1.6 ± 0.3
Coding (min)	1.6 ± 0.2	1.6 ± 0.2	1.6 ± 0.3	1.5 ± 0.2
	1.5 ± 0.5	1.7 ± 0.7	1.6 ± 0.6	1.6 ± 0.6
Div. Attention	41.3 ± 14.1	44.8 ± 15.3	45.5 ± 16.0	46.5 ± 20.2
(Delay) (ms)	45.0 ± 12.0	50.5 ± 16.6	50.4 ± 19.2	47.1 ± 14.5
	37.8 ± 12.1	40.2 ± 12.8	41.3 ± 16.0	39.7 ± 13.4
Div. Attention	3.1 ± 1.0	3.9 ± 1.0	3.4 ± 1.1	3.6 ± 1.6
(Excur.) (units)	3.3 ± 1.6	4.1 ± 1.3	3.5 ± 1.2	3.6 ± 1.0
	3.5 ± 1.0	3.6 ± 2.0	3.4 ± 1.5	3.2 ± 1.4
Div. Attention	2.7 ± 0.8	3.1 ± 0.8	2.7 ± 0.9	2.7 ± 0.8
(Reaction Time)	2.8 ± 0.8	3.1 ± 0.9	2.8 ± 0.7	2.8 ± 0.8
	2.7 ± 1.2	3.1 ± 1.2	2.8 ± 1.3	2.6 ± 1.0
Subjective Q1	0.9 ± 0.3	0.3 ± 0.5	0.3 ± 0.5	0.7 ± 0.5
	0.9 ± 0.3	0.2 ± 0.4	0.3 ± 0.5	0.7 ± 0.5
	0.8 ± 0.4	0.2 ± 0.4	0.3 ± 0.5	0.5 ± 0.5
Subjective Q2	0.8 ± 0.4	0.4 ± 0.5	0.4 ± 0.5	0.4 ± 0.5
	0.8 ± 0.4	0.5 ± 0.5	0.4 ± 0.5	0.5 ± 0.5
	0.7 ± 0.5	0.4 ± 0.5	0.4 ± 0.5	0.2 ± 0.4
Subjective Q3	2.0 ± 1.9	5.2 ± 2.4	5.2 ± 2.4	3.4 ± 2.5
	2.3 ± 1.9	5.0 ± 2.0	4.6 ± 2.2	3.1 ± 2.0
	1.4 ± 1.0	4.5 ± 2.3	4.3 ± 2.2	3.3 ± 2.0

Subjective Q4	1.8 ± 1.4	4.9 ± 2.3	5.1 ± 2.5	3.0 ± 2.2
	2.2 ± 2.0	4.7 ± 2.1	4.3 ± 2.0	2.7 ± 1.9
	1.1 ± 1.6	3.5 ± 2.5	3.5 ± 2.0	2.5 ± 1.1
Subjective Q5	7.1 ± 2.0	4.7 ± 2.1	4.6 ± 2.3	6.0 ± 2.3
	6.8 ± 1.7	4.4 ± 2.2	4.9 ± 2.0	6.1 ± 2.1
	7.6 ± 1.1	5.5 ± 2.5	5.9 ± 1.7	6.0 ± 2.0
Subjective Q6	0 ± 0	5.0 ± 2.3	4.5 ± 2.2	2.3 ± 1.9
	0.1 ± 0.4	4.8 ± 2.3	3.9 ± 2.0	2.0 ± 1.9
	0.1 ± 0.3	5.3 ± 2.5	4.3 ± 2.1	2.6 ± 2.0
Subjective Q7	1.8 ± 1.6	5.4 ± 2.1	4.9 ± 2.3	3.3 ± 2.3
	2.1 ± 2.0	4.8 ± 2.3	4.4 ± 2.0	3.0 ± 2.1
	1.2 ± 1.4	3.7 ± 1.9	3.7 ± 2.2	3.5 ± 2.0
Subjective Q8	-	0.2 ± 0.4	0.3 ± 0.5	0.3 ± 0.5
	-	0.2 ± 0.4	0.2 ± 0.4	0.2 ± 0.4
	-	0.2 ± 0.4	0.2 ± 0.4	0.5 ± 0.5

3.5. RESULTS BY $ALDH_2$ GENOTYPING

3.5.1. BREAKDOWN OF SUBJECTS BY $ALDH_2$

Heterozygote subjects made up 49% of the $ALDH_2$ genotype, Homo22 subjects comprised 12% of the total, and the remainder possessed Homo11 genotypes (Fig.32).

The gene frequency calculated for the ALDH2-1 allele was found to be 63.5% and 36.5% for the ALDH2-2 allele respectively.

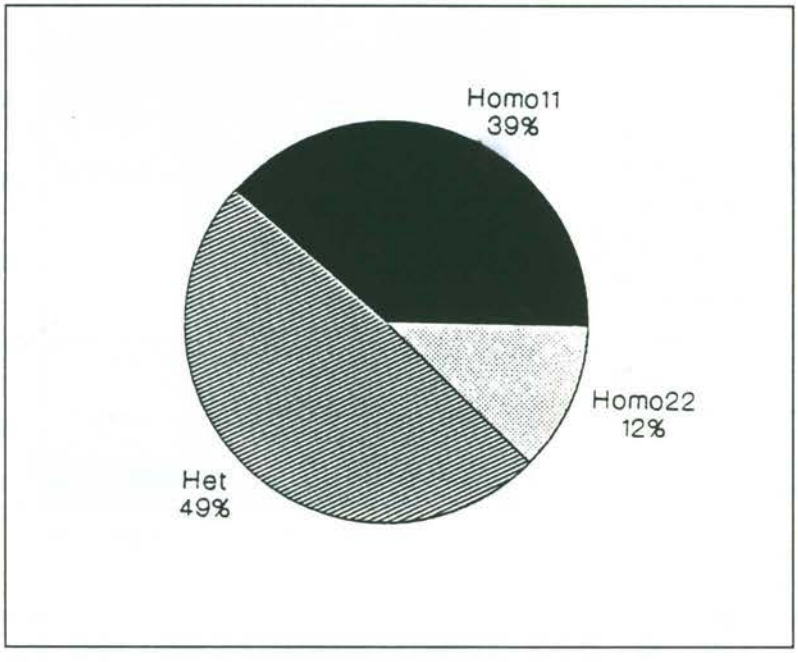


Fig.32: Breakdown of subjects based on $ALDH_2$ enzyme type.

3.5.2. DRINKING DETAILS

ALDH Homo22 subjects reported drinking ethanol only 1-2 times a month (46%) or even less frequently (54%).

The Het group had 44% of subjects who drank less than 1-2 times a month or not at all, 29% who drank 1-2 times a month and 23% drank 1-2 times a week. Drinking 3-4 times a week was indicated by 4% of the subjects in this group.

Non-drinkers and those who consumed ethanol less than 1-2 times a month made up 42% of Homo11 subjects, 39% of this group reported drinking 1-2 times a month, and 20% consumed ethanol 1-2 times a week (Fig.33). However, using non-parametric tests, these findings did not reach levels of significance ($p>0.05$).

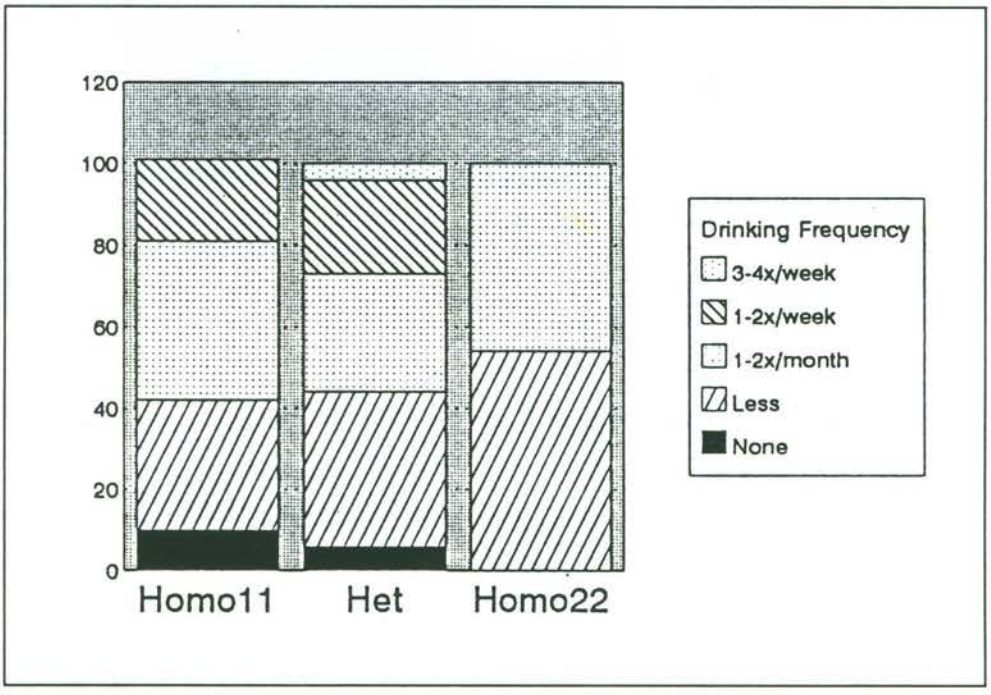


Fig.33: Drinking habits based on $ALDH_2$ genotype: How often subjects consume ethanol.

The preferred choice of beverage for subjects in all three groups was beer (31-45% of subjects). However, spirits rated equally with beer (31%) for the Homo22 subjects. This, as mentioned earlier, might be due to the fact that many subjects in this

group do not usually consume ethanol, and when they do, it is for a particular celebratory occasion, hence the preferred 'choice' of spirits (Fig.34).

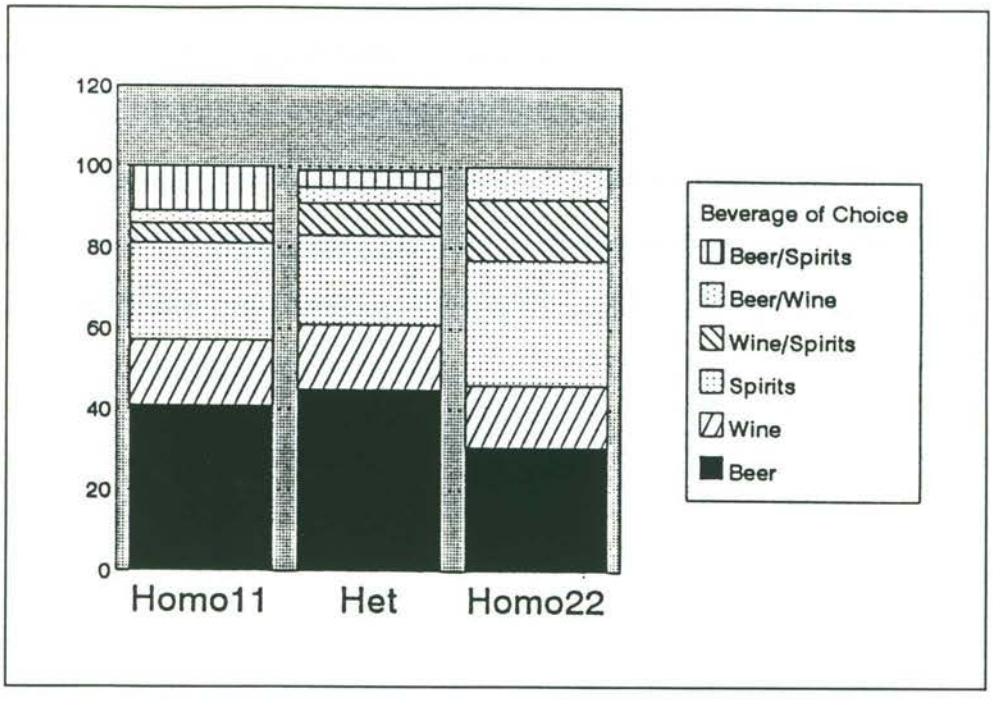


Fig.34: Drinking habits by $ALDH_2$ genotype: Choice of beverage preferred.

As expected, the $ALDH_2$ Homo11 group had the highest proportion of subjects who reported that they usually drank two or more drinks per session (59%). Also, in this group, 28% of subjects usually drank one drink whilst only 13% reported that they consumed less than this amount (Fig.35).

In contrast, the $ALDH_2$ Homo22 group had a higher proportion of subjects reporting that they usually consumed less than one standard drink per session (31%). A further 46% of this group reported that they averaged one drink per session, although it should be noted that some of these subjects added that they usually were not able to finish even a single drink and thus their real ethanol consumption was less than one drink.

The Het group had the highest proportion of subjects who reported consuming, on average, one drink per session.

It should be noted here that the usual time-course for drinking was not recorded. Therefore, although subjects might have reported that they consumed one standard drink, it is not known whether this drink was consumed over one minute or over the entire night. The differences did not reach levels of statistical significance with non-parametric tests ($p>0.05$) although, if the significance level was taken at $p<0.10$, a difference could be detected between the Het and Homo22 groups.

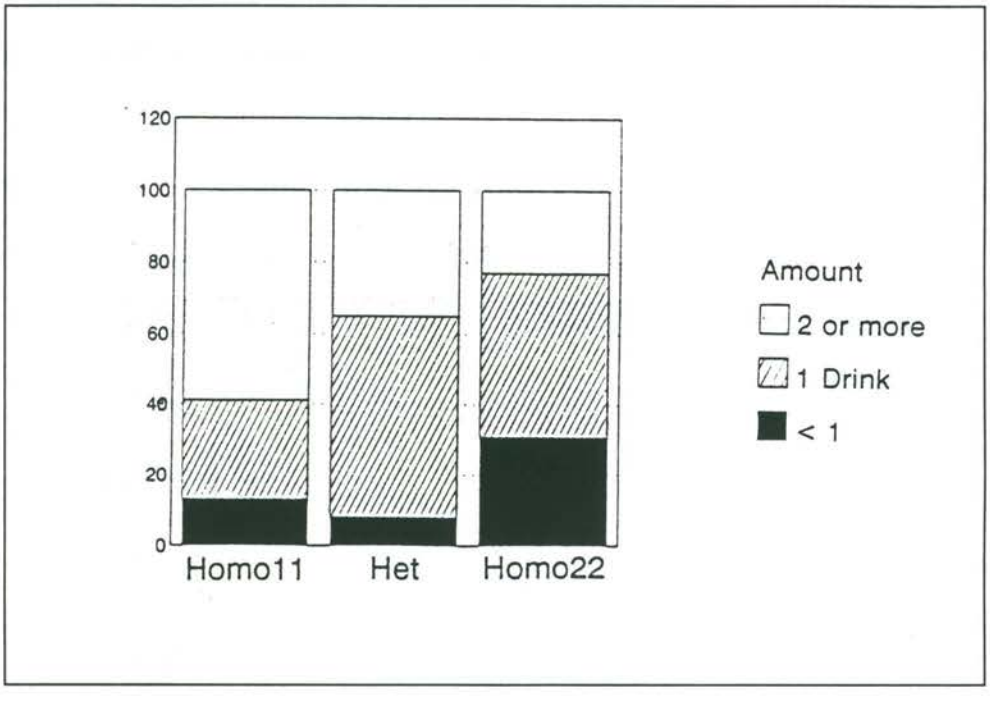


Fig.35: Quantity of ethanol consumed per session -by $ALDH_2$ type.

3.5.3. BREATH ETHANOL CONCENTRATION CURVES

Significant differences ($F_{6,306}$=3.28, p<0.005) were detected among the three groups in the pharmacokinetic parameters before and after ethanol (Fig.36).

Post-hoc analysis revealed that the highly significant differences were present throughout the 120 min test period. In Homo22 subjects differences with t=0 were found at 15, 60 and 120 minutes after ethanol ($q_{9,306}$=24.85, $q_{6,306}$=13.67 & $q_{3,306}$=4.44, p<0.005); Het subjects at 15 ($q_{10,306}$=28.42, p<0.005), 60 ($q_{7,306}$=14.58, p<0.005) and 120 minutes ($q_{4,306}$=4.48, p<0.005); and the Homo11 subjects at 15 ($q_{8,306}$=22.87, p<0.005), 60 ($q_{5,306}$=13.42, p<0.005) and 120 minutes after ethanol consumption ($q_{2,306}$=2.78, p<0.005).

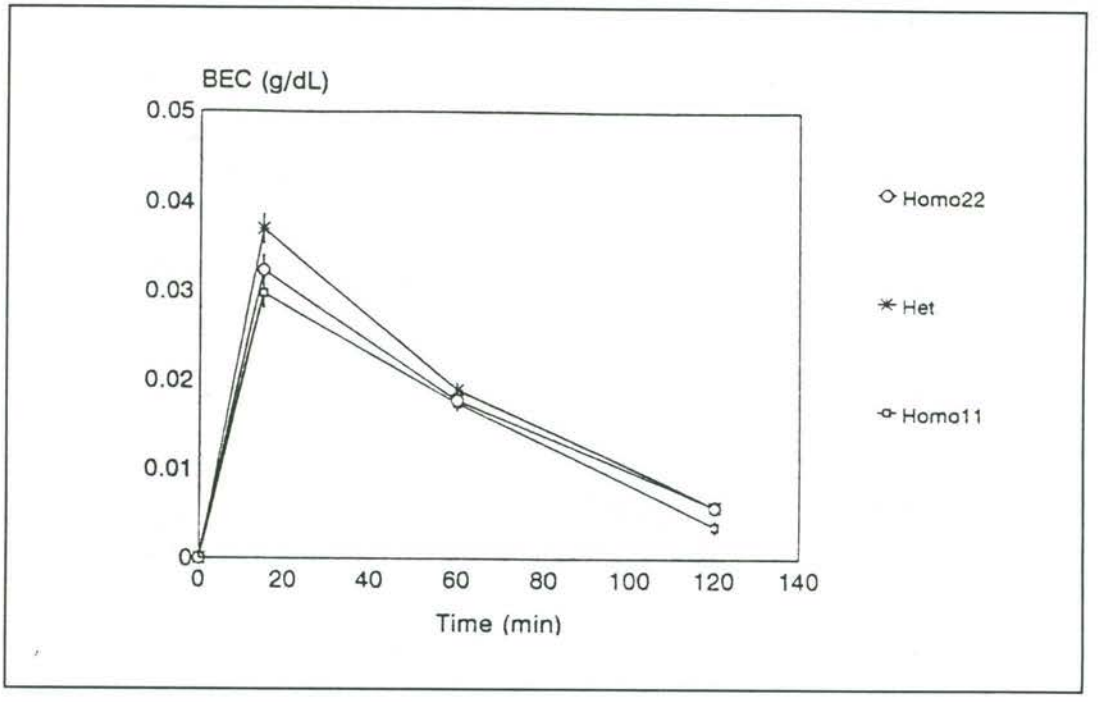


Fig.36: Breath ethanol concentration (g dl^{-1}) by $ALDH_2$ genotype.

(N.B. Only four data points, corresponding to the time-points for blood ethanol measurements, are shown for clarity -see later section for full graph)

3.5.3.1. PEAK BEC

The mean peak BEC attained was found to be 0.030 g dl^{-1} for the Homo11 and 0.037 g dl^{-1} for the Het groups, compared with 0.034 g dl^{-1} for the Homo22 subjects.

3.5.3.2. TIME TO PEAK

All three groups (Homo11, Het and Homo22) peaked at 15 min after ethanol.

3.5.3.3. ELIMINATION RATE

The mean rate of decline of BEC was calculated, using linear regression, to be 0.0147 ± 0.001 (SEM) g dl^{-1} hr^{-1} for the Homo11 subjects, whilst the rate was 0.0152 ± 0.001 (SEM) g dl^{-1} hr^{-1} for the Homo22 group. Het subjects exhibited the highest rate of decline at 0.0171 ± 0.002 (SEM) g dl^{-1} hr^{-1} (Fig.37).

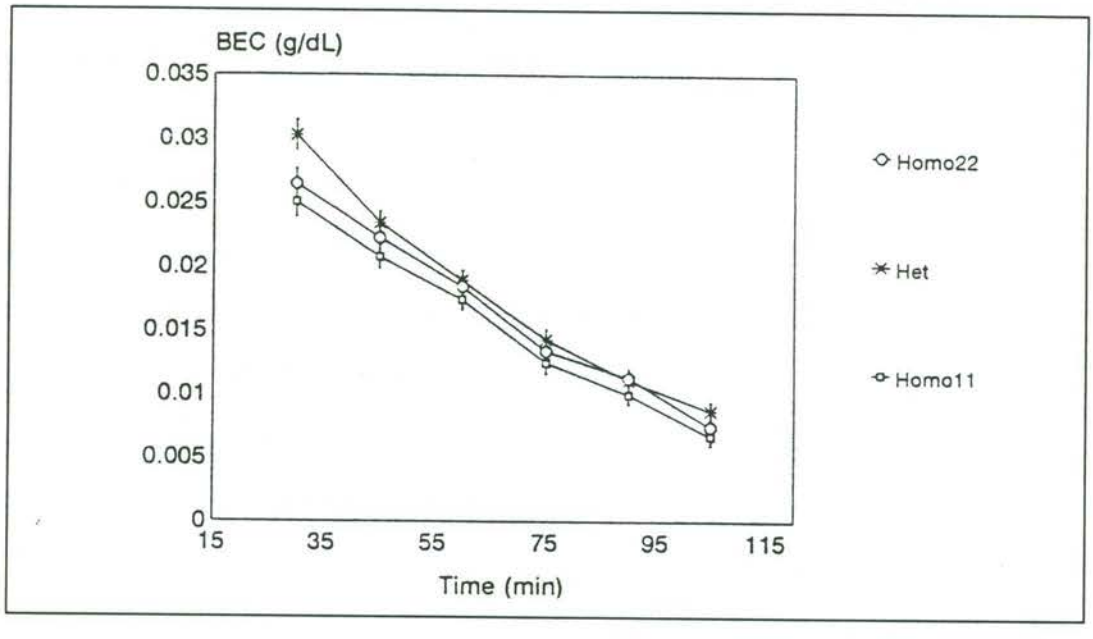


Fig.37: Ethanol elimination curve by $ALDH_2$ genotype.

3.5.3.4. VOLUME OF DISTRIBUTION

The mean volumes of distribution were calculated to be 58.6, 50.8 and 57.6 litres for the $ALDH_2$ Homo11, Het and Homo22 groups respectively.

3.5.3.5. AREA UNDER THE CURVE

The mean areas under the curve were 0.122 ± 0.0002 (SEM), 0.147 ± 0.0002 (SEM) and 0.137 ± 0.0005 (SEM) g dl^{-1} min for Homo11, Het and Homo22 groups respectively.

3.5.4. BIOCHEMICAL VARIABLES

Significant effects of $ALDH_2$ genotype were found in only two of the biochemical variables which were measured (acetaldehyde and acetate concentrations). Differences in the other biochemical variables (including those of pyruvate, lactate and plasma ethanol concentrations) did not reach statistical significance.

3.5.4.1. BLOOD ACETALDEHYDE

A highly significant time by group difference ($F_{6,306}$=4.32, p<0.005) was found for acetaldehyde concentrations (Fig.38). Both Homo22 and Het subjects showed a significant increase in acetaldehyde levels over time up to 120 min after ethanol consumption. The greatest increase was found in the Homo22 group. Homo11 subjects exhibited an insignificant increase in acetaldehyde levels.

Post-hoc analysis revealed that the acetaldehyde concentrations of Homo22 subjects were significantly different from pre-ethanol values at 15 ($q_{9,306}$=10.65, p<0.05), 60 ($q_{11,306}$=11.57, p<0.05) and 120 minutes after ethanol ($q_{10,306}$=10.79, p<0.05). Likewise, the Het subjects had statistically significant differences in acetaldehyde concentrations from pre-ethanol values throughout the test session (15

min ($q_{8,306}$=5.33, $p<0.05$), 60 min ($q_{4,306}$=5.17, $p<0.05$) and 120 min ($q_{6,306}$=5.63, $p<0.05$)) after ethanol.

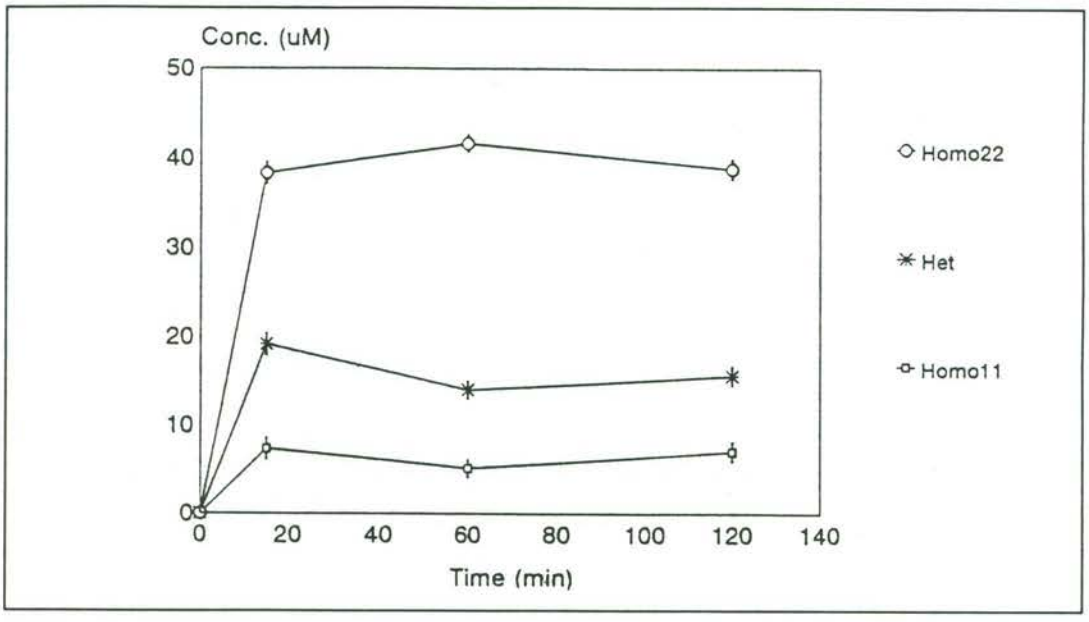


Fig.38: Acetaldehyde concentration (μM) by $ALDH_2$ genotype.
(N.B. Blank corrections accounted for in measurements)

3.5.4.2. PLASMA ACETATE

A highly significant difference ($F_{2,102}$=5.66, $p<0.005$) was found among the three $ALDH_2$ groups with the highest values found for the Homo11 subjects (Fig.39).

The Homo22 and Het groups had similar acetate values, although Homo22 subjects exhibited a fall in acetate concentration at t_{60}, in contrast to Het subjects who did not.

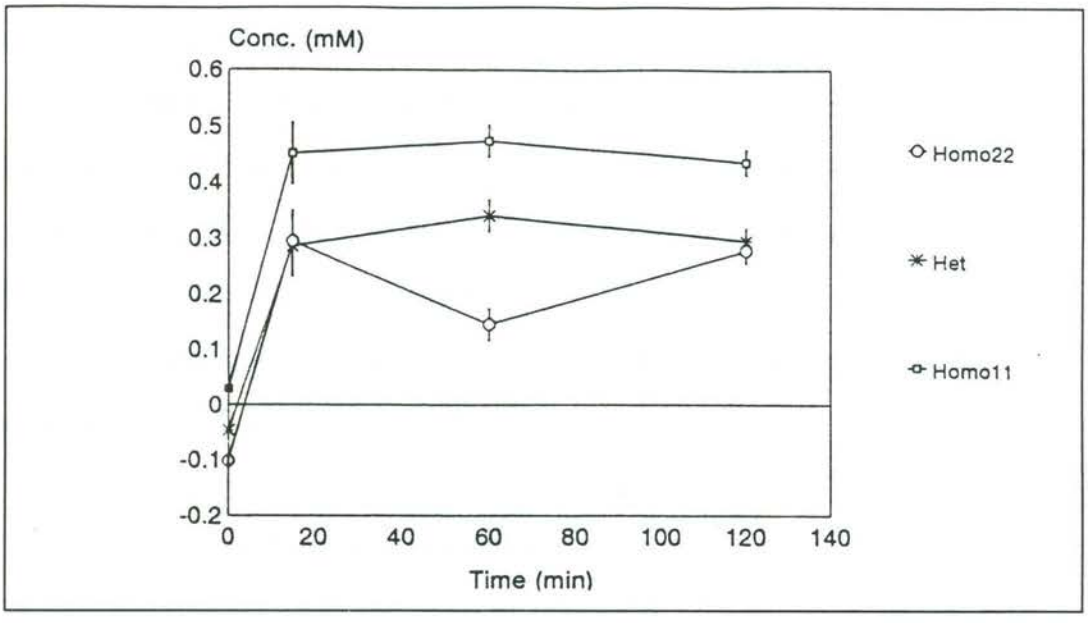


Fig.39: Acetate concentration (mM) by $ALDH_2$ genotype.

3.5.4.3. PLASMA ETHANOL

$ALDH_2$ Het subjects had the highest plasma ethanol concentrations, followed by Homo22 and Homo11 subjects. The differences among the groups did not reach statistical significance (see section 3.5.8. for data tables).

3.5.4.4. BLOOD LACTATE

The $ALDH_2$ Homo22 group had the highest lactate levels overall, which further increased after ethanol consumption to a peak value at t_{120}.

The response of the Het subjects was similar in that there was an increase in lactate concentrations with the highest levels also occurring at t_{120}.

With Homo11 subjects, however, although there was an increase in lactate concentration at t_{15}, this was followed by a decrease to near pre-ethanol values by t_{120}.

The mean lactate concentration value was not significantly affected by $ALDH_2$ genotype (see section 3.5.8. for data tables).

3.5.4.5. BLOOD PYRUVATE

$ALDH_2$ Homo22 subjects had the highest pyruvate levels overall. The responses of the Homo22 and Het subjects were similar, in that the pyruvate concentrations measured fell sharply after ethanol consumption and, by t_{60}, they had risen again towards pre-ethanol levels.

The differences among the groups did not reach statistical significance however (see section 3.5.8. for data tables).

3.5.4.6. BLOOD LACTATE:PYRUVATE RATIO

Differences in the lactate:pyruvate ratio by $ALDH_2$ genotype did not reach levels of statistical significance (see section 3.5.8. for data tables).

3.5.5. PHYSIOLOGICAL VARIABLES

Significance differences among the groups occurred for (facial temperature and heart rate), but not all measures (systolic and diastolic blood pressure).

3.5.5.1. HEART RATE

A highly significant heart rate increase was found among the three $ALDH_2$ groups tested ($F_{2,102}=10.99$, $p<0.005$).

Both the Homo22 and Het subjects had higher heart rates than the Homo11 subjects (Fig.40). The increase in heart rate after ethanol consumption was greater in the Homo22 and Het groups than in the Homo11 group.

The heart rates of all three groups remained elevated for up to one hour after ethanol consumption, after which time recovery to pre-ethanol values commenced.

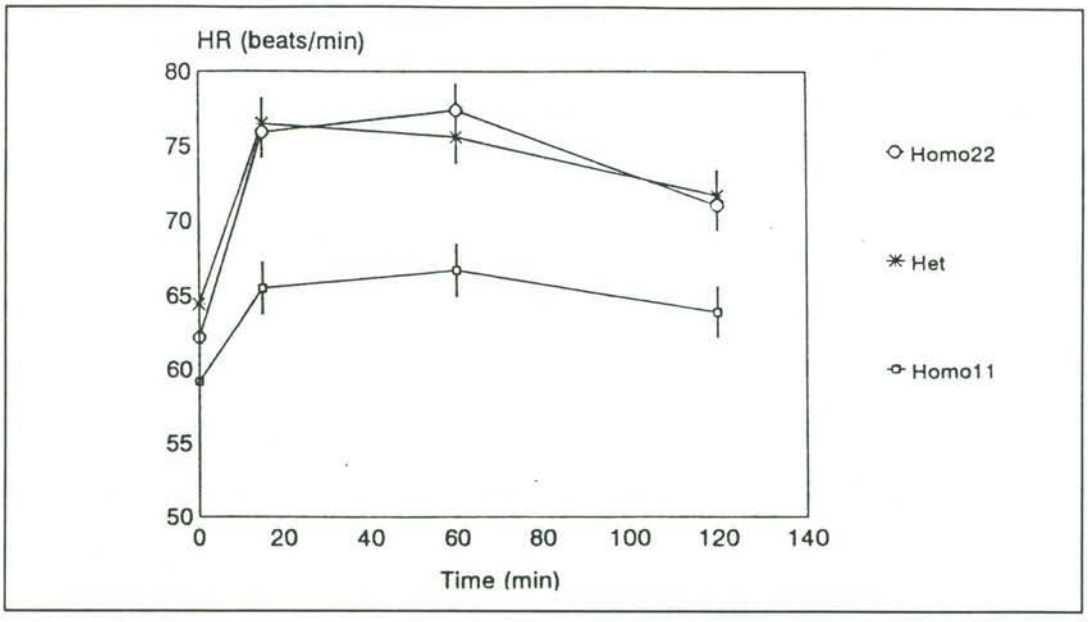


Fig.40: Heart rate by $ALDH_2$ genotype.

3.5.5.2. BLOOD PRESSURE

In the Het and Homo11 groups, systolic blood pressure fell after ethanol consumption, reaching its lowest value at t_{60} and then increasing again at t_{120}.

The Homo22 subjects exhibited an initial increase in systolic blood pressure after ethanol, although by t_{60}, the blood pressure had fallen to a level which was not significantly different from those of the other two groups.

Overall, the differences among the groups did not reach statistical significance (see section 3.5.8. for data tables).

The diastolic blood pressure response to ethanol was similar for both Het and Homo22 subjects. That is, the diastolic blood pressure fell immediately after ethanol and then increased again, although not quite to pre-ethanol values.

The differences in diastolic blood pressure among the groups did not reach statistical significance (see section 3.5.8. for data tables).

3.5.5.3. FACIAL TEMPERATURE

A highly significant difference was found in the time by group interaction for the facial temperature variable ($F_{6,306}$=8.67, p<0.005) -Fig.41. *Post-hoc* analysis revealed that the facial temperature of Homo22 subjects was significantly increased after ethanol at 15 ($q_{12,306}$=14.46, p<0.05), 60 ($q_{11,306}$=14.08, p<0.05) and 120 minutes after ethanol ($q_{6,306}$=6.18, p<0.05). Likewise, the Het subjects had significantly higher facial temperatures at 15 ($q_{9,306}$=11.98, p<0.05), 60 ($q_{8,306}$=11.66, p<0.05) and 120 minutes after ethanol ($q_{7,306}$=7.97, p<0.05) compared to pre-ethanol values. The Homo11 subjects, however, had significantly increased facial temperatures at 15 ($q_{2,306}$=3.82, p<0.05) and 60 ($q_{5,306}$=4.46, p<0.05) minutes after ethanol consumption although by 120 min after ethanol, the Homo11 subjects were not significantly affected.

Differences in the responses of the three $ALDH_2$ groups were highly significant ($F_{2,102}$=5.27, p<0.005).

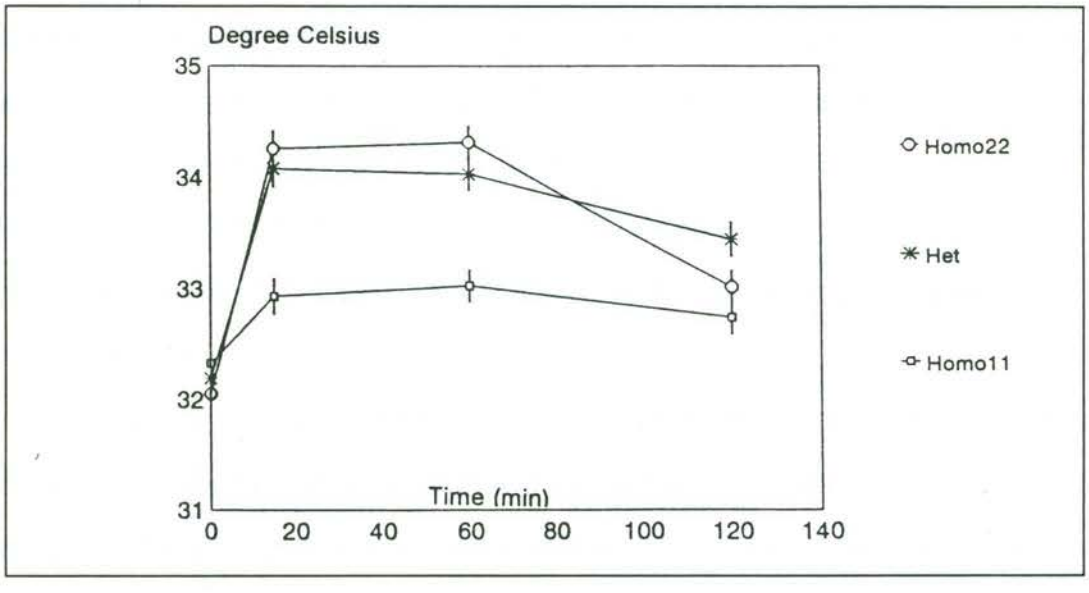


Fig.41: Facial temperature by $ALDH_2$ genotype.

Temperature increased in both the Het and Homo22 subjects 15 min after ethanol consumption. The Homo22 group attained slightly higher temperatures than the Het group, both at t_{15} and t_{60}. However, by t_{120}, the Homo22 subjects had exhibited a sudden fall in temperature (Fig.41).

3.5.6. PSYCHOMOTOR VARIABLES

The $ALDH_2$ Homo22 subjects demonstrated the greatest post-ethanol psychomotor impairment in all of the tests.

Psychomotor performance was significantly different among the three $ALDH_2$ groups in the DSC and the divided attention delay and excursion tasks.

Differences in the CFFF threshold and standing steadiness tests, did not reach statistical significance among the three groups.

3.5.6.1. DIVIDED ATTENTION

Delay A significant ($F_{2,101}=4.75$, $p<0.05$) difference was found among the three $ALDH_2$ groups for divided attention delay, although the group order was unexpected (Fig.42). A highly significant ($F_{6,303}=3.02$, $p<0.01$) time by group difference from pre-ethanol values was also detected in the delay of the Homo22 group at 15 ($q_{11,303}=8.66$, $p<0.05$), 60 ($q_{10,303}=8.12$, $p<0.05$) and 120 ($q_{9,303}=5.07$, $p<0.05$) minutes after ethanol. However, the Het and Homo11 groups were not significantly affected by ethanol on this variable.

Of all the groups, the Homo22 subjects exhibited the greatest impairment after ethanol, this being most obvious at the first post-ethanol time-point (t_{15}). This increase in divided attention delay time continued for the duration of the test session, although as time progressed, the increases in delay were not as great as in the first fifteen minutes. Recovery to pre-ethanol levels did not occur in Homo22 subjects.

The Homo11 and Het subjects responded similarly after ethanol, demonstrating a small increase in response, followed by a plateau effect until t_{60}, after which time recovery towards pre-ethanol values was evident.

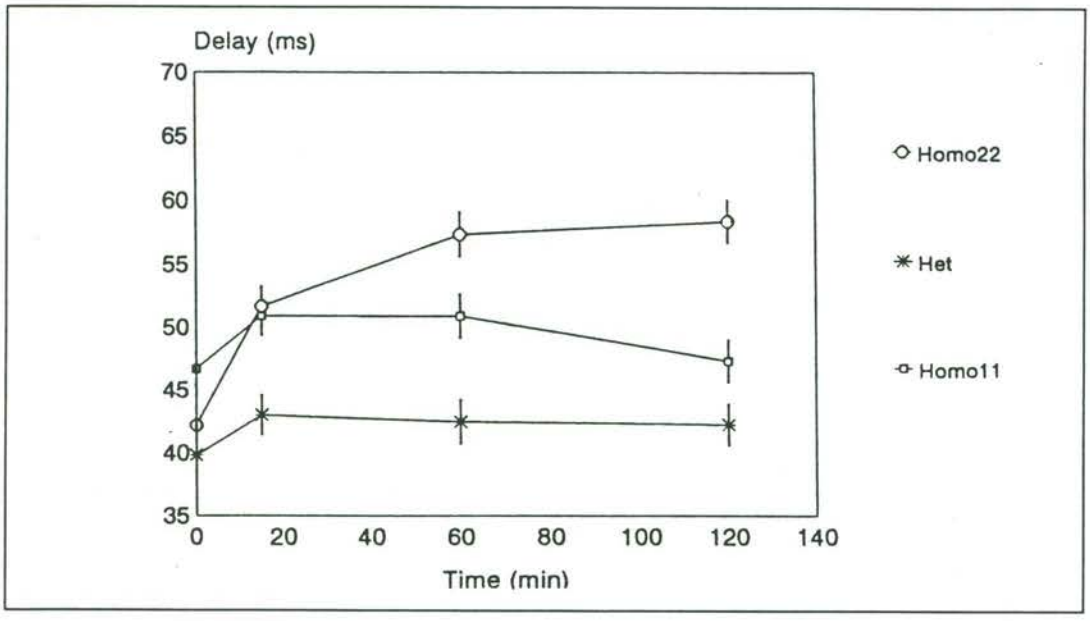


Fig.42: Divided attention delay by $ALDH_2$ genotype.

Excursion A significant difference was found among the three $ALDH_2$ groups for divided attention excursion ($F_{2,101}=4.19$, $p<0.05$) both before and after ethanol, indicating an effect unrelated to ethanol consumption. The group order was not as expected, with Homo22 subjects having the greatest overall excursion values, followed by Homo11 and Het subjects (Fig.43).

On the whole, all three groups produced a similar pattern of response after ethanol characterized by an initial increase in excursion, followed by a recovery towards pre-ethanol values at t_{60}, then by another increase in excursion (at t_{120}) - although Het subjects produced a decrease in excursion at t_{120}.

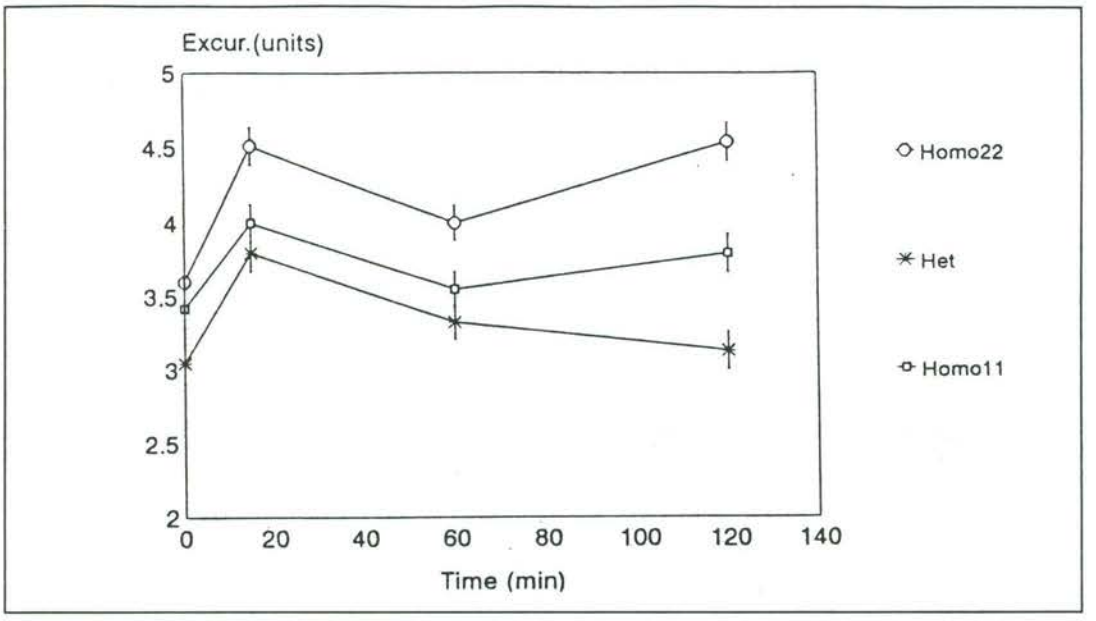


Fig.43: Divided attention excursion by $ALDH_2$ genotype.

Reaction Time Differences among the groups for divided attention peripheral reaction time results did not reach statistical significance (see section 3.5.8. for data tables). However, the Homo22 subjects had the longest reaction times of the three groups tested, followed by the Het and then the Homo11 subjects. This trend was noted both before *and* after ethanol, hence the effects appeared to be group- rather than ethanol-related.

Responses from all three groups were similar, with an increase in reaction time after ethanol followed by a decrease and then a gradual recovery to pre-ethanol values at t_{120}. Only Homo22 subjects failed to exhibit a recovery to pre-ethanol values. Instead, compared with the effect at t_{60}, reaction times were further decreased at t_{120}.

3.5.6.2. DIGIT SYMBOL CODING

A significant difference was found among the three $ALDH_2$ groups ($F_{2,101}$=3.98, $p<0.05$), the Homo22 group having the greatest overall DSC reaction times, followed by Het and then Homo11 subjects (Fig.44).

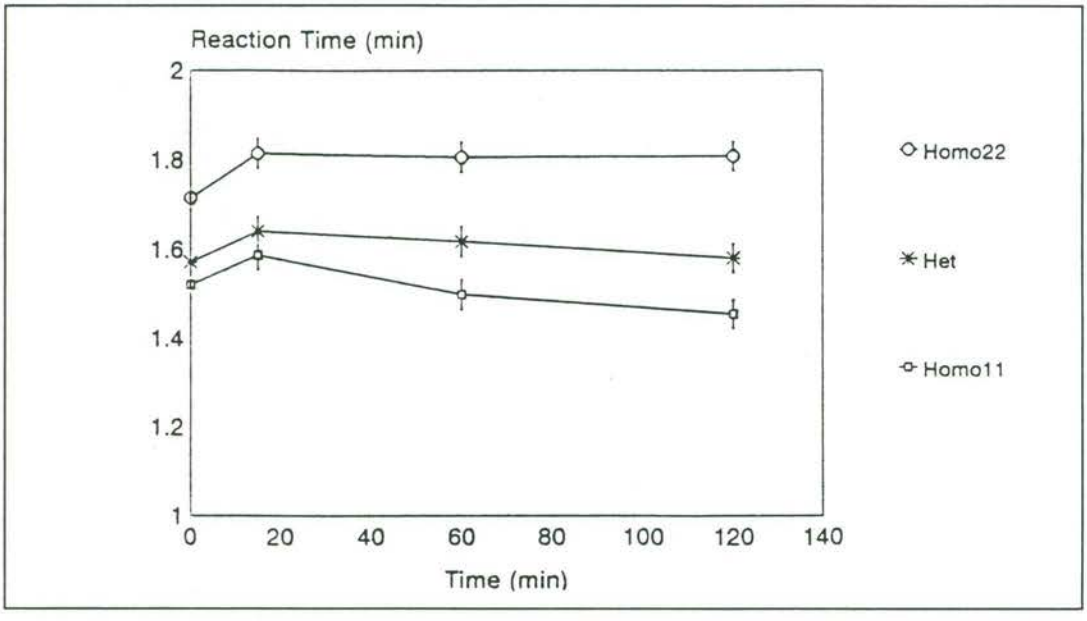


Fig.44: Digit symbol coding reaction time by $ALDH_2$ genotype.

The time by group difference was also found to be significant ($F_{6,303}$=2.13, $p<0.05$). In the Homo22 subjects, this effect lasted for up to 120 min after ethanol. *Post-hoc* analysis revealed that the Homo22 subjects had significantly different reaction times compared to the pre-ethanol values at 15 ($q_{4,303}$=3.89, $p<0.05$), 60 ($q_{3,303}$=3.66, $p<0.05$) and 120 minutes after ethanol ($q_{2,303}$=3.56, $p<0.05$). There were no significant differences detected between pre- and post-ethanol values for the Het and Homo11 subjects.

In all three groups, the response to ethanol challenge consisted of an increase at t_{15}, followed by a gradual decrease towards pre-ethanol values.

3.5.6.3. STANDING STEADINESS

A strong trend towards significance ($F_{6,306}$=1.99, p=0.067) was found for post compared to pre-ethanol values (Fig.45).

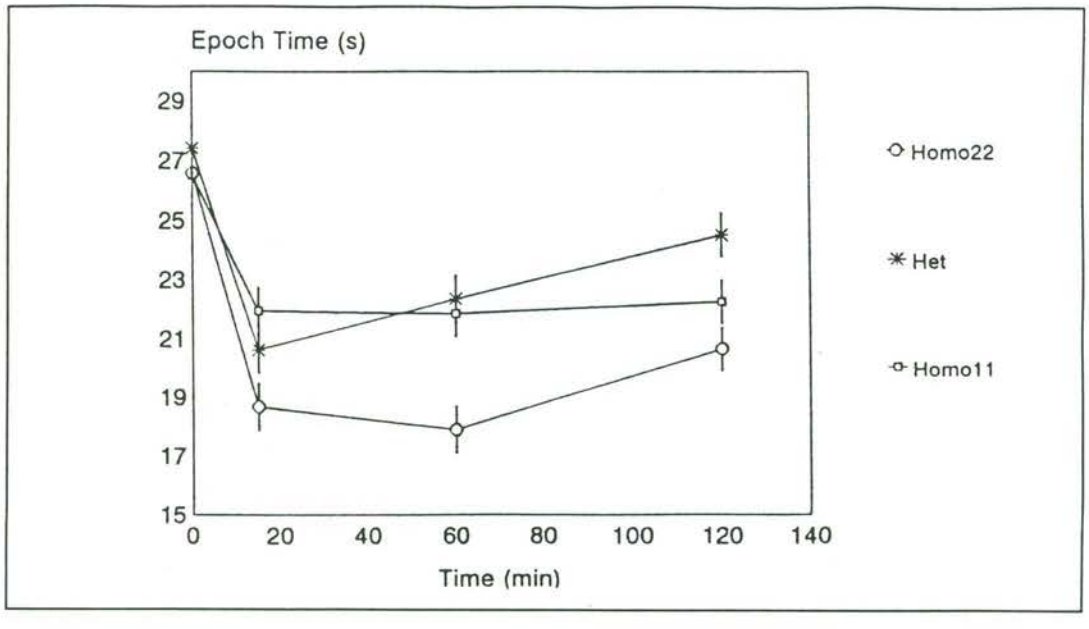


Fig.45: Standing steadiness by $ALDH_2$ genotype.

Impairment after ethanol was most severe among the three groups at t_{15}, and deteriorated further at t_{60} for all groups except the Het group (which began recovery to pre-ethanol values). The greatest effect was seen in the Homo22 subjects who still remained affected up to 120 min after ethanol, although by this time, recovery towards pre-ethanol values was apparent.

A similar pattern of response was found for both the Het and Homo11 groups, although the effect was not as great as in the Homo22 subjects.

There were no significant differences detected among the three groups tested.

3.5.6.4. CRITICAL FLICKER FUSION FREQUENCY

A highly significant ($F_{6,303}$=3.84, $p<0.005$) time by group interaction was found for the CFFF variable (Fig.46). This difference was attributable to the Homo22 group, beginning 60 min after ethanol ingestion ($q_{12,303}$=8.62, $p<0.05$) and continuing up to 120 min ($q_{11,303}$=7.79, $p<0.05$). Homo11 and Het subjects did not exhibit any statistically significant differences after ethanol.

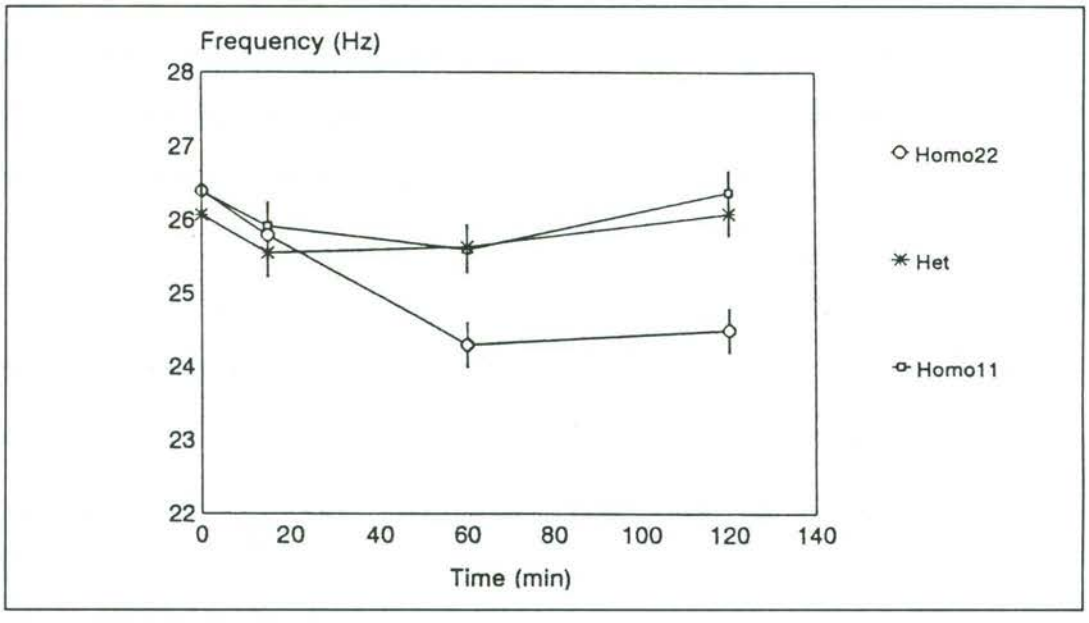


Fig.46: Critical flicker fusion frequency threshold by $ALDH_2$ genotype.

In general, the time-course of the responses of all three groups was similar in that after ethanol, the CFFF threshold value decreased to 60 min, by which time recovery had begun in all groups. The main difference was found in the Homo22 subjects who exhibited a large fall in CFFF at t_{60}.

3.5.7. SUBJECTIVE MEASURES OF INTOXICATION

Most of the responses to the subjective questions reached statistical significance (reported below).

In general, across the three $ALDH_2$ groups the Homo11 subjects were affected least by ethanol, returning to pre-ethanol values by 120 min. The Homo22 subjects were the most affected group, also being affected for the longest duration. The Het subjects exhibited responses which fell in between those of the other two groups. This order was found to be consistent across all the subjective questions and the greatest effect occurred predominantly at 15 min after ethanol consumption.

All three groups reported that they would not drive a car (Q1) 15-20 min after ethanol consumption, even though their BEC levels at that time were only around 0.030 g dl^{-1}.

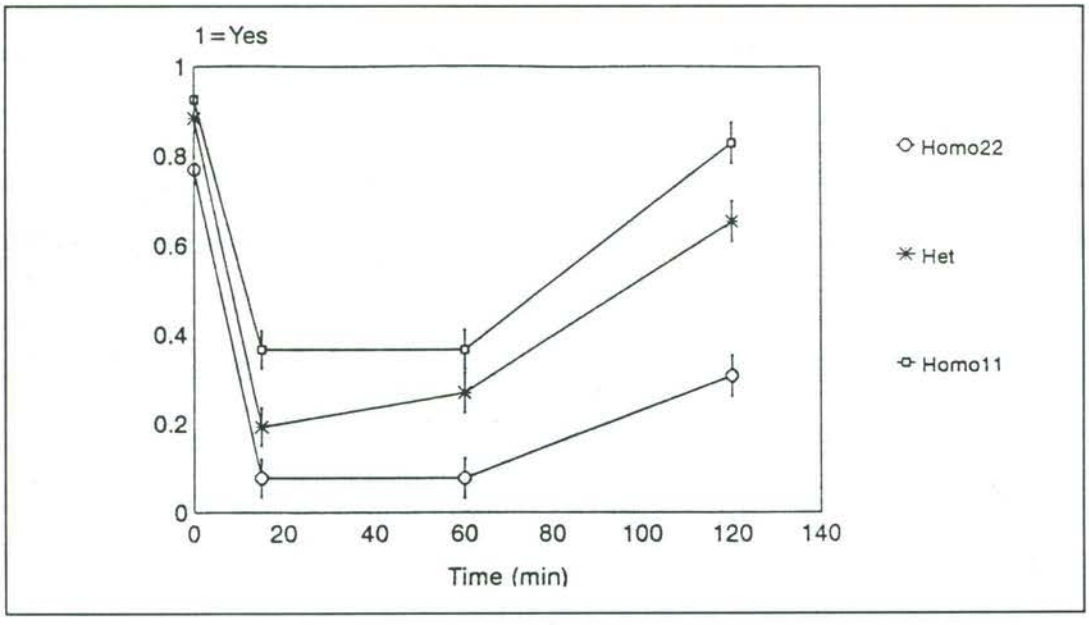


Fig.47: 'Would you drive a car now?' subjective response by $ALDH_2$ genotype.

The Homo22 group had the greatest response ($F_{2,103}$=6.72, p<0.005) although, as with the other two groups, this response decreased (as more subjects began to answer that they would be prepared to drive a car) by 120 min (Fig.47).

After ethanol, Homo22 subjects ($F_{6,309}$=2.42, p<0.05) reported that they would not accept another alcoholic drink from friends (Q2), this decision being especially evident 15 min after drinking ($q_{1,306}$=10.57, p<0.05), and did not change markedly at 120 min. Homo11 and Het groups had a similar, although attenuated, response after ethanol, with more subjects answering 'yes' at 120 min. Homo11 subjects were least affected by ethanol ($F_{2,103}$=5.87, p<0.005) -Fig.48.

All three $ALDH_2$ groups reported feeling drowsier (Q3) after consuming ethanol ($F_{6,306}$=2.73, p<0.05), with the Homo22 group being the most affected (5.7). This response ($q_{8,306}$=24.79, p<0.05) was continued even up to 120 min after ethanol (4.6), by which time the responses of the Het and the Homo11 groups had returned almost to their pre-ethanol values (2.1). The Homo11 group was the least affected of the three groups and the response of the Het group fell in between those of the other two groups when compared by group basis ($F_{2,102}$=5.66, p<0.005) -Fig.49.

In Q4, all subjects reported feeling clumsier after ethanol consumption ($F_{6,306}$=3.24, p<0.005). The Het and Homo22 subjects reported feeling more clumsy (5.4) and for a longer time than the Homo11 subjects (3.9). By 120 min, the Homo11 group had returned to pre-ethanol levels (1.9), the Het group was almost back to normal (2) but the Homo22 group was still affected at 4.3. Significant differences were detected among the three groups tested $F_{2,102}$=6.48, p<0.005 (Fig.50).

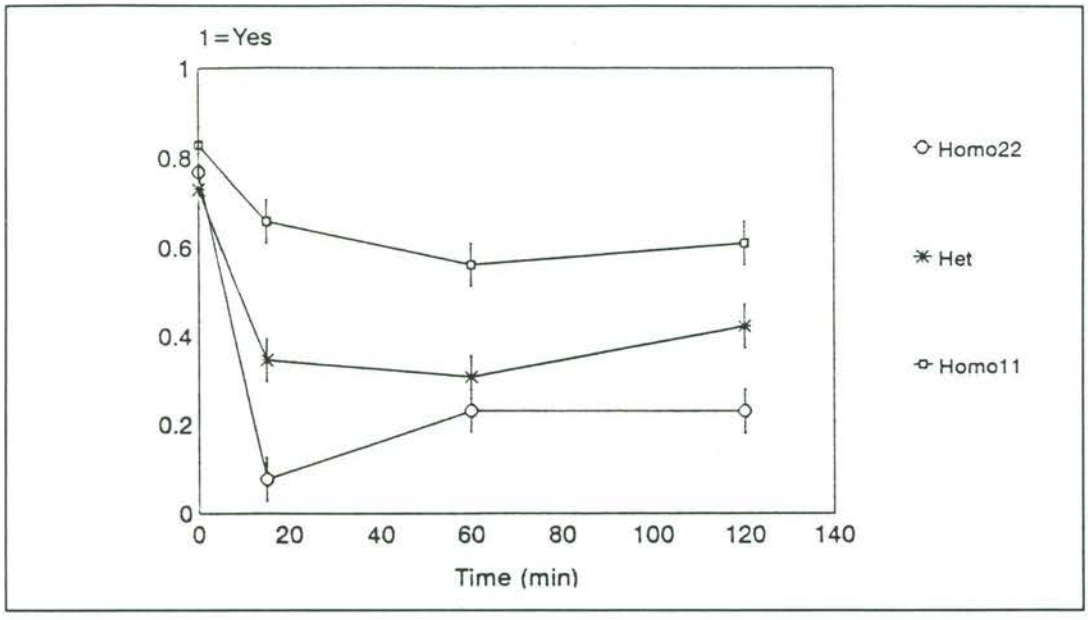


Fig.48: 'Another drink?' subjective response by $ALDH_2$ genotype.

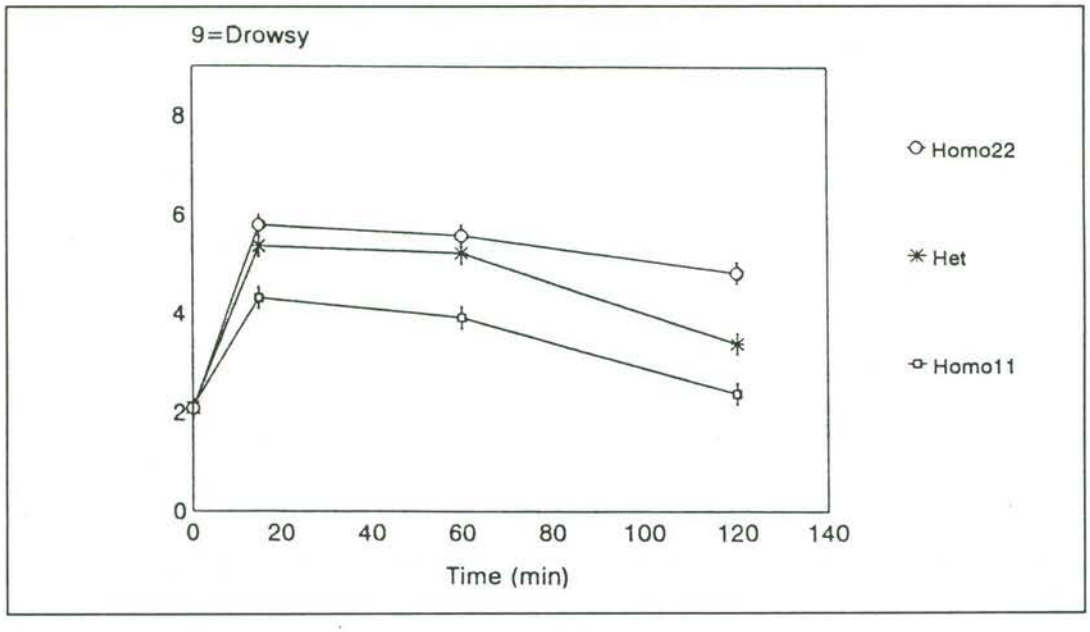


Fig.49: 'Do you feel alert or drowsy?' response by $ALDH_2$ genotype.

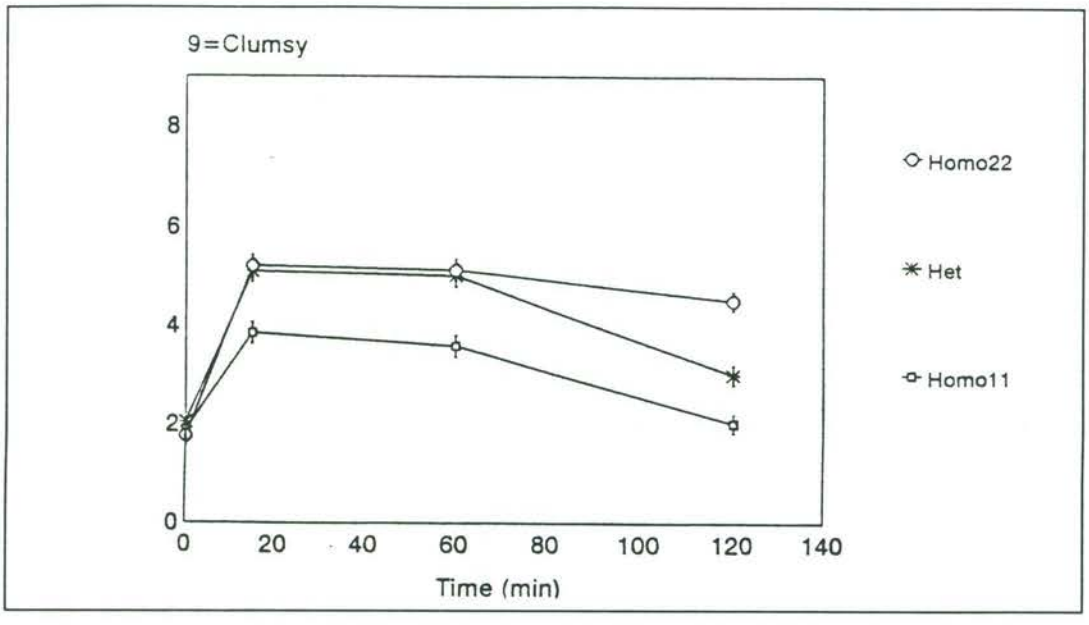


Fig.50: 'Do you feel coordinated or clumsy?' response by $ALDH_2$ genotype.

All three $ALDH_2$ groups reported a decrease in concentration levels (Q5) after ethanol ($F_{6,306}$=2.47, $p<0.05$). The Homo22 group reported the greatest effect (3.9), followed by the Het group (4.4). The Homo11 group was the least affected group (5.2) which also returned most rapidly to pre-ethanol values (Fig.51).

As expected, the perceived level of intoxication (Q6) increased after ethanol consumption ($F_{6,306}$=4.19, $p<0.005$). These responses were in the same order as the other subjective responses. That is, the Homo22 subjects were the most affected group followed by the Het and the Homo11 groups. Homo11 subjects were the least intoxicated group at 120 min after ethanol -$F_{2,102}$=11.51, $p<0.05$ (Fig.52).

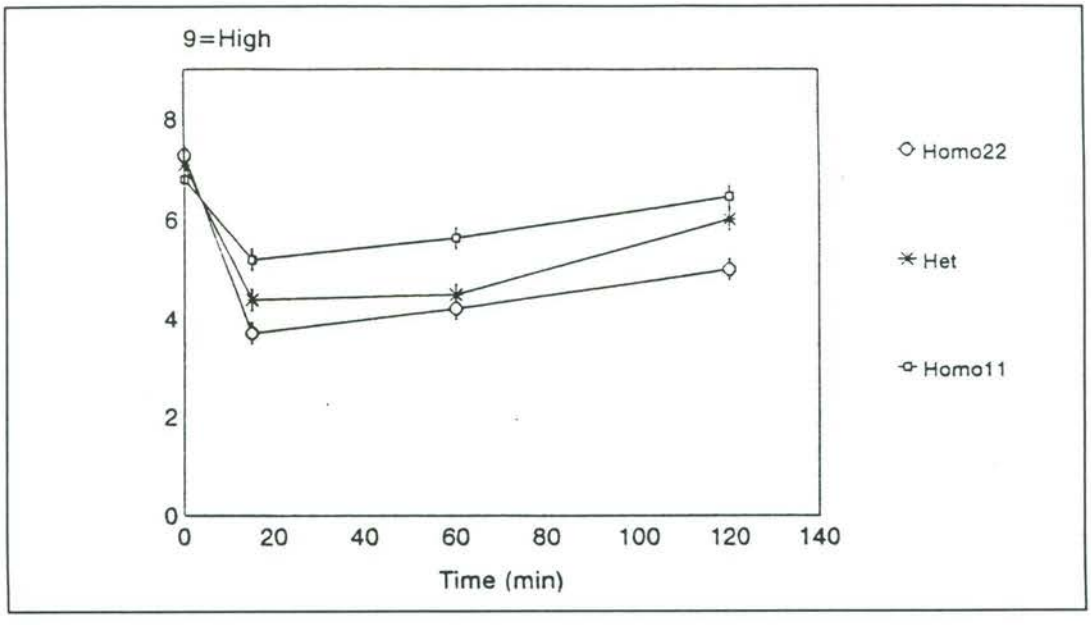


Fig.51: 'Is your concentration high or low?' response by $ALDH_2$ genotype.

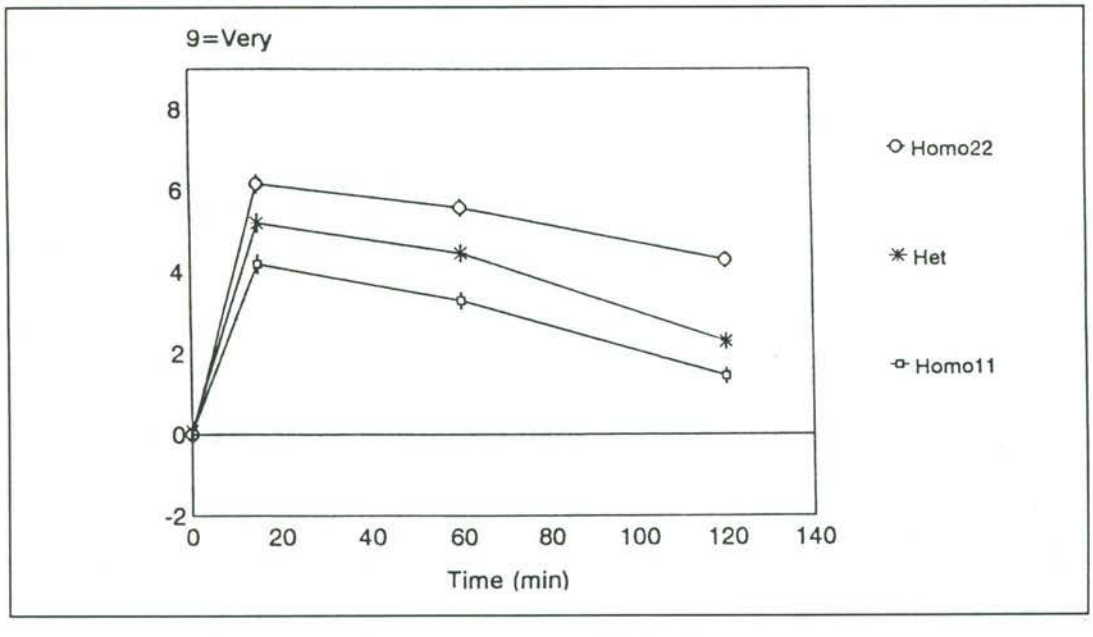


Fig.52: 'How intoxicated do you feel?' response by $ALDH_2$ genotype.

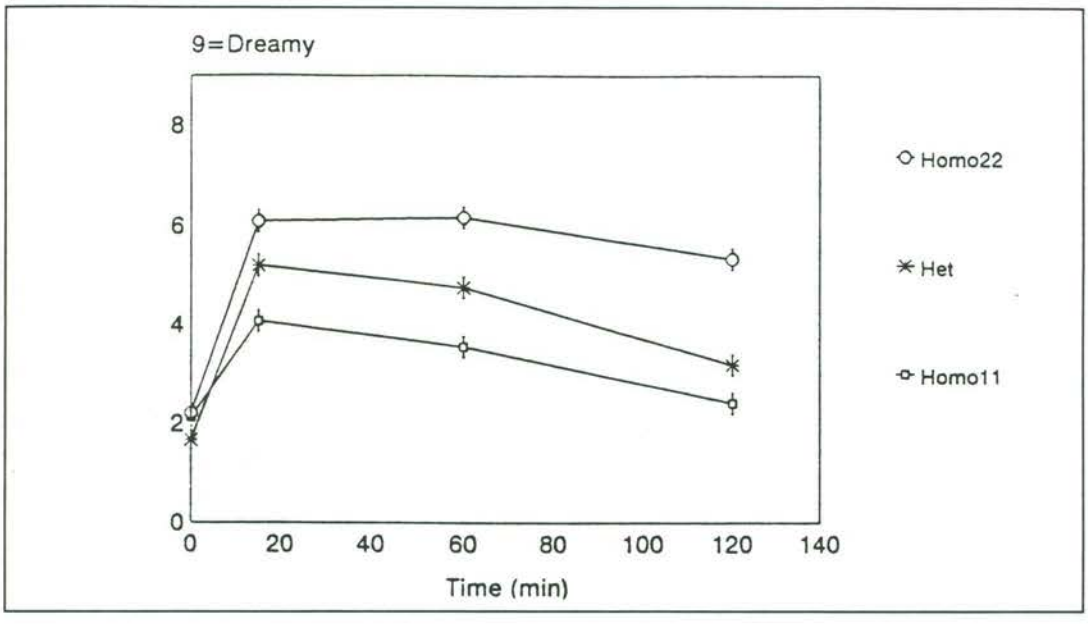


Fig.53: 'Do you feel attentive or dreamy?' response by $ALDH_2$ genotype.

When asked if they felt attentive or dreamy (Q7), Homo11 subjects had a lower response and did not report feeling as dreamy as the other two groups ($F_{2,102}$=9.12, $p<0.005$). By 120 min, the Homo11 group had returned almost to normal levels while the Het and Homo22 groups were still affected. Differences compared with pre-ethanol values were also highly significant $F_{6,306}$=4.03, $p<0.005$ (Fig.53).

The Homo22 group experienced the most severe headache (Q8) after ethanol. This response was the greatest at t_{120} although, in general, over the course of the whole session (from 15-120 min after ethanol), the Homo22 group reported the most headaches compared to the Homo11 and Het groups ($F_{2,103}$=7.35, $p<0.005$) -Fig.54.

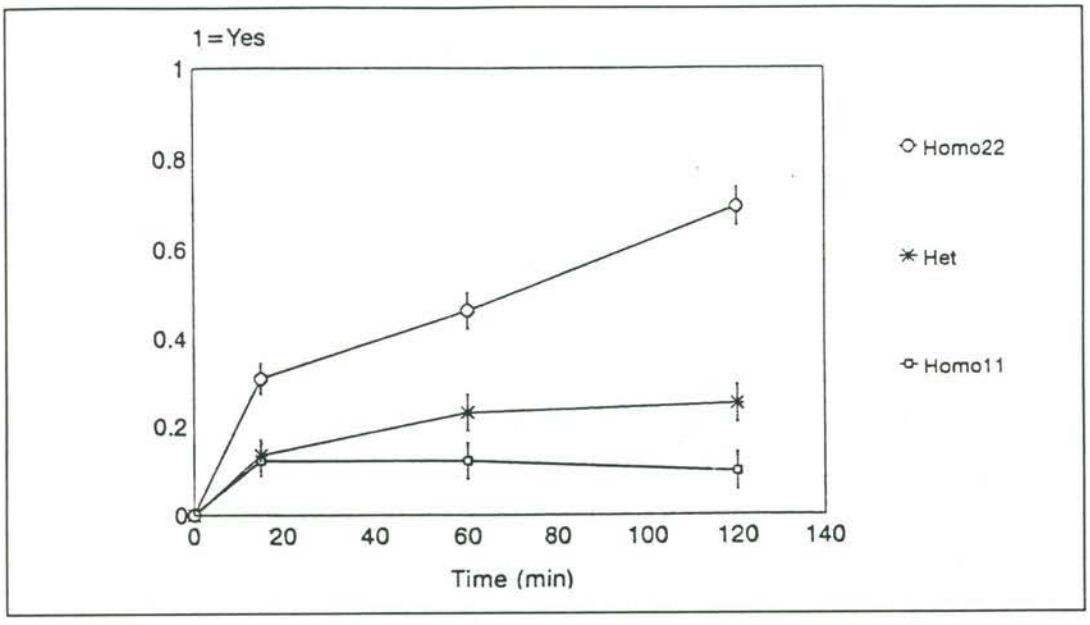


Fig.54: 'Do you experience a headache?' response by $ALDH_2$ genotype.

Of the three groups, the Homo22 subjects felt the most nauseated (Q9) after ethanol. This effect occurred mainly in the time period from 60-120 min after ethanol, and was most marked at t_{120} ($F_{4,206}$=5.35, $p<0.005$). By 120 min after ethanol, the responses of both the Het and Homo11 groups had returned to pre-ethanol values (Fig.55).

It is of interest to note that at t_{15}, Het and Homo11 subjects reported more intense nausea than Homo22 subjects. However, the nausea experienced by the Homo22 subjects increased progressively over time.

Reported hangover-like symptoms (Q10) were most striking in the Homo22 group, this effect increased from t_{15} and was the greatest at 120 min after ethanol ($F_{4,206}$=3.51, $p<0.005$). Homo11 and Het subjects did not report many hangover-

like symptoms after ethanol. Differences among the three groups were statistically significant $F_{2,103}=3.95$, $p<0.05$ (Fig.56).

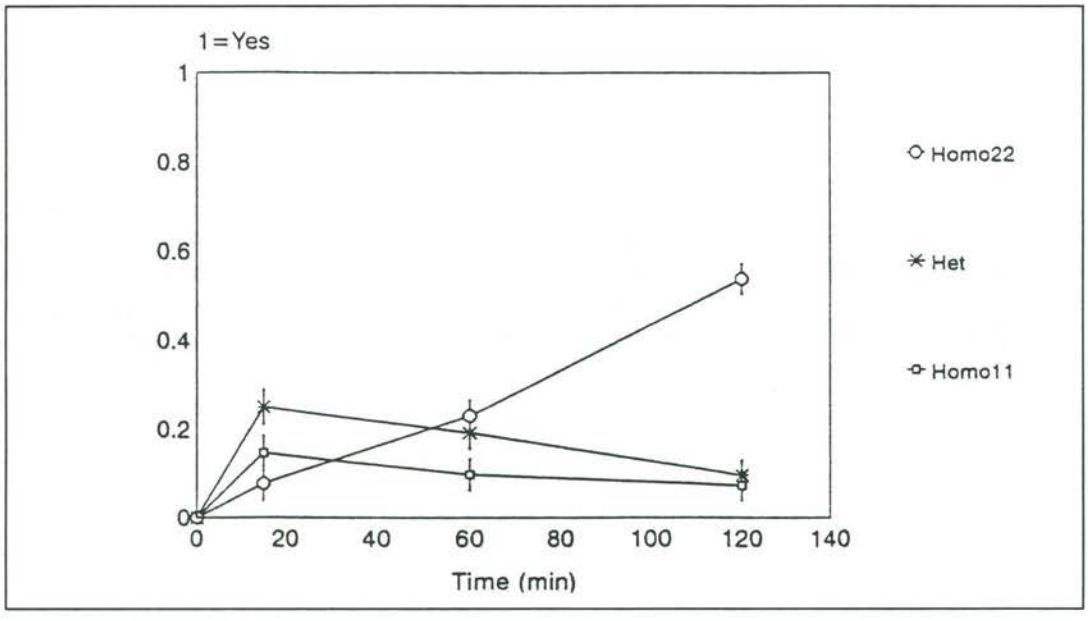


Fig.55: 'Stomach uneasy or sick?' response by $ALDH_2$ genotype.

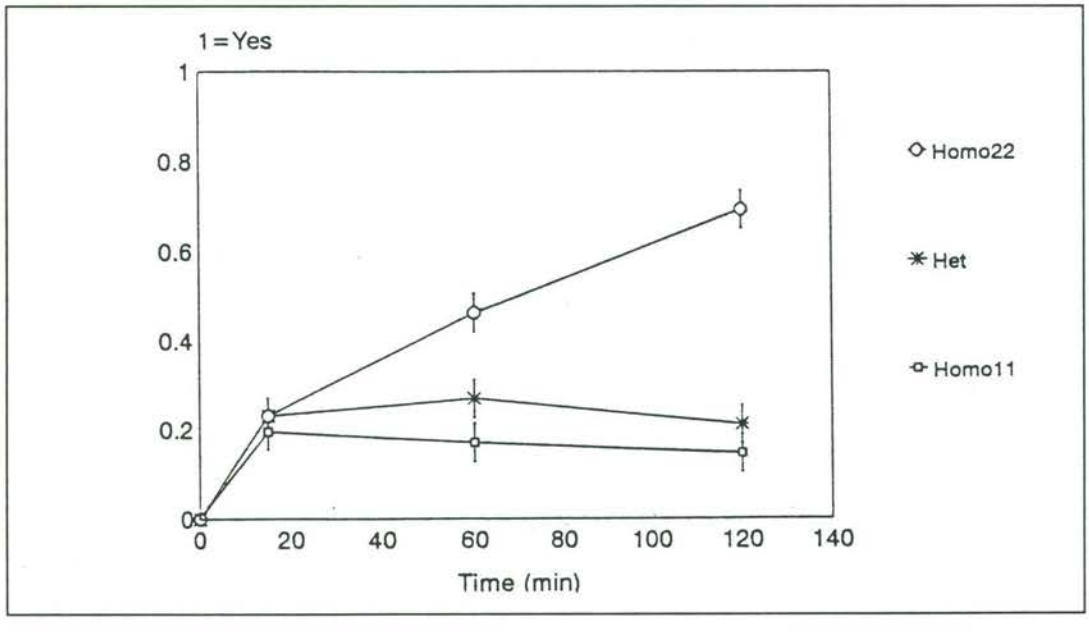


Fig.56: 'Any hangover-like effects?' response by $ALDH_2$ genotype.

3.5.8. SUMMARY OF OTHER RESULTS BY $ALDH_2$ GENOTYPE (NON-SIGNIFICANT)

The results for each variable below are listed in the following manner for each time-point (t_0, t_{15}, t_{60}, t_{120}):

Homo11 mean ± SD; n=43

Het mean ± SD; n=50

Homo22 mean ± SD; n=14

VARIABLE	t_0	t_{15}	t_{60}	t_{120}
Ethanol (mM)	0 ± 0	8.3 ± 2.0	8.5 ± 1.5	5.3 ± 1.3
	0 ± 0	9.3 ± 3.0	8.3 ± 1.7	5.4 ± 1.6
	0 ± 0	9.1 ± 2.5	8.8 ± 1.5	5.7 ± 1.3
Lactate (mM)	1.5 ± 1.2	1.8 ± 1.2	1.7 ± 0.9	1.6 ± 0.7
	1.3 ± 0.9	1.6 ± 0.8	1.7 ± 0.8	1.7 ± 1.0
	1.5 ± 1.0	2.0 ± 1.0	2.0 ± 1.2	2.4 ± 1.5
Pyruvate (mM)	0.06 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.02
	0.05 ± 0.02	0.04 ± 0.03	0.04 ± 0.02	0.04 ± 0.02
	0.06 ± 0.02	0.04 ± 0.01	0.05 ± 0.02	0.05 ± 0.02
Lac:Pyr Ratio	24.9 ± 14.2	43.9 ± 22.1	46.1 ± 41.3	46.6 ± 44.6
	30.1 ± 38.2	60.6 ± 97.1	46.9 ± 28.0	61.5 ± 117.1
	24.3 ± 12.5	48.1 ± 17.2	43.1 ± 13.9	45.1 ± 17.8
Blood Pressure	112.2 ± 12.0	108.6 ± 12.4	104.8 ± 11.1	106.5 ± 14.1
(Sys) (mmHg)	111.6 ± 13.2	109.4 ± 15.3	106.8 ± 13.7	104.4 ± 13.3
	109.6 ± 13.5	112.2 ± 14.0	106.8 ± 12.4	107.5 ± 11.4
Blood Pressure	71.0 ± 10.5	70.8 ± 10.4	67.5 ± 9.8	68.7 ± 9.7
(Dia) (mmHg)	73.0 ± 11.3	68.1 ± 11.4	66.5 ± 11.4	67.9 ± 10.0
	73.4 ± 6.9	63.6 ± 8.8	65.3 ± 9.4	66.3 ± 6.6

Div. Attention	2.6 ± 0.7	2.9 ± 0.8	2.6 ± 0.8	2.6 ± 0.7
(Reaction Time)	2.7 ± 0.8	3.1 ± 0.9	2.8 ± 0.9	2.8 ± 0.9
	3.2 ± 1.0	3.4 ± 0.9	3.0 ± 1.0	2.8 ± 0.8

3.6. RESULTS BY DEGREE OF FLUSH

Initially, flush rating of subjects on a scale of 0-6 was used to rate each subject on the basis of his/ her flushing pattern. Based on the results of this rating scale, subjects were then divided into three main groups for statistical analysis. These groups were designated as follows: 'NF' for Non-Flushers, 'FF' for those Facial-Flushers rating between 1-4 and 'LF' for those rating 5, 5.5 or 6 (Lobster-Flushers).

3.6.1. BREAKDOWN OF SUBJECTS BY DEGREE OF FLUSH AND GENOTYPE

3.6.1.1. BY $ALDH_2$

Non-flushers accounted for 45% of the $ALDH_2$ Homo11 subjects compared with only 8% of the Het subjects (Fig.57).

There were no non-flushers in the Homo22 group. Instead, this group had subjects who rated 4 or higher on the subjective flushing scale. 77% of the Homo22 group flushed intensely over the entire body and sometimes the limbs as well (ratings of 5, 5.5 and 6: these subjects constituted the LF group). These findings were highly significant ($p<0.005$) among Homo11-Het and Homo11-Homo22 groups.

3.6.1.2. BY ADH_2

Comparison of the subjects by ADH_2 genotype (Fig.58) indicated that subjects assessed subjectively on the flush scale as rating 5 or above (the more severe flushers), demonstrated corresponding percentage increases across the Homo11 (35%), Het (41%) and Homo22 (63%) groups respectively. These findings, however, did not reach statistical significance ($p>0.05$).

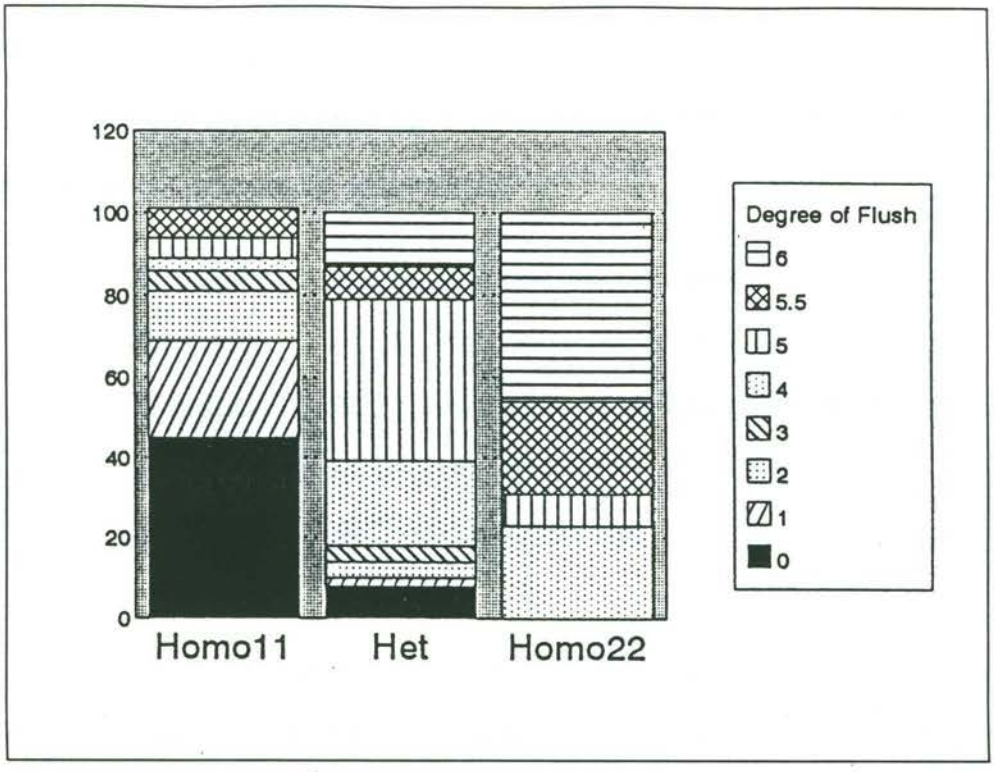


Fig.57: Degree of flush by $ALDH_2$ Homo11, Het, Homo22 genotype.

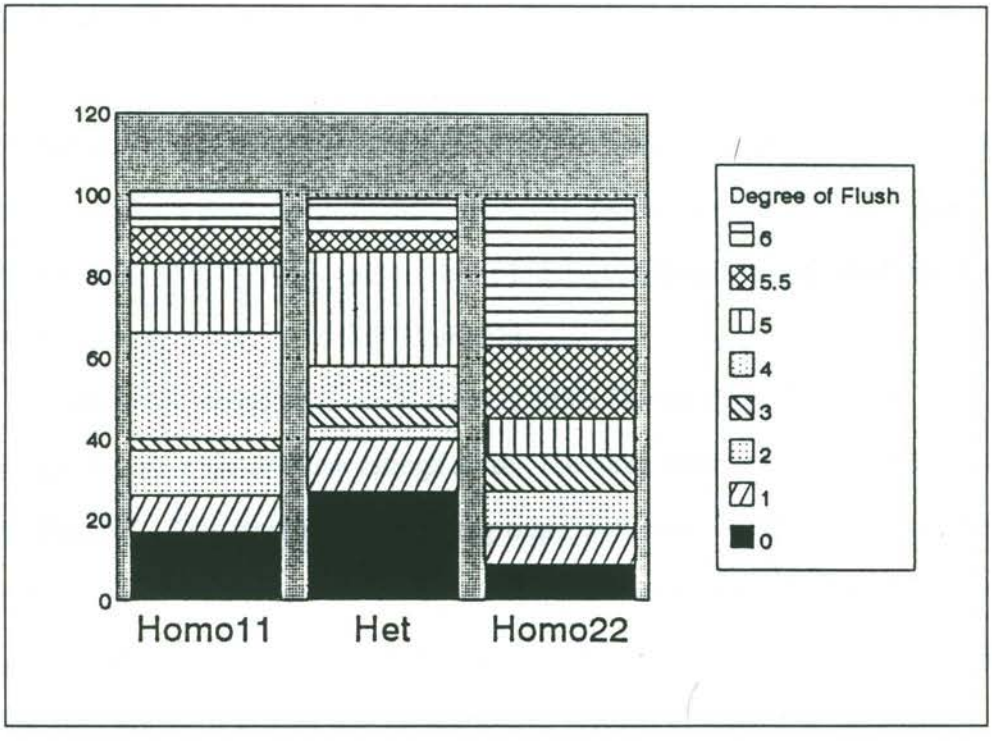


Fig.58: Degree of flush by ADH_2 Homo11, Het, Homo22 genotype.

3.6.1.3. BY $ALDH_2$/ADH_2

Summary of the five groupings for the various $ALDH_2$/ ADH_2 combination genotypes (further discussed in section 3.7.):

GroupA	$ALDH_2$ Homo11/ ADH_2 Homo11
GroupB	$ALDH_2$ Het/ ADH_2 Homo11
GroupC	$ALDH_2$ Homo22/ ADH_2 Homo11, Het or Homo22
GroupD	$ALDH_2$ Homo11/ ADH_2 Het
GroupE	$ALDH_2$ Het/ ADH_2 Het

Subjects rating 5 or more on the subjective flushing scale were mainly found in GroupC (82%) and GroupE (74%). As explained in more detail later, GroupC subjects comprised those people that possessed the ALDH Homo22/ ADH Homo11, Het or Homo22 enzymes, whilst GroupE where those people who had the ALDH Het/ ADH Het enzymes. The lowest proportions of non-flushers were also found in these two groups (0% and 4% respectively) -Fig.59.

When the subjects were categorized into the original *nine* different $ALDH_2$/ ADH_2 combination genotypes (Table3), the mean degree of flush for each group increased across the $ALDH_2$ groups (Homo11< Het< Homo22) and, similarly, down the ADH_2 groups (Homo11< Het< Homo22).

It should be noted that where there is either Het or Homo22 ALDH, the ADH_2 groups have higher flush ratings across the three ADH_2 groups from Homo11, Het to Homo22. This increase across the groups, however, is dependent upon the $ALDH_2$ type and the increase is not apparent in subjects with the $ALDH_2$ Homo11 type. Although the differences are not as great in the ADH_2 groups as for the $ALDH_2$ groups, they are nevertheless real differences ($p<0.005$) of statistical significance.

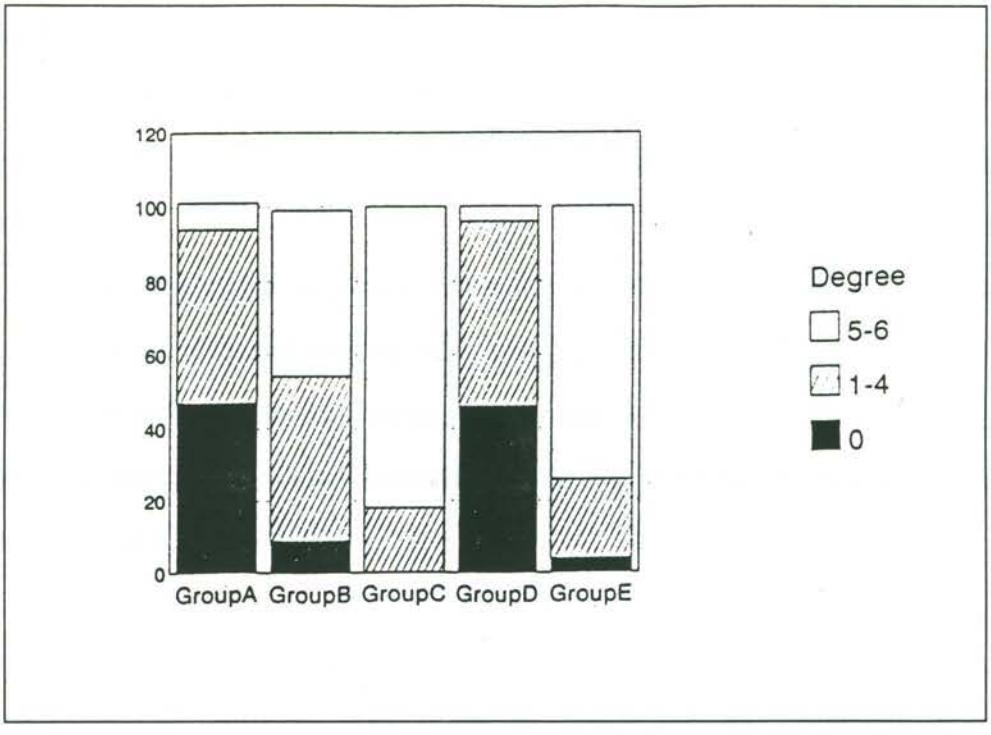


Fig.59: Degree of flush by $ALDH_2$/ ADH_2 combination genotype.

	$ALDH_2$ Homo11	**$ALDH_2$ Het**	**$ALDH_2$ Homo22**	**mean**
ADH_2 Homo11	1.3 SEM=0.42	4 SEM=0.36	5.1 SEM=0.46	3.5
ADH_2 Het	0.9 SEM=0.24	4.6 SEM=0.25	5.4 SEM=0.33	3.6
ADH_2 Homo22	1.3 SEM=0.88	5.7 SEM=0.20	5.8 SEM=0.25	4.3
mean	1.2	4.8	5.4	3.8

Table 3: Breakdown of subjects into their $ALDH_2$/ ADH_2 genotype combinations by average degree of flush.

3.6.2. DRINKING DETAILS

Most LF and FF subjects (77%) consumed ethanol 1-2 times a month or less, or not at all; the remaining 23% of subjects reported that they drank ethanol more often than this.

More NF subjects reported that they drank ethanol only 1-2 times a month or less, or not at all compared with the other two groups. 87% of subjects indicated that they drank ethanol 1-2 times a month (35%) or less (39%) or not at all (13%) -Fig.60. However, when tested with non-parametric tests, these findings did not reach levels of statistical significance ($p>0.05$).

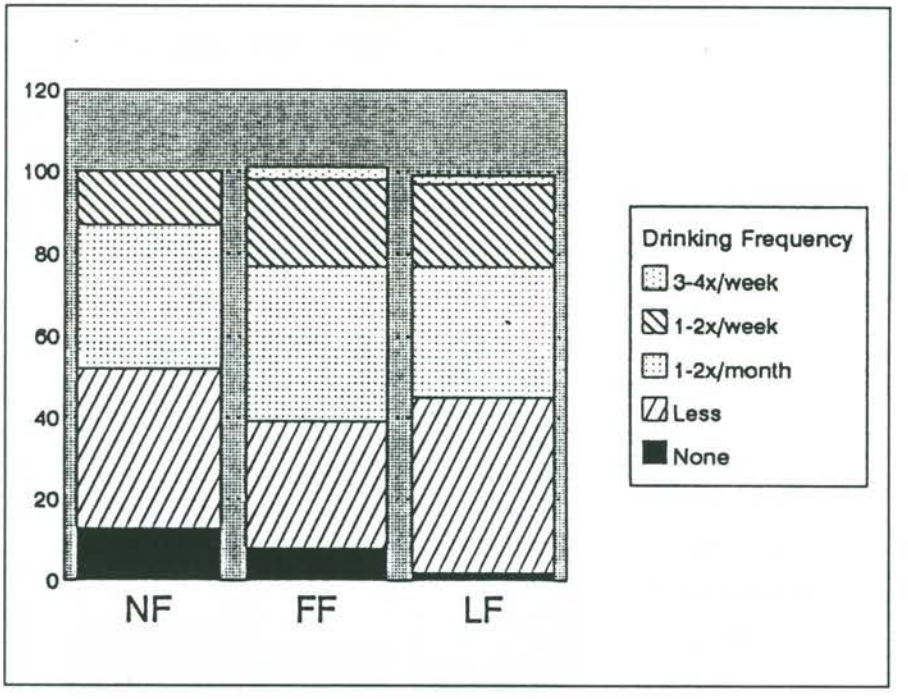


Fig.60: Drinking habits by degree of flush: How often subjects drink ethanol.

The choice of beverage preferred was predominantly beer in all three degree of flush groups (Fig.61). The high proportion of people reportedly consuming spirits

in the LF Group might be attributable to the fact that most of these subjects did not consume ethanol very often, but when they did, the occasion was a celebratory one where spirits were consumed.

The average amount of ethanol consumed by subjects was reported to be greater than one standard drink for the majority of NF and FF subjects. In these two groups, less than 10% of subjects claimed to consume less than one drink per session (9%). In contrast, in the LF group, only 32% of subjects reported that they consumed two or more drinks per session, compared with 53% and 45% in the FF and NF groups respectively (Fig.62). These results are not surprising as the LF subjects would be expected to have a stronger adverse reaction to ethanol and so would tend to consume less. The results, when tested non-parametrically, were not of statistical significance ($p>0.05$).

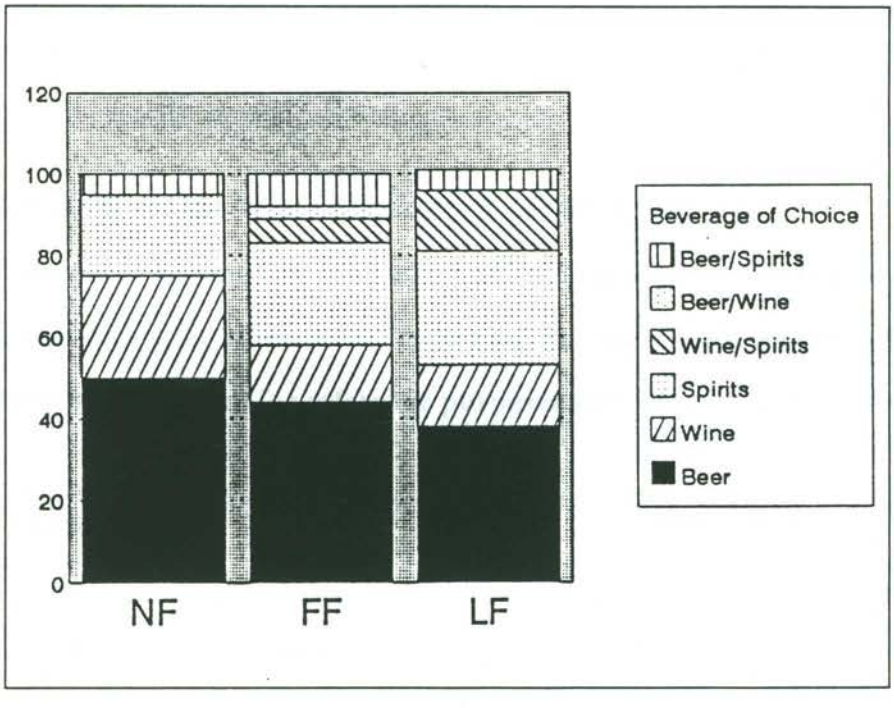


Fig.61: Choice of Beverage by $ALDH_2$/ ADH_2 genotype.

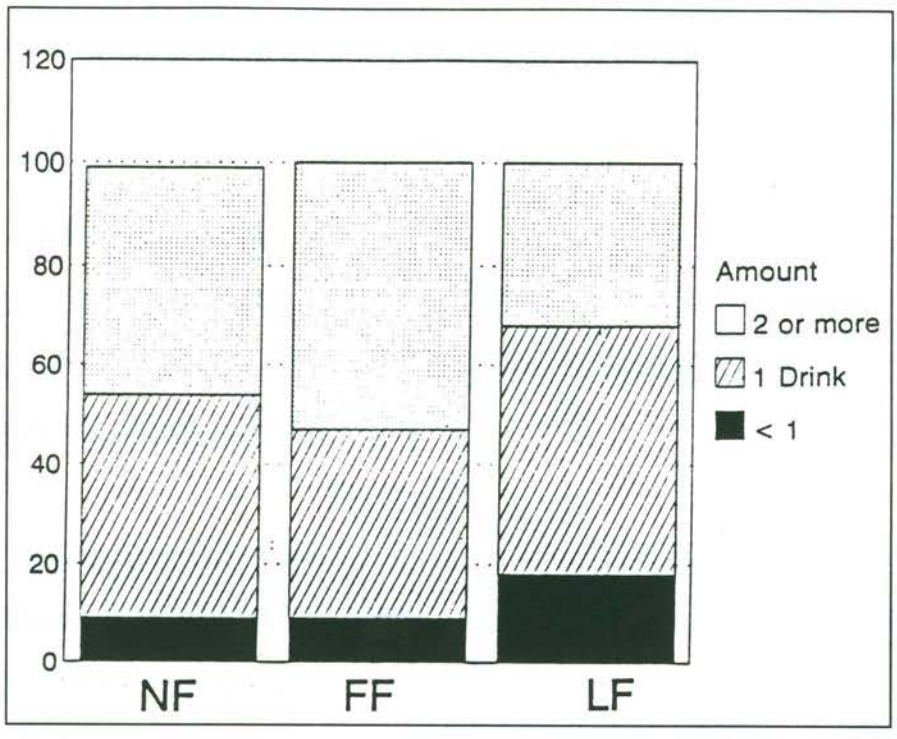


Fig.62: Quantity of ethanol usually consumed -by Degree of Flush groups.

3.6.3. BREATH ETHANOL CONCENTRATION CURVES

Differences attained by the Degree of Flush groups in the ethanol pharmacokinetics after ethanol challenge were not statistically significant although results are given for reference purposes (see section 3.6.8. for data).

3.6.3.1. PEAK BEC

The mean BEC values were found to be 0.033 g dl^{-1} for both the LF and FF groups while the NF group peaked at a value of 0.032 g dl^{-1}.

3.6.3.2. TIME TO PEAK

Mean peak BECs were reached 15 min after ethanol consumption in all three groups.

3.6.3.3. ELIMINATION RATE

Using linear regression methods, the LF subjects had a mean ethanol elimination rate of 0.0161 g dl^{-1} hr^{-1}, FF subjects had a rate of 0.0159 g dl^{-1} hr^{-1} whilst the NF subjects had a rate of 0.0150 g dl^{-1} hr^{-1}.

3.6.3.4. VOLUME OF DISTRIBUTION

The mean volumes of distribution were 55.4, 54.4 and 57.6 litres for the LF, FF and NF subjects respectively.

3.6.3.5. AREA UNDER THE CURVE

The LF group had the smallest mean area under the curve (0.129 ± 0.014 (SEM) g dl^{-1} min). The mean area for the FF group was 0.135 ± 0.015 (SEM) g dl^{-1} min, and 0.131 ± 0.019 (SEM) g dl^{-1} min for the NF group.

3.6.4. BIOCHEMICAL VARIABLES

Acetate concentrations were highly significantly different among the three groups. There were also highly significant differences for the time by group interactions for acetaldehyde concentrations.

None of the other biochemical variables, including plasma ethanol, lactate and pyruvate levels, were found to be significantly different among the groups.

3.6.4.1. BLOOD ACETALDEHYDE

Acetaldehyde levels were highly significantly different in the LF group compared to pre-ethanol values ($F_{6,306}$=3.45, $p<0.005$); the increase in acetaldehyde concentration began soon after drinking and continued up to 120 min after ethanol (Fig.63).

The rate of rise of acetaldehyde concentration after ethanol consumption was the greatest in the LF group and the acetaldehyde concentrations remained elevated throughout the entire test session.

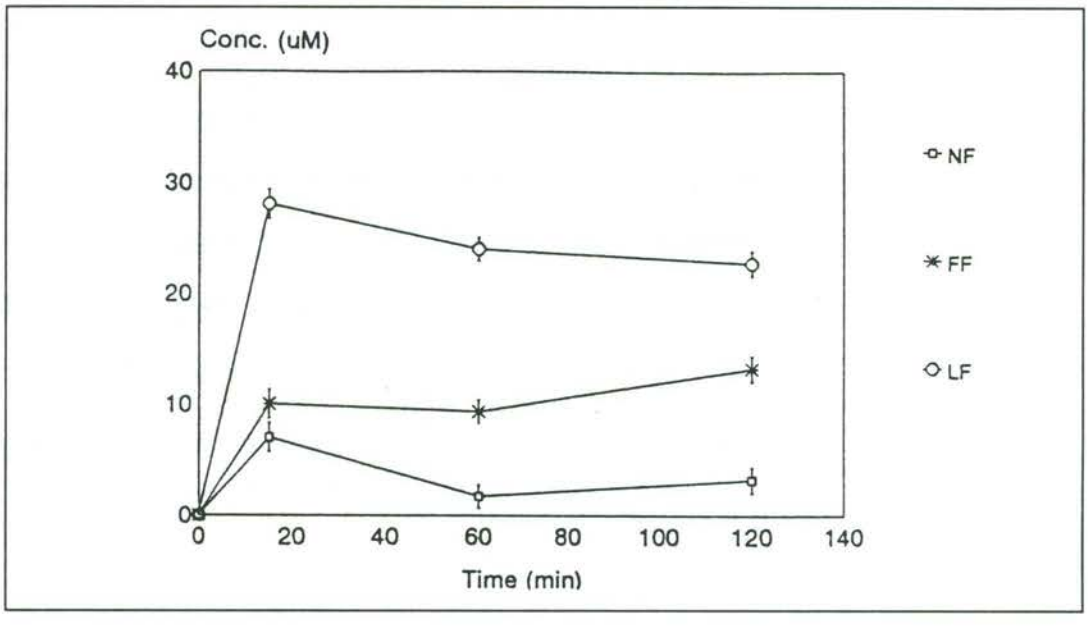


Fig.63: Acetaldehyde concentrations (μM) by degree of flush group.
(N.B. Blank corrections accounted for in measurements)

The FF group exhibited an intermediate increase in acetaldehyde concentration compared with the two other group, this was maximal at t_{120}.

The NF subjects had the lowest acetaldehyde concentrations of all the three groups; these subjects attained their peak levels at t_{15} and the level declined thereafter.

Post-hoc analysis revealed that the LF group subjects were highly significantly different from the pre-ethanol values at 15 ($q_{12,306}$=9.36, $p<0.005$), 60 ($q_{8,306}$=8.0, $p<0.005$) and 120 ($q_{7,306}$=7.58, $p<0.005$) minutes after ethanol consumption. The differences found in NF and FF subjects after ethanol were not significant.

3.6.4.2. PLASMA ACETATE

Acetate levels were highly significantly different among the three groups ($F_{2,102}$=7.57, $p<0.005$) -Fig.64.

The LF group had the lowest acetate concentration among the groups at all times. When considered together with the acetaldehyde levels among the groups, a build-up of acetaldehyde, coupled with a slower production of acetate, is a clear characteristic of the LF group, facilitating strong flushing patterns.

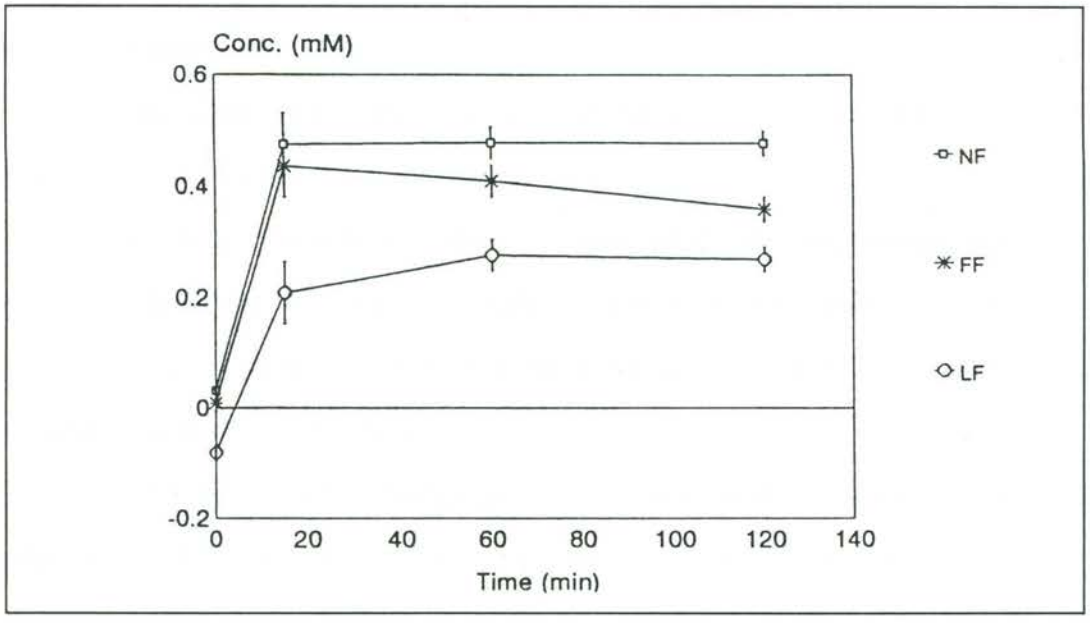


Fig.64: Acetate concentrations (mM) by degree of flush group.

3.6.4.3. PLASMA ETHANOL

Differences found among the three groups for plasma ethanol levels were not statistically significant (see section 3.6.8. for data tables). The FF subjects had the highest BECs, followed by the LF subjects, with the NF subjects having the lowest BEC values.

3.6.4.4. BLOOD LACTATE

Differences among the groups for lactate levels were not statistically significant (see section 3.6.8. for data tables).

In general, all three groups exhibited an increase in lactate concentration after ethanol. The lactate concentrations of the NF and FF groups declined towards pre-ethanol values by t_{120}.

In LF subjects, however, there was an increase in lactate concentration after ethanol consumption, which continued for the duration of the test session with the highest lactate concentration being attained at t_{120}.

The NF subjects had the lowest lactate concentrations of the three groups.

3.6.4.5. BLOOD PYRUVATE

Group differences in the pyruvate levels measured did not reach statistical significance (see section 3.6.8. for data tables).

All three groups demonstrated a decrease in pyruvate concentration after ethanol consumption. This was followed by a slight increase towards pre-ethanol values at t_{15} in the case of FF and LF subjects. The pyruvate levels of NF subjects remained low after t_{15} and showed no recovery.

The LF group had the highest pyruvate concentrations overall, followed by FF subjects. The NF group had the lowest pyruvate levels of all three groups.

3.6.4.6. BLOOD LACTATE:PYRUVATE RATIO

Differences in the lactate:pyruvate ratio by degree of flush did not reach levels of statistical significance (see section 3.6.8. for data tables).

3.6.5. PHYSIOLOGICAL VARIABLES

There were highly significant group differences in facial temperature. Differences in heart rate were also found to be highly significant across the three groups.

Systolic and diastolic blood pressure did not differ significantly among the groups.

3.6.5.1. HEART RATE

After ethanol, the heart rate was highly significantly increased in all three groups ($F_{2,102}$=15.9, $p<0.005$) -Fig.65.

The LF group attained the highest heart rates, followed by the FF group and then the NF group. No significant differences in the time by group interaction was detected among the groups after ethanol.

The response to ethanol was similar in all three groups, that is, the heart rate increased after ethanol consumption, plateaued until t_{60}, and then decreased gradually towards pre-ethanol levels.

It is of interest to note that there was a pre-ethanol difference in heart rate among the groups (Fig.65). This might have arisen from anxiety in the people who flushed and had experienced the AFR in the past, causing elevated heart rates even before the drinking commenced.

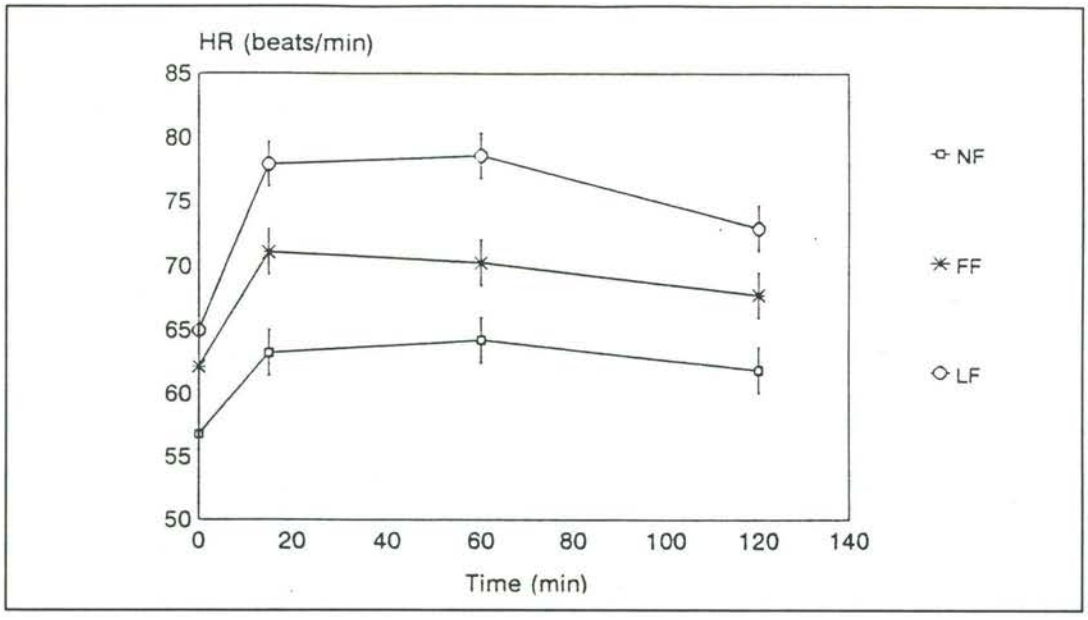


Fig.65: Heart rate by degree of flush group.

3.6.5.2. BLOOD PRESSURE

After ethanol, systolic blood pressure was found to decrease over time. The group differences for systolic blood pressure were not statistically significant (see section 3.6.8. for data tables).

Diastolic blood pressure increased after ethanol in NF subjects, although after t_{15}, the blood pressure fell in all groups. Recovery occurred in FF and LF subjects by t_{120}. NF subjects had the highest diastolic blood pressures overall, and LF subjects exhibited the greatest fall in diastolic blood pressure immediately after ethanol. Group differences in diastolic blood pressure were not statistically significant (see section 3.6.8. for data tables).

3.6.5.3. FACIAL TEMPERATURE

Facial temperature was highly significantly different among the three groups ($F_{2,102}=5.66$, $p<0.005$) -Fig.66. The time by group interaction was also found to be highly significant ($F_{6,306}=9.81$, $p<0.005$).

The facial temperature rose in all three groups after ethanol. The LF subjects recorded the highest facial temperatures, which remained elevated throughout the whole test session.

After ethanol, the NF subjects had smaller increases in temperature than the other two groups.

Post-hoc analysis revealed that the LF subjects were significantly different from the other groups tested at 15 ($q_{12,306}$=15.52, $p<0.005$), 60 ($q_{11,306}$=14.61, $p<0.005$) and 120 minutes after ethanol ($q_{9,306}$=8.28, $p<0.005$) and the FF subjects at 15 ($q_{8,306}$=10.28, $p<0.005$), 60 ($q_{10,306}$=12.06, $p<0.005$) and 120 ($q_{5,306}$=8.55, $p<0.005$) minutes after ethanol consumption. NF subjects did not show any significant differences after ethanol.

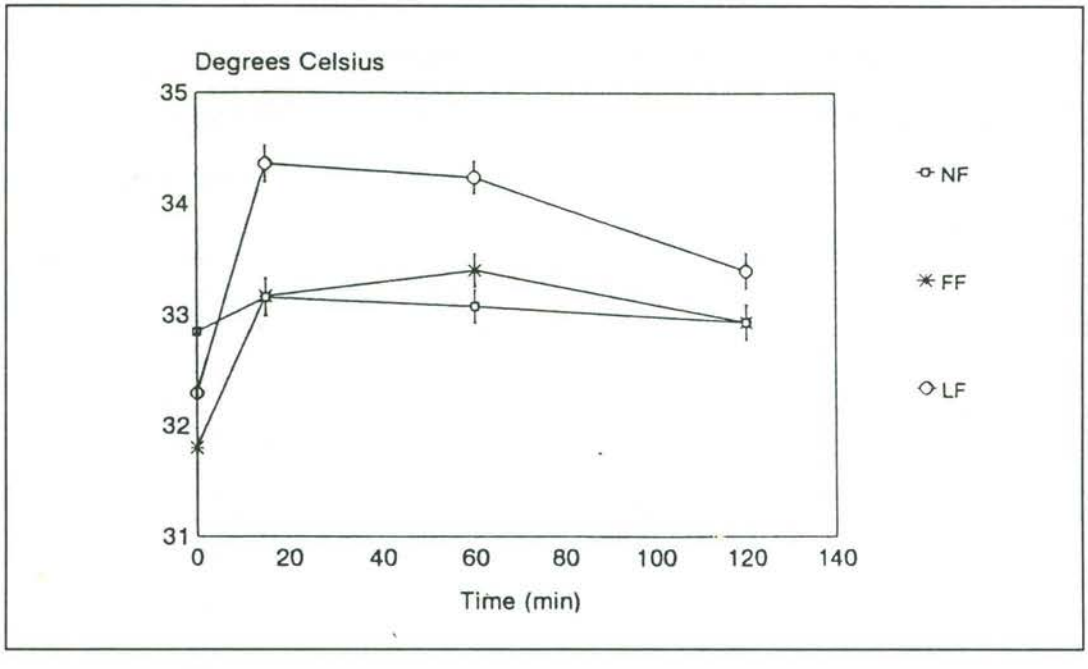


Fig.66: Facial temperature by degree of flush group.

3.6.6. PSYCHOMOTOR VARIABLES

A highly significant divided attention delay difference was found among the various groups and a strong trend was also detected for the DSC task.

Group differences in the other psychomotor variables investigated, including CFFF threshold, standing steadiness, divided attention excursion and reaction time tasks, did not reach statistical significance.

3.6.6.1. DIVIDED ATTENTION

Delay A highly significant time by group difference was found among the groups for the divided attention delay task ($F_{6,303}$=3.38, p<0.005) -Fig.67.

The NF group delay time decreased 15 min after ethanol consumption. This result is in direct contrast to that of the FF and LF groups who had increased delays 15 min after ethanol.

Post-hoc differences, compared with pre-ethanol values, at 120 min after ethanol were found for the LF group ($q_{9,303}$=4.48, p<0.005) which exhibited increased delay time.

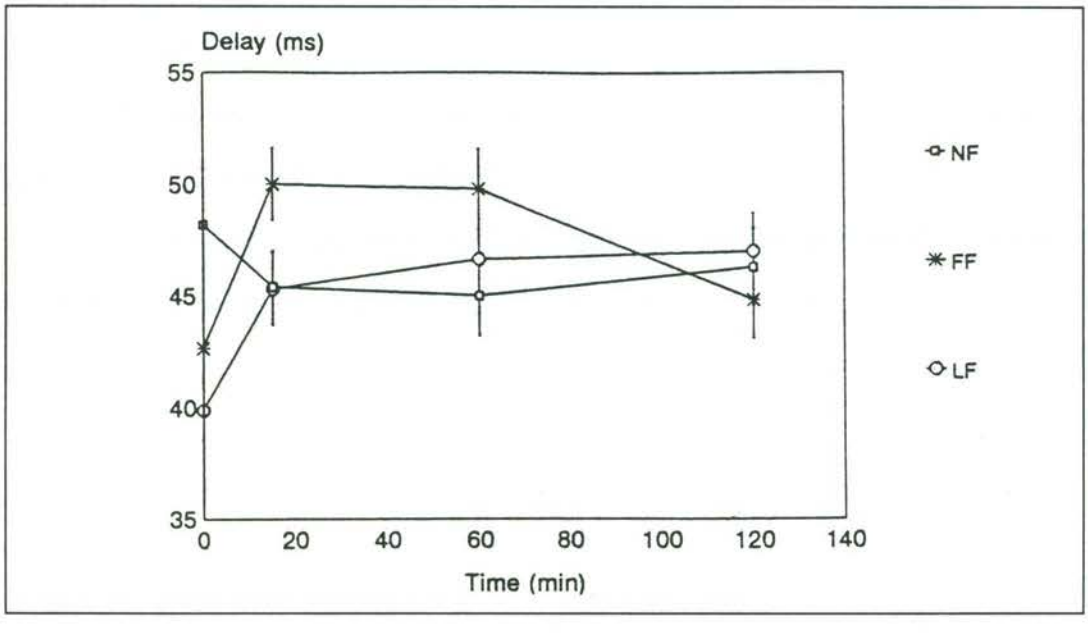


Fig.67: Divided attention delay by degree of flush group.

Excursion Group differences found in divided attention excursion were not statistically significant (see section 3.6.8. for data tables).

After ethanol consumption, there was an increase in excursion followed by a subsequent decrease towards pre-ethanol values. At t_{120} there was a second, smaller, increase in divided attention excursion. The FF subjects did not exhibit the second increase; instead, they continued their recovery towards pre-ethanol levels. The NF subjects had the lowest overall excursion values.

Reaction Time Divided attention peripheral reaction time differences among the groups did not reach statistical significance (see section 3.6.8. for data tables).

In general, after ethanol, subjects' reaction times briefly increased at t_{15} but then subsequently fell towards initial levels. The FF and the LF groups had similar responses, with reaction times measured being higher than that of the NF group.

3.6.6.2. DIGIT SYMBOL CODING

Group differences in the DSC task performance did not reach statistical significance, although there was a strong trend towards significance ($F_{6,303}=2.05$, $p=0.059$) -Fig.68.

After ethanol, the reaction time of all three groups increased briefly at t_{15}, and then decreased towards pre-ethanol values.

The LF subjects had the slowest reaction times overall, and the NF subjects the fastest. The FF subjects had intermediate responses in between the other two groups.

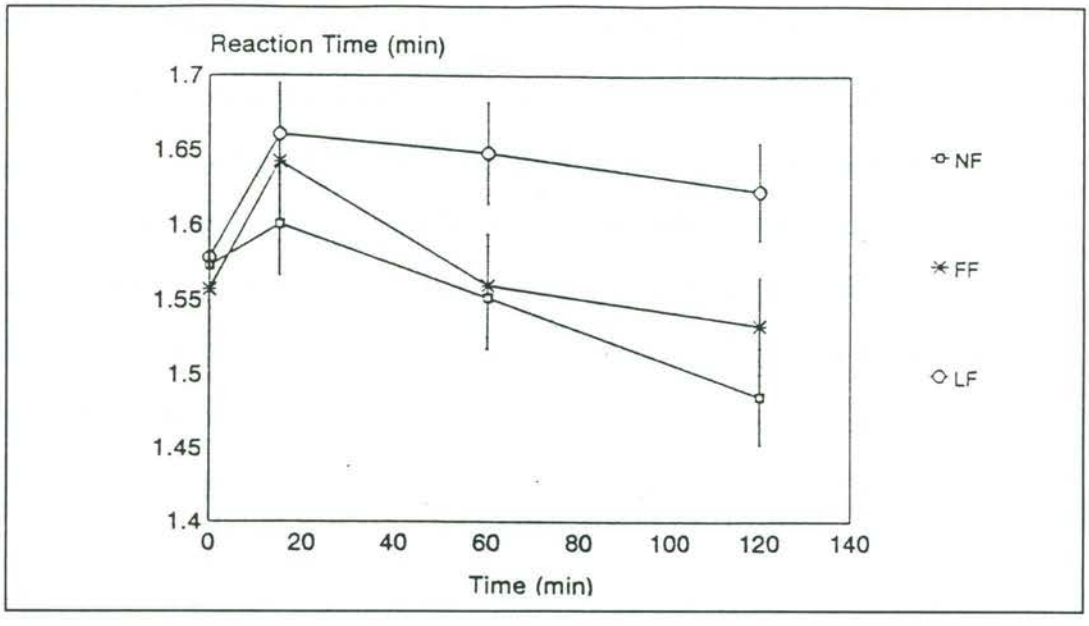


Fig.68: Digit symbol coding reaction time by degree of flush group.

3.6.6.3. STANDING STEADINESS

Group differences in standing steadiness were not statistically significant (see section 3.6.8. for data tables). A fall in standing steadiness was seen in all subjects after ethanol; the NF group being the least affected of the three groups.

3.6.6.4. CRITICAL FLICKER FUSION FREQUENCY

Differences found among the groups for CFFF threshold were not statistically significant (see section 3.6.8. for data tables).

Ethanol caused a decrease in the CFFF threshold of all groups tested, with the lowest values occurring at the t_{60} time-point. Recovery ensued thereafter.

The NF subjects had higher overall CFFF values than the other two groups which were essentially similar in their responses.

3.6.7. SUBJECTIVE MEASUREMENT OF INTOXICATION

Most of the responses to the questions reached levels of statistical significance. As expected, the NF subjects were least affected by ethanol, returning to pre-ethanol values rapidly after a peak effect at t_{15}. In contrast, the LF group showed the greatest effect after ethanol for the longest time. The FF subjects exhibited an intermediate response. In all cases, the effect was greatest 15 min after ethanol.

Subjects reported that they would not drive a car (Q1) after ethanol (Fig.69), although by 120 min their reluctance had dissipated. The LF group was the worst affected group, followed by the FF group. The NF group was the least affected by ethanol ($F_{2,103}=7.10$, $p<0.005$).

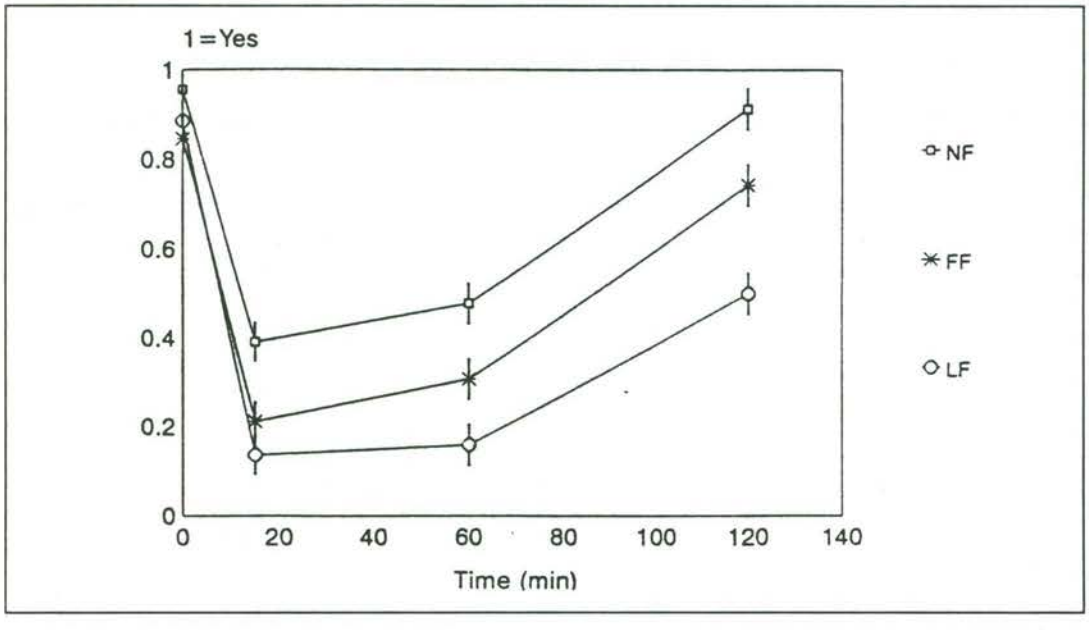


Fig.69: 'Would you drive a car now?' subjective response.

Similarly, the LF group reported that they would not accept another drink (Q2), this effect lasting for the whole 120 min testing period (Fig.70). The FF and NF groups were also affected, although not to the same degree as the LF group ($F_{2,103}$=5.18, p<0.005).

Drowsiness (Q3) increased after ethanol in all three groups and reached significant levels ($F_{2,102}$=7.84, p<0.005). The LF group had the greatest effect (5.8), followed by the FF group (4.8) and finally the NF group (4.0) -this effect occurred at t_{15}. After 120 min, only the LF group remained drowsy (4.6) compared with the FF and the NF groups, who had essentially recovered (2.7 and 2.0 respectively) to pre-ethanol values (Fig.71).

Clumsiness (Q4) after ethanol was most marked in the LF group (5.3), this effect lasted for up to 120 min after ethanol (4.1) -Fig.72. The FF and the NF groups experienced their greatest effect at 15 min after ethanol, but by 120 min they had returned to near normal values (2.3 and 1.7 respectively) -$F_{2,102}$=7.73, p<0.005.

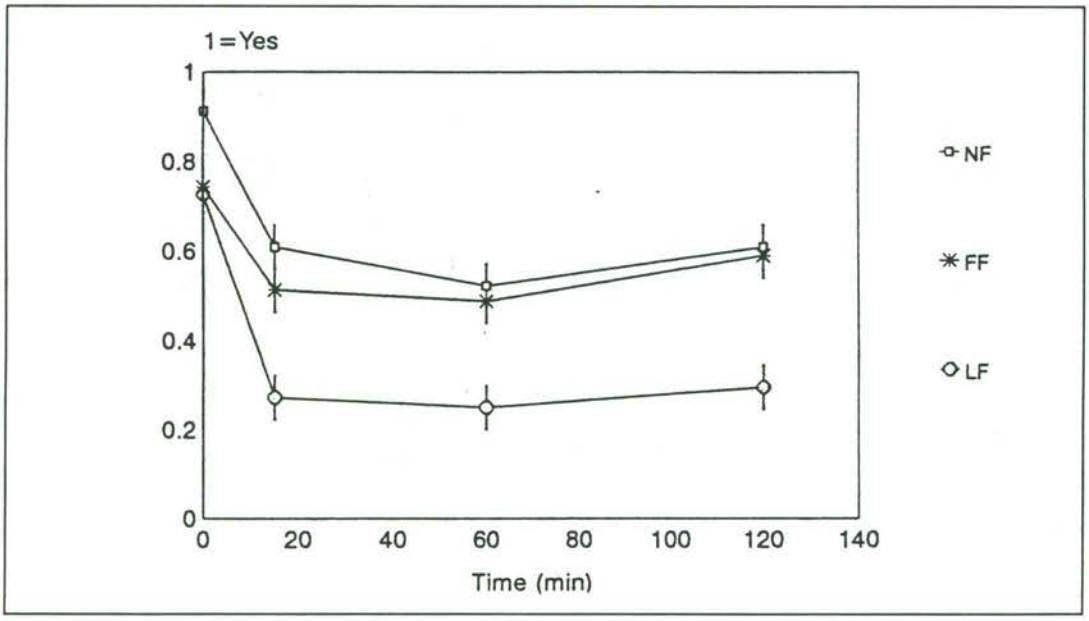


Fig.70: 'Would you accept another drink?' subjective response.

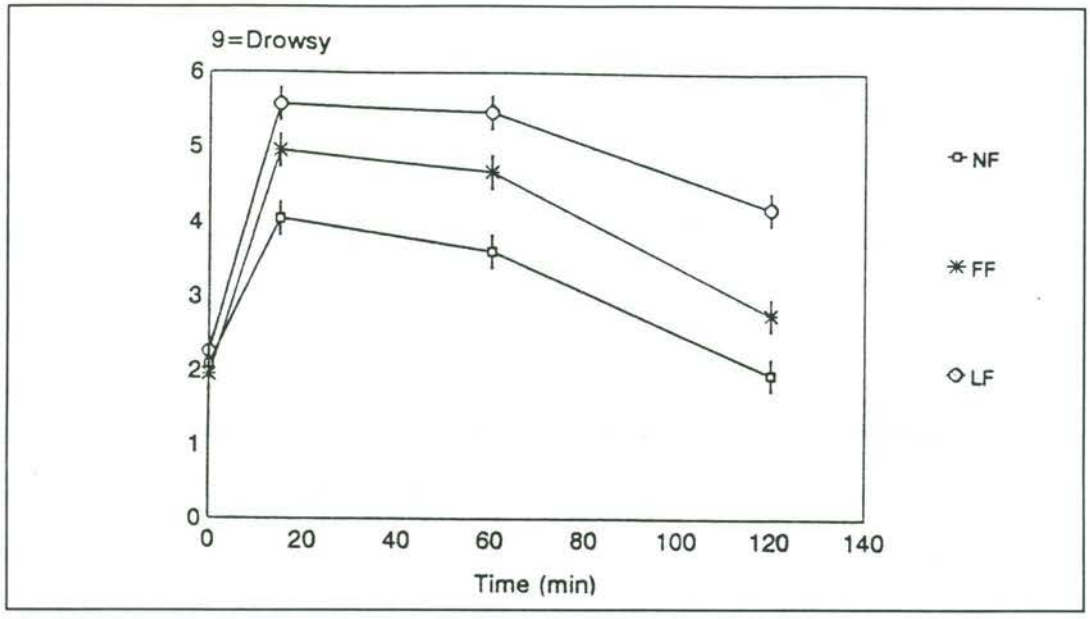


Fig.71: 'Do you feel alert or drowsy?' subjective response.

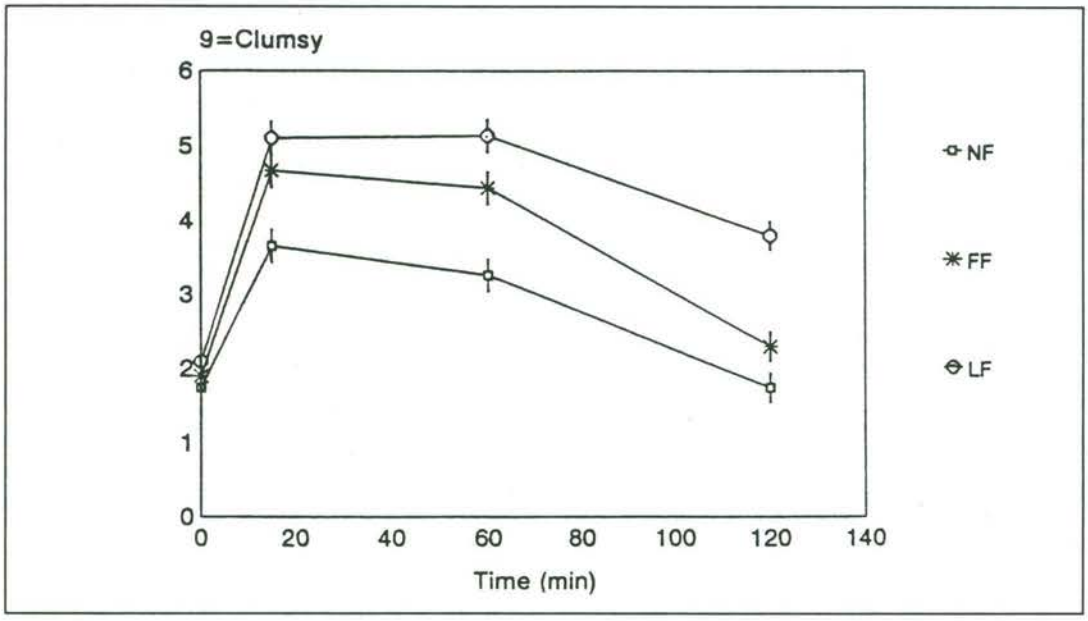


Fig.72: 'Do you feel co-ordinated or clumsy?' subjective response.

Feelings of intoxication (Q6) -Fig.73 -were reported to be the highest in the LF subjects (5.9), followed by the FF (4.5) and NF (3.9) groups, these results were highly significant ($F_{2,102}$=13.82, p<0.005). This effect was greatest at 15 min after ethanol, and by 120 min after ethanol, the LF group still reported the greatest degree of intoxication (3.7) compared to the other two groups (1.4).

Attentiveness (Q7) of the subjects decreased after ethanol consumption. The LF group was the most affected group (5.5), this effect lasted for up to 120 min after ethanol. In contrast, the responses of the two other groups were greatest at t_{15} and then decreased back towards normal values (at a faster rate than the LF group) (Fig.74). These differences were highly significant $F_{2,102}$=7.25, p<0.005.

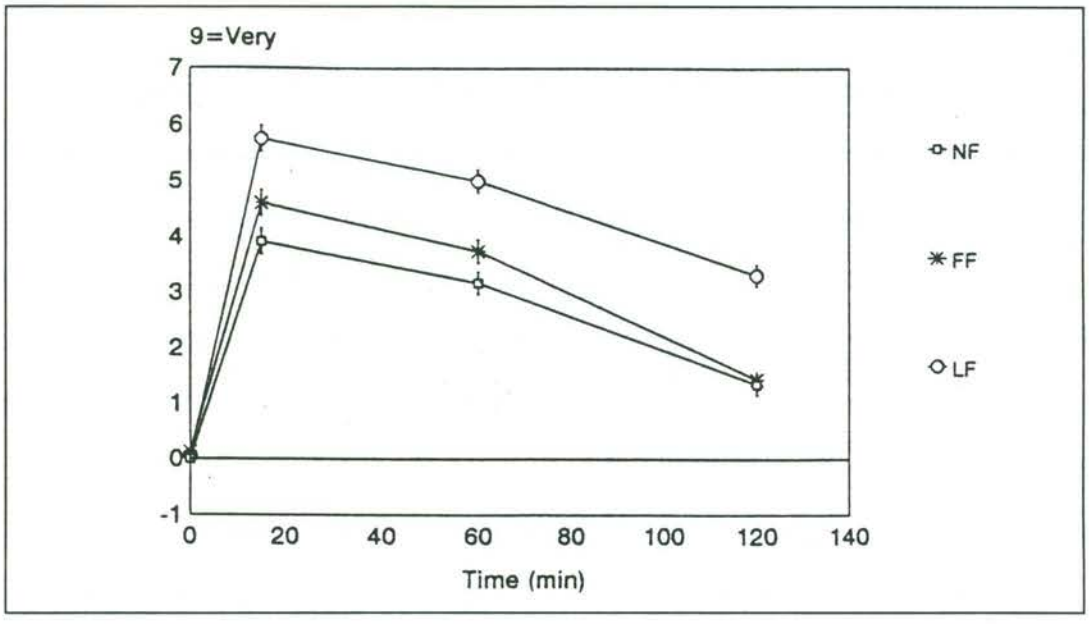


Fig.73: 'How intoxicated do you feel?' subjective response.

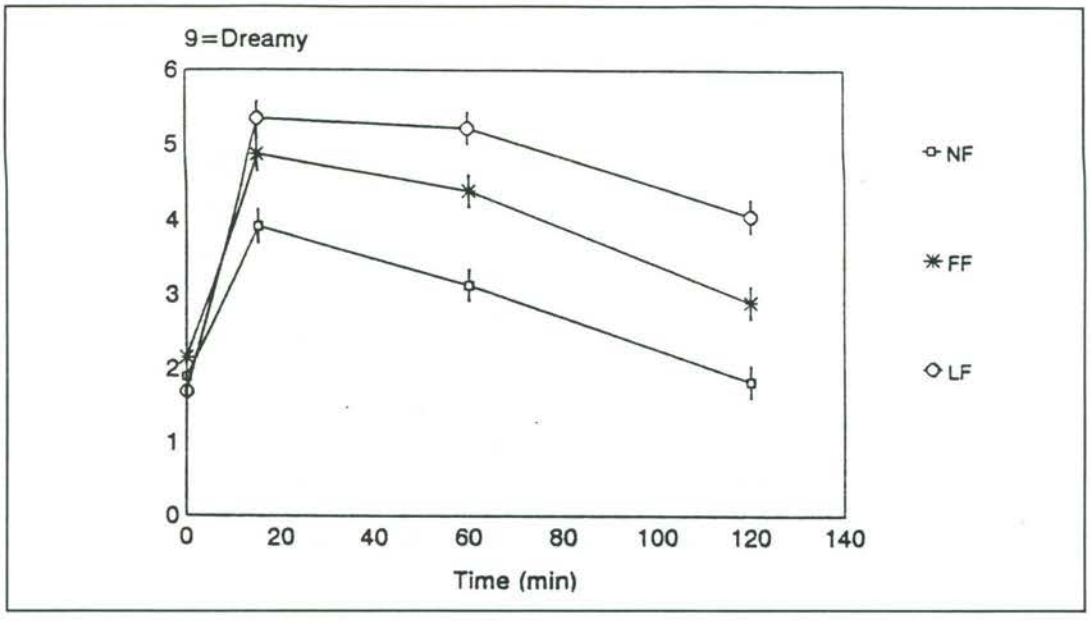


Fig.74: 'Do you feel attentive or dreamy?' subjective response.

LF subjects reported the most headaches after ethanol consumption (Q8), increasing as time progressed up to a maximum at 120 min. The response of the FF group increased after ethanol but, by 120 min, the response had begun to decrease towards initial values. The least affected subjects were the NF group, their values were not as high as either the LF or FF groups, and by 120 min they had also returned close to pre-ethanol values ($F_{2,103}=3.74$, $p<0.05$) -Fig.75.

It should also be noted that after ethanol, all three groups reported an increase in headache although by t_{60}, a divergence in response saw the NF and FF groups report a decrease in this effect, whilst the LF group continued to report an increase in the severity of headaches which persisted until the end of the test session.

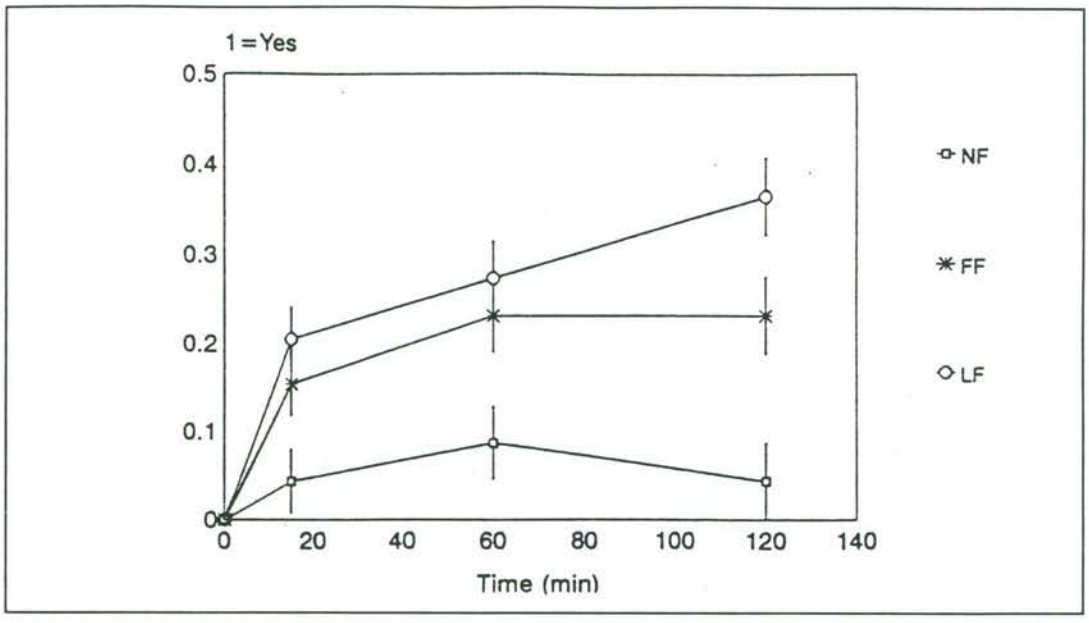


Fig.75: 'Do you experience a headache?' subjective response.

Nausea (Q9) was reported by all three groups 15 min after ethanol consumption. The FF group had the greatest response at t_{15}, followed by the LF and the NF groups. The response was similar in all three groups at t_{60} and, by 120 min the LF group was clearly the most affected compared with the other two groups which had returned to near normal values ($F_{4,206}=2.97$, $p<0.05$) Fig.76.

Hangover-like effects (Q10) were the most marked for the LF group 60-120 min after ethanol, although 15 min after ethanol, the FF group was the worst affected. By 120 min, hangover-like effects were infrequently reported by the NF and FF groups ($F_{4,206}=2.49$, $p<0.05$) -Fig.77.

Group differences regarding levels of concentration perceived by subjects (Q5) was the only subjective response which did not reach statistical significance.

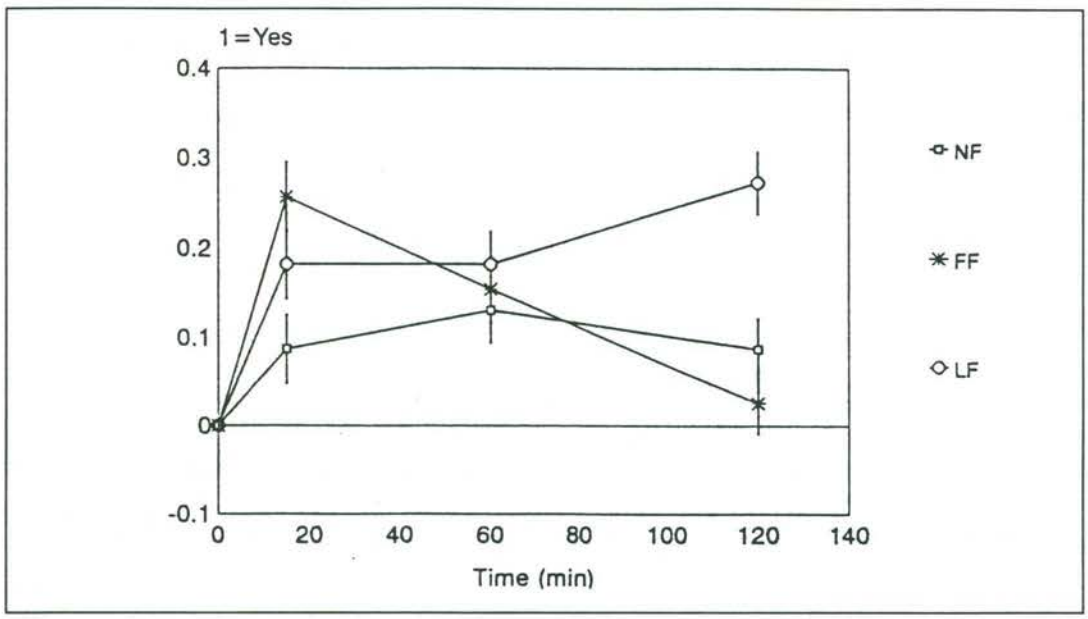


Fig.76: 'Does your stomach feel uneasy or sick?' subjective response.

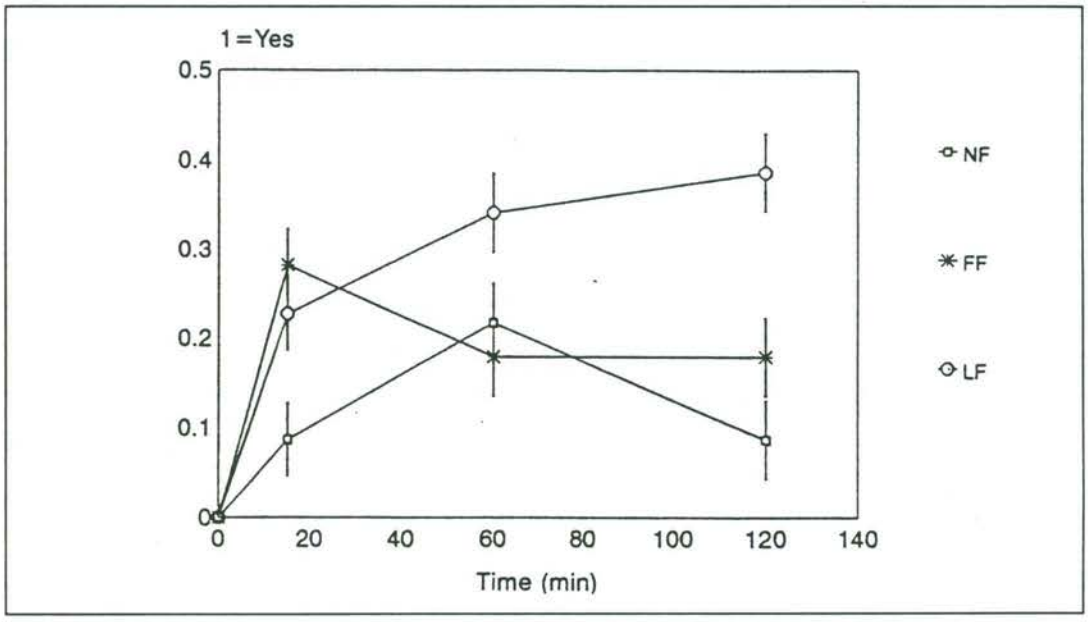


Fig.77: 'Any hangover-like effects?' subjective response.

3.6.8. SUMMARY OF OTHER RESULTS BY DEGREE OF FLUSH (NON-SIGNIFICANT)

The results for each variable below are listed in the following manner for each time-point (t_0, t_{15}, t_{60}, t_{120}):

NF mean ± SD; n=23

FF mean ± SD; n=39

LF mean ± SD; n=44

VARIABLE	t_0	t_{15}	t_{60}	t_{120}
Ethanol (mM)	0 ± 0	8.4 ± 2.5	8.3 ± 1.6	5.1 ± 1.4
	0 ± 0	9.4 ± 2.5	8.8 ± 1.7	5.8 ± 1.4
	0 ± 0	8.7 ± 2.8	8.2 ± 1.3	5.1 ± 1.4
Lactate (mM)	1.3 ± 1.3	1.7 ± 1.2	1.6 ± 0.8	1.5 ± 0.7
	1.5 ± 1.0	1.8 ± 1.1	1.8 ± 0.9	1.7 ± 1.0
	1.3 ± 1.0	1.7 ± 0.8	1.7 ± 1.0	1.9 ± 1.0
Pyruvate (mM)	0.05 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.02
	0.05 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.02
	0.06 ± 0.02	0.04 ± 0.03	0.05 ± 0.02	0.05 ± 0.02
Lac:Pyr Ratio	24.0 ± 13.1	49.2 ± 23.4	47.6 ± 25.1	54.3 ± 56.7
	35.3 ± 43.6	65.5 ± 111.3	51.7 ± 45.7	67.6 ± 135.2
	22.1 ± 11.0	43.0 ± 19.9	40.4 ± 17.9	41.2 ± 15.8
Blood Pressure	114.0 ± 10.5	111.4 ± 9.1	107.0 ± 10.1	106.1 ± 13.0
(Sys) (mmHg)	110.5 ± 14.0	106.6 ± 15.1	103.2 ± 13.2	104.3 ± 14.9
	111.3 ± 12.7	110.9 ± 14.9	108.1 ± 12.8	106.5 ± 12.2
BEC (g dl^{-1})	0 ± 0	0.032 ± 0.010	0.019 ± 0.004	0.004 ± 0.004
	0 ± 0	0.035 ± 0.012	0.019 ± 0.006	0.005 ± 0.005
	0 ± 0	0.033 ± 0.013	0.018 ± 0.007	0.006 ± 0.005

CFFF (Hz)	26.8 ± 3.1	26.7 ± 4.4	26.4 ± 3.5	26.8 ± 3.3
	25.9 ± 2.7	25.3 ± 3.0	25.2 ± 2.9	26.0 ± 3.1
	26.2 ± 2.7	25.6 ± 2.8	25.2 ± 3.0	25.6 ± 2.8
Standing	25.7 ± 8.8	21.8 ± 8.8	22.4 ± 8.3	23.7 ± 7.5
Steadiness (s)	27.2 ± 8.9	19.9 ± 9.3	21.6 ± 9.1	23.2 ± 8.6
	27.4 ± 5.2	21.4 ± 7.2	21.3 ± 7.4	22.9 ± 6.3
Div. Attention	3.2 ± 1.0	3.8 ± 1.3	3.1 ± 0.7	3.5 ± 1.1
(Excur.) (units)	3.3 ± 1.4	4.0 ± 1.3	3.8 ± 1.5	3.5 ± 1.4
	3.2 ± 1.5	4.0 ± 1.3	3.4 ± 1.0	3.6 ± 1.4
Div. Attention	2.5 ± 0.7	2.8 ± 0.9	2.4 ± 0.6	2.6 ± 0.9
(Reaction Time)	2.8 ± 0.7	3.1 ± 0.8	2.8 ± 0.9	2.8 ± 0.8
	2.8 ±0.9	3.2 ± 0.9	2.9 ± 0.9	2.7 ± 0.8

3.7. RESULTS BY $ALDH_2$/ ADH_2 GENOTYPE COMBINED

3.7.1. BREAKDOWN OF SUBJECTS BY $ALDH_2$ & ADH_2 COMBINATIONS

The subjects were initially classified into nine different groups based on their combination of $ALDH_2$/ ADH_2 genotypes. However, due to small numbers in some of the cells and for statistical analysis purposes, some groups were further recombined to form a total of five groups according to the combination of $ALDH_2$ and ADH_2 enzymes which they possessed (Table4). From the table below it can be seen that two of the initial nine groups could not be appropriately combined with other groups, therefore, they were omitted to facilitate interpretation of the results.

	$ALDH_2$ Homo11	**$ALDH_2$ Het**	**$ALDH_2$ Homo22**
ADH_2 Homo11	GroupA n=11	GroupB n=19	GroupC n=5
ADH_2 Het	GroupD n=26	GroupE n=29	GroupC n=6
ADH_2 Homo22	~~GroupG n=4~~	~~GroupH n=5~~	GroupC n=2

Table 4: Breakdown of subjects into their $ALDH_2$/ ADH_2 genotype combinations.

The highest number of divisions possible, from a practical point of view and allowing comparisons among the different combination genotypes (taking statistical requirements for balanced cell numbers into consideration), was five, based mainly on the widely reported influence of $ALDH_2$. All subjects possessing the $ALDH_2$ Homo22 genotype made up GroupC subjects. Because of the small number of subjects in the

cells when the ADH_2 genotype was accounted for, no distinction was made among the three possible ADH_2 combinations. However, $ALDH_2$ Het subjects could be separated on the basis of their ADH_2 genotype with $ALDH_2$ Het/ ADH_2 Homo11 and $ALDH_2$ Het/ ADH_2 Het groups being represented by Groups B & E respectively.

$ALDH_2$ Homo11/ ADH_2 Het subjects were classified as one group (GroupD), whilst the $ALDH_2$ Homo11/ ADH_2 Homo11 subjects comprised another (GroupA).

A summary of the five groupings for the various $ALDH_2$/ ADH_2 combination genotypes is as follows:

GroupA	$ALDH_2$ Homo11/ ADH_2 Homo11
GroupB	$ALDH_2$ Het/ ADH_2 Homo11
GroupC	$ALDH_2$ Homo22/ ADH_2 Homo11, Het or Homo22
GroupD	$ALDH_2$ Homo11/ ADH_2 Het
GroupE	$ALDH_2$ Het/ ADH_2 Het

GroupA subjects made up 10% of total subjects tested, GroupB, GroupC, GroupD and GroupE were 18%, 12%, 24% and 27% respectively. 9% of total subjects tested were omitted from this section of the analysis for reasons mentioned previously.

The degree of flush produced by subjects according to the $ALDH_2$/ ADH_2 combination genotypes is indicated in section 3.6.1.3.

3.7.2. DRINKING DETAILS

GroupA subjects reported the greatest incidence (47%) of drinking (3-4 times a week), only GroupB and GroupE contained subjects who drank so often and even then, they comprised only 5% and 4.4% of subjects in each group respectively. 33% of all subjects in this group drank less than 1-2 times a month or did not consume ethanol at all.

77% of the GroupB subjects drank 1-2 times a month or less, however, another 18% of this group reported drinking 1-2 times a week and some, as mentioned above, drank 3-4 times a week (5%).

GroupC had 46% of subjects who reported that they drank ethanol 1-2 times a month, although most subjects in this group drank even less frequently (54%).

Nearly 70% of subjects in GroupE drank 1-2 times a month or less, or not at all. The remainder of this group reported drinking 1-2 times a week (26%), or more often at 3-4 times a week (4.4%).

76% of GroupD subjects drank 1-2 times a month or less, or not at all. A further 24% of this group indicated that they consumed ethanol 1-2 times a week (Fig.78).

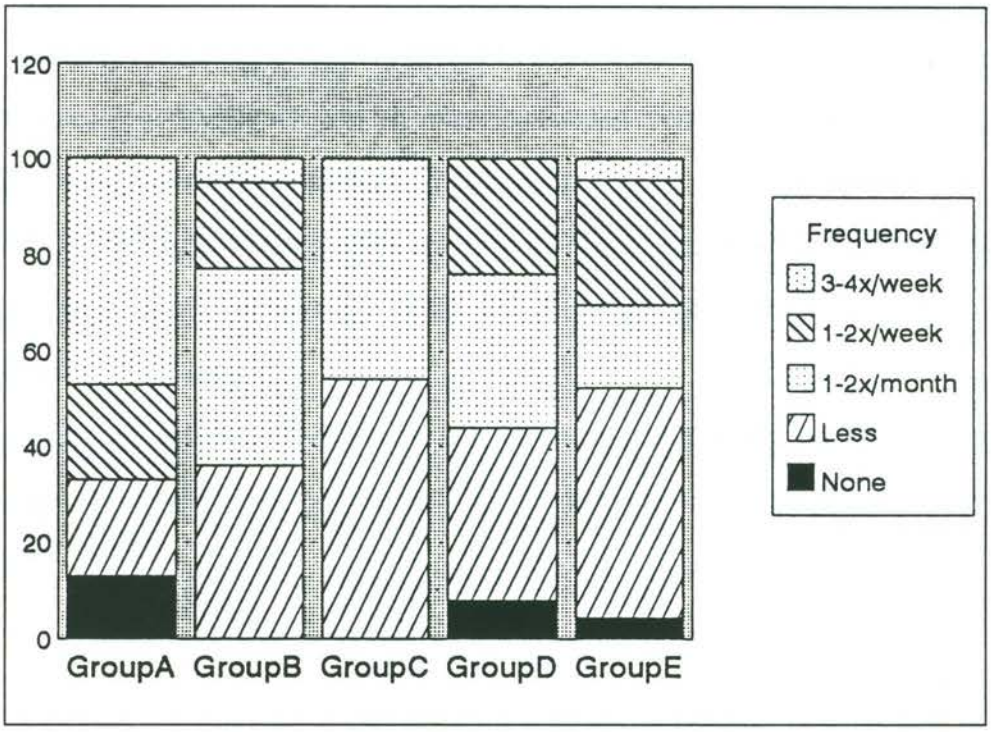


Fig.78: Frequency of drinking by $ALDH_2$/ ADH_2 genotype.

It should be noted that the two categories 'less' and 'none' can be considered as having a degree of overlap in that some subjects may have consumed 'none' but reported their drinking frequency as 'less'. These findings, using non-parametric tests, did not reach levels of statistical significance ($p>0.05$).

GroupA subjects clearly preferred beer (62%) to other alcoholic beverages when drinking (Fig.79).

Similarly, GroupB subjects also drank beer (41%) although 27% of this group reported spirits as their preferred choice of beverage.

GroupC subjects reported the same preferences for beer and spirits (31%). This unexpectedly high proportion of spirits might be due to the relative inexperience of the GroupC drinkers. That is, when subjects were asked about the beverage which they normally consumed, subjects referred to as a reference point, the last special festive occasion -usually when spirits were consumed. Wine was the beverage of choice for just over 15% of subjects in this group.

GroupD subjects nominated spirits and beer (35% and 30% respectively) as their preferred choice of beverage, although 17% of subjects reported that they preferred to drink wine.

GroupE subjects chose beer overwhelmingly (59%) as the preferred beverage, although 18% of subjects reported that they preferred spirits, while 5% preferred wine.

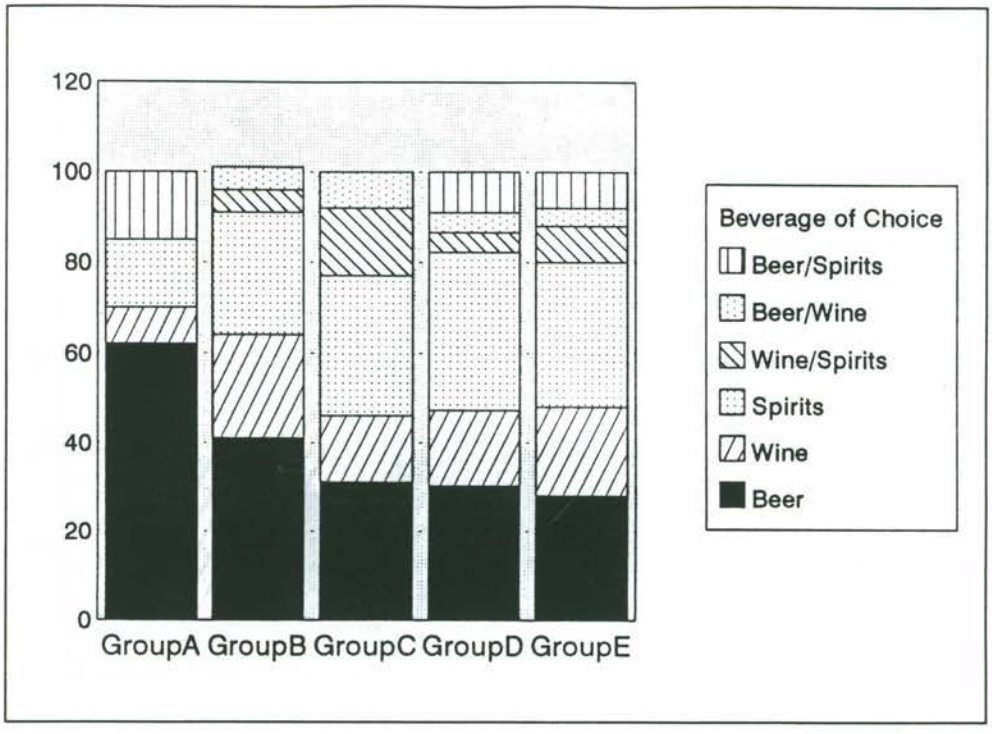


Fig.79: Drinking habits based on $ALDH_2$/ ADH_2 genotype: Choice of beverage preferred.

From Fig.80, it can be seen that GroupC subjects had the highest proportion of subjects who reported that they usually consumed less than one standard drink per drinking session (33%). This finding might be attributable to the more unpleasant adverse effects experienced after ethanol consumption by the subjects in this group. Groups D, A, B and E followed in order with 12.5%, 7%, 5% and 4% of subjects respectively, consuming less than one standard drink each time they drank.

Groups D and B had the highest percentage of subjects consuming the equivalent of two or more standard drinks per session (62.5% and 62% respectively), although GroupE and GroupA were very similar with 61% and 60% respectively. In contrast, only 31% of GroupC subjects consumed two or more drinks per session. The findings were not of statistical significance ($p>0.05$) with non-parametric tests.

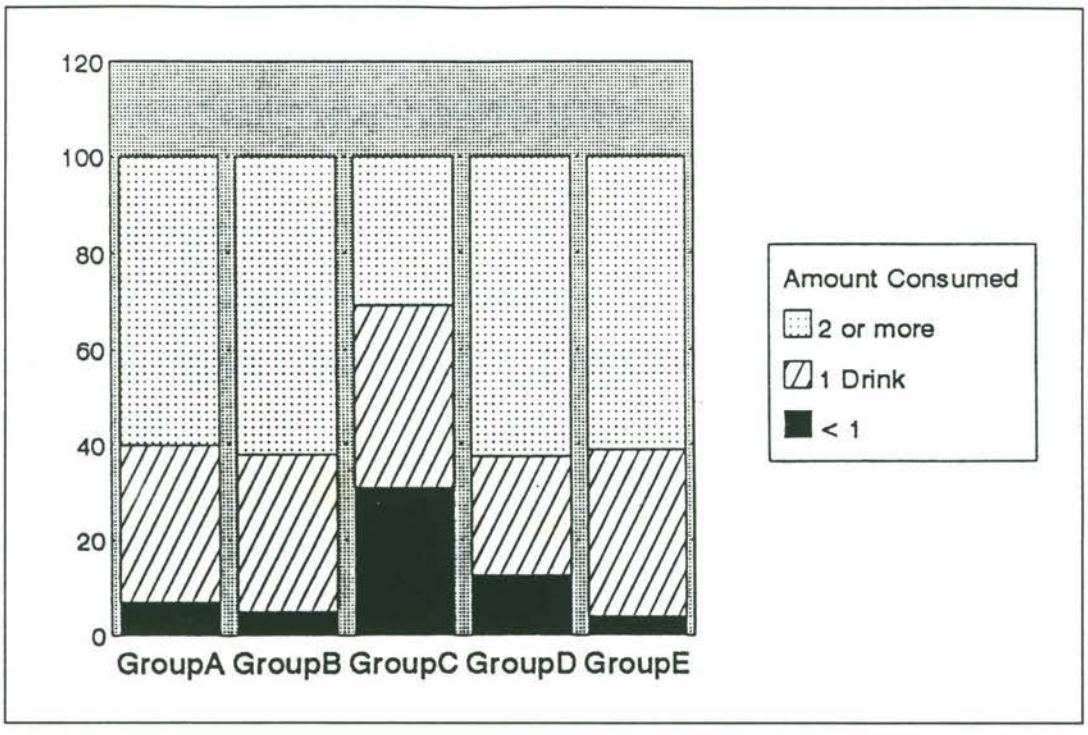


Fig.80: Quantity of ethanol usually consumed -by $ALDH_2$/ ADH_2 combination genotypes.

3.7.3. BREATH ETHANOL CONCENTRATION CURVES

Highly significant differences were found both among the five groups tested ($F_{4,91}$=3.94, p<0.005) and for the interaction of time by group ($F_{12,273}$=3.57, p<0.005) -Fig.81.

Post-hoc analysis revealed that GroupA subjects were significantly affected, compared with pre-ethanol values, when tested at 15 ($q_{12,273}$=27.18, p<0.005), 60 ($q_{9,273}$=18.55, p<0.005) and 120 minutes after ethanol ($q_{4,273}$=4.91, p<0.005). GroupB subjects were also significantly different at 15 ($q_{16,273}$=43.05, p<0.005), 60 ($q_{11,273}$=20.74, p<0.005) and 120 minutes after ethanol ($q_{6,273}$=7.05, p<0.005). GroupC subjects at 15 ($q_{14,273}$=32.31, p<0.005), 60 ($q_{8,273}$=17.77, p<0.005) and 120 minutes after ethanol ($q_{5,273}$=5.77, p<0.005). GroupD at 15 ($q_{13,273}$=30.85, p<0.005), 60 ($q_{8,273}$=16.73, p<0.005) and 120 minutes ($q_{2,273}$=2.92, p<0.005); and

GroupE subjects at 15 ($q_{15,273}$=34.52, $p<0.005$), 60 ($q_{10,273}$=18.59, $p<0.005$) and 120 ($q_{4,273}$=5.59, $p<0.005$) minutes after ethanol

3.7.3.1. PEAK BEC

The highest mean peak BEC occurred in GroupB subjects (0.041 g dl^{-1}), followed by GroupE & Group C (0.034 g dl^{-1}), GroupD (0.031 g dl^{-1}) and GroupA (0.030 g dl^{-1}).

3.7.3.2. TIME TO PEAK

The mean time taken for each peak BEC was found to be fifteen minutes for all five groups.

3.7.3.3. ELIMINATION RATE

The highest mean elimination rate, calculated by linear regression, occurred in GroupB subjects (0.0181 ± 0.002 (SEM) g dl^{-1} hr^{-1}), followed by GroupE (0.0162 ± 0.001 (SEM) g dl^{-1} hr^{-1}), GroupC (0.0161 ± 0.001 (SEM) g dl^{-1} hr^{-1}), GroupA (0.0152 ± 0.001 (SEM) g dl^{-1} hr^{-1}) and GroupD (0.0147 ± 0.001 (SEM) g dl^{-1} hr^{-1}) subjects.

3.7.3.4. VOLUME OF DISTRIBUTION

The mean volumes of distribution were calculated to be 59.9, 57.9, 57.6, 55.0 and 46.5 litres for GroupA, GroupD, GroupC, GroupE and GroupB respectively.

3.7.3.5. AREA UNDER THE CURVE

The mean areas under the curve were 0.159 ± 0.024 (SEM), 0.139 ± 0.021 (SEM), 0.131 ± 0.026 (SEM), 0.131 ± 0.024 (SEM) and 0.120 ± 0.017 (SEM) g dl^{-1} min for the five groups: GroupB, GroupE, GroupC, GroupA and GroupD respectively.

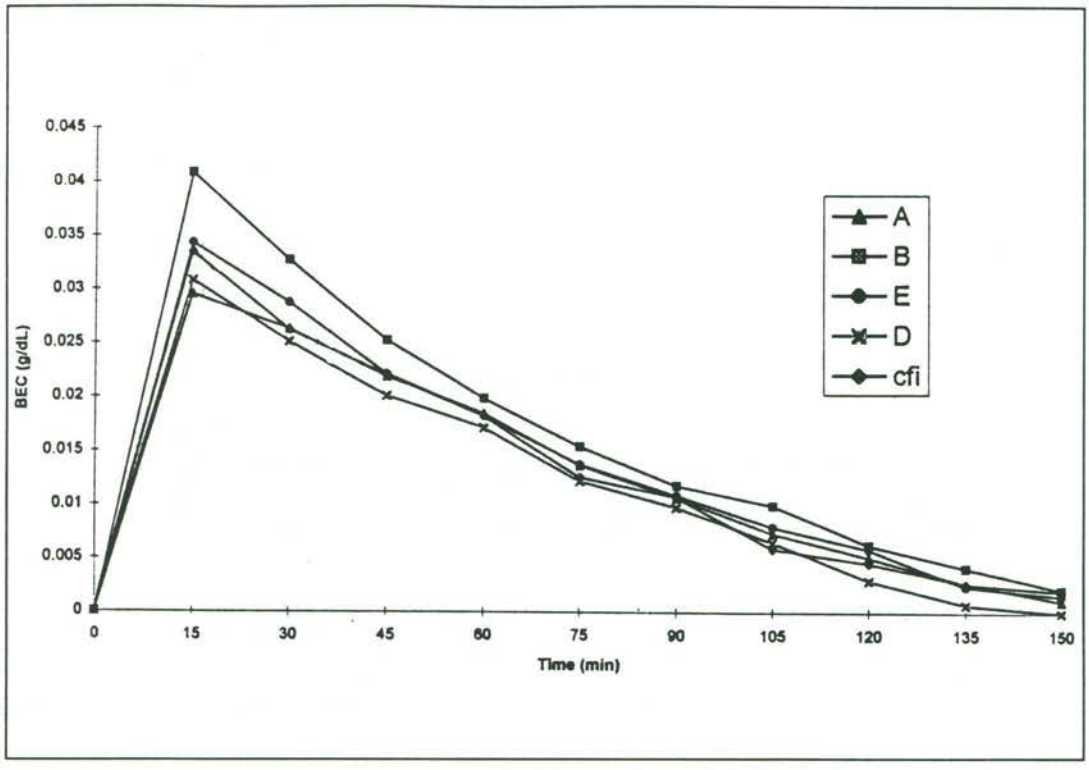


Fig.81: BEC (g dl^{-1}) by $ALDH_2$/ ADH_2 genotype combinations.

3.7.4. BIOCHEMICAL VARIABLES

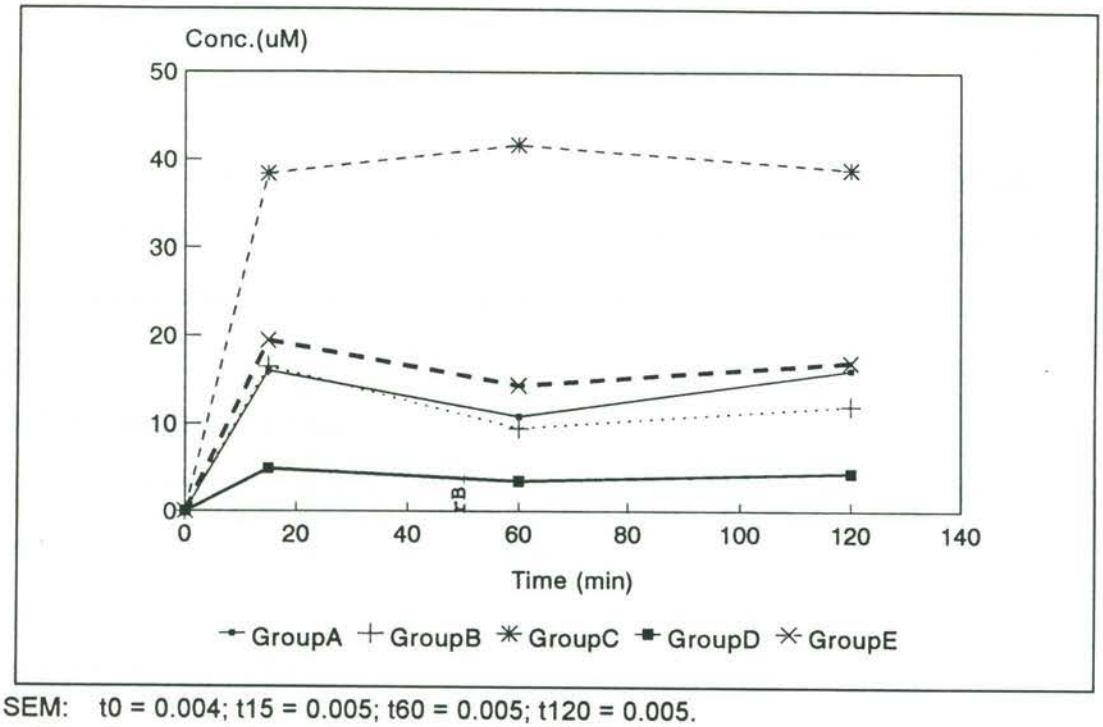
Significant group differences were encountered in the blood acetaldehyde and plasma ethanol concentrations.

Differences in the other variables including acetate, lactate and pyruvate concentrations did not achieve statistical significance.

3.7.4.1. BLOOD ACETALDEHYDE

Significant differences were detected ($F_{12,273}$=2.11, $p<0.05$) in the interaction of time by group -Fig.82.

Post-hoc analysis revealed that the GroupC subjects were significantly different from the other groups at 15 ($q_{17,273}$=6.61, $p<0.05$), 60 ($q_{19,273}$=7.18, $p<0.05$) and 120 ($q_{18,273}$=6.69, $p<0.05$) minutes after ethanol consumption. Differences among the other groups did not reach significance.



SEM: t0 = 0.004; t15 = 0.005; t60 = 0.005; t120 = 0.005.

Fig.82: Acetaldehyde concentration (μM) by $ALDH_2$/ ADH_2 genotype.
(N.B. Blank corrections accounted for in measurements)

In all groups, a rise in acetaldehyde concentration was seen immediately after ethanol. After this time, the acetaldehyde levels fell in GroupA, GroupB. GroupD and GroupE subjects, but continued to rise in GroupC subjects.

GroupA and GroupB subjects, comprising ADH_2 Homo11 and either $ALDH_2$ Homo11 or Het genotypes respectively, produced similar acetaldehyde concentrations at most time-points, although at 120 min, GroupA subjects had higher acetaldehyde concentrations than GroupB subjects.

GroupC subjects exhibited the highest and most rapid rates of increase in acetaldehyde concentrations overall. These highly elevated acetaldehyde concentrations were evident for the duration of the testing session, even at the final timepoint (t_{120}).

GroupD subjects, a combination of ADH_2 Het and $ALDH_2$ Homo11 genotypes, had the lowest acetaldehyde concentrations overall. A small increase was

observed immediately after ethanol consumption but, thereafter, the acetaldehyde concentration plateaued at this level for the rest of the test session.

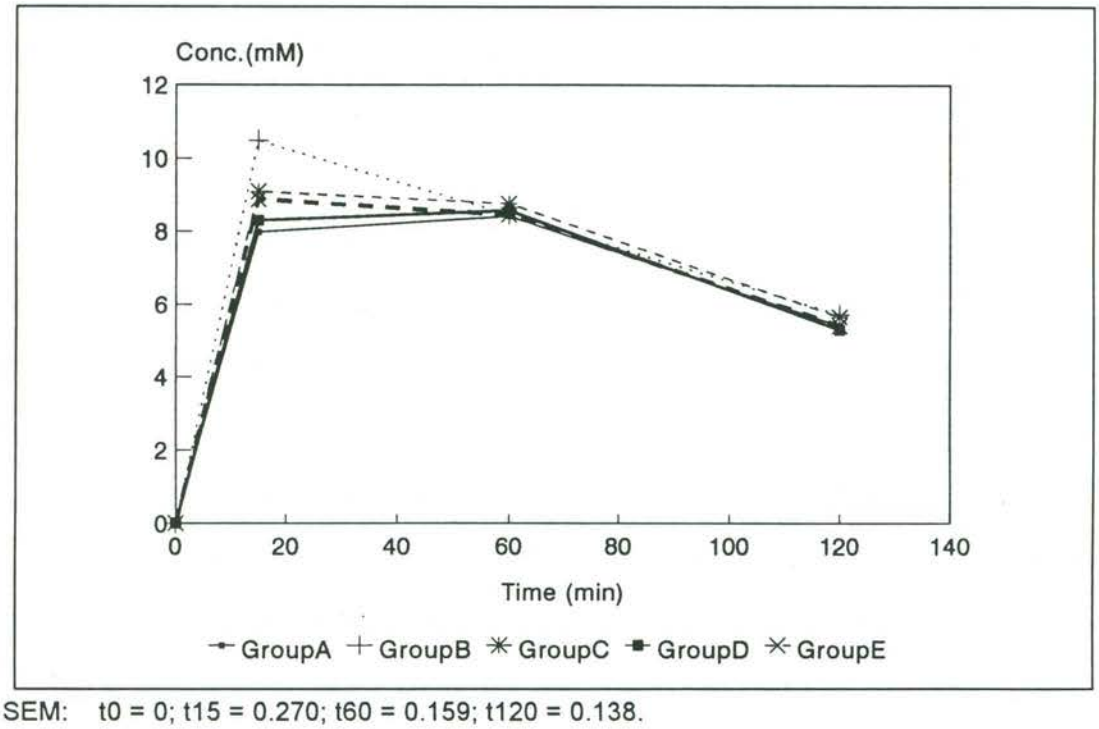
Subjects in GroupE exhibited the second largest increase in acetaldehyde levels (after ethanol) after GroupC subjects. The highest concentration was reached 15 min after ethanol (as with all groups except GroupC).

3.7.4.2. PLASMA ACETATE

Differences in acetate concentrations by degree of flush did not reach levels of statistical significance (see section 3.7.8. for data tables).

3.7.4.3. PLASMA ETHANOL

Differences among the plasma ethanol concentrations were not significantly different among the five groups, although differences among the pre- and post-ethanol results were statistically significant ($F_{12,273}$=1.90, $p<0.05$) -Fig.83.



SEM: t0 = 0; t15 = 0.270; t60 = 0.159; t120 = 0.138.

Fig.83: Ethanol concentration (mM) by $ALDH_2$/ ADH_2 genotype.

GroupB subjects had the highest plasma ethanol concentrations 15 min after ethanol consumption, in contrast with GroupA subjects who had the lowest ethanol concentrations overall.

As expected, a rise in plasma ethanol levels occurred 15 min after drinking, peaking at this time-point for most groups except GroupA and GroupD (these two groups peaked at 60 min after ethanol). After t_{15}, the plasma ethanol levels began to fall and by 120 min, although ethanol concentrations had fallen to much lower levels, ethanol was still present in the blood of subjects in all groups.

Post-hoc analysis revealed that GroupA subjects were significantly different from the pre-ethanol values at 15 ($q_{7,273}=17.58$, $p<0.05$), 60 ($q_{9,273}=18.52$, $p<0.05$) and 120 minutes after ethanol ($q_{3,273}=11.92$, $p<0.05$). GroupB subjects at 15 ($q_{16,273}=23.08$, $p<0.05$), 60 ($q_{11,273}=18.63$, $p<0.05$) and 120 minutes after ethanol ($q_{6,273}=12.61$, $p<0.05$). GroupC subjects at 15 ($q_{15,273}=20.03$, $p<0.05$), 60 ($q_{13,273}=19.28$, $p<0.05$) and 120 minutes after ethanol ($q_{5,273}=12.47$, $p<0.05$). GroupD subjects at 15 ($q_{8,273}=18.29$, $p<0.05$), 60 ($q_{12,273}=18.87$, $p<0.05$) and 120 minutes ($q_{2,273}=11.66$, $p<0.05$); and GroupE subjects at 15 ($q_{14,273}=19.55$, $p<0.05$), 60 ($q_{10,273}=18.6$, $p<0.05$) and 120 ($q_{4,273}=11.94$, $p<0.05$) minutes after ethanol consumption.

3.7.4.4. BLOOD LACTATE

A rise in lactate concentration was seen immediately after ethanol consumption in all groups, which returned to near pre-ethanol values only in GroupA subjects.

GroupA subjects had peak lactate levels at t_{15}, which declined thereafter.

Lactate concentrations in GroupB and GroupE subjects peaked at t_{60} and remained elevated for the duration of the test session. GroupE subjects exhibited a plateau effect after t_{60}, whilst GroupB subjects demonstrated a fall in lactate concentration after this timepoint.

GroupC subjects had the highest lactate concentrations of all five groups; the lactate concentration rose after ethanol, reaching a peak value at t_{120}.

Differences in lactate concentrations among the groups were not statistically significant (see section 3.7.8. for data tables).

3.7.4.5. BLOOD PYRUVATE

All groups showed a decrease in pyruvate concentrations immediately after ethanol consumption. Recovery towards pre-ethanol levels began by t_{15}, although a complete recovery to pre-ethanol values was not achieved in any of the groups tested by the end of the test session.

GroupC subjects had the highest pyruvate levels both before and after ethanol, and GroupB subjects had the lowest levels.

Differences in the pyruvate concentrations did not reach statistical significance (see section 3.7.8. for data tables).

3.7.4.6. BLOOD LACTATE:PYRUVATE RATIO

Differences in the lactate:pyruvate ratio by ADH_2 genotype did not reach levels of statistical significance (see section 3.7.8. for data tables).

3.7.5. PHYSIOLOGICAL VARIABLES

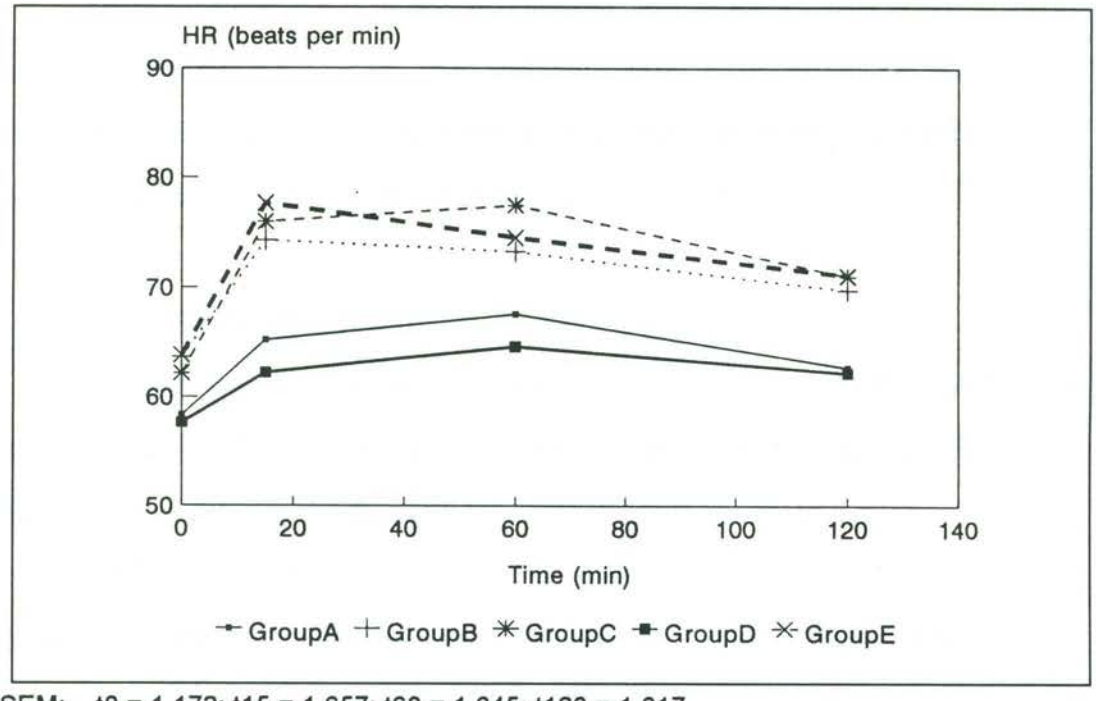
The facial temperature and heart rate variables were significantly different among the five groups but differences in systolic and diastolic blood pressure did not reach significance.

3.7.5.1. HEART RATE

Heart rate was highly significantly different among the five genotype combination groups ($F_{4,91}=7.20$, $p<0.005$), although no significant differences were found for the time by group interaction effect (Fig.84).

In all five groups, there was a sudden increase in heart rate after ethanol consumption which was followed by another smaller rise at t_{60}. By t_{120}, the heart rate had returned towards pre-ethanol levels.

GroupA and GroupD subjects responded similarly to ethanol and little difference could be discerned between the responses of the two groups, except that GroupA subjects had higher heart rates at the t_{15} and t_{60} timepoints.



SEM: t0 = 1.172; t15 = 1.657; t60 = 1.645; t120 = 1.617.

Fig.84: Heart rate by $ALDH_2$/ ADH_2 combination genotypes.

GroupB and GroupE subjects responded in a similar manner, although a decrease, rather than an increase, in heart rate was evident between t_{15}-t_{60}.

GroupC subjects attained the highest heart rates of all groups tested, the peak effect occurring at t_{60} after ethanol consumption. The fastest heart rates at the end of the test session were also found in GroupC subjects, followed by Groups E and B, and then Groups A and D respectively.

3.7.5.2. BLOOD PRESSURE

Systolic blood pressure was not significantly different among the groups, however, GroupC subjects had the highest values overall. In general, systolic blood pressure decreased immediately after ethanol consumption (except in GroupC subjects where the decrease occurred at t_{60}), and the final blood pressure reading (t_{120}) was lower than pre-ethanol values for all groups (see section 3.7.8. for data tables).

Diastolic blood pressure was also not found to be significantly different among the groups and, like the systolic blood pressure, values decreased after ethanol. However, unlike systolic blood pressure, diastolic blood pressure, in general, increased again at t_{60} in most groups (see section 3.7.8. for data tables).

3.7.5.3. FACIAL TEMPERATURE

A significant difference was found among the five $ALDH_2$/ ADH_2 combination groups ($F_{4,91}=3.07$, $p<0.05$) -Fig.85. A highly significant time by group interaction effect was also detected for facial temperature ($F_{12,273}=5.87$, $p<0.005$).

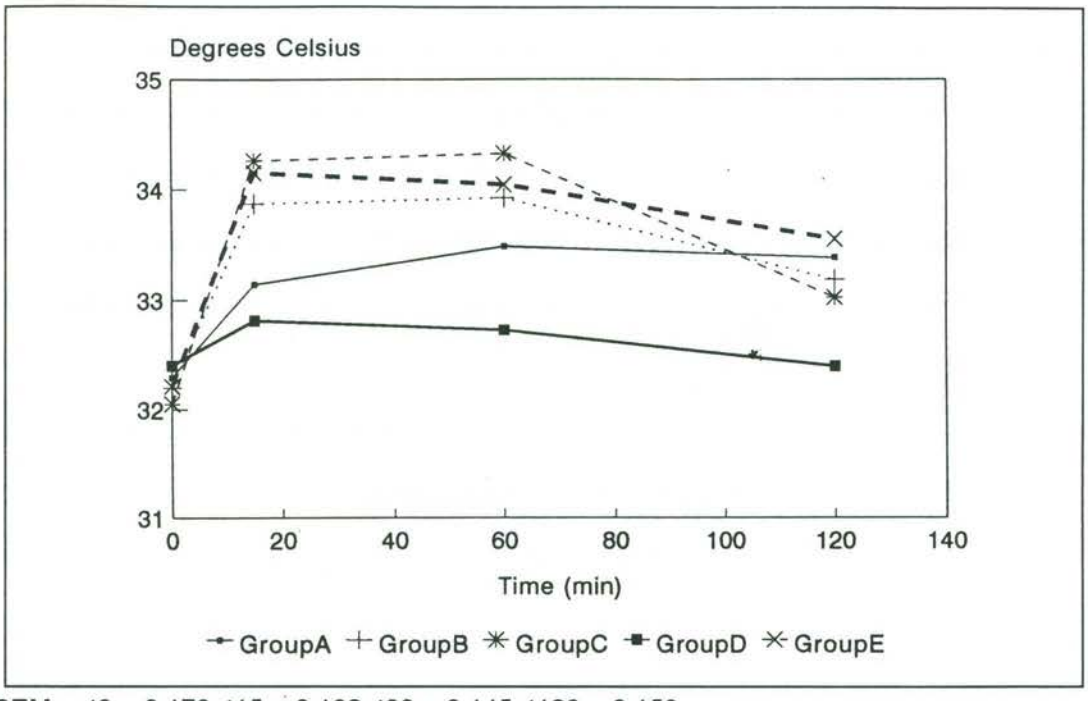
All five groups exhibited a rise in facial temperature after ethanol which reached the highest values at t_{60} (except GroupD and GroupE subjects who peaked earlier at t_{15}). This was followed by a fall in temperature towards pre-ethanol values by the end of the session.

The highest temperature was seen in GroupC subjects fifteen minutes after ingestion of ethanol, followed by GroupE, GroupB, GroupA and GroupD subjects.

GroupA subjects were the only group who did not demonstrate a return of facial temperature towards normal levels by the end of the test session.

GroupB and GroupE subjects produced similar responses to ethanol, and they did not exhibit as sharp a fall in temperature as GroupC subjects in the second hour after drinking.

GroupC subjects had the highest temperature readings for up to one hour after drinking. In the second hour, as the facial temperature started to fall, these subjects no longer had the highest temperatures.



SEM: t0 = 0.178; t15 = 0.162; t60 = 0.145; t120 = 0.153.

Fig.85: Temperature by $ALDH_2$/ ADH_2 combination genotypes.

GroupD subjects had the lowest facial temperatures overall, and the initial increase in temperature after ethanol was only small compared with those of the other groups.

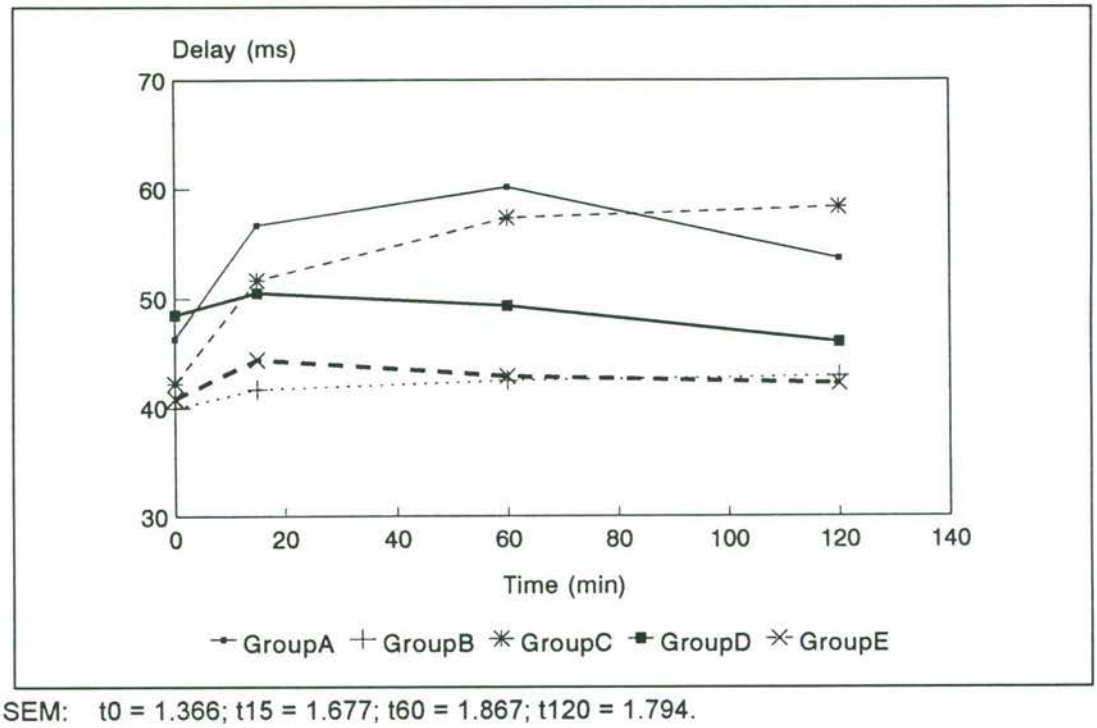
Post-hoc analysis detected that even GroupA subjects had significantly increased temperatures at 60 ($q_{10,273}$=5.22, $p<0.05$) and 120 minutes after ethanol ($q_{9,273}$=4.79, $p<0.05$). GroupB subjects produced significant differences at 15 ($q_{14,273}$=7.32, $p<0.05$) and 60 ($q_{15,273}$=7.53, $p<0.05$) minutes. GroupC subjects at 15 ($q_{19,273}$=9.67, $p<0.05$) and 60 ($q_{20,273}$=9.92, $p<0.05$) minutes; and GroupE subjects at 15 ($q_{16,273}$=8.54, $p<0.05$), 60 ($q_{15,273}$=8.03, $p<0.05$) and 120 ($q_{12,273}$=5.87, $p<0.05$) minutes after ethanol consumption also reached levels of statistical significance. *Post-hoc* differences in GroupD subjects were not statistically significant.

3.7.6. PSYCHOMOTOR VARIABLES

Performance in the divided attention delay and excursion tasks and the digit symbol coding task exhibited significant differences among the various groups. The CFFF threshold task also exhibited a significant difference for the time by group interaction effect, although differences in standing steadiness and divided attention reaction time responses were not statistically significant.

3.7.6.1. DIVIDED ATTENTION

Delay A highly significant time by group interaction was found for the divided attention delay task ($F_{12,270}$=2.27, p<0.005) -Fig.86.



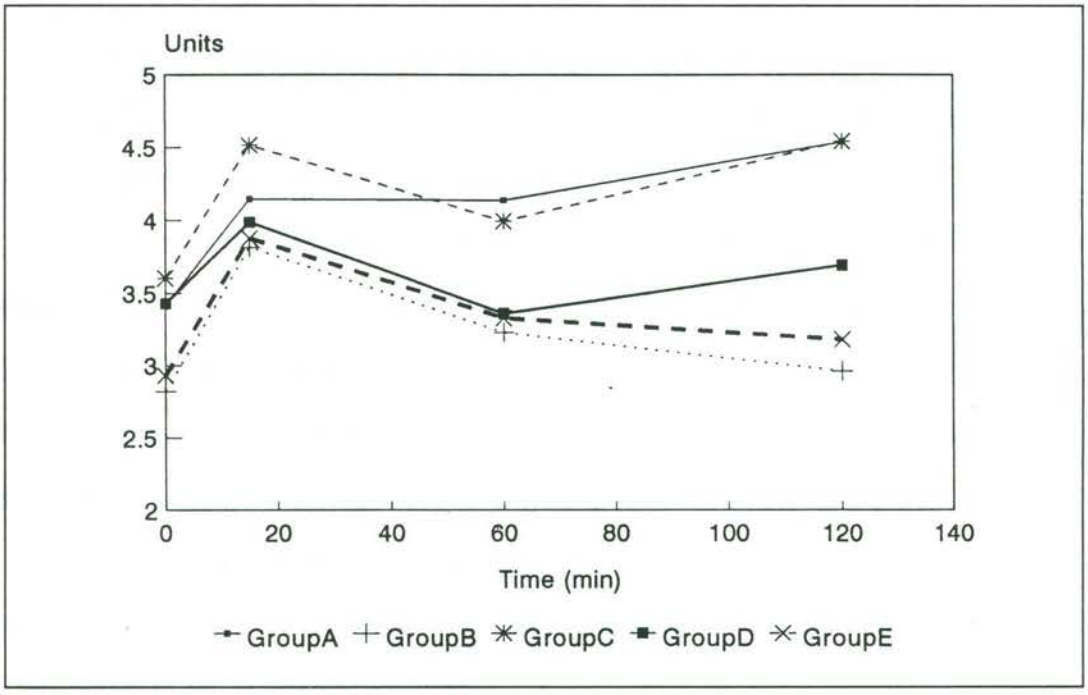
SEM: t0 = 1.366; t15 = 1.677; t60 = 1.867; t120 = 1.794.

Fig.86: Divided attention delay by $ALDH_2$/ ADH_2 combination genotypes.

After ethanol, the divided attention delay time increased in all five groups ($F_{4,90}$=2.70, p<0.05). GroupD and GroupE subjects were most affected 15 min after ethanol, although performance had returned towards pre-ethanol levels by t_{120}. GroupA and GroupC subjects were most affected at t_{60} and t_{120} respectively. All groups, except GroupC, had recovered towards pre-ethanol values by t_{120}.

Excursion Divided attention excursion was significantly different among the five groups tested ($F_{4,90}$=2.95, p<0.05) -Fig.87.

The responses of all groups to ethanol were similar in that there was an increase in excursion immediately after ethanol consumption. This was followed in most groups, except GroupA, by a fall in excursion value at t_{60}, followed by another smaller rise in excursion measured at the end of the session.



SEM: t0 = 0.118; t15 = 0.129; t60 = 0.124; t120 = 0.137.

Fig.87: Divided attention excursion by $ALDH_2$/ ADH_2 combination genotypes.

Reaction Time Divided attention peripheral reaction time was not found to be significantly different among the groups, and the response pattern after ethanol consumption was similar in all groups tested.

In general, after ethanol there was an increase in reaction time, reaching a maximum value at t_{15}, followed by a decrease which continued until t_{120}.

GroupC subjects demonstrated an increase in reaction time at t_{15} but, later, the reaction time decreased to below pre-ethanol values at both t_{60} and t_{120}. Groups B and E had a greater increase in reaction time than GroupC, these values also decreasing by t_{60} but, at t_{120} increasing again. This is in contrast with GroupA and GroupD -these two groups plateaued at t60 after falling from the increased reaction time at t_{15} (see section 3.7.8. for data tables).

3.7.6.2. DIGIT SYMBOL CODING

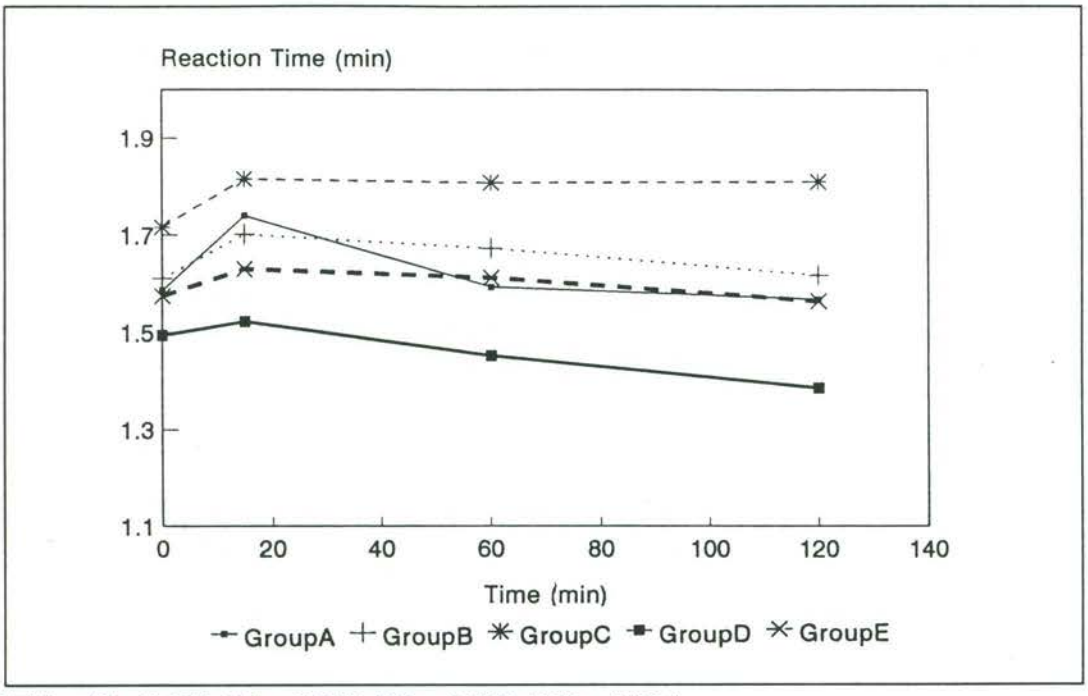
The DSC reaction time increased after ethanol in all five groups (Fig.88).

A significant group difference was detected in performance of the digit symbol coding task ($F_{4,90}=2.60$, $p<0.05$).

Groups B, D and E had very similar responses to ethanol in that the changes in reaction time which occurred were minor compared with those which occurred in GroupC and GroupA subjects. The three groups each produced an increase in reaction time after ethanol, but this was short-lived and reaction time decreased thereafter.

GroupC subjects had unusually high DSC reaction times; this was evident even before the ethanol challenge, and their values remained high, with a plateau effect between the t_{15}-t_{60} timepoints.

GroupA subjects exhibited the greatest increase in reaction time after ethanol, and also had the greatest decrease in reaction time after the t_{15} timepoint.



SEM: t0 = 0.030; t15 = 0.035; t60 = 0.036; t120 = 0.034.

Fig.88: Digit symbol coding by $ALDH_2$/ ADH_2 combination genotypes.

3.7.6.3. STANDING STEADINESS

Differences among the groups in the standing steadiness task performance were not statistically significant (see section 3.7.8. for data tables).

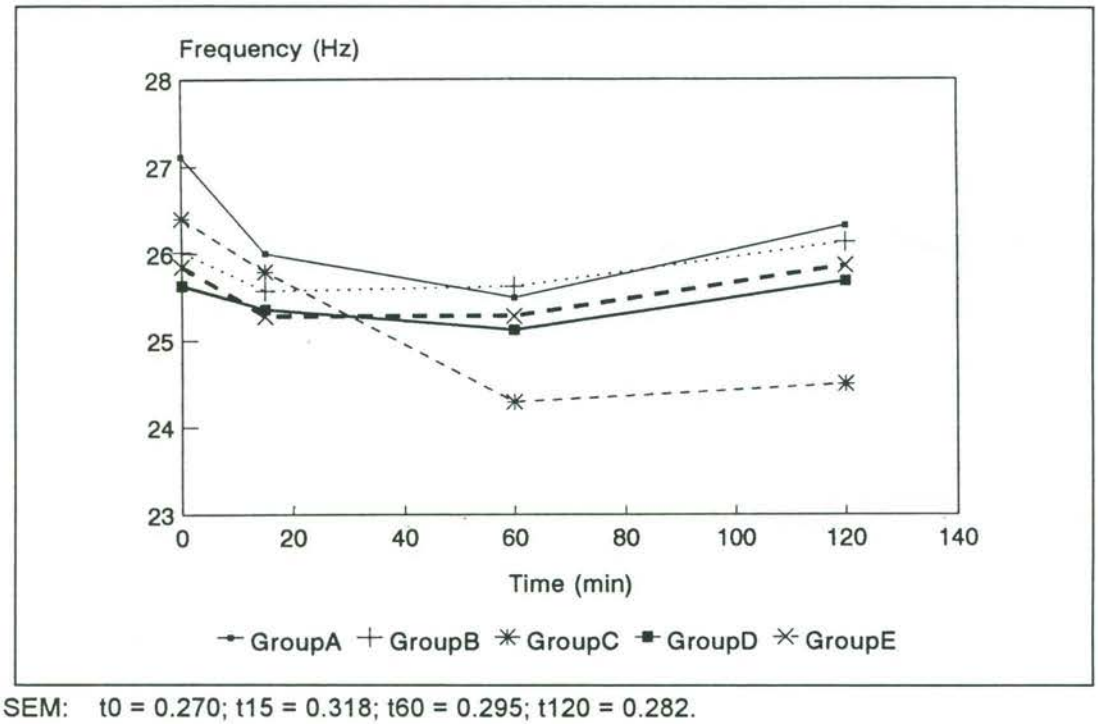
After ethanol, standing steadiness decreased in all five groups. Recovery towards the pre-ethanol values also began very quickly (15 min after ethanol consumption) in Groups B and E.

Groups A, C and D remained affected, with lower standing steadiness values, until t_{60}, at which time recovery commenced.

3.7.6.4. CRITICAL FLICKER FUSION FREQUENCY

Performance in the CFFF threshold task exhibited a statistically significant time by group interactive effect ($F_{12,273}=2.20, p<0.05$) -Fig.89. All five groups

demonstrated a decrease in threshold values after ethanol consumption, falling further at t_{60} and then recovering towards pre-ethanol values.



SEM: t0 = 0.270; t15 = 0.318; t60 = 0.295; t120 = 0.282.

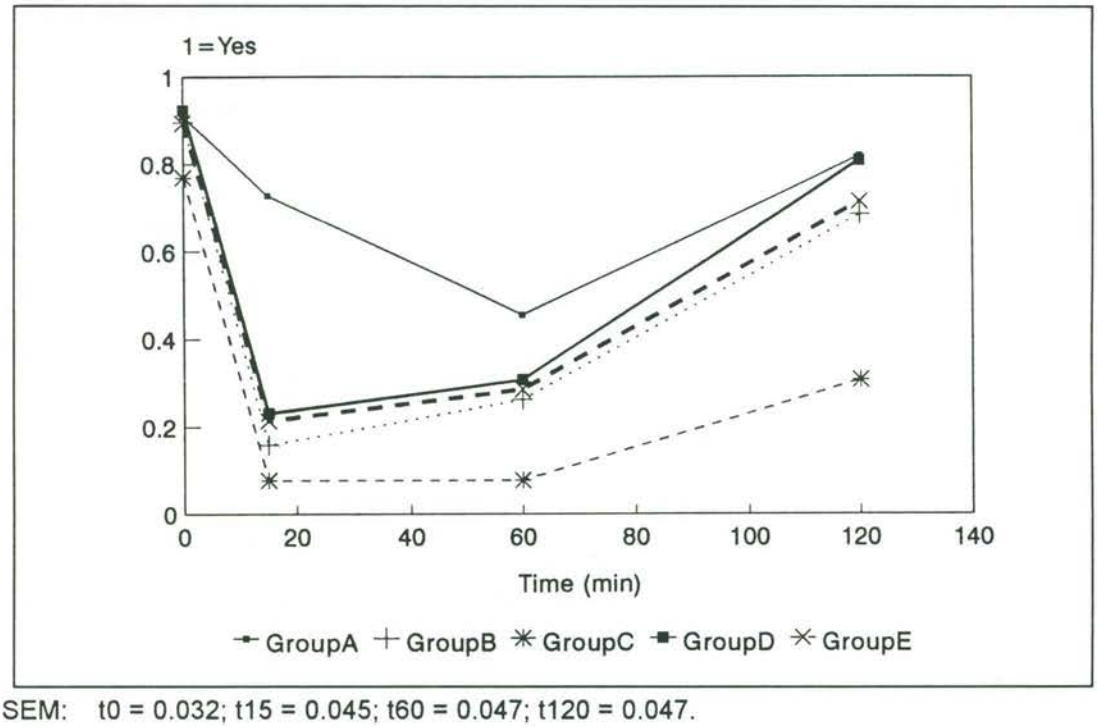
Fig.89: CFFF threshold by $ALDH_2$/ ADH_2 genotype.

GroupC subjects had the greatest decrease in threshold value, which fell rapidly between t_{15} and t_{60}. The lowest threshold value attained was at t_{60} by GroupC subjects.

3.7.7. SUBJECTIVE MEASURES OF INTOXICATION

Most of the responses to the subjective questions reached statistical significance (reported below). In general, GroupA and GroupD subjects were the least affected by ethanol. GroupB and GroupC subjects were the most affected groups after ethanol, with GroupC subjects being affected for the longest length of time. GroupE subjects reported intermediate responses.

All five $ALDH_2$/ ADH_2 combination groups reported that they would not drive a car (Q1) 15 min after consuming ethanol. GroupC had the greatest response in this respect ($F_{4,92}$=3.65, $p<0.005$) although, as with the other groups, recovery was apparent by 120 min (Fig.90).

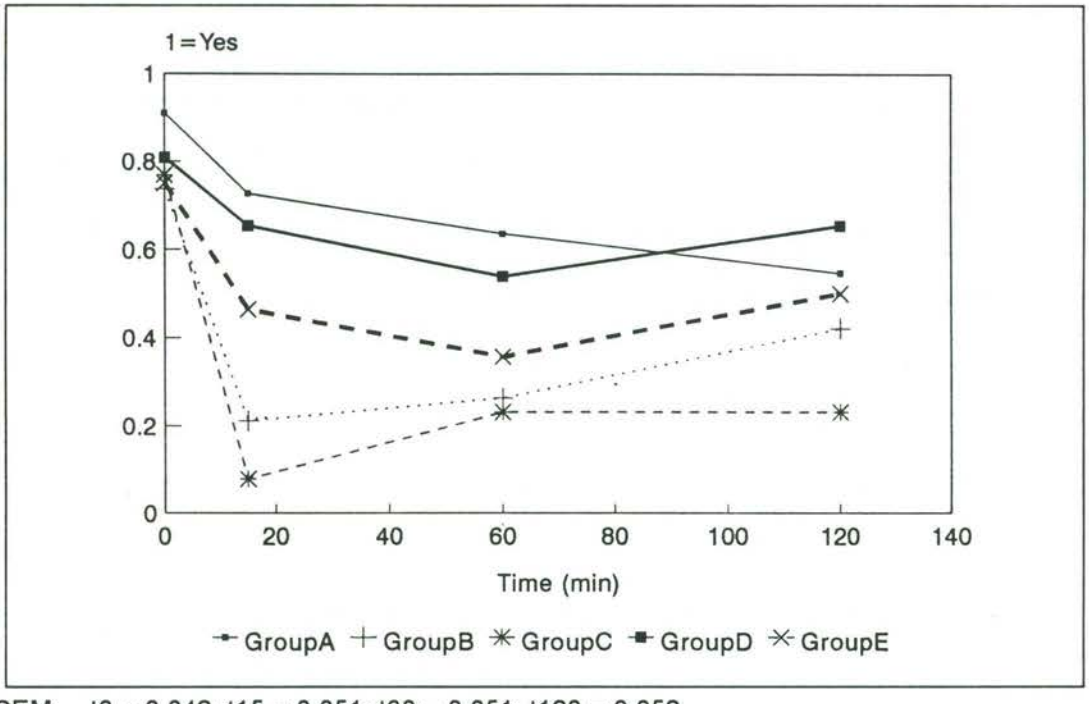


SEM: t0 = 0.032; t15 = 0.045; t60 = 0.047; t120 = 0.047.

Fig.90: 'Would you drive a car now?' subjective response.

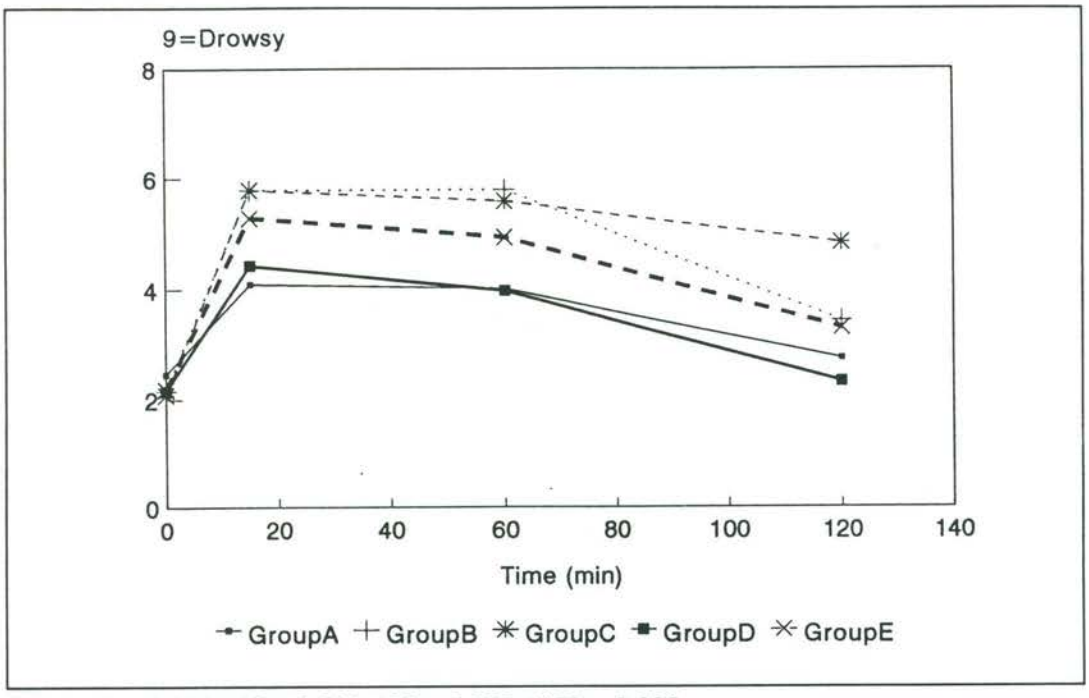
After ethanol, GroupC and GroupB subjects ($F_{4,92}$=3.04, $p<0.05$) reported that they would not accept a second alcoholic drink from friends (Q2). This decision was most marked 15 min after drinking, and was more evident (after a brief recovery at t_{60}) for GroupC subjects at 120 min, although it was less evident for GroupB subjects at 120 min. The other groups were also affected by the ethanol challenge, however their responses were not as great (Fig.91).

All groups reported feeling drowsy (Q3) after consuming ethanol ($F_{4,91}$=2.59, $p<0.05$), with GroupB being the worst-affected (5.8). This effect remained up to 120 min after ethanol, by which time GroupB and most of the other groups had almost returned to their pre-ethanol values. GroupC continued to remain drowsy at 120 min after ethanol consumption (Fig.92).



SEM: t0 = 0.042; t15 = 0.051; t60 = 0.051; t120 = 0.052.

Fig.91: 'Would you accept another drink?' subjective response.

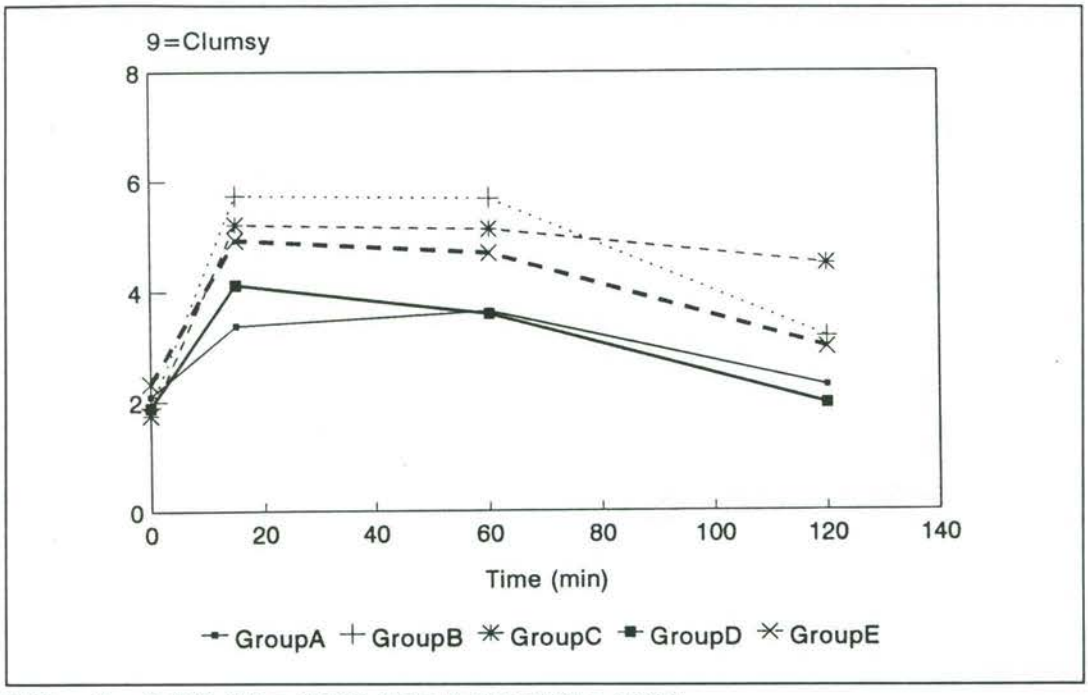


SEM: t0 = 0.191; t15 = 0.221; t60 = 0.230; t120 = 0.223.

Fig.92: 'Do you feel alert or drowsy?' subjective response.

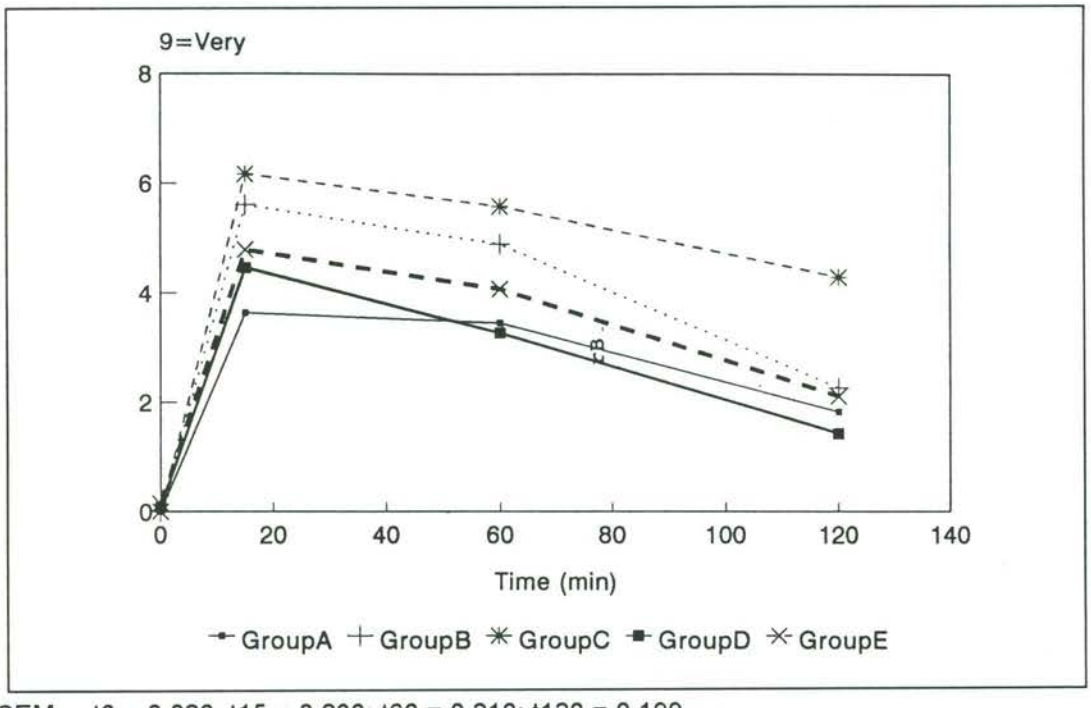
Subjects reported feeling clumsier after ethanol consumption ($F_{4,91}$=3.37, $p<0.05$; $F_{12,273}$=2.34, $p<0.005$) -Q4 (Fig.93). GroupB subjects perceived themselves as the most clumsy (5.7), with GroupA and GroupD subjects being less affected (3.6 and 4 respectively). GroupC subjects were still feeling clumsy at 120 min (3.9).

The level of intoxication reported (Q6) after ethanol consumption increased as expected ($F_{4,91}$=5.38, $p<0.005$; $F_{12,273}$=2.54, $p<0.005$). GroupA and GroupD subjects considered themselves to be the least intoxicated overall, in contrast with GroupC and GroupB subjects who were the most affected by ethanol (Fig.94).



SEM: t0 = 0.177; t15 = 0.222; t60 = 0.230; t120 = 0.206.

Fig.93: 'Do you feel co-ordinated or clumsy?' subjective response.



SEM: t0 = 0.028; t15 = 0.233; t60 = 0.210; t120 = 0.199.

Fig.94: 'How intoxicated do you feel?' subjective response.

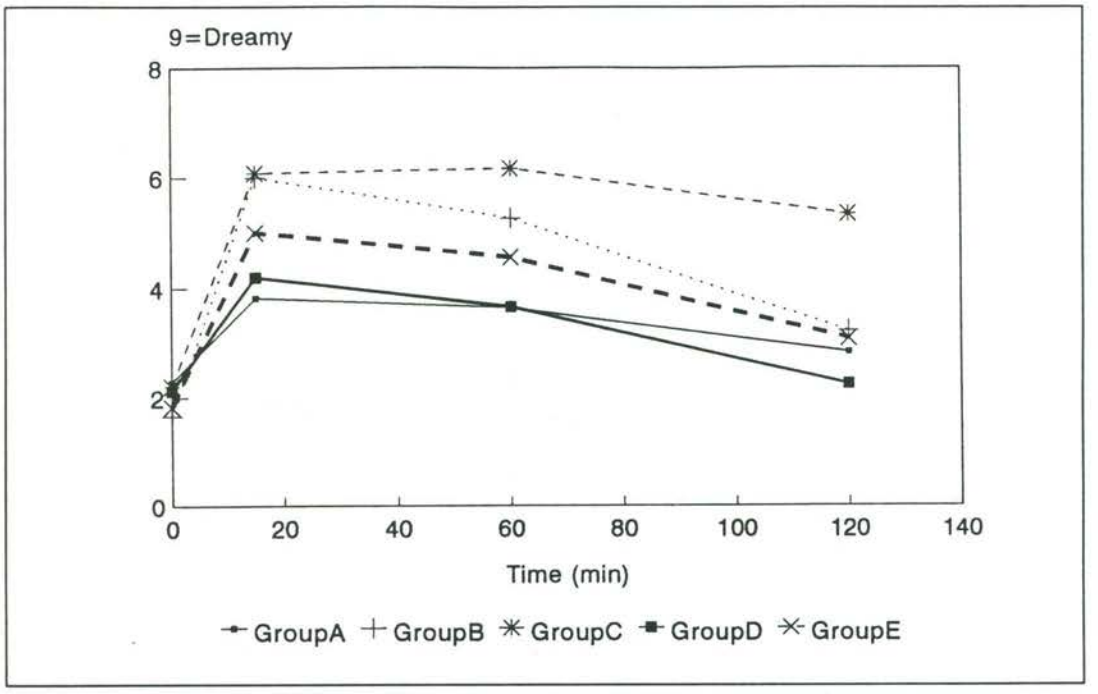
Similarly, when asked if they felt attentive or dreamy (Q7), GroupA and GroupD subjects reported feeling less dreamy than the other groups ($F_{4,91}$=4.46, p<0.005). GroupB and GroupC subjects were the most affected, GroupC subjects reported the greatest dreaminess at 60 min and 120 min after ethanol ($F_{12,273}$=2.39, p<0.005) -Fig.95.

GroupC was clearly the most affected by headache (Q8) after ethanol ($F_{4,92}$=3.86, p<0.005), this response was most marked at t_{120} (Fig.96).

GroupB subjects reported feeling most nauseated (Q9) 15 min after ethanol ($F_{8,184}$=4.12, p<0.005). However, by 120 min, GroupC subjects reported feeling the worst after ethanol; at this time, the GroupB subjects had responses almost back to normal (Fig.97).

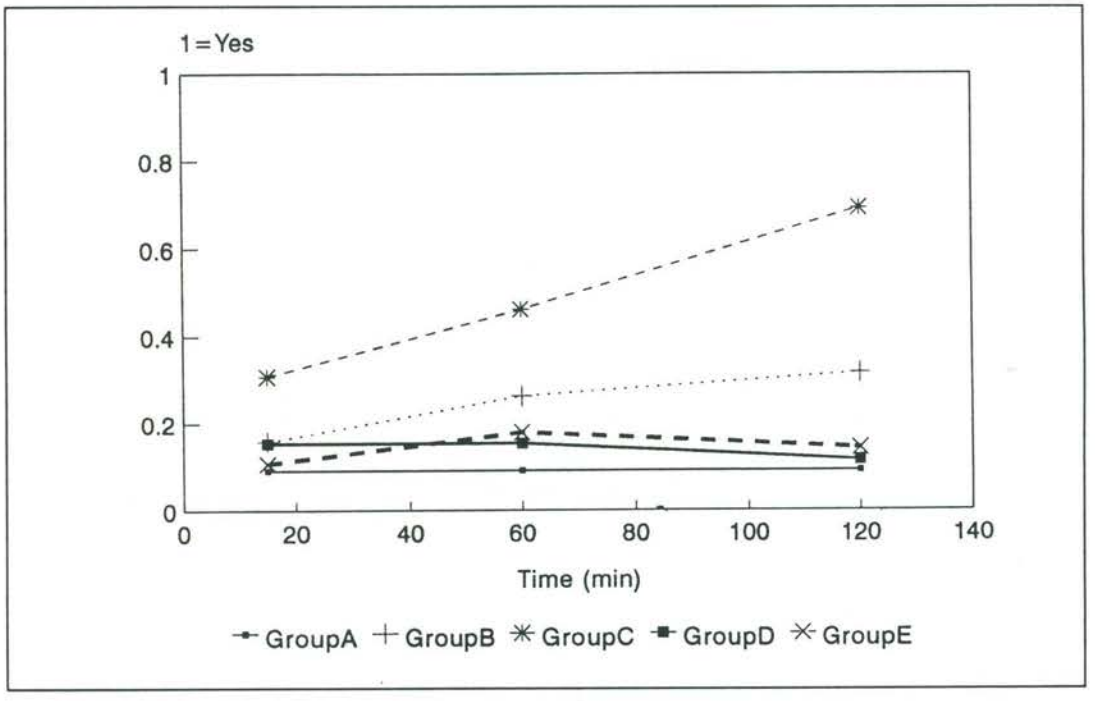
Reported hangover-like effects (Q10) -Fig.98 -were the greatest for GroupA subjects at t_{15}. This effect persisted until t_{120}, by which time GroupC subjects were the worst affected group ($F_{4,92}$=4.49, p<0.005; $F_{8,184}$=3.13, p<0.005).

The response which probed the perceived level of concentration of subjects (Q5) was not significantly different among the five groups.



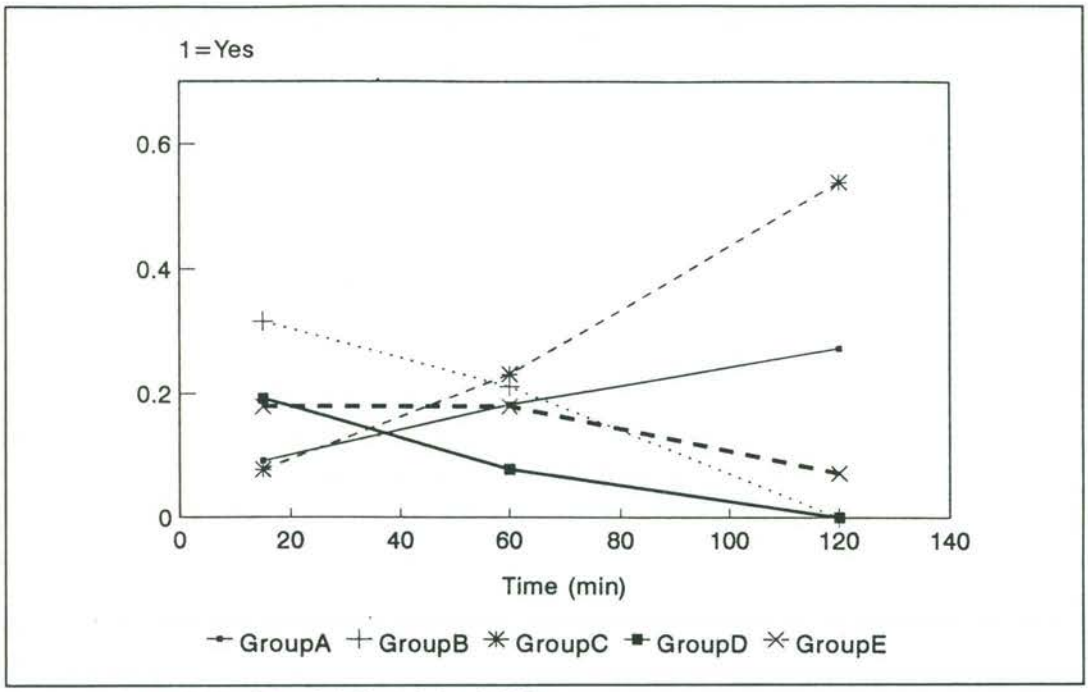
SEM: t0 = 0.190; t15 = 0.233; t60 = 0.222; t120 = 0.223.

Fig.95: 'Do you feel attentive or dreamy?' subjective response.



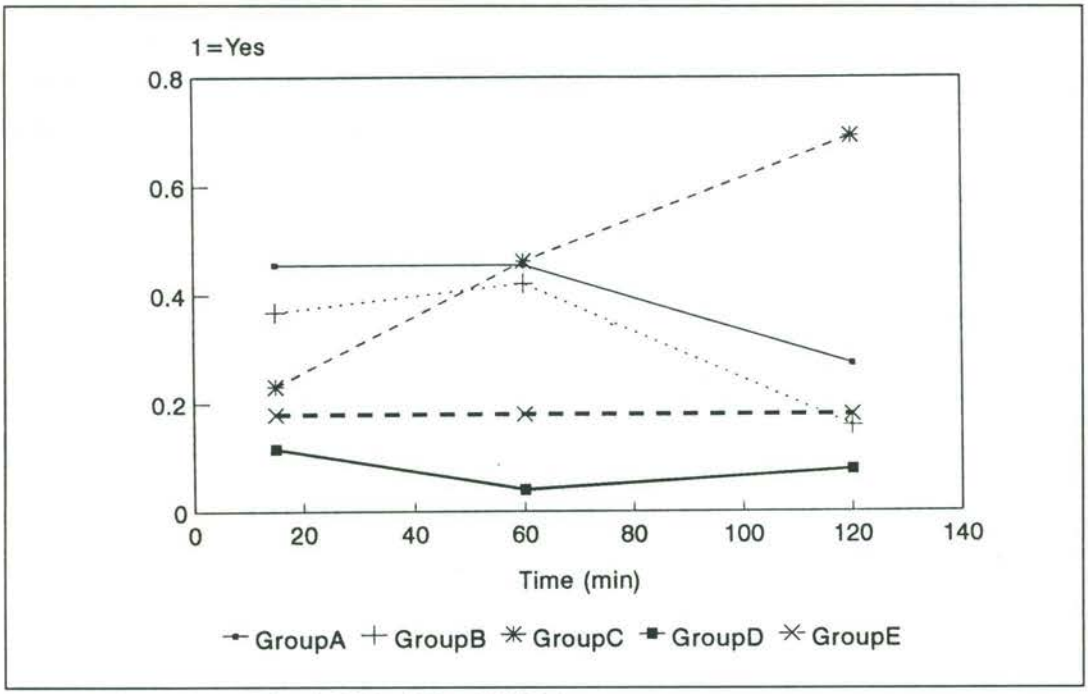
SEM: t15 = 0.037; t60 = 0.042; t120 = 0.044.

Fig.96: 'Do you experience a headache?' subjective response.



SEM: t15 = 0.040; t60 = 0.038; t120 = 0.033.

Fig.97: 'Does your stomach feel uneasy or sick?' subjective response.



SEM: t15 = 0.044; t60 = 0.045; t120 = 0.043.

Fig.98: 'Any hangover-like effects?' subjective response.

3.7.8. SUMMARY OF OTHER RESULTS BY $ALDH_2$/ ADH_2 COMBINED GENOTYPE (NON-SIGNIFICANT)

The results for each variable below are listed in the following manner for each time-point (t_0, t_{15}, t_{60}, t_{120}):

GroupA mean ± SD; n=15

GroupB mean ± SD; n=22

GroupC mean ± SD; n=13

GroupD mean ± SD; n=25

GroupE mean ± SD; n=23

VARIABLE	t_0	t_{15}	t_{60}	t_{120}
Acetate (mM)	0.03 ± 0.14	0.45 ± 0.25	0.49 ± 0.33	0.50 ± 0.34
	-0.02 ± 0.09	0.40 ± 0.28	0.36 ± 0.24	0.35 ± 0.25
	-0.10 ± 0.16	0.30 ± 0.15	0.15 ± 0.51	0.28 ± 0.12
	0.02 ± 0.10	0.42 ± 0.16	0.43 ± 0.17	0.39 ± 0.18
	-0.04 ± 0.13	0.22 ± 0.99	0.34 ± 0.20	0.27 ± 0.19
Lactate (mM)	1.5 ± 1.4	1.9 ± 1.3	1.7 ± 0.9	1.6 ± 0.8
	1.4 ± 1.0	1.7 ± 1.0	1.8 ± 1.0	1.7 ± 1.1
	1.5 ± 1.0	2.0 ± 1.0	2.0 ± 1.2	2.4 ± 1.5
	1.4 ± 1.2	1.7 ± 1.2	1.8 ± 1.0	1.6 ± 0.7
	1.2 ± 0.9	1.5 ± 0.6	1.6 ± 0.7	1.6 ± 0.9
Pyruvate (mM)	0.06 ± 0.02	0.04 ± 0.02	0.04 ± 0.01	0.04 ± 0.02
	0.05 ± 0.02	0.03 ± 0.01	0.04 ± 0.02	0.04 ± 0.03
	0.06 ± 0.02	0.04 ± 0.01	0.05 ± 0.02	0.05 ± 0.02
	0.05 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.02
	0.05 ± 0.02	0.04 ± 0.03	0.04 ± 0.02	0.04 ± 0.02

Lac:Pyr Ratio	23.4 ± 17.1	50.1 ± 32.0	37.4 ± 10.2	39.2 ± 15.1
	42.7 ± 60.0	84.9 ± 156.4	52.4 ± 36.5	99.4 ± 189.4
	24.3 ± 12.5	48.1 ± 17.2	43.1 ± 13.9	45.1 ± 17.8
	26.2 ± 14.0	40.9 ± 16.5	42.7 ± 23.4	47.2 ± 51.9
	23.1 ± 12.2	50.1 ± 26.7	45.2 ± 22.4	41.2 ± 18.5
Blood Pressure	113.6 ± 12.3	109.6 ± 10.2	102.7 ± 12.9	108.1 ± 14.6
(Sys) (mmHg)	107.1 ± 11.5	106.6 ± 15.0	105.8 ± 15.8	103.5 ± 13.9
	109.6 ± 13.5	112.2 ± 14.0	106.8 ± 12.4	107.5 ± 11.4
	110.8 ± 10.8	106.7 ± 11.2	105.2 ± 10.3	106.0 ± 14.9
	113.8 ± 13.4	109.3 ± 15.4	106.0 ± 12.1	103.9 ± 11.5
Blood Pressure	74.0 ± 10.6	72.2 ± 12.7	68.0 ± 13.3	72.2 ± 8.8
(Dia) (mmHg)	72.2 ± 12.5	67.9 ± 9.0	66.5 ± 10.6	68.2 ± 10.2
	73.4 ± 6.9	63.6 ± 8.8	65.3 ± 9.4	66.3 ± 6.6
	69.3 ± 10.7	71.1 ± 9.5	67.9 ± 8.8	68.4 ± 9.7
	72.4 ± 10.2	67.7 ± 12.4	65.9 ± 11.4	67.4 ± 9.5
Standing	24.7 ± 6.5	20.3 ± 8.1	20.3 ± 6.7	20.9 ± 8.0
Steadiness (s)	29.4 ± 10.0	20.2 ± 9.5	21.6 ± 9.6	25.7 ± 9.2
	26.6 ± 6.2	18.7 ± 6.8	17.9 ± 6.7	20.7 ± 7.9
	26.4 ± 6.9	21.8 ± 8.2	20.9 ± 5.6	21.9 ± 4.8
	25.7 ± 7.0	20.1 ± 8.6	21.8 ± 8.4	23.4 ± 7.3
Div. Attention	2.7 ± 0.5	3.1 ± 0.8	2.9 ± 0.7	2.9 ± 0.7
(Reaction Time)	2.8 ± 0.8	3.2 ± 0.8	2.8 ± 0.9	2.9 ± 0.9
	3.2 ± 1.0	3.4 ± 0.9	3.0 ± 1.0	2.8 ± 0.8
	2.5 ± 0.7	2.8 ± 0.8	2.5 ± 0.7	2.5 ± 0.7
	2.7 ± 0.7	3.1 ± 0.9	2.7 ± 0.8	2.8 ± 0.9

Subjective Q5	6.5 ± 2.1	5.1 ± 2.6	5.6 ± 2.0	6.4 ± 1.9
	7.2 ± 1.9	4.6 ± 2.0	4.2 ± 2.4	6.1 ± 2.3
	7.3 ± 1.8	3.7 ± 2.0	4.2 ± 2.3	5.0 ± 2.6
	6.9 ± 1.7	5.1 ± 2.1	5.5 ± 1.9	6.5 ± 2.1
	7.0 ± 1.7	4.1 ± 2.3	4.6 ± 1.9	6.1 ± 1.9

3.8. RESULTS BY GENDER

3.8.1. BREAKDOWN OF SUBJECTS

3.8.1.1. BY $ALDH_2$

The $ALDH_2$ genotype breakdown is shown in Fig.99. Most subjects (male and female) were found to be $ALDH_2$ Het (45% and 55% respectively), although no significant differences were detected for the breakdown of subjects by $ALDH_2$ type ($p>0.05$).

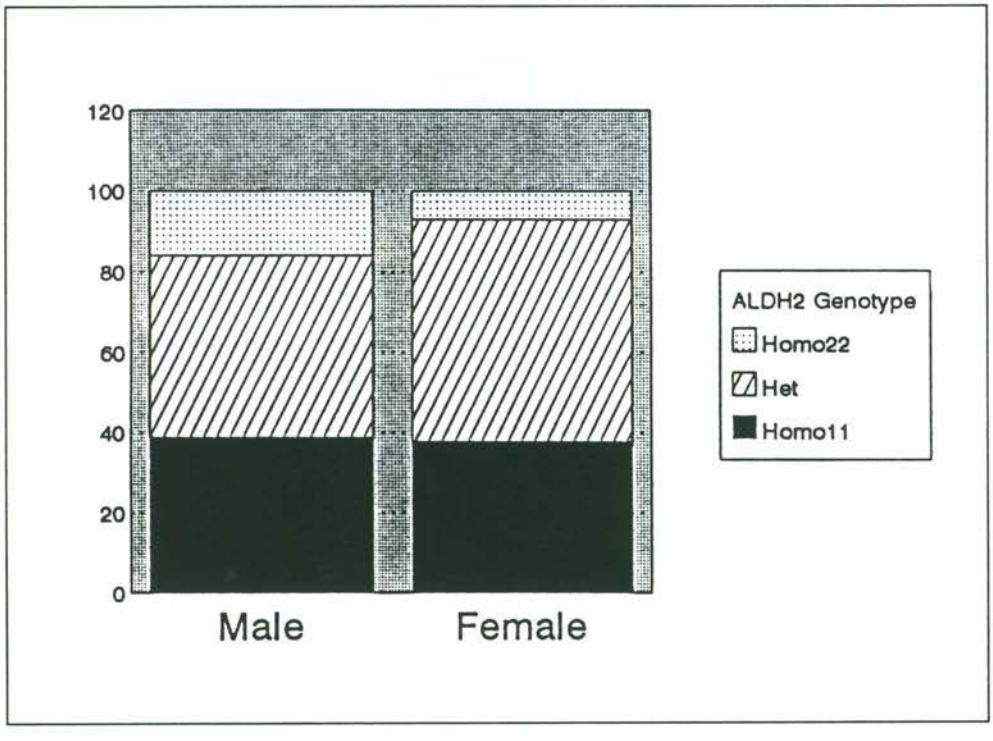


Fig.99: Gender breakdown of $ALDH_2$ genotype subjects.

3.8.1.2. BY ADH_2

Similarly to the gender breakdown of $ALDH_2$ genotype subjects, that of subjects by ADH_2 genotype resulted in the finding that the majority were of the Het

group (Fig.100). There were no significant differences detected among the different groups ($p>0.05$).

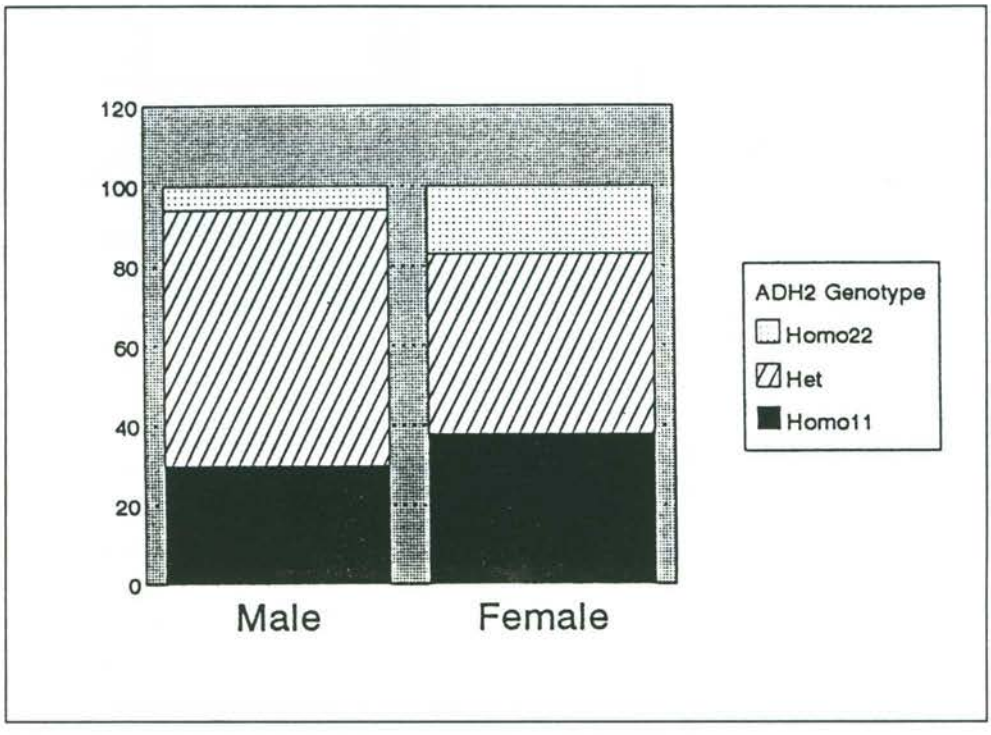


Fig.100: Gender breakdown of ADH_2 genotype subjects.

3.8.1.3. BY DEGREE OF FLUSH

28% of male subjects did not flush, and 42% were rated in the LF group. This LF result is similar to those of the female subjects (43%), although only 12% of female subjects did not flush after ethanol (Fig.101). No significant differences among the groups were detected ($p>0.05$).

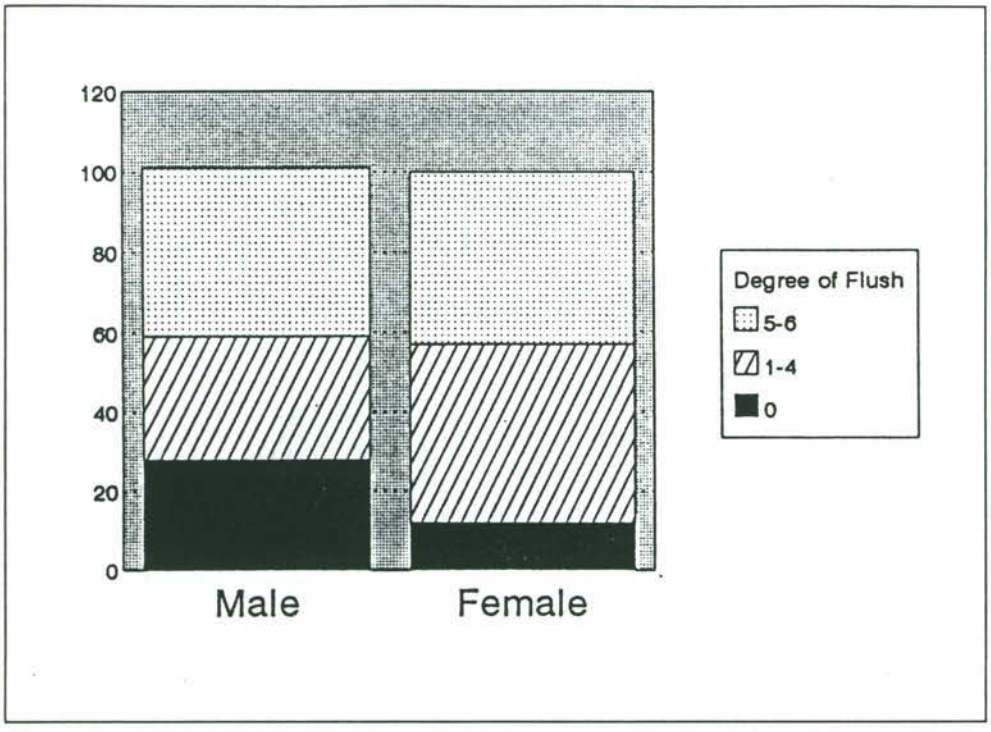


Fig.101: Gender breakdown of degree of flush subjects.

3.8.2. DRINKING DETAILS

Female subjects drank slightly less frequently than their male counterparts. 81% of the female subjects drank 1-2 times a month or less, or not at all, whilst male subjects drinking ethanol 1-2 times a month or less, or not at all comprised 78% of subjects in this group (Fig.102). However, these findings did not reach levels of statistical significance ($p>0.05$) with non-parametric tests.

Male subjects generally preferred to drink beer (56%), whilst female subjects preferred to drink spirits (33%) -Fig.103.

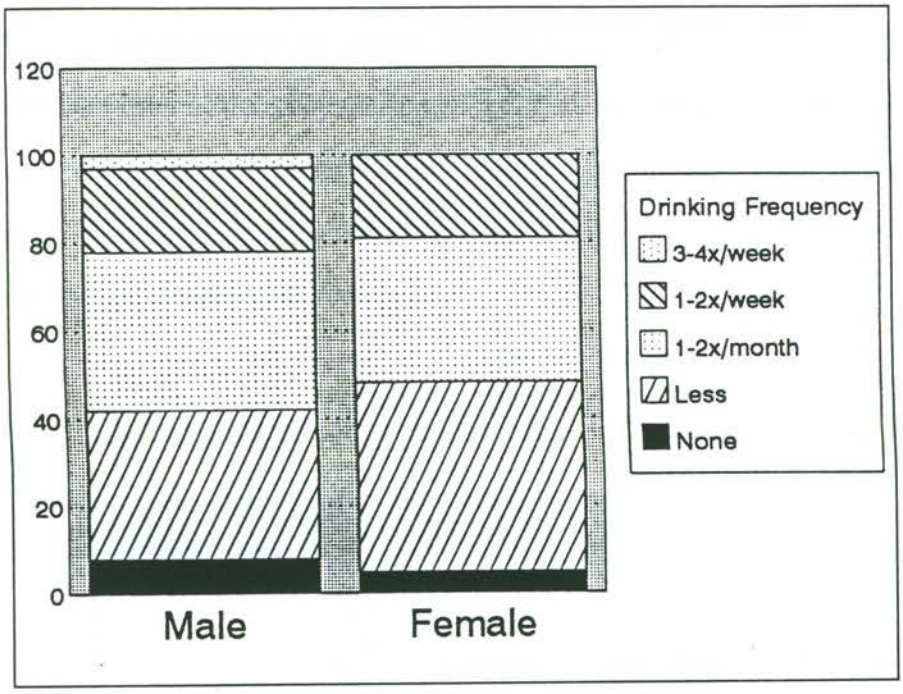


Fig.102: Drinking habits by gender: How often subjects consume ethanol.

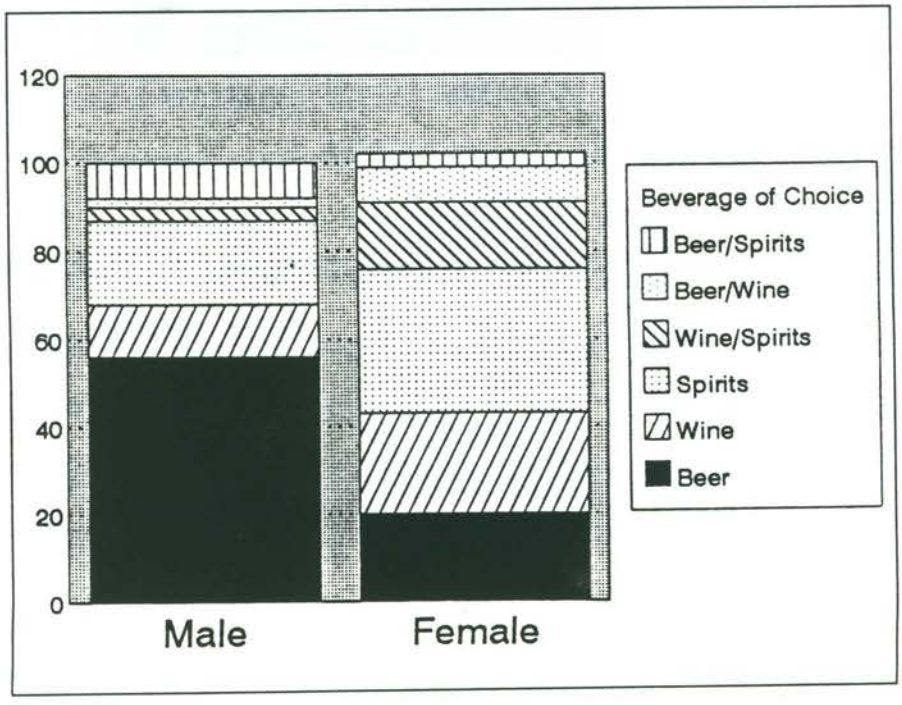


Fig.103: Drinking habits by gender: Choice of beverage preferred.

The amount of ethanol habitually consumed can be seen in Fig.104. 17% of female subjects reported that they consumed less than one standard drink per session, whilst only 10% of the male subjects tested reported such 'low' levels of ethanol consumption. The findings, using non-parametric tests, were not significantly different between males and females ($p>0.05$).

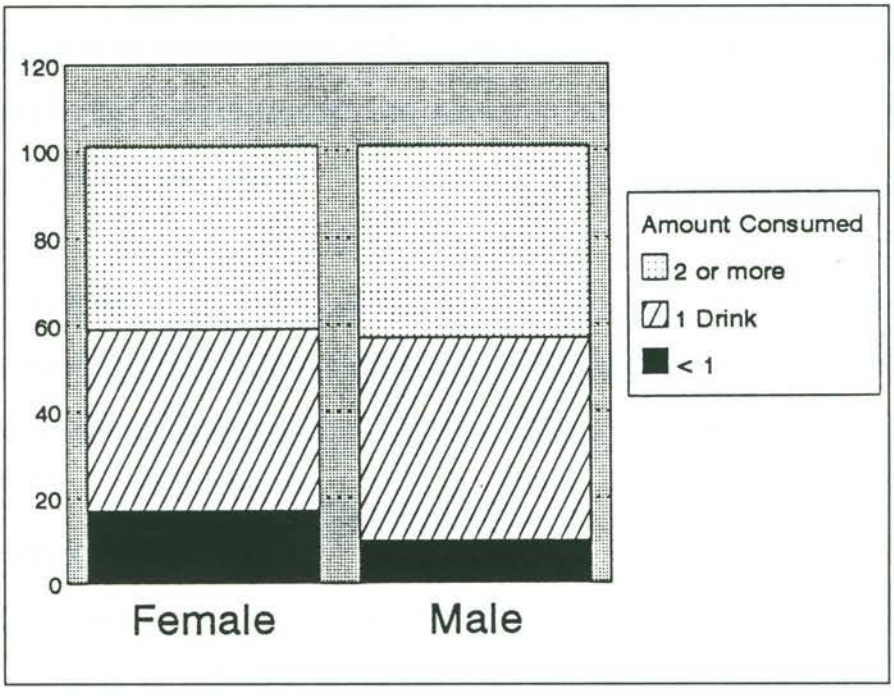


Fig.104: Amount of ethanol consumed -by Gender.

3.8.3. BREATH ETHANOL CONCENTRATION CURVES

Gender-related differences in ethanol pharmacokinetic variables were not statistically significant, although they are included here for reference purposes (see section 3.8.8. for data tables).

As a result of the above findings (detailed below), the correction factor in the amount of ethanol which was given to female subjects thus appears to have been appropriate.

It should be noted that the stage of menstrual cycle of female subjects was not recorded, therefore assessment as to whether or not these different phases affected the female subjects was not possible.

3.8.3.1. PEAK BEC

The mean peak BEC levels obtained were similar for both females and males, both groups having a peak response of 0.034 g dl^{-1}.

3.8.3.2. TIME TO PEAK

The mean time to peak BEC was 15 min after ethanol in both male and female subjects.

3.8.3.3. ELIMINATION RATE

Mean differences between the ethanol elimination rates, calculated by linear regression, did not reach statistical significance, being 0.0163 ±0.001 (SEM) and 0.0157 ± 0.001 (SEM) g dl^{-1} hr^{-1} for females and males respectively.

3.8.3.4. VOLUME OF DISTRIBUTION

Female subjects had a lower mean volume of distribution (47.7 litres) compared with those of the males (58.9 litres).

3.8.3.5. AREA UNDER THE CURVE

The mean area under the curve was calculated to be 0.136 g dl^{-1} min for both the female (SEM=0.015) and male (SEM=0.012) subjects.

3.8.4. BIOCHEMICAL VARIABLES

Of the biochemical measures taken, the only gender-related difference to reach statistical significance was the pyruvate concentration.

Differences in the other variables (lactate, acetate, plasma ethanol and acetaldehyde) were not statistically significant.

3.8.4.1. BLOOD ACETALDEHYDE

Differences in the acetaldehyde levels were not statistically significant (see section 3.8.8. for data tables). Both groups showed an increase in acetaldehyde concentration after ethanol, the male subjects having higher levels than the females.

3.8.4.2. PLASMA ACETATE

Gender-related differences found in acetate concentrations did not reach statistical significance (see section 3.8.8. for data tables). Both groups initially responded to ethanol in the same way. After t_{15}, however, male and female subjects diverged in their responses, with the male subjects having higher levels of acetate. Acetate concentrations also decreased slightly after t_{60}.

3.8.4.3. PLASMA ETHANOL

Group differences in plasma ethanol concentrations measured did not reach statistical significance (see section 3.8.8. for data tables). The response after ethanol consumption was similar for both groups up to t_{15}, after which time a divergence occurred. Male subjects had higher plasma ethanol levels than the female subjects. Recovery towards pre-ethanol values began after t_{60}.

3.8.4.4. BLOOD LACTATE

Differences attributable to gender in lactate levels were not statistically significant (see section 3.8.8. for data tables). Both sexes had an increase in lactate concentration after ethanol. The female subjects had the highest lactate concentrations overall.

3.8.4.5. BLOOD PYRUVATE

Statistically significant gender-related differences in pyruvate concentrations were found ($F_{1,102}$=6.27, $p<0.05$) -Fig.105. The male subjects had lower pyruvate concentration than the female subjects. In general, the consumption of ethanol caused the pyruvate levels to decrease immediately after drinking (t_{15}).

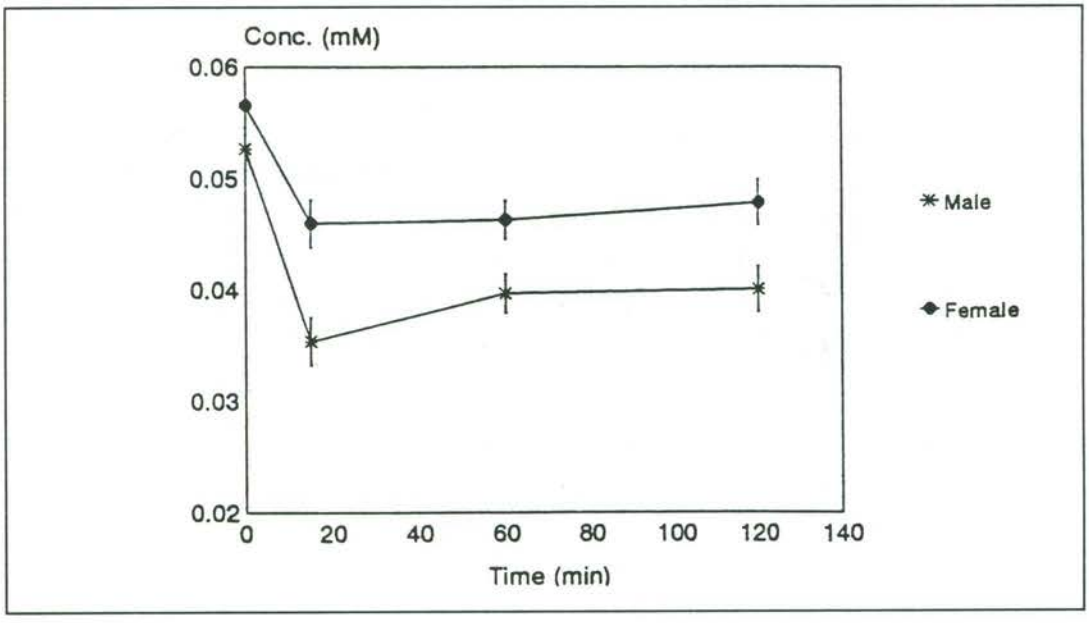


Fig.105: Pyruvate concentrations (mM) by gender.

3.8.4.6. BLOOD LACTATE:PYRUVATE RATIO

Differences in the lactate:pyruvate ratio by gender did not reach levels of statistical significance (see section 3.8.8. for data tables).

3.8.5. PHYSIOLOGICAL VARIABLES

Highly significant gender-related differences were found for systolic blood pressure and facial temperature after ethanol. Changes in heart rate after ethanol also differed significantly between the sexes.

No significant differences were seen for diastolic blood pressure, however.

3.8.5.1. HEART RATE

A significant gender-related difference was found for heart rate ($F_{1,103}=5.64$, $p<0.05$) -Fig.106. Female subjects had higher heart rates than males in the pre-ethanol measurements and recorded higher heart rates after ethanol.

In all subjects, the heart rate increased after ethanol. Recovery was faster in male subjects (by t_{15}) compared to females, in whom recovery began later (at t_{60}).

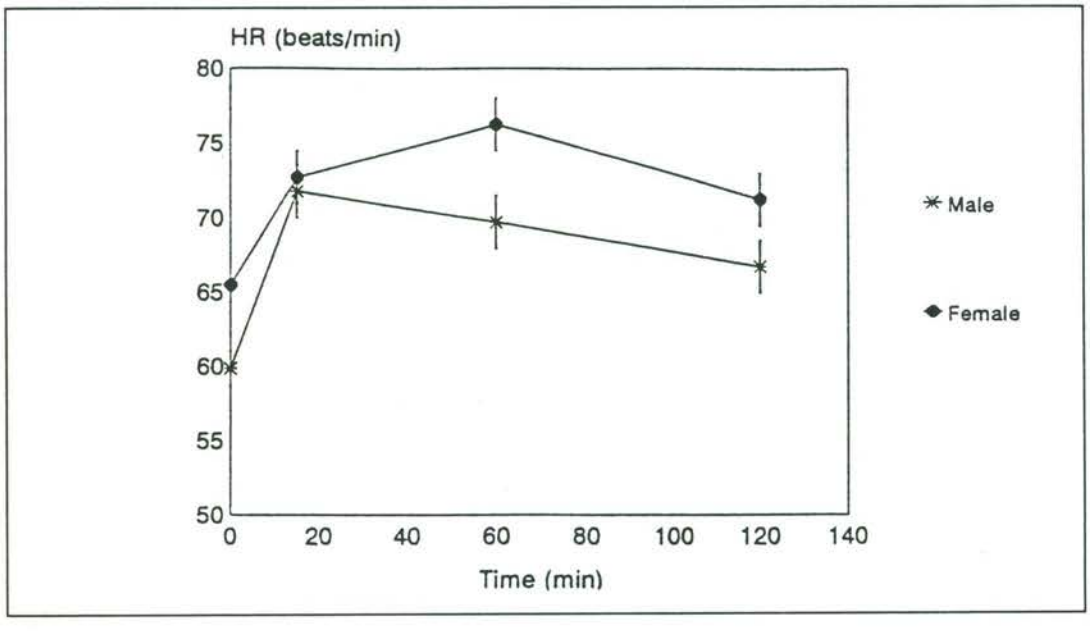


Fig.106: Heart rate by gender.

3.8.5.2. BLOOD PRESSURE

There was a highly significant difference between the systolic blood pressure of males and females ($F_{1,103}$=25.04, $p<0.005$). Males had a higher mean systolic blood pressure than that of female subjects, even before ethanol was given. The systolic blood pressure did not change markedly after ethanol, but there was a slight fall in blood pressure in both males and females (Fig.107).

Male-female differences in diastolic blood pressure did not reach statistical significance (see section 3.8.8. for data tables). After ethanol, the diastolic blood pressure fell to a trough at t_{60}, after which time an increase occurred in both groups. Male subjects had higher overall blood pressures than female subjects, the changes associated with this group also being much greater than the other group.

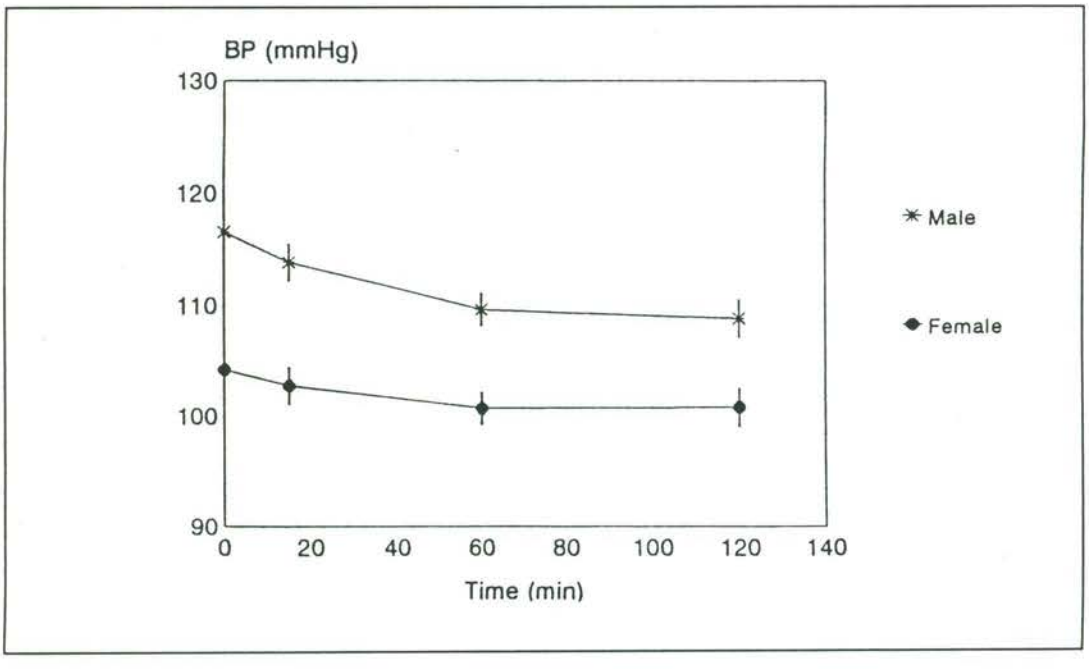


Fig.107: Systolic blood pressure by gender.

3.8.5.3. FACIAL TEMPERATURE

A highly significant time by group interaction was detected in facial temperature ($F_{3,309}$=6.04, $p<0.005$) -Fig.108. Male subjects generally exhibited slightly higher temperatures than their female counterparts, although the increase in temperature after ethanol was greater for female subjects.

Post-hoc analysis revealed that the male subjects were highly significantly affected after ethanol treatment at 15 ($q_{8,309}$=10.09, $p<0.005$), 60 ($q_{6,309}$=9.52, $p<0.005$) and 120 ($q_{4,309}$=5.81, $p<0.005$) minutes. Female subjects were also highly significantly affected at 15 ($q_{5,309}$=16.3, $p<0.005$), 60 ($q_{7,309}$=17.63, $p<0.005$) and 120 ($q_{3,309}$=11.03, $p<0.005$) minutes after ethanol

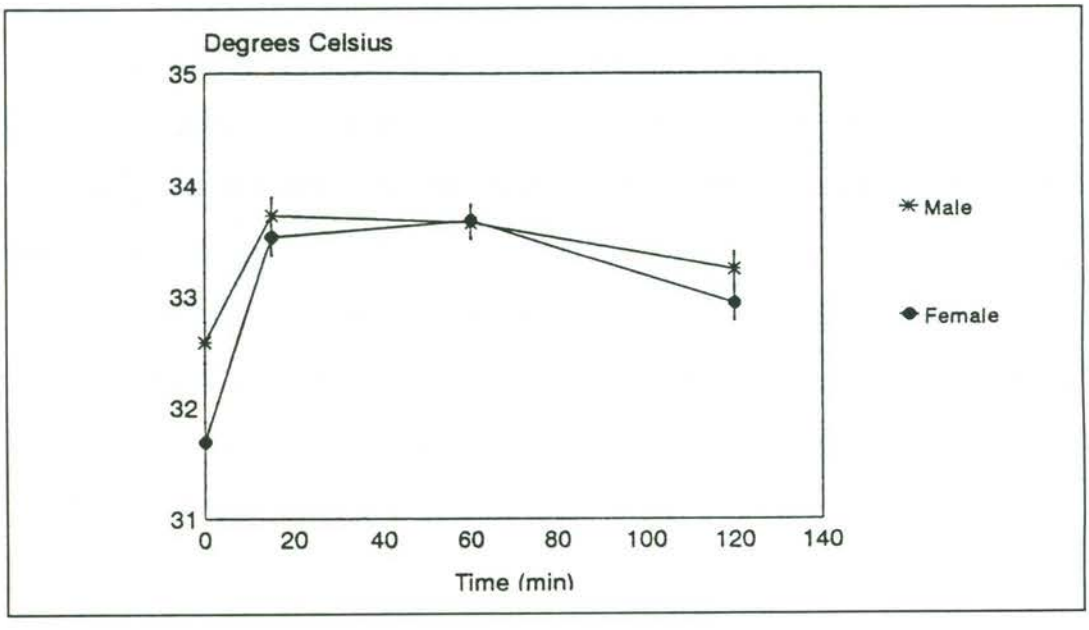


Fig.108: Facial temperature by gender.

3.8.6. PSYCHOMOTOR VARIABLES

Highly significant male-female differences were found for the standing steadiness, divided attention delay and reaction time tasks. A significant difference in the time by group interaction effect was also found for the delay component.

Other psychomotor variables measured, including CFFF threshold, DSC and divided attention excursion values, showed no significant gender-related differences.

3.8.6.1. DIVIDED ATTENTION

Delay A highly significant gender-related difference was found ($F_{1,102}=10.5$, $p<0.005$) in divided attention delay and the time by group interaction reached significance ($F_{3,306}=2.65$, $p<0.05$).

Female subjects had higher overall delay times than male subjects and the change after ethanol was greater than in the male subjects. After ethanol, both males and females demonstrated an increase in delay times; male subjects showing a small recovery at t_{60} (Fig.109).

Post-hoc analysis revealed that the male subjects were not significantly impaired after ethanol although the female subjects exhibited significant impairment at 15 ($q_{6,306}=4.87$, $p<0.05$), 60 ($q_{8,306}=6.11$, $p<0.05$) and 120 ($q_{7,306}=5.77$, $p<0.05$) minutes after ethanol.

Excursion Gender-related divided attention excursion differences were not statistically significant (see section 3.8.8. for data tables). In general, there was an increase in excursion after ethanol with recovery beginning at t_{15}. Male subjects were initially affected more than the females, but their recovery was faster and performance returned closer to pre-ethanol values than that of the female subjects. Female subjects exhibited a second increase in excursion at t_{120}.

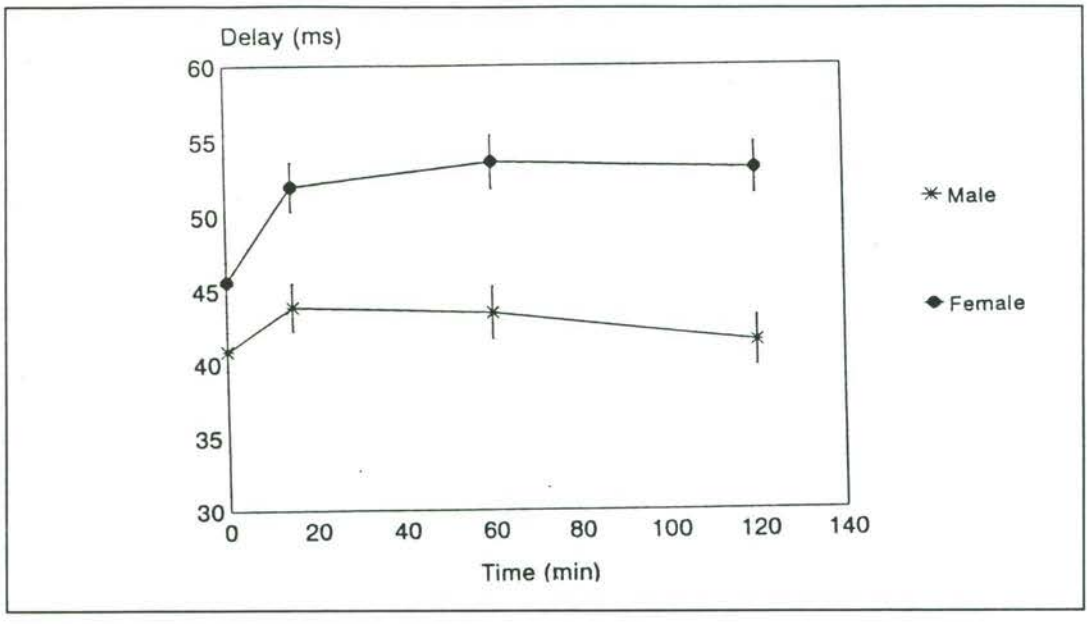


Fig.109: Divided attention delay by gender.

Reaction Time A highly significant gender-related difference ($F_{1,102}=8.85$, $p<0.005$) was found in the divided attention peripheral reaction time task. The reaction times of female subjects were longer than those of the males at all time-points, both before and after ethanol.

The overall pattern of response however was similar in that, after ethanol, an increase in reaction time occurred in both males and females. Peak effect occurred at t_{15} after ethanol, after which time the reaction times improved towards pre-ethanol levels (Fig.110).

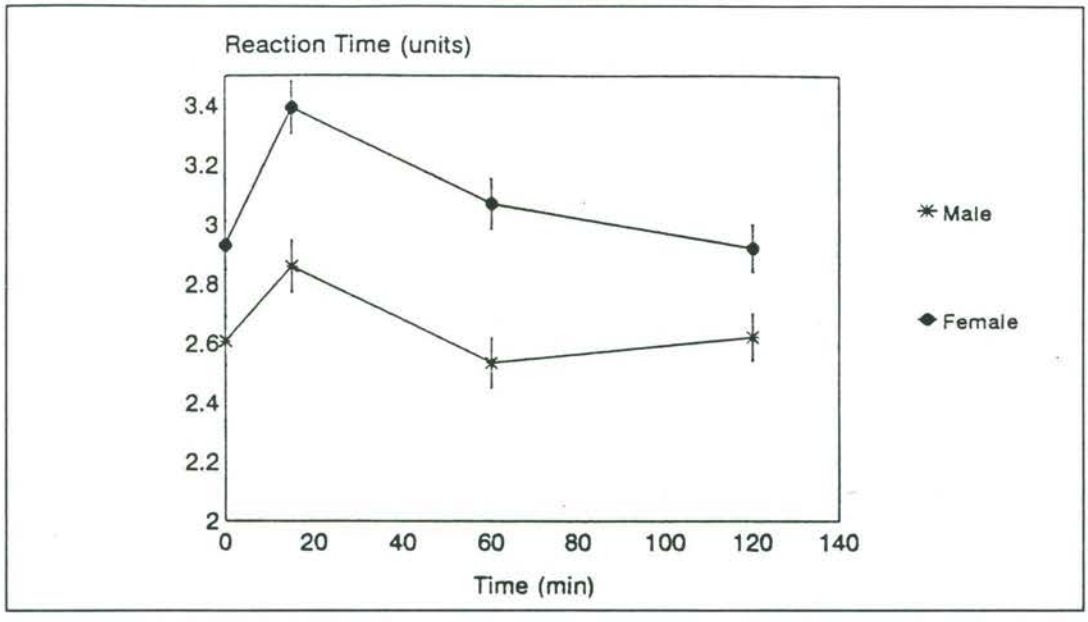


Fig.110: Divided attention reaction time by gender.

3.8.6.2. DIGIT SYMBOL CODING

Gender-related differences found in the DSC reaction time did not reach statistical significance (see section 3.8.8. for data tables). After ethanol, a transient increase in DSC reaction time was seen in both males and females followed by a prompt recovery towards pre-ethanol values. Female subjects were affected to a greater extent than the males. Females had longer DSC reaction times immediately after ethanol, which remained for the duration of the test session.

3.8.6.3. STANDING STEADINESS

A highly significant gender-related difference was found ($F_{1,103}=10.37$, $p<0.005$) for standing steadiness (Fig.111). Female subjects were steadier, with longer epoch times, than their male counterparts although the basic pattern of response of both sexes was similar. Ethanol consumption caused all subjects to become less steady, with the worst performance at t_{15}. After this time, recovery towards initial values occurred in both sexes.

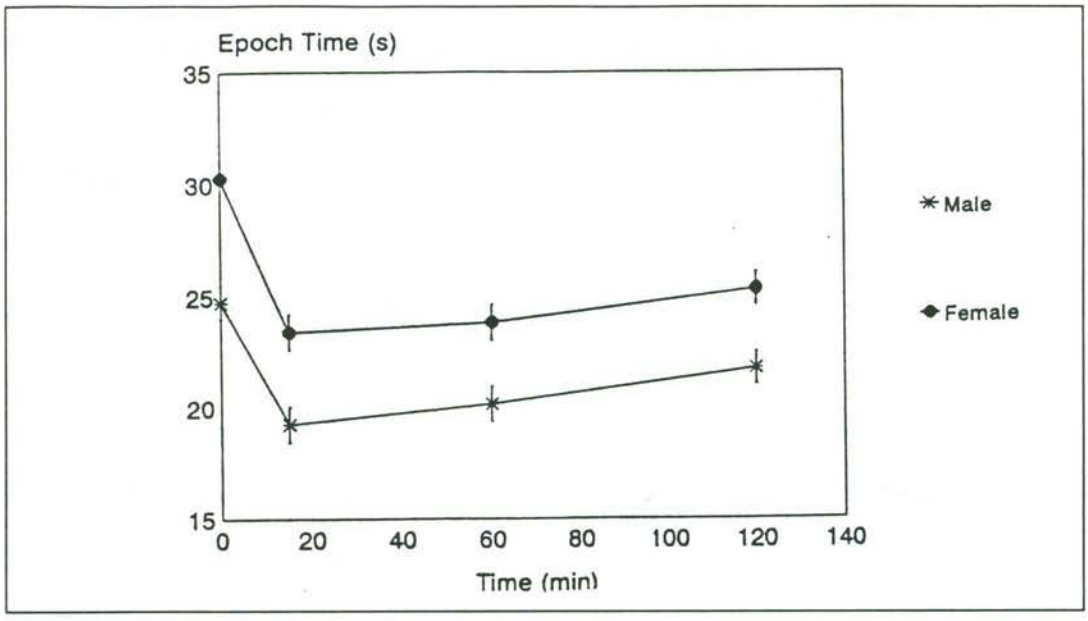


Fig.111: Standing steadiness by gender.

3.8.5.4. CRITICAL FLICKER FUSION FREQUENCY

Male-female differences found for CFFF threshold values were not statistically significant (see section 3.8.8. for data tables). In general, after ethanol consumption, the CFFF threshold decreased to its lowest level at t_{60}, and then returned back to near-normal levels by t_{120}. Male subjects had the highest overall CFFF threshold values, and exhibited greater changes after ethanol.

3.8.7. SUBJECTIVE MEASURES OF INTOXICATION

No significant differences were found between the two sexes in the subjective responses to questions which probed as to whether or not they would like another drink and their levels of alertness, co-ordination, concentration, intoxication, attentiveness, headache, nausea or hangover-like effects.

A significant difference ($F_{1,104}$=4.27, $p<0.05$) was detected between the males and females in the response to the question on whether or not the subject would drive a car after drinking (Q1). Both groups initially responded by saying 'no', although by t_{120}, both groups were less likely to answer in the negative (Fig.112).

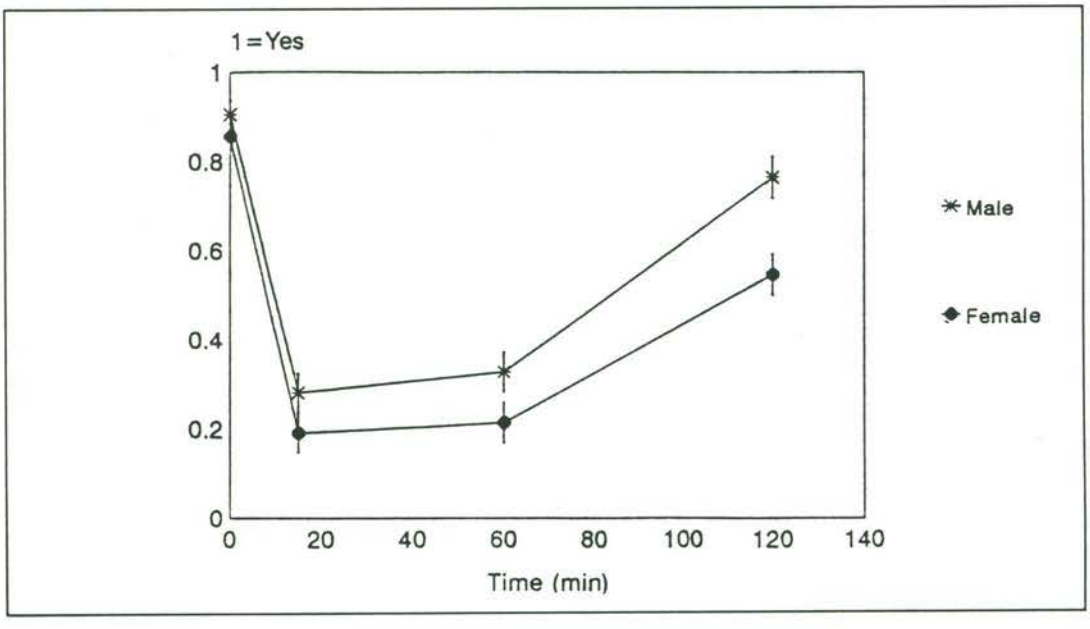


Fig.112: 'Would you drive a car now?' subjective response based on gender.

Female subjects were more likely to answer 'no' after drinking, this effect was still evident at t_{120} min. This difference might have been due to a general lack of confidence in driving of the female subjects (many were quite young and

inexperienced drivers), or it might have been due to the generally more careful and cautious nature of female subjects compared to male subjects (female subjects during testing tended to want to be more sure of the procedures than their male counterparts who just wanted to finish the session quickly and see their results).

3.8.8. SUMMARY OF OTHER RESULTS BY GENDER (NON-SIGNIFICANT)

The results for each variable below are listed in the following manner for each time-point (t_0, t_{15}, t_{60}, t_{120}):

Male mean ± SD; n=64

Female mean ± SD; n=42

VARIABLE	t_0	t_{15}	t_{60}	t_{120}
Acetaldehyde	37.2 ± 34.8	56.7 ± 53.1	51.8 ± 49.8	54.1 ± 47.4
(μM)	36.3 ± 41.4	49.0 ± 44.9	48.8 ± 43.9	48.4 ± 50.3
Acetate (mM)	-0.02 ± 0.14	0.35 ± 0.68	0.37 ± 0.33	0.36 ± 0.23
	-0.03 ± 0.15	0.36 ± 0.25	0.36 ± 0.21	0.33 ± 0.21
Ethanol (mM)	0 ± 0	8.8 ± 2.8	8.5 ± 1.5	5.6 ± 1.2
	0 ± 0	9.0 ± 2.5	8.3 ± 1.7	5.0 ± 1.7
Lactate (mM)	1.3 ± 1.0	1.6 ± 1.0	1.7 ± 0.8	1.7 ± 1.0
	1.5 ± 1.1	1.8 ± 1.0	1.8 ± 1.0	1.8 ± 1.0
Lac:Pyr Ratio	27.8 ± 35.0	59.1 ± 88.8	46.3 ± 26.1	61.7 ± 111.2
	26.9 ± 15.4	43.4 ± 22.9	45.9 ± 40.5	42.3 ± 23.7
Blood Pressure	74.6 ± 10.7	69.3 ± 11.7	67.0 ± 10.6	69.3 ± 10.2
(Dia) (mmHg)	68.8 ± 9.3	67.6 ± 9.6	66.3 ± 10.5	66.1 ± 8.0
BEC (g dl^{-1})	0 ± 0	0.034 ± 0.012	0.018 ± 0.006	0.005 ± 0.005
	0 ± 0	0.033 ± 0.012	0.019 ± 0.006	0.005 ± 0.005
CFFF (Hz)	26.7 ± 2.8	26.0 ± 3.2	25.7 ± 3.3	26.4 ± 3.1
	25.6 ± 2.7	25.4 ± 3.5	25.1 ± 2.8	25.4 ± 2.8
Digit Symbol	1.6 ± 0.3	1.6 ± 0.3	1.6 ± 0.3	1.5 ± 0.3
Coding (min)	1.6 ± 0.3	1.7 ± 0.4	1.6 ± 0.4	1.6 ± 0.4
Div. Attention	3.2 ± 1.2	4.0 ± 1.2	3.4 ± 1.2	3.4 ± 1.2
(Excur.) (units)	3.4 ± 1.5	3.9 ± 1.4	3.7 ± 1.2	3.8 ± 1.5

Subjective Q2	0.8 ± 0.4	0.5 ± 0.5	0.41 ± 0.5	0.52 ± 0.5
	0.7 ± 0.5	0.4 ± 0.5	0.38 ± 0.5	0.40 ± 0.5
Subjective Q3	2.1 ± 1.8	5.0 ± 2.2	4.6 ± 2.3	3.0 ± 2.3
	2.0 ± 1.8	5.1 ± 2.2	5.0 ± 2.2	3.4 ± 2.0
Subjective Q4	1.9 ± 1.6	4.7 ± 2.1	4.3 ± 2.2	2.6 ± 1.9
	2.0 ± 2.1	4.5 ± 2.4	4.7 ± 2.2	3.1 ± 2.0
Subjective Q5	6.9 ± 1.7	4.8 ± 2.1	5.2 ± 2.0	6.4 ± 2.1
	7.2 ± 1.7	4.3 ± 2.3	4.5 ± 2.2	5.6 ± 2.1
Subjective Q6	0.08 ± 0.3	4.8 ± 2.3	4.1 ± 2.0	2.0 ± 1.9
	0.05 ± 0.2	5.2 ± 2.4	4.2 ± 2.1	2.4 ± 2.0
Subjective Q7	1.9 ± 1.7	4.7 ± 2.3	4.3 ± 2.1	2.9 ± 2.2
	1.9 ± 2.0	5.2 ± 2.2	4.7 ± 2.3	3.5 ± 2.0
Subjective Q8	-	0.13 ± 0.33	0.17 ± 0.38	0.19 ± 0.39
	-	0.19 ± 0.40	0.29 ± 0.46	0.33 ± 0.48
Subjective Q9	-	0.17 ± 0.38	0.14 ± 0.35	0.14 ± 0.35
	-	0.21 ± 0.42	0.19 ± 0.40	0.14 ± 0.35
Subjective	-	0.20 ± 0.41	0.27 ± 0.45	0.19 ± 0.39
Q10	-	0.24 ± 0.43	0.24 ± 0.43	0.33 ± 0.48

3.9. ADDITIONAL RESULTS

3.9.1. CORRECTIONS FOR t=0

After the t=0 values were subtracted from the results at other times during the test session, the findings were found to be essentially similar to those obtained *without* correcting for the t=0 value. A brief summary of the results obtained, after taking the t=0 values into account, for the biochemical aspect of the study is given below.

3.9.1.1. Effect of ADH_2 genotype

Breath ethanol levels

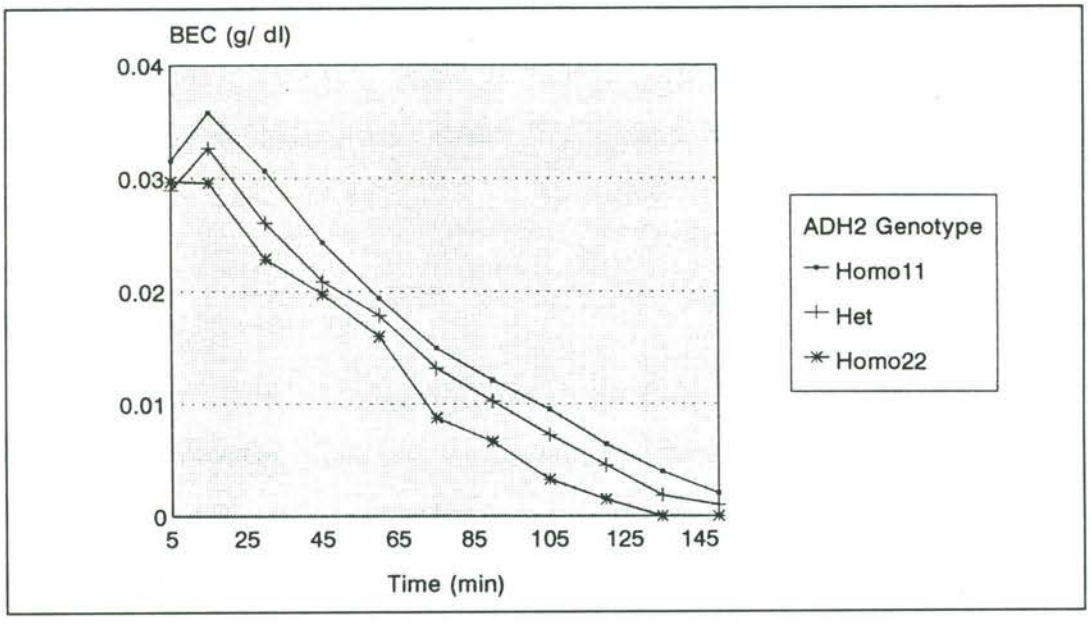


Fig.113: Mean breath ethanol readings (expressed as calculated blood ethanol concentration in g dl^{-1}) by ADH_2 type.

ADH_2 type had a significant effect ($F_{2,91}=3.67$, $p<0.05$) on the breath ethanol readings in the repeated measures ANOVA covering all post-ethanol times, and there

were significant differences at 30, 45, 105 and 135 minutes. It can be seen that the breath ethanol curves were more or less parallel, with the Homo11 having the highest mean at all times and the Homo22 being lowest at all times (Fig.113).

Plasma ethanol levels

Plasma ethanol values tended to reaffirm the breath results but showed only borderline statistical significance ($F_{2,101}$=2.94, p=0.057). As with the breath ethanol results, Homo11 subjects showed the highest values and Homo22 subjects had the lowest.

Blood Acetaldehyde/ Plasma Acetate

ADH_2 genotype had no significant effects on blood acetaldehyde ($F_{2,102}$=0.10, p>0.05) or plasma acetate ($F_{2,98}$=0.46, p>0.05) concentrations after ethanol.

Blood Lactate:Pyruvate Ratio

Before ethanol consumption, in the fasting state, there was a significant difference between the ADH_2 genotype groups for blood pyruvate ($F_{2,102}$=3.32, p<0.05), but not for lactate or the lactate:pyruvate ratio. Pyruvate concentration was found to be highest in the Homo22 group and lowest in the Het group. After ethanol there was no significant effect of ADH_2 genotype on lactate, pyruvate or on the lactate:pyruvate ratio.

3.9.1.2. Effect of $ALDH_2$ genotype

Breath ethanol levels

Breath ethanol readings showed a borderline-significant difference ($F_{2,91}$=2.98, p=0.056) among the three $ALDH_2$ groups. The value for Homo11 subjects was on average 0.0026 g dl^{-1} lower than those for the Het and Homo22 groups (which were similar in their values) -Fig.114.

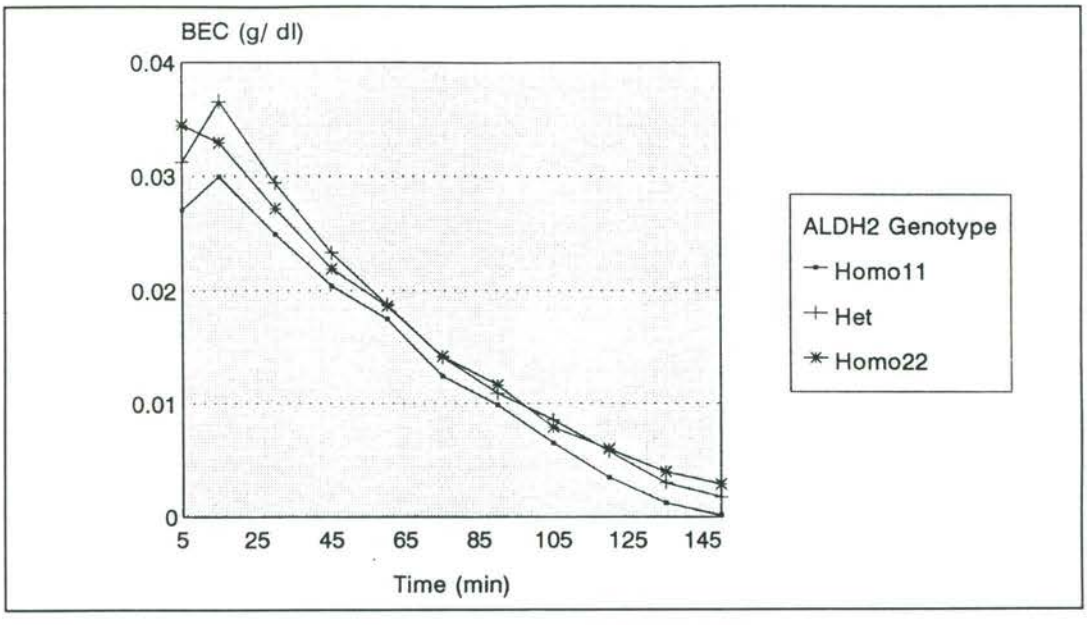


Fig.114: Mean breath ethanol readings (expressed as calculated blood ethanol concentration in g dl^{-1}) by $ALDH_2$ type.

Plasma ethanol levels

The plasma ethanol results showed no significant group-related differences ($F_{2,101}=0.80$, $p>0.05$).

Blood Acetaldehyde

A highly significant difference ($F_{2,102}=7.23$, $p<0.005$) was found in the blank-corrected acetaldehyde measurements. Both Homo22 and Het groups had increases in acetaldehyde levels, compared to Homo11, up to 120 minutes after ethanol. The greatest increase was found in the Homo22 group (Fig.115).

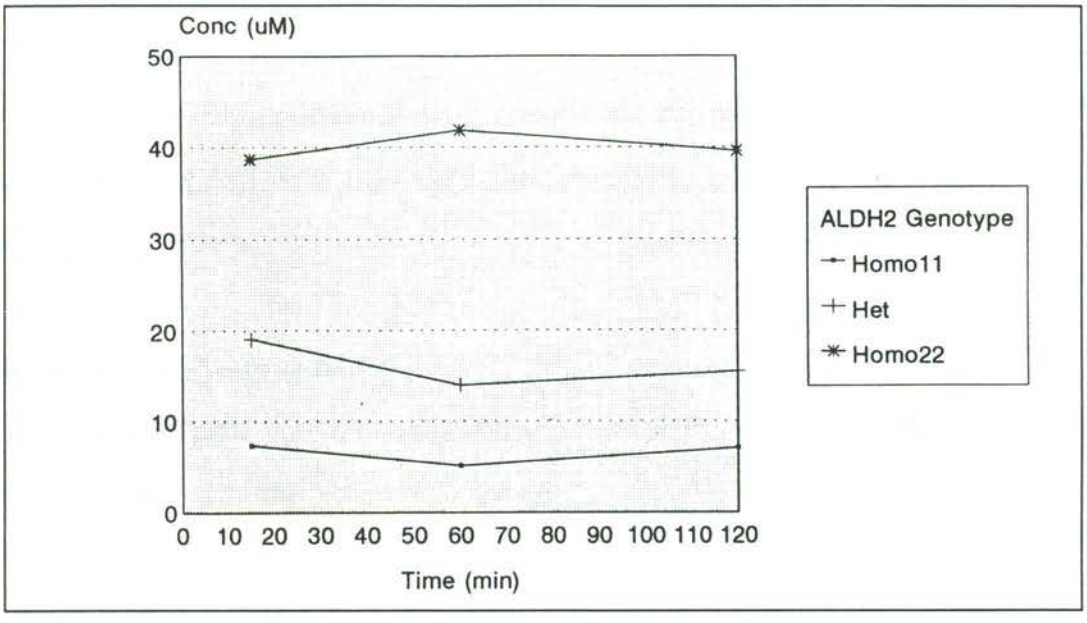


Fig.115: Blood acetaldehyde concentration after ethanol consumption by $ALDH_2$ genotype.

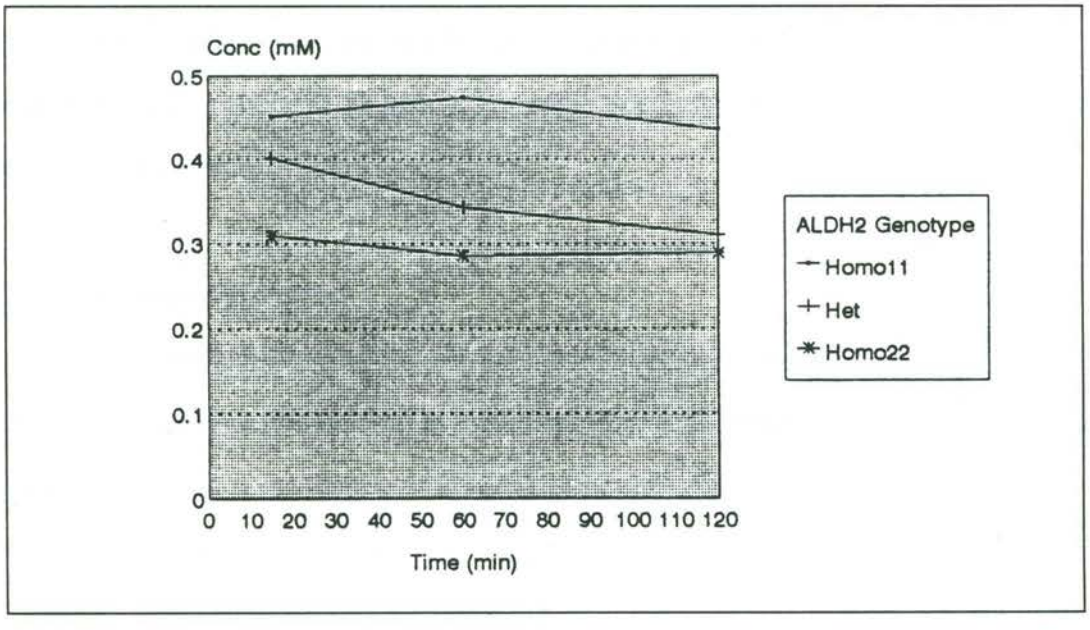


Fig.116: Plasma acetate concentration after ethanol consumption by $ALDH_2$ genotype.

Plasma Acetate

Acetate concentrations showed a significant difference ($F_{2,98}$=4.30, $p<0.05$) among the three $ALDH_2$ groups, the highest values being found for the Homo11 subjects (Fig.116).

Blood Lactate:Pyruvate Ratio

$ALDH_2$ genotype had no significant effects on lactate or pyruvate concentrations or on the lactate:pyruvate ratio.

3.9.1.3. $ALDH_2$/ ADH_2 interactions

There was no significant association between ADH_2 and $ALDH_2$ genotype frequencies in this group of subjects (chi-square=1.88, 4df, $p>0.05$). When the subjects were divided into three groups according to their $ALDH_2$ genotypes, no significant effects of ADH_2 type on acetaldehyde or acetate concentrations after ethanol could be shown in any $ALDH_2$ group. Due to the small number of subjects with ADH2-22 or ALDH2-22 genotypes, some interaction of the type found for flush intensity cannot be ruled out.

3.9.1.4. Gender differences

No significant differences were found between men and women in ADH_2 or $ALDH_2$ genotype frequencies, in the breath or plasma ethanol values, acetaldehyde, acetate, lactate or in the degree of flushing, although differences in pyruvate levels were found to be significant ($F_{1,102}$=7.19, $p<0.01$).

3.9.2. CORRELATIONS

3.9.2.1. Rate of decrease in BEC by ADH_2 and $ALDH_2$ genotype

Derivation of the pharmacokinetic variables C_0, V_{max} and K_m for individual subjects' breath ethanol readings was not successful. In many cases, the estimated error was greater than the estimated value. Similarly, estimation of these variables from the average breath ethanol concentrations for each ADH_2 genotype group did not produce very reliable results. For all subjects regardless of genotype, the estimated values were 34.4 mg $100ml^{-1}$ for C_0, 19.4 mg $100ml^{-1}$ $hour^{-1}$ for V_{max} and 4.5 mg $100ml^{-1}$ (0.98 mM) for K_m.

The rate of decrease in blood ethanol was estimated for each subject by averaging the rate of fall over the three time periods 30-90, 45-105 and 60-120 minutes. With this procedure, no significant difference in rate of decline in breath ethanol reading by $ALDH_2$ or ADH_2 genotype could be detected (ADH_2 type, $F_{2,99}=0.81$ $p>0.05$, $ALDH_2$ type, $F_{2,99}=1.38$ $p>0.05$).

3.9.2.2. Rate of ethanol metabolism and steady-state metabolite concentrations

Faster metabolism of ethanol in a linear metabolic pathway, such as that from ethanol to acetyl CoA, should lead to higher steady-state levels of acetaldehyde, acetate, and NADH (represented by the lactate:pyruvate ratio) when metabolite fluxes and enzyme kinetics are considered.

In the current study we tested whether this flux-concentration dependence could be shown in a group of over 100 individual subjects. The results were statistically significant for lactate:pyruvate ratio -there was a significant correlation with rate of ethanol metabolism ($r=0.233$, $p<0.05$). For mean acetate values the correlation ($r=0.191$) was only marginally significant ($p=0.064$).
Ethanol metabolism rate was not significantly correlated with the steady-state acetaldehyde levels (the mean of t_{15}, t_{60} and t_{120} values); $r=0.005$, $p>0.05$.

3.9.2.3. Acetaldehyde concentration vs flush intensity

Since the AFR is thought to be caused by acetaldehyde accumulation, the correlation between flush intensity and acetaldehyde concentration (mean of the t_{15}, t_{60} and t_{120} values) was tested. When all subjects were taken into consideration, a significant correlation was found ($r=0.337$, $p<0.001$).

However, because variation in acetaldehyde concentration is mainly due to $ALDH_2$ genotype, the correlation was also tested separately for the three $ALDH_2$ groups to see whether variation in acetaldehyde not caused by $ALDH_2$ genotype was important in determining the degree of flush. No significant effect was found in the ALDH2-11 group ($r=0.133$, $p>0.05$) but in the $ALDH_2$ heterozygotes there was a significant correlation ($r=0.315$, $p<0.05$). In the ALDH2-22 group the correlation was higher but, due to smaller numbers, it was not statistically significant ($r=0.410$, $n=12$, $p>0.05$).

3.9.3. EFFECT OF GENOTYPE ON FLUSH INTENSITY

The $ALDH_2$ genotype had a highly significant effect on the intensity of the flush reaction ($p<0.0001$, Kruskal-Wallis test), although neither gender nor ADH_2 genotype showed any significant effects.

However, the effect of ADH_2 genotype varied according to the subjects' $ALDH_2$ type (Fig.117). In the subjects who were either ALDH2-11 or -22 homozygotes, ADH_2 type had no significant effect on the intensity of flushing but in the $ALDH_2$ heterozygotes there was a significant difference detected ($p<0.01$, Kruskal-Wallis test).

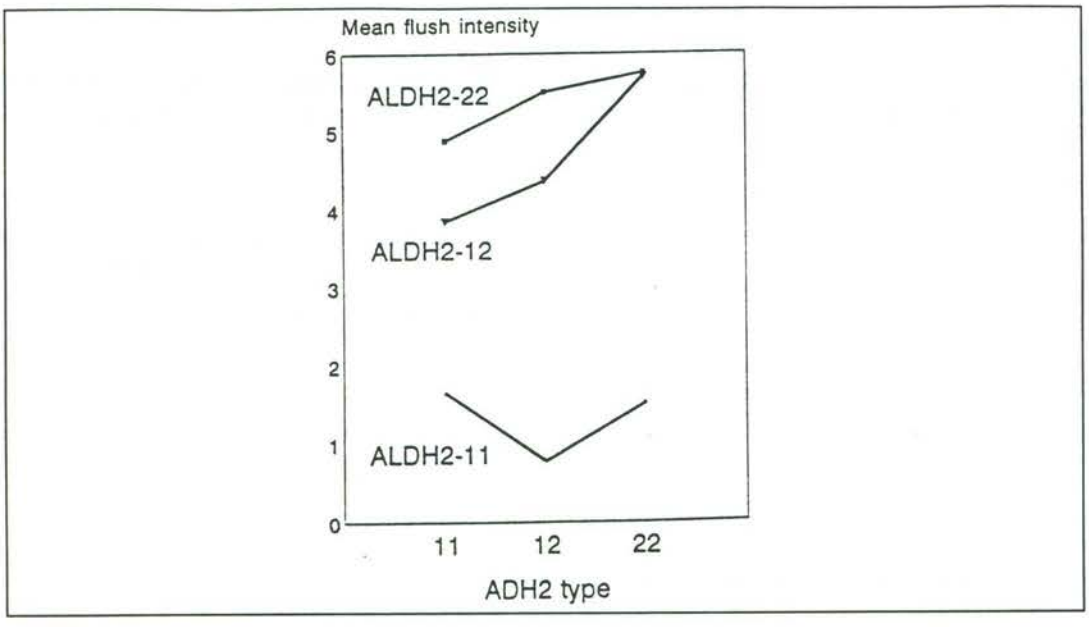


Fig.117: The effects of ADH_2 type on mean flush intensity in the different $ALDH_2$ groups.

3.10. SUMMARY OF SIGNIFICANT RESULTS

GROUP TESTED	PARAMETER	GROUP	TIME BY GROUP
ADH_2			
	BIOCHEMISTRY		
	Pyruvate	Significant	n.s.
	PHYSIOLOGY		
	Heart rate	Highly significant	n.s.
	PSYCHOMOTOR		
	CFFF	Strong trend towards significance.	n.s.
	Standing steadiness	Significant	n.s.
	SUBJECTIVE		
	Q9	n.s.	Significant
	Q10	Significant	Significant
$ALDH_2$			
	BEC	Significant	Highly significant
	BIOCHEMISTRY		
	Acetaldehyde	n.s	Highly significant
	Acetate	Highly significant	n.s.
	PHYSIOLOGY		
	Temperature	Highly significant	Highly significant
	Heart rate	Highly significant	n.s.

	PSYCHOMOTOR		
	CFFF	n.s.	Highly significant
	DSC	Significant	Significant
	Standing steadiness	n.s.	Strong trend towards significance.
	Divided Attention - Delay	Significant	Highly significant
	Excursion	Significant	n.s.
	SUBJECTIVE		
	Q1	Highly significant	n.s.
	Q2	Highly significant	Significant
	Q3	Highly significant	Significant
	Q4	Highly significant	Highly significant
	Q5	n.s.	Significant
	Q6	Highly significant	Highly significant
	Q7	Highly significant	Highly significant
	Q8	Highly significant	Significant
	Q9	n.s.	Highly significant
	Q10	Significant	Highly significant
DEGREE OF FLUSH	BIOCHEMISTRY		
	Acetaldehyde	n.s.	Highly significant
	Acetate	Highly significant	n.s.
	PHYSIOLOGY		
	Temperature	Highly significant	Highly significant
	Heart rate	Highly significant	n.s.
	PSYCHOMOTOR		
	DSC	n.s.	Strong trend towards significance
	Divided Attention - Delay	n.s.	Highly significant

	SUBJECTIVE		
	Q1	Highly significant	n.s.
	Q2	Highly significant	n.s.
	Q3	Highly significant	Significant
	Q4	Highly significant	Significant
	Q6	Highly significant	Highly significant
	Q7	Highly significant	Highly significant
	Q8	Significant	n.s.
	Q9	n.s.	Significant
	Q10	n.s.	Significant
$ALDH_2$/ ADH_2 COMBINED	BEC	Highly significant	Highly significant
	BIOCHEMISTRY		
	Acetaldehyde	n.s.	Significant
	Plasma Ethanol	n.s.	Significant
	PHYSIOLOGY		
	Temperature	Significant	Highly significant
	Heart Rate	Highly significant	n.s.
	PSYCHOMOTOR		
	CFFF	n.s.	Significant
	DSC	Significant	n.s.
	Divided Attention -		
	Delay	Significant	Highly significant
	Excursion	Significant	n.s.

SUBJECTIVE		
Q1	Highly significant	n.s.
Q2	Significant	n.s.
Q3	Significant	n.s.
Q4	Significant	Highly significant
Q6	Highly significant	Highly significant
Q7	Highly significant	Highly significant
Q8	Highly significant	n.s.
Q9	n.s.	Highly significant
Q10	Highly significant	Highly significant

GENDER

BIOCHEMISTRY		
Pyruvate	Significant	n.s.
PHYSIOLOGY		
Temperature	n.s.	Highly significant
Systolic blood pressure	Highly significant	n.s.
Heart rate	Significant	n.s.
PSYCHOMOTOR		
Standing steadiness	Highly significant	n.s.
Divided Attention - Delay	Highly significant	Significant
Reaction time	Highly significant	n.s.
SUBJECTIVE		
Q1	Significant	n.s.

ADDITIONAL FINDINGS (t=0 corrected)	**BEC**		
	ADH_2	Significant	n.s.
	$ALDH_2$	Strong trend towards significance.	n.s.
	Acetaldehyde		
	$ALDH_2$	Highly significant	n.s.
	Plasma Ethanol		
	ADH_2	Strong trend towards significance.	n.s.
	Pyruvate		
	GENDER	Significant	n.s.

n.s. = not significant

Chapter 4.

DISCUSSION & CONCLUSIONS

Flushing after the consumption of only modest doses of ethanol is well-known to be common among people of Asian descent and can be a highly unpleasant and incapacitating experience which could be manifest as an increase in traffic-crash risk, extending to other potentially hazardous situations. Not all Asians flush after ethanol, however, and evidence that the flush reaction depends, to a large extent, on the aldehyde dehydrogenase ($ALDH_2$) genotype of the individual has been reviewed in Chapter 1.

Having determined the $ALDH_2$ and alcohol dehydrogenase (ADH_2) genotypes of a large (n=110) cohort of volunteers of full or partial Asian descent, they were subjected to a low-dose (0.3 g kg^{-1}) ethanol challenge. The nature and extent of the flush reaction was monitored over time, as were the effects of ethanol on driving-related psychomotor performance and physiological (facial temperature, blood pressure and heart rate), biochemical and subjective responses. Biochemical measures included the concentrations of ethanol and acetate in plasma and acetaldehyde, lactate and pyruvate in blood. Changes in these parameters after ethanol were related to $ALDH_2$ and ADH_2 genotype(s), 'Degree of Flush' and to gender. In particular, it was intended to compare differences between:

- the three ADH_2 groups, and to determine if responses could be graded according to allele type (Homozygote-11 (Homo11), Heterozygote (Het) or Homozygote-22 (Homo22)).

- the three $ALDH_2$ groups, and to determine if responses could be graded according to allele type (Homo11, Het or Homo22).

- the observer-rated 'Degree of Flush' groups, and to determine if responses could be rated according to the designated groups.

- the combinations of the $ALDH_2$ and ADH_2 enzymes, and to determine which of these combinations was associated with the greatest response to ethanol.

- male and female subjects in terms of susceptibility to ethanol.

- drinking patterns and a number of demographic variables including country of origin and the extent of assimilation into the host country.

Also, it was of interest to consider whether any of the differences found could be attributed to diet, other drug interactions or cultural/ social differences not investigated above.

A discussion of the results of this study and other related studies conducted in the past follows below. Before proceeding, it should be noted here that several aspects of this study have not been investigated by others in the past and so previous literature may be lacking.

This Chapter is organized into several sections, beginning with a discussion of aspects of the experimental procedure and methodology, including various difficulties which were encountered and resolved. The findings of this study are discussed in terms of 'Degree of Flush' and genotype, ethanol pharmacokinetics and biochemical, physiological, psychomotor and subjective responses. A General Discussion of broader issues, including ethanol sensitivity in Asians, ADH_2 genotype and the risk of ethanol dependence and future research in this area is also given towards the end of this Chapter. A Conclusion, with important practical implications for the findings of this study, completes this thesis.

4.1. METHODOLOGICAL CONSIDERATIONS

The ADH_2 and $ALDH_2$ Combination Genotype Groups

Originally, when this study was planned, it was intended to ethanol challenge equal numbers of subjects in the nine possible ADH_2 and $ALDH_2$ combination groups. In practice, however, this aim proved to be unachievable because some subjects, notably those with $ALDH_2$ Homo22 and/ or ADH_2 Homo22 genotypes, were few in number. Low numbers in some of the cells created potential difficulties for statistical analysis and it proved necessary to redefine the groups. The following five groups were compared:

GroupA	$ALDH_2$ Homo11/ ADH_2 Homo11
GroupB	$ALDH_2$ Het/ ADH_2 Homo11
GroupC	$ALDH_2$ Homo22/ ADH_2 Homo11, Het or Homo22
GroupD	$ALDH_2$ Homo11/ ADH_2 Het
GroupE	$ALDH_2$ Het/ ADH_2 Het

It is not known whether $ALDH_2$ Homo22 and/ or ADH_2 Homo22 genotypes are really uncommon in the target population or whether these individuals would be inherently unlikely to volunteer for an ethanol study because of previous unpleasant experiences with the drug. If the latter case pertains, it might be viewed as 'protective' influence against excessive ethanol consumption. Most of the subjects who participated in this study were teetotalers or very occasional drinkers and some of them, especially those of the $ALDH_2$ Homo22 genotype, found the experience to be sufficiently aversive to preclude ethanol consumption in the future. Some of the subjects who were most affected by ethanol did have previous drinking experience but they generally consumed small amounts of ethanol with food over a long period and then slept, thus minimizing any reaction which might otherwise have occurred.

Some Issues with the Experimental Methodology

The Questionnaire

One of the questionnaire responses, concerning the quantity of ethanol habitually consumed per drinking session, posed problems of interpretation in that the time-frame of drinking was not specified. It emerged in conversation with the subjects that although for some, drinking sessions lasted for several hours, others habitually consumed a single drink over a much shorter period. More precise drinking details were therefore elicited from the subjects during the testing sessions.

The menstrual cycle

A deliberate decision was made not to establish the menstrual cycle stage of female subjects on the grounds of perceived cultural sensitivity.

Acetaldehyde estimation in blood

Acetaldehyde concentrations are technically difficult to measure in blood (Eriksson, 1980) and methods used in the past have been problematic both because of the artifactual production of acetaldehyde, and also because of the rapid disappearance and/ or binding of acetaldehyde to various blood constituents after sample withdrawal. Acetaldehyde can form stable adducts with many proteins including haemoglobin (Stevens, Fantl, Newman, Sims, Cerami & Peterson, 1981) and plasma membrane proteins (Barry, Williams & McGivan, 1987) and such reactions would inevitably influence the acetaldehyde concentration. In this study, it was found necessary for the pre-ethanol acetaldehyde concentrations to be subtracted from the post-ethanol values before a statistically significant effect of $ALDH_2$ type could be identified.

HPLC was normally carried out within 24 hrs of sample extraction. On one occasion, however, a malfunction of the HPLC system necessitated refrigeration of the samples (wrapped in Parafilm) for a further 48 hours. There was no apparent effect on the samples.

Genotyping

The main problems which were encountered in the genotyping of subjects related to the length of time required to process each sample (approximately two days for ADH_2 genotyping and one day for $ALDH_2$ -not including DNA extraction time). It should be noted, however, that compared with methods used in the past, the present procedures used were fast and reliable.

Some contamination at the PCR amplification stage also occurred from time to time. This was easily detected by the use of controls (i.e. amplification of the whole mixture *without* the presence of subject DNA); isolating each reagent to try to trace the source of contamination. This procedure was very time consuming, however.

Nervousness?

The subject's feeling of anxiety and of 'not knowing what to expect' at the start of the testing session might have affected the subject's initial response so that the adverse effects of the low-dose of ethanol administered were not readily detectable. However, a practice period was allowed before the actual testing session commenced to minimize the probability of this occurring.

4.2. DEGREE OF FLUSH AND GENOTYPE FINDINGS

(a) Grouped by ADH_2 genotype

The relationship between flushing pattern and ADH_2 genotype has not been as extensively studied as that with the $ALDH_2$ enzyme. Possession of the inactive form of ALDH is well-known to be associated with the alcohol flush reaction (AFR) but there is little information concerning the relationship between ADH_2 genotype and the propensity to flush after ethanol.

In this study, the flushing intensity was found to increase across the three ADH_2 Homo11, Het and Homo22 groups, with the Homo22 subjects exhibiting the greatest degree of flush. This finding is in accord with the suggestion of Thomasson, Zeng, Mai, McGarvy, Deka & Li (1994) that possession of the ADH_2 genotype might result in an intensification of the alcohol flush reaction.

It is possible that the rate of ethanol metabolism in ADH_2 subjects might differ according to whether they are Homo22, Het or Homo11. That is, if the rate of ethanol elimination is relatively rapid (as in ADH_2 Homo22 subjects), the metabolism of ethanol to acetaldehyde would also be expected to be rapid. If this was combined with a slow metabolism of acetaldehyde to acetate (as in $ALDH_2$ Homo22 subjects) this could result in a build-up of acetaldehyde. This is a possible explanation for the highly intense flushing seen in this study when ethanol was given to subjects with the $ALDH_2$ and ADH_2 Homo22 enzyme types. Support for this hypothesis is given by the findings of Shibuya, Yasunami & Yoshida (1989) who reported that the allele frequency of ADH2-2 was higher in flushing subjects than in non-flushing subjects.

(b) Grouped by $ALDH_2$ genotype

In this study, the degree of flush was greatest in the $ALDH_2$ Homo22 subjects, intermediate in the Het and lowest in those with the Homo11 genotype. This finding is consistent with those of Enomoto *et al.* (1991) and Wall *et al.* (1992) who reported that Homo22 subjects suffered more severe adverse reactions to ethanol than Het subjects.

The adverse effects experienced and reported by Homo22 subjects in this study included severe tachycardia, which developed soon after the ethanol challenge, and was quickly followed by headache, nausea and syncope (presumably due to postural hypotension). The Het subjects reported similar but much less severe effects after ethanol. The Het subjects did not faint and the tachycardia and blood pressure changes were much less pronounced than in the Homo22 subjects.

Contrary to what might have been anticipated from the results of previous studies (e.g. Harada *et al.*, 1982; Goedde *et al.*, 1983), in this study it was found that more than half of the $ALDH_2$ Homo11 subjects also experienced some degree of facial flushing after ethanol. However, the subjectively rated degree of flush, although variable, was generally not as intense (most subjects rated only 1-2) as that in the Het and Homo22 subjects. This finding is consistent with that of Thomasson *et al.* (1993) who mentioned that some Asians with the $ALDH_2$ Homo11 genotype reported flushing after ethanol. The relatively mild nature of the reaction might also explain that flushing *per se* does not have the same deterrent effects on drinking in Homo11 as in the Homo22 or Het subjects.

(c) Grouped by $ALDH_2$/ ADH_2 combination genotype

In the past, cumbersome genotyping techniques appear to have hindered the investigation of the relationship between the alcohol flush reaction and combinations of the two ADH_2 and $ALDH_2$ enzymes. The introduction of PCR technology has facilitated genotyping to the extent that the characterisation of both ADH_2 and $ALDH_2$ enzymes of a large number of subjects became a practical possibility. The combination groups are shown below:

GroupA	$ALDH_2$ Homo11/ ADH_2 Homo11
GroupB	$ALDH_2$ Het/ ADH_2 Homo11
GroupC	$ALDH_2$ Homo22/ ADH_2 Homo11, Het or Homo22

GroupD $ALDH_2$ Homo11/ ADH_2 Het

GroupE $ALDH_2$ Het/ ADH_2 Het

If only the $ALDH_2$ enzyme is considered, GroupC subjects would be expected to experience the most severe flushing response. This prediction was borne out in the findings of this study where GroupC subjects exhibited the highest average degree of flush compared with those in the other groups.

By comparing the responses of GroupA and GroupD subjects (both with $ALDH_2$ Homo11 type), the influence of the ADH_2 Homo11 and Het genotypes on the degree of flush could be examined (Table 3). It was found that the degree of flush of ADH_2 Het subjects was less marked than that of Homo11 subjects. Similarly, by comparing the responses of GroupB subjects with those of GroupE (both with $ALDH_2$ Het type), it was found that the degree of flush of ADH_2 Het subjects was *higher* than that in Homo11 subjects. Hence, it can be inferred that the degree of flush is influenced to some extent by the $ALDH_2$ genotype.

When the subjects were grouped according to the original *nine* different combinations of $ALDH_2$/ ADH_2 genotypes (Table 3), the average degree of flush rating increased across the three $ALDH_2$ groups, from Homo11 to Het to Homo22, irrespective of the ADH_2 genotype. It is interesting to note that the ADH_2 groups also had increasing flush ratings across the three groups, from Homo11 to Het to Homo22, although the inter-group differences were not as great as for the $ALDH_2$ groups. As indicated above, however, the increase in flush rating across the groups is dependent on the $ALDH_2$ type and the increase is not apparent in subjects with the active ALDH ($ALDH_2$ Homo11) type.

(d) Grouped by Gender

No statistically significant gender-related differences in the subjectively rated degree of flush after ethanol were identified. This finding is in accord with that of

Wolff (1973). However, it should be noted that significant differences between males and females were found when facial temperature, which is also a component of the flushing response, was measured (see section 4.5).

4.3. ETHANOL PHARMACOKINETICS

Initially, the pseudolinear part of the blood ethanol concentration (BEC) curve was used to estimate the rate of ethanol elimination by linear regression. The BEC readings which were used in these calculations were taken between 30 and 105 minutes after drinking.

However, because of the low dosage of ethanol which had to be given and, in consequence, the low BECs which were attained, it was found that some subjects had already reached zero BEC by 105 min. Linear regression on these subjects' BEC readings would have yielded misleading ethanol elimination rates. Therefore, it was decided to re-calculate the individual rates of ethanol elimination by averaging the observed decreases in BEC between 30 and 90, 45 and 105, and 60 and 120 minutes after ethanol. This procedure minimized the influence of early readings, when ethanol absorption and distribution might have been incomplete in some subjects, and of later readings, when some subjects had very low BEC values and thus a diminished rate of decline.

The pharmacokinetic parameters C_0, K_m and V_{max} were estimated by fitting the data to the integrated form of the Michaelis-Menten equation:

$$(C_0 - C) + (K_m . \ln(C_0/ C)) = V_{max} . t$$

This information is clinically relevant because there is wide individual variation in rate of ethanol metabolism and any consequent variation in metabolite levels could affect susceptibility to ethanol-induced liver damage.

(a) Grouped by ADH_2 genotype

The effects of ADH_2 type on the breath/ BECs were subtle. The pseudolinear portions of the BEC curves for each of the three groups were essentially parallel and differences between the ADH_2 genotype groups did not reach statistical significance. ADH_2 Homo11 subjects had the highest mean BECs throughout the session and the ADH_2 Homo22 subjects had the lowest mean. Similarly, no significant group

differences were found for any of the other ethanol pharmacokinetic variables. Thomasson, Li & Crabb (1990) and Thomasson *et al.* (1993), who used the slope of the pseudolinear part of the BEC curve in their estimations, also reported non-significant differences among groups with different (Homo22 and Het) ADH_2 genotypes. However, the numbers in their groups were small and both groups possessed the $ALDH_2$ Homo11 genotype.

For reasons which have already been discussed, it was decided to re-calculate the rates of ethanol elimination using the method described above. When a repeated measures ANOVA was carried out, a significant difference was detected between the three ADH_2 groups. It should be noted at this point that the plasma ethanol concentrations (discussed in a later section) were similar to the breath ethanol concentrations, although the group difference showed only a strong trend towards significance.

Apart from the activities of the ADH and ALDH enzymes, variations in the mean ethanol concentrations which are attained after a given dose of ethanol could also be due to differences in volume of distribution and/ or in the extent of pre-systemic (hepatic or gastric) metabolism. An association between ADH_2 genotype and volume of distribution or body composition is unlikely and there was no difference in body weight according to ADH_2 genotype. Therefore, a difference in early ethanol metabolism remains as the most probable explanation of the differences which were identified.

The ADH enzymes which are found in the stomach are predominantly ADH_3 and ADH_7, rather than ADH_2 (Jornvall & Hoog, 1995), so variation in the properties of these gastric enzymes does not provide any obvious explanation. However, in the initial stages of ethanol metabolism, while the ethanol is being absorbed from the duodenum and passing *via* the portal vein to the liver, the concentration of ethanol in

the blood perfusing the liver will be quite high -certainly much higher than the levels which are found in peripheral blood at that time or later. Under such circumstances, the higher V_{max} of ADH2-2 and its greater resistance to inhibition by NADH (Bosron & Li, 1987) might lead to a greater or more sustained initial burst of hepatic ethanol metabolism.

Data obtained from *in vitro* experiments on purified ADH variants suggests that the enzyme encoded by the ADH_2 Homo11 gene has a much lower V_{max} and a lower K_m for ethanol than that encoded by ADH_2 Homo22. The accepted figures are 9.2 and 400 for V_{max}, and 0.049 and 0.94 mM for K_m, respectively (Bosron & Li, 1987). However, the difference in V_{max} for the different genes clearly does not lead to differences in the rate of disappearance of ethanol from the circulation, and it was not possible to demonstrate any difference in K_m among the three ADH_2 genotype groups. It is important to note that subjects also possess ADH_1 and ADH_3 enzymes and thus the effects of ADH_2 are not exclusive and necessarily dominant; the other ADH enzymes may also influence the ethanol elimination rate.

Only a few other studies were identified which have detailed the effects of ADH_2 genotype in Asian subjects on ethanol pharmacokinetic variables. Of these, more recently, Thomasson *et al.* (1993) did not detect any significant ADH_2 mediated effects (between ADH_2 Het and Homo22 subjects) on the ethanol elimination rate. It is possible that effects might have been present but not detected, although it seems likely that the rate of ethanol metabolism, and its concentration-dependence in the latter part of the BEC curve, is not predictable from the *in vitro* properties of this rate-limiting enzyme.

(b) Grouped by $ALDH_2$ genotype

A significant difference was found in this study between the BEC curves for the three $ALDH_2$ groups, although the group order was unexpected. It was considered initially that the group order of the findings would increase from Homo11 to Het to

Homo22 but this did not occur. The $ALDH_2$ Het subjects attained the highest peak BEC (0.037 g dl^{-1}) followed by Homo22 (0.034 g dl^{-1}) and Homo11 (0.030 g dl^{-1}) groups respectively. Peak BECs were reached by all groups 15 min after ethanol consumption.

The ethanol elimination rates, calculated from the slope of the linear part of the BEC curve, were also determined in this study. $ALDH_2$ Homo22 subjects demonstrated significantly lower (0.015 g dl^{-1} hr^{-1}) ethanol elimination rates than Het subjects (0.017 g dl^{-1} hr^{-1}). This was possibly due to a high acetaldehyde build-up in the Homo22 subjects which inhibited the ADH reaction. Similar ethanol elimination rates were also obtained by Thomasson *et al.* (1993). When the ethanol elimination rates were re-calculated, only a strong trend towards significance was detected among the three $ALDH_2$ groups, although the group order was essentially similar.

Enomoto *et al.* (1991) found no significant difference between the three different $ALDH_2$ genotypes when blood ethanol levels were studied. However, only a small dose of ethanol was administered to their subjects (0.1 g kg^{-1}) and the number of subjects was small.

The ethanol elimination rates of ALDH deficient subjects were not immediately elevated by increasing the ethanol dose given, in contrast to the effect in subjects with the normal ALDH enzyme whose ethanol elimination rates increased with increasing BEC (Mizoi, Kogame, Fukunaga, Ueno, Adachi & Fujiwara, 1985). This difference was postulated to have occurred because of product inhibition, that is, the $ALDH_2$ Homo22, found in deficient subjects, caused the accumulation of acetaldehyde, which in turn, inhibited the conversion of more ethanol to acetaldehyde. Likewise, Thomasson *et al.* (1993) found that the presence of ALDH2-2 was associated with a slower ethanol metabolism rate and the most intense flushing of the groups which were studied. This was also suggested to be a result of product

inhibition of ADH by the high acetaldehyde concentrations attained by subjects with this allele.

In this study, the Homo11 group had a lower mean ethanol elimination rate (0.015 g dl^{-1} hr^{-1}) than either the Het or Homo22 groups and because the Het and the Homo22 groups had higher acetaldehyde concentrations, the product inhibition concept does not appear to hold.

Asian subjects, in this study, with the normal ALDH had slower ethanol elimination rates than those with the deficient ALDH enzyme. Again this finding is in contrast with those of a previous report (Thomasson *et al.* 1993). However, the ethanol elimination rates in the normal $ALDH_2$ subjects were similar to those reported in the past for Caucasian subjects (e.g. Harger & Forney, 1963). Slower ethanol elimination rates could be due to an atypical ADH affecting ethanol metabolism and/ or steady-state acetaldehyde concentrations, causing a product inhibition effect in some of the subjects (Mizoi, Adachi, Fukunaga, Kogame, Ueno, Nojo & Fujiwara, 1987).

(c) Grouped by 'Degree of Flush'

In this study, no statistically significant differences in the BEC curves were detected among the subjects who were grouped according to the degree of flush. The LF group had the highest rate of elimination compared to the other two groups, and the peak BEC was reached 15 min after ethanol consumption in all three groups.

The degree of flush for each subject was found to be strongly dependent upon the $ALDH_2$ type. As a result, it was of interest to find that the BEC curves of subjects who were grouped according to the degree of flush were in the expected order (based on $ALDH_2$ genotype) from LF> FF> NF. This is in contrast to the BEC curves for subjects grouped according to $ALDH_2$ genotype (see previous section) which were not. It is therefore possible that other factors, apart from the $ALDH_2$ genotype,

influenced the BEC curve. However, it should be pointed out that the differences found between the groups were not statistically significant.

The findings of this study corroborate those of Mizoi *et al.* (1979) who also failed to detect any significant differences between flushers and non-flushers in the maximum BEC attained or rate of ethanol elimination. Ewing *et al.* (1974) also found no evidence to suggest that Asian subjects absorbed or eliminated ethanol at a different rate from Caucasian subjects.

(d) Grouped by $ALDH_2$/ ADH_2 combination genotype

Highly significant differences were found among the ethanol pharmacokinetic parameters for groups of subjects with the five different combinations of $ALDH_2$ and ADH_2 enzymes.

On comparison of the different $ALDH_2$/ ADH_2 genotypes categorized in this study, it was found that GroupB ($ALDH_2$ Het/ ADH_2 Homo11) subjects had the fastest elimination rate, and GroupD ($ALDH_2$ Homo11/ ADH_2 Het) subjects had the slowest.

Similarly, GroupB subjects attained the highest BEC peak, although GroupA ($ALDH_2$ Homo11/ ADH_2 Homo11) subjects had the lowest peak value of all five groups.

In contrast, Thomasson *et al.* (1993) reported that subjects in their study, who would have been classified as GroupD subjects in this study, had slightly faster rates of ethanol elimination and a more intense flushing response than GroupE ($ALDH_2$ Het/ ADH_2 Het) subjects.

(e) Grouped by Gender

Gender-related differences in the response to ethanol have been widely reported in the past (Jones & Jones, 1984; Hindmarch, Bhatti, Starmer, Mascord, Kerr & Sherwood, 1992). When challenged with the same dose of ethanol, the

females generally attained higher BECs than males. This has been mainly attributed to the higher fat:body water ratio in females (Hindmarch *et al.*, 1992), although, since ethanol interacts with the GABA (γ-aminobutyric acid) receptor ionophore, differences that may exist between male and female GABA neurotransmitter systems may also play a part (Hunt, 1983; Johnston, 1990).

In this study, the gender-related differences between the subjects were not statistically significant; this was due to the fact that corrections were made in the dosage given to female subjects in order to correct for the fat:body water ratio.

4.4. BIOCHEMICAL VARIABLES

(a) Grouped by ADH_2 genotype

It was originally proposed by Stamatoyannopoulos *et al.* (1975) that individuals with the ADH_2 Homo22 type were able to metabolize ethanol faster those with other ADH_2 genotypes, leading to higher metabolite concentrations. In this study, however, such differences were not detected, despite variations in flushing intensity among the different ADH_2 genotypes. The failure to establish a definitive link between acetaldehyde levels and flushing intensity in the different ADH_2 genotype groups was unexpected. However, it should be noted that von Wartburg, Gennari, Muellener & Wermuth (1988), in a low-dose ethanol study, were also unable to demonstrate variations in acetaldehyde production due to ADH polymorphisms.

The only effect of ADH_2 genotype on ethanol metabolites which was encountered occurred in blood pyruvate concentrations. The ADH_2 Homo22 subjects had significantly higher pyruvate levels than the other two groups before ethanol consumption but the difference was no longer apparent after the ethanol challenge. This finding is difficult to interpret and might have been due to chance (the probability of the observed difference occurring by chance was 4%, and there was no trend across genotypes).

The acetate concentrations did not differ overall among the ADH_2 genotype groups (even after allowing for $ALDH_2$ type) and neither did the lactate:pyruvate ratio. The lack of significant inter-group differences is consistent with the minimal influence which ADH_2 type was found to have on the BEC peak attained and the ethanol elimination rate. This has already been discussed.

(b) Grouped by $ALDH_2$ genotype

There have been many studies on $ALDH_2$ variation in Asian subjects (Harada *et al.*, 1982; Goedde *et al.*, 1983; Enomoto *et al.*, 1991; Higuchi *et al.*, 1992;

Thomasson *et al.*, 1993), although most have not attempted to comprehensively address the biotransformation of ethanol.

Enomoto *et al.* (1991), reported that after ethanol, $ALDH_2$ Homo22 subjects attained significantly higher blood acetaldehyde levels than $ALDH_2$ Homo11 subjects. In this study, it was also found that both significantly ($p<0.005$) higher acetaldehyde concentrations and the most severe flush reactions occurred in the $ALDH_2$ Homo22 subjects after ethanol. Significantly ($p<0.005$) higher acetaldehyde levels were also attained by subjects with the $ALDH_2$ Het isozyme, compared with the other groups. In Homo22 subjects, acetaldehyde levels reached a peak of 41.7 μM, 60 min after ethanol, while those of the Het subjects reached an earlier (15 min after drinking) and much lower peak (19.2 μM). These responses should be compared with those of the Homo11 subjects who attained a maximum blood acetaldehyde concentration of only 7.4 μM, again 15 min after drinking. The $ALDH_2$ Homo11 subjects in this study attained higher acetaldehyde levels than have been previously reported, but this may reflect the method of analysis. In this study, the analysis was based on the detection of a fluorescent derivative of acetaldehyde by HPLC, a technique which was considered (Ung-Chhun & Collins, 1987; Peterson & Polizzi, 1987) to give more accurate results than gas chromatographic methods.

The blood acetaldehyde levels found in this study are generally in accord with the concentration ranges reported in previous studies (Harada *et al.*, 1981; Crabb *et al.*, 1989), although some groups have reported acetaldehyde levels as high as 100 μM in ALDH-deficient subjects (Enomoto *et al.*, 1991). The findings of this study provide further evidence in support of the proposition that $ALDH_2$ Homo22 subjects are likely to experience the greatest increase in acetaldehyde levels after ethanol when compared with the response of Het subjects. Although Homo11 subjects exhibited an increase in mean acetaldehyde levels after ethanol, the difference from pre-ethanol values did not reach significance.

The dose of ethanol which was used in this study was very low (0.3 g kg^{-1}) but it was considered to represent the upper limit of tolerability for the more sensitive subjects. It should be noted that a number of previous studies (Zorzano, Del Arbol & Herrera, 1989; Meier-Tackmann, Leonhardt, Agarwal & Goedde, 1990) have suggested that an ethanol challenge dose should be higher than 0.4 g kg^{-1} so that blood acetaldehyde levels can be measured reliably. However, these comments appear to relate to studies in both Caucasians and Asians and the question of analytical sensitivity appears to have been resolved by the use of HPLC and fluorescence detection.

As indicated earlier, there is a lack of information regarding the effects of $ALDH_2$ genotype on the other metabolic consequences of ethanol consumption. In this study, it was found that plasma acetate levels were significantly affected by $ALDH_2$ genotype. The Homo11 group attained the highest acetate concentrations after ethanol and the Homo22 group, the lowest. The higher acetate concentrations attained by the Homo11 subjects is possibly due to a more rapid production of acetate in the presence of the active, as opposed to the inactive, $ALDH_2$ enzyme.

The conversion of acetaldehyde to acetate by the $ALDH_2$ enzyme is influenced by the $ALDH_2$ enzyme genotype -Homo11, Het or Homo22. Thus, if $ALDH_2$ Homo22 subjects metabolize acetaldehyde more slowly than Het or Homo11 subjects, their acetate concentrations would also be expected to increase more slowly, resulting in inter-group differences in post-ethanol acetate concentrations. However, in a linear pathway such as ethanol/ acetaldehyde/ acetate/ acetyl CoA, the rate of conversion of each compound into its successor must be equal if the concentrations of the intermediates are to be maintained in the same proportion. Given that the rate of decrease in ethanol concentration was similar among the $ALDH_2$ groups and that the acetaldehyde concentrations did not increase continuously over time, the rates of production of acetate should be equal in all three $ALDH_2$ groups. Because there is no

reason to expect an increased capacity to metabolize acetate in $ALDH_2$ deficient subjects, their lower steady-state acetate values were difficult to interpret.

Inter-group differences in the other biochemical metabolites were not statistically significant before or after ethanol.

(c) Grouped by 'Degree of Flush'

Based on the 'Degree of Flush', the LF group (with the most severe flushing symptoms) attained the highest acetaldehyde concentrations after ethanol. The acetaldehyde concentrations of this group remained higher than those of other groups throughout the test session. The rate of increase of acetaldehyde concentration in the LF group was very rapid, rising to a peak value within fifteen minutes of ethanol consumption. The FF group had the next highest acetaldehyde concentration after ethanol, although the levels attained by this group were much lower than those of the LF group. The NF group exhibited a small, insignificant increase in acetaldehyde concentration fifteen minutes after ethanol, however this response was short-lived and acetaldehyde levels declined shortly thereafter. These findings can be related to the $ALDH_2$ genotype because the LF group consisted mainly of $ALDH_2$ Homo22 subjects (hence the Lobster Flushing description) whereas the FF group was composed of mainly $ALDH_2$ Het subjects and the NF group were mainly $ALDH_2$ Homo11 subjects.

NF subjects attained the highest acetate concentrations of the three flushing groups. FF subjects exhibited a similar rapid increase in acetate levels but they did not attain as high concentrations as the NF subjects. The LF group attained the lowest levels of acetate after ethanol consumption.

As already discussed, the conversion of acetaldehyde to acetate is influenced by the $ALDH_2$ genotype. Subjects with the $ALDH_2$ Homo22 genotype would be expected to metabolize acetaldehyde more slowly than either Het or Homo11 subjects. The findings obtained in this study support such a hypothesis.

'Degree of Flush' differences in the other ethanol metabolites did not reach statistical levels of significance.

(d) Grouped by $ALDH_2$/ ADH_2 combination genotype

Among the five different combinations of $ALDH_2$/ ADH_2 groups which were tested in this study, GroupC (comprising all subjects with $ALDH_2$ Homo22/ ADH_2 Homo11, Het or Homo22 genotypes) had the greatest increase of acetaldehyde concentration after ethanol. GroupC subjects also had much higher acetaldehyde concentrations throughout the total testing period, compared with the other groups. The differences were highly significant, but it should be noted that GroupC subjects were only grouped by $ALDH_2$ genotype. The other groups also had increased acetaldehyde concentrations after ethanol consumption but the concentrations attained were not as high as those in GroupC.

Although GroupB subjects ($ALDH_2$ Het/ ADH_2 Homo11) had the highest peak plasma ethanol concentrations at the 15 min timepoint and GroupA ($ALDH_2$ Homo11/ ADH_2 Homo11) subjects had the lowest plasma ethanol concentrations overall, the inter-group differences were not statistically significant.

Differences among the five groups for the other biochemical measures, including acetate, lactate and pyruvate, did not reach levels of statistical significance.

(e) Grouped by Gender

There were no significant gender-related differences in acetaldehyde.

Previous studies have also found that acetaldehyde levels in blood did not differ significantly between the two sexes (e.g. Helander, Lowenmo & Johansson, 1993). However, Redmond & Cohen (1972) found a gender difference in the amounts of acetaldehyde in the expired air of mice which had received large intraperitoneal

doses of ethanol. This difference appeared to be sex hormone-related and was no longer evident when the male mice were castrated.

Although, as expected, the concentration of acetate in plasma increased after ethanol in both male and female subjects, no significant gender-related differences were detected. These findings are in accord with those of previous studies (Israel *et al.*, 1994).

Both males and females exhibited a similar decrease in pyruvate levels 15 min after ethanol consumption, which was followed by a small increase to a plateau. It was considered to be interesting that the female subjects in this study had significantly higher pyruvate concentrations than the males and the difference persisted throughout the entire testing session.

Thus it can be seen that a low dose of ethanol had a number of significantly different biochemical effects in Asian subjects who were grouped by gender, 'Degree of Flush', ADH_2 genotype, $ALDH_2$ genotype and $ALDH_2$/ ADH_2 combination genotypes. Many of these differences could be related to $ALDH_2$ type and its influence on the rate of ethanol metabolism. These results provide previously unpublished information concerning a number of the biochemical correlates of human ethanol metabolism.

4.5. PHYSIOLOGICAL VARIABLES

Blood flow in the skin of the face and extremities has been found to increase after ethanol consumption (Gillespie, 1967), these effects of ethanol can be explained by changes in the peripheral circulation (Yap, 1990). Martin *et al.* (1985) also reported increased facial skin temperature, and blood pressure, after ethanol in Caucasian subjects.

In previous studies on Caucasian subjects, ethanol has been shown to elevate heart rates (Nelson, Laden & Carlson, 1979; Martin *et al.*, 1985). This effect can be attributed, in part, to the release of catecholamines from the adrenal medulla by ethanol and acetaldehyde (Pohorecky & Brick, 1988).

(a) Grouped by ADH_2 genotype

In this study, the heart rate, both before and after ethanol, was higher in ADH_2 Homo22 subjects than in the other two groups which were tested. The differences were highly significant and have been attributed to an increased rate of production of acetaldehyde (Stamatoyannopoulos *et al.*, 1975) in ADH_2 Homo22 subjects compared to either Het or Homo11 subjects. The resultant increase in acetaldehyde concentration was then thought to cause an increase in heart rate *via* release of catecholamines from the adrenal medulla. However, in this study, such an effect could not be confirmed because the acetaldehyde concentrations by ADH_2 genotype were not significantly different and the Homo22 subjects attained the lowest acetaldehyde concentrations. Consideration of the reasons for differences in heart rate in the resting state was beyond the scope of this thesis.

Group differences in facial temperature and blood pressure were not statistically significant.

(b) Grouped by $ALDH_2$ genotype

The findings of this study indicate that both $ALDH_2$ Homo22 and Het subjects had highly significantly different heart rates from Homo11 subjects. These differences pertained both before and after ethanol and persisted throughout the test session. The heart rates of the $ALDH_2$ Homo22 and Het subjects remained elevated compared to pre-ethanol values, even at 120 min after ethanol.

Mizoi *et al.* (1985) also found an increased pulse rate after ethanol in their studies on subjects who had been genotyped for ALDH.

Facial temperature was highly significantly increased after ethanol in both the Homo22 and Het groups compared with the Homo11 subjects. The facial temperature remained elevated after ethanol and did not decline to pre-ethanol levels by the end of the test session. Previous studies, including those on the Antabuse reaction, have attributed these effects (involving both heart rate and facial temperature) to acetaldehyde and the results of this study support this suggestion.

Mizoi *et al.* (1985) found that a decrease in diastolic blood pressure occurred in an ALDH-deficient group after ethanol, but was not evident in normal subjects.

In this study, systolic and diastolic blood pressure did not change significantly after ethanol. There was a small and insignificant fall in diastolic blood pressure after ethanol in all three groups (especially the Homo22 genotype). The failure to detect any significant changes in blood pressure after ethanol is perhaps reflective of the low dose (0.3 g kg^{-1}) of ethanol which was given to subjects in this study. Mizoi *et al.* (1985) gave considerably larger doses (0.4-2 g kg^{-1}) of ethanol to their subjects.

(c) Grouped by 'Degree of Flush'

After ethanol, the LF group, as expected, attained the highest facial temperatures and heart rates which remained above pre-ethanol values for the duration of the test session. FF subjects had the next highest readings, followed by NF

subjects. The differences were highly significant and were reminiscent of the results which were obtained when subjects were grouped according to $ALDH_2$ type.

Previously, Ewing *et al.* (1974) reported a fall in systolic blood pressure of more than 10 mmHg occurring only in Asian subjects, when both Asian and Caucasian subjects received an ethanol dose. A significant difference from pre-ethanol values was also noted for heart rate at 30 and 60 minutes after ethanol, the Asian group attaining the higher readings.

Reed & Hanna (1986) also found that, compared to Caucasian subjects, Asian subjects experienced a greater increase heart rate and a greater decrease in blood pressure after ethanol.

The present study did not detect any significant group-related differences in either systolic or diastolic blood pressure, before or after ethanol.

(d) Grouped by $ALDH_2$/ ADH_2 combination genotype

After ethanol, GroupC ($ALDH_2$ Homo22/ ADH_2 Homo11, Het or Homo22) developed the highest heart rates of all the groups tested, exhibiting a peak response at 60 min after ethanol. The difference in the response from those of the other groups was highly significant. However, as indicated earlier, it should be noted that GroupC subjects were essentially grouped by the $ALDH_2$ (Homo22) genotype.

GroupE ($ALDH_2$ Het/ ADH_2 Het) also developed a sudden tachycardia within 15 min of the ethanol dose. However, the heart rate then began to decline slowly but remained slightly higher than in the other groups for the duration of the testing session.

Similarly, the highest facial temperatures recorded in this study after ethanol was also in GroupC subjects, although the response of GroupE was nearly as high.

These differences reached statistical significance. It is interesting to note that GroupC subjects experienced a sudden fall in temperature at 120 min after ethanol which coincided with the onset of self-reported feelings of chill and nausea.

Differences among the other physiological variables which were monitored were not statistically significant.

(e) Grouped by Gender

The female subjects in this study had significantly higher heart rates than the males, both before and after ethanol. After ethanol, both males and females exhibited increased heart rates; the male subjects recovering sooner than the females.

The mean systolic blood pressure of the male subjects was highly significantly greater than that of the females before the ethanol dose was given. After ethanol, the systolic blood pressure fell slightly and insignificantly in both groups.

A highly significant gender-related effect for systolic blood pressure in Caucasian subjects has been well-documented (Martin *et al.*, 1985) and has been attributed to the influence of oestrogen (Sherwood, 1989).

A highly significant difference was detected for facial temperature, with both groups demonstrating increased facial temperatures after ethanol. Male subjects had slightly higher temperatures than female subjects, this might have been, at least in part, attributable to the higher proportion of male subjects (77%) who were also $ALDH_2$ Homo22 genotype rather than a true gender-related difference.

Differences in the other physiological variables which were measured did not reach statistical significance.

4.6. PSYCHOMOTOR PERFORMANCE VARIABLES

There is very little information available on differences in the psychomotor effects of ethanol on individuals with different genotypes. This has resulted in a tacit classification of all individuals into a single group (overwhelmingly Caucasian) as far as ethanol-induced impairment of psychomotor performance is concerned. From the results which have been presented so far, the inappropriateness of such a blanket classification is well-evident. This is especially true for those who possess the deficient version of the ALDH enzyme and report highly unpleasant and incapacitating effects from what would be normally regarded as very 'modest' amounts of ethanol.

Until now there have been very few studies on the psychomotor effects of ethanol in Asian subjects and the results presented in this thesis provide new information.

Ethanol, because of its non-specific sedative properties, affects many central processes including motor sensory and cognitive functions (Goldstein, 1983). Even low doses of ethanol have been consistently shown to produce such effects (Moskowitz & Robinson, 1988) and the basic deficits are considered to be on information-processing (Rohrbaugh, Stapleton, Parasraman, Frohwein, Eckhardt & Linnoila, 1987) and the ability to time-share efficiently among several tasks. For example, in studies on Caucasian subjects, ethanol doses as low as 0.5 g kg^{-1} have been shown to result in significantly increased reaction times (Linnoila, 1973). Considerable inter-individual variability is usually a feature of the ethanol effect, which is also subject to acute and chronic tolerance (Moskowitz, Burns & Williams, 1985). Taken together with an ethanol-induced reduction of inhibition, which leads to increase in aggression and risk-taking behaviour, such performance deficits translate to an increased potential for motor vehicle crashes and other accidents.

The results which are presented in this study clearly indicate that low-dose ethanol (0.3 g kg^{-1}) in **Asian** subjects, producing peak BEC readings of less than 0.04 g dl^{-1}, causes significant impairment of the psychomotor performance of some subjects, which is influenced by their $ALDH_2$ and ADH_2 genotype.

(a) Grouped by ADH_2 genotype

Even before the ethanol challenge, there was a significant difference in body sway among the three ADH_2 groups, with Homo22 being steadier than the other two groups. This group difference was maintained after ethanol. All three groups were less steady 15 min after ethanol and this was followed by a progressive recovery towards pre-ethanol levels of performance by Het and Homo11 subjects. A slight recovery by the Homo22 group at 60 min was followed by deterioration at 120 min which coincided with subjective feelings of nausea in some subjects.

Differences among the three ADH_2 groups for the critical flicker fusion frequency (CFFF) threshold measure approached statistical significance, with the Homo22 subjects having higher thresholds than the other two groups. Again, these differences were apparent both before and after ethanol.

Differences among the other psychomotor variables did not reach significance.

(b) Grouped by $ALDH_2$ genotype

Significant differences were found among the three $ALDH_2$ groups in the divided attention (excursion) measure, with the Homo22 subjects exhibiting the greatest impairment after ethanol. The excursion increased 15 min after ethanol and recovery was apparent by the next time point (t_{60}). Although recovery continued in the Homo11 group, both the Het and Homo22 subjects were more impaired at 120 min. This effect might have been a correlate of the overwhelming feelings of fatigue or sleepiness which were reported by many of these subjects.

The response of the $ALDH_2$ Homo22 subjects to ethanol in the delay measure of the divided attention task was highly significantly greater than those of the other groups and remained so for the whole of the testing session. The Homo22 subjects exhibited the greatest delay after ethanol and this effect increased over time, with no recovery apparent by 120 min. Only the Homo11 subjects showed signs of recovery at 120 min.

Significant group differences were found in performance in the digit symbol coding (DSC) task both before and after ethanol. $ALDH_2$ Homo22 subjects had the longest reaction times overall, followed by the Het subjects and then the Homo11 subjects. After ethanol, the Homo11 group recovered more rapidly than the other groups.

Differences among the $ALDH_2$ groups in their responses to ethanol in the standing steadiness task almost reached significance. Again, the greatest ethanol effect occurred in the Homo22 subjects and the Homo11 subjects were the least affected. Although the performance of Het subjects had begun to return towards pre-ethanol levels at t_{60}, that of the Homo22 subjects was still adversely affected.

A highly significant difference among the three $ALDH_2$ groups was found for the critical flicker fusion frequency threshold. The Homo22 group was impaired to a greater extent than the other two groups, especially in the last hour of the experiment.

(c) Grouped by 'Degree of Flush'

Both FF and LF groups exhibited a significant increase in divided attention delay time 15 min after ethanol, as opposed to the performance of the NF group which improved slightly. The FF group had higher delay times than the LF subjects, both before and after ethanol, except at 120 min after ethanol when the order was

reversed. This pattern of response followed a similar time-course to many of the subjective measures.

There were no significant group differences in the digit symbol coding reaction times before ethanol. After ethanol, there was a strong trend towards a greater impairment in the LF group.

No group differences were detected in this study in performance in the standing steadiness test, although Truitt *et al.* (1984) found that body sway in Asian subjects was affected by low doses of ethanol to a greater extent in subjects who flushed than in those who did not.

(d) Grouped by $ALDH_2$/ ADH_2 combination genotype

GroupC ($ALDH_2$ Homo22/ ADH_2 Homo11, Het or Homo22) subjects had longer digit symbol coding reaction times than those of the other groups, both before and after ethanol. Reaction times increased after ethanol in all groups, with the greatest effect occurring 15 min after the ethanol dose.

In the divided attention delay task, GroupC subjects were the most affected after ethanol and were impaired for up to 120 min after ethanol challenge. Although the performance of the other groups had started to return to pre-ethanol levels by 60 min, the highest delay occurred in GroupC subjects at 120 min. Again, this pattern of impairment after ethanol is similar to that of the self-report data.

No significant group differences were detected in performance on the other psychomotor tasks.

(e) Grouped by Gender

Female subjects have often been reported to have much higher divided attention delay and reaction times than their male counterparts (e.g. Mills & Bisgrove, 1983). These effects are usually apparent both before and after ethanol ingestion. The differential gender-related effects of ethanol on psychomotor performance are not simple, however. For example, speed-accuracy trade-off, whereby female subjects tended to maintain a constant error rate whilst sacrificing speed in response, and the reverse is true for males, has been reported by Jennings *et al.* (1976) and Martin *et al.* (1985).

The findings for performance in the divided attention task are in agreement with those of other groups which have found that females exhibit greater impairment than males at similar BEC levels (Mills & Bisgrove, 1983). In females it has also been found that ethanol induced deficits in psychomotor performance are exacerbated during the follicular phase of the menstrual cycle (Linnoila *et al.*, 1980; MacTavish, 1992). For reasons which relate to cultural sensitivity, a decision was made not to record the menstrual cycle phase in this study.

A highly significant difference was detected for gender in performance in the standing steadiness test, with the females exhibiting less body sway than the males.

In this study, the critical flicker fusion threshold was not found to be significantly different between males and females. However, the necessarily low dose of ethanol given to subjects might have been too low to produce any measurable effects. A consensus opinion is that findings with ethanol doses which produce BECs of less than 0.07 g dl^{-1} (in Caucasian subjects) have been 'controversial' (Starmer, 1988) and most studies have failed to demonstrate gender-related differences in CFF thresholds (Walls, Mascord & Starmer, 1992).

Therefore in terms of genotype, in each case the $ALDH_2$ Homo22 subjects exhibited the greatest post-ethanol psychomotor impairment. The $ALDH_2$ and ADH_2 genotype also appears to affect various aspects of psychomotor performance. The influence of the ADH_2 genotype was often apparent both *before and after*, and that of the $ALDH_2$ genotype *after*, ethanol. Subjects who had the $ALDH_2$ Homo22 genotype were more severely affected, after even a low dose of ethanol, than subjects of other $ALDH_2$ genotypes.

4.7. SUBJECTIVE MEASURES OF INTOXICATION

(a) Grouped by ADH_2 genotype

ADH_2 Homo22 subjects reported experiencing greater malaise/ nausea, this was especially obvious late in the course of the session (up to two hours after ethanol consumption).

Similarly, the most severe 'hangover' effects were reported by the Homo22 subjects at 120 min. Although, intuitively, it was expected that this effect would have been most marked when the BEC was rising to or at peak, for Homo22 subjects, the sensation appeared to increase over the course of the testing session, with the worst effect being reported at 120 min. This response pattern should be viewed as being in sharp contrast to those in ADH_2 Homo11 and Het subjects who reported the most severe 'hangover' effect soon after ethanol consumption with a progressive decline in intensity thereafter.

(b) Grouped by $ALDH_2$ genotype

Overall, the $ALDH_2$ Homo22 subjects experienced the most severe subjective responses to ethanol, reporting higher feelings of drowsiness, clumsiness, lower concentration levels, greater feelings of intoxication and more intense feelings of dreaminess than either the Het or Homo11 groups.

Headache was also experienced by $ALDH_2$ Homo22 subjects with an onset shortly after ethanol had been ingested and continuing throughout the entire test session.

As with the ADH_2 groups, the $ALDH_2$ Homo22 subjects reported the highest incidence of malaise/ nausea at 120 min after ethanol consumption.

These findings are in general agreement with those of Wall *et al.* (1992) who noted that Asian flushers possessing the ALDH2-2 allele reported experiencing more intense feelings of intoxication than their non-flushing counterparts, despite attaining equivalent BECs.

(c) Grouped by 'Degree of Flush'

As expected, LF subjects were the most affected by ethanol and the non-flushers were the least affected group. LF subjects considered themselves to be drowsier, clumsier, dreamier and more intoxicated after ethanol than either the FF or the NF subjects.

The most severe headaches were mainly reported by the LF group and these were usually worst at the t_{120} timepoint.

Similarly, the LF group reported the most severe malaise/ nausea and 'hangover' effects at 120 minutes after ethanol ingestion, whereas the FF group reported their worst experiences 15 min after ethanol, the intensity of the effects declining over time thereafter.

Ewing *et al.* (1974) reported the occurrence of greater symptoms of discomfort in Asian subjects after ethanol compared with the effects in a control group of Caucasian subjects.

(d) Grouped by $ALDH_2$/ ADH_2 combination genotype

The groups which were most affected after ethanol consumption were GroupC ($ALDH_2$ Homo22/ ADH_2 Homo11, Het or Homo22) and GroupB ($ALDH_2$ Het/ ADH_2 Homo11) who reported feeling the drowsiest, clumsiest, dreamiest and the most intoxicated of all the five groups tested. The least affected group was GroupA ($ALDH_2$ Homo11/ ADH_2 Homo11).

In terms of headache, GroupC was clearly the most affected group and the severity of the headache increased over time throughout the test session. GroupA subjects reported the fewest and mildest headaches.

Similarly, the reported incidence and severity of malaise/ nausea and 'hangover' effects were reported to be the greatest in GroupC subjects and these effects were most marked at 120 min after ethanol.

(e) Grouped by Gender

Female subjects felt more affected by ethanol than their male counterparts, and in response to the question as to whether they would drive a car or not, female subjects were more likely than males to respond in the negative. These findings may be related to previous drinking experience. For cultural reasons, Asian females are less likely than males to consume ethanol regularly and, in consequence, are less likely to have developed little or no tolerance to the drug. It is reasonable to suppose that the perceived effects of ethanol would have been commensurably greater in these subjects and would have led to a stronger disinclination to drive.

Subjectively perceived impairment appears to be a near-uniform experience in the Asian subjects who volunteered to take part in this study, even after such a low dose of ethanol. More important is the extent to which the severity of the effects varied among individuals according to their ADH_2 and $ALDH_2$ genotype(s).

In general, subjects who flushed after ethanol (especially the LF flushers) experienced longer-lasting and more severe adverse effects. In consequence, these subjects expressed a greater reluctance to drive and were less willing to take risks than the other subjects. In a real-life situation, it might be anticipated that a severe adverse reaction to ethanol might act as a powerful disincentive for an affected person to attempt to drive a motor vehicle, operate machinery or work in a dangerous

environment. This is despite the fact that these individuals are subjectively and objectively more impaired after ethanol than others.

4.8. DRINKING DETAILS

It should be emphasized that the volunteers in this study, whether they flushed or not, were generally *not* regular consumers of ethanol. When the subjects did drink, they predominantly favoured beer. The flushing subjects made up 78% of the subjects tested.

(a) Grouped by ADH_2 genotype

In terms of genotype groupings, by ADH_2 genotype, the Homo22 group consumed the least amount of ethanol (usually less than one drink per session) and had the highest percentage of non-drinkers compared to the Homo11 and Het groups.

(b) Grouped by $ALDH_2$ genotype

Grouping by $ALDH_2$ genotype indicated that the Homo22 subjects reported drinking less often and in smaller amounts of ethanol than Homo11 or Het subjects. This was a predictable consequence of the unpleasant symptoms associated with the alcohol flush reaction. The $ALDH_2$ Homo11 subjects (59%) usually consumed two or more drinks per session.

(c) Grouped by 'Degree of Flush'

Based on the 'Degree of Flush', the Lobster Flusher group consumed the least amount of ethanol per drinking session (one drink or less) compared with Non Flusher and Facial Flusher groups.

(d) Grouped by $ALDH_2$/ ADH_2 combination genotype

A combination of $ALDH_2$ Homo22/ ADH_2 Homo11, Het or Homo22 (GroupC) genotype meant that subjects consumed less ethanol (one or less than one drink per session), less frequently (less than 1-2 times per month) than the other combination groups.

(e) Grouped by Gender

Female subjects, on the whole, reported that they consumed ethanol slightly less frequently than their male counterparts. The number of subjects consuming less than one standard drink per session was also greater for female subjects as opposed to the males. These findings, though not reaching levels of statistical significance, are in accord with previous findings (Weatherspoon *et al.* 1994).

These findings provide further evidence that the genotype(s) and the degree of flush which an individual experiences after ethanol may influence that person's drinking habits. Various other factors such as peer pressure and socio-cultural implications may also impact on drinking habits.

4.9. REVIEW OF THE MAJOR FINDINGS OF THIS STUDY

(a) Grouped by ADH_2 genotype

After ethanol, pyruvate concentrations, heart rate, CFFF threshold and standing steadiness are all differentially affected according to the ADH_2 genotype a person possesses. However, although ADH_2 genotype was found to have an influence on the intensity of the AFR in subjects with $ALDH_2$ deficiency (see below), variations in acetaldehyde concentration which could be attributed to ADH_2 polymorphisms were not encountered.

An interesting but inexplicable finding was that ADH_2 Homo22 subjects had a higher standing steadiness and CFFF threshold than either Het or Homo11 subjects; this was apparent both before and after ethanol consumption.

(b) Grouped by $ALDH_2$ genotype

The $ALDH_2$ genotype was found to exert an influence on the ethanol pharmacokinetics, acetaldehyde concentration, acetate concentration, facial temperature, heart rate, CFFF threshold, DSC reaction time, standing steadiness and divided attention delay and excursion tasks.

After ethanol, the performance of $ALDH_2$ Homo22 subjects was more impaired in the divided attention delay, excursion, and DSC reaction time tasks than those of either Het or Homo11 subjects. The $ALDH_2$ Homo22 group also exhibited the most severe deficits in standing steadiness and CFFF threshold after ethanol.

(c) Grouped by 'Degree of Flush'

Grouping by subjectively-rated 'Degree of Flush' was found to be associated with post-ethanol differences in acetaldehyde concentration, acetate concentration, DSC reaction time, divided attention delay, facial temperature and heart rate.

Impairment of performance in the delay measure of the divided attention task was greatest for the FF group, although by t_{120}, the LF group were the most

affected. In the DSC reaction time task, the LF subjects exhibited the greatest impairment.

It is generally agreed that $ALDH_2$ Homo22 and Het subjects attain higher concentrations of acetaldehyde after a given dose of ethanol than those with the Homo11 genotype. While this is the immediate cause of the AFR, it is not certain whether the $ALDH_2$ genotype alone entirely accounts for the variation in individual reactions to ethanol. This is probably the case within Asian groups, where $ALDH_2$ deficiency is common. Two aspects of the results presented in this thesis emphasize this uncertainty. Firstly, a number of subjects who were of the $ALDH_2$ Homo11 genotype (i.e. they possessed the fully-active ALDH2-1 enzyme) exhibited flushing of the face or body after ethanol. Secondly, genetic variation at a second locus (ADH_2) was associated with differences in the severity of the AFR in $ALDH_2$ Het subjects.

The ADH_2 genotype was found to affect flush intensity despite a lack of effect on acetaldehyde concentrations. This effect was dependent upon the $ALDH_2$ genotype. That is, after ethanol, $ALDH_2$ Homo11 subjects flushed only mildly irrespective of ADH_2 type, whereas for subjects with the $ALDH_2$ Homo22 enzyme, the flush intensity and duration increased from ADH_2 Homo11 subjects, through ADH_2 Het to the greatest reaction in ADH_2 Homo22 subjects. This gene-gene interaction presumably occurs because of an initially higher rate of ethanol metabolism in ADH_2 Het and Homo22 subjects, producing acetaldehyde which can readily be metabolized in $ALDH_2$ Homo11 subjects but not in those with lower $ALDH_2$ activity.

As expected, flush intensity was also significantly correlated with post-ethanol acetaldehyde concentrations. Although the $ALDH_2$ genotype accounted for most of this association in the cohort as a whole the correlation was still significant for the $ALDH_2$ Het subjects alone. This demonstrates that within-genotype group variation in acetaldehyde concentration does occur and may affect the intensity of the AFR.

(d) Grouped by $ALDH_2$/ ADH_2 combination genotype

Previous studies have only genotyped subjects on one of the ADH_2 or $ALDH_2$ enzyme types, and combinations of these two groups have yet to be studied in any detail. The findings of the present study suggest that BEC curves, acetaldehyde concentrations, acetate concentrations, plasma ethanol levels, DSC reaction time, divided attention delay and excursion measures, facial temperature and heart rate are all likely to be influenced by the $ALDH_2$/ ADH_2 combination genotype.

GroupA ($ALDH_2$ Homo11/ ADH_2 Homo11) subjects were more impaired by ethanol as evidenced by the increase in delay time and excursion value in the divided attention tasks. In the DSC task, GroupC ($ALDH_2$ Homo22/ ADH_2 Homo11, Het or Homo22) subjects had longer reaction times both before and after ethanol. The reason for this is unclear.

(e) Grouped by Gender

The gender of the subject was found to influence pyruvate concentrations (F>M), facial temperature (M>F), systolic blood pressure (M>F), heart rate (F>M), standing steadiness (F>M), divided attention delay (F>M) and reaction time (F>M) after ethanol. Female subjects also had longer epoch times in the standing steadiness task, but this difference was apparent both before and after ethanol.

4.10. GENERAL DISCUSSION

The rate of ethanol consumption in Asia has traditionally not been as high as that in the West. However, as industrial expansion and economic development accelerates and the traditional Asian lifestyle becomes more Westernized, many changes including an increase in ethanol consumption, have become apparent. Differences in the responses between Caucasian and Asian subjects to ethanol were first scientifically documented over fifty years ago (LaBarre, 1946). Since then, research in this general area has been sporadic and along two separate lines; the molecular biology of the ethanol metabolizing enzymes and acetaldehyde concentrations in Asian subjects who flush after ethanol. Given that the flushing reaction is a highly unpleasant experience with a potential for the impairment of skills performance, it is surprising that research into the behavioural consequences of the flushing reaction has not received more attention. Almost invariably, studies on the pharmacokinetics and psychomotor effects of ethanol have used Caucasian subjects or failed to specify the ethnicity of subjects. A recent study on the pharmacokinetics of low-dose (0.3 g kg^{-1}) ethanol (Fraser, Rosalki, Gamble & Pounder, 1995) used subjects of whom 20% were stated to be Asian, yet the researchers failed to distinguish between the responses of the two groups. A practical question, which will need to be answered, is whether advice concerning the incapacitating effects of ethanol will need to be specifically directed towards Asian populations as their drinking habits change. The information presented in this thesis provides information which links the ADH_2 and $ALDH_2$ genotype of Asian subjects with the metabolic and behavioural consequences of the consumption of a very modest dose of ethanol.

On a molecular level, the availability of new techniques, which permit relatively fast and convenient genotyping on blood samples, has allowed the testing of gene loci for either association or linkage with important biochemical, physiological, psychomotor processes or risk of disease. ADH_2 and $ALDH_2$ provide obvious candidate genes for studies on ethanol metabolism and on ethanol-related diseases

(including ethanol dependence), and the results of this study include findings which have not previously been presented.

4.10.1. Group differences

Ethnic minority groups within the same Asian country may possess varying prevalencies of ALDH deficiency, which might, in part, explain regional differences in drinking habits. For example, the Korean and Mongolian groups in the north of China have been found to have ALDH deficiency frequencies of 25-30%, whereas the Zhuang and Han groups were 45-50% deficient (Goedde, Benkmann, Kriese, Bogdanski, Agarwal, Du, Chen, Cui, Yuan & Xu, 1984). Although the individual subjects in this study grew up in various different countries, they were predominantly of Han ethnic Chinese ancestral origin and the cohort can be considered to be relatively homogenous in this context. The extent to which the ethnic culture and heritage had been retained by individual subjects after growing up in other countries (both Asian and non-Asian) -including Australia -was much more difficult to assess. Hence, the question of whether or not differences in drinking habits are due to cultural and/ or socio-environmental influences cannot be resolved from these results.

Similarly, there is no conclusive evidence in the literature to suggest that the unpleasant physiological symptoms experienced after ethanol ingestion have helped to develop the Asian cultural values which discourage drinking or *vice versa*. A lively debate on this subject has persisted for many years without any conclusive outcome. A reasonable consensus would be that both factors are probably operative.

4.10.2. Ethanol sensitivity in Asians

Previous studies on racial differences in the sensitivity of response to ethanol consumption, together with the findings which are presented in this thesis, provide strong evidence that the Asian response to ethanol is different to that in Caucasians.

The need for specific advice to Asians is exemplified by the comment of a Lobster Flushing female subject who stated that, although she knew that she flushed and felt sleepy after ethanol, the severity of her psychomotor response to the challenge dose of ethanol, when she was not allowed to sleep, was completely unexpected and would act as a powerful disincentive against further ethanol consumption. Similar comments were made by several other LF subjects, which suggests that the ability of low doses of ethanol to produce incapacitating effects in certain Asian groups is not widely appreciated, even among those who are likely to experience such a reaction.

In a study of the drinking habits of Asian and Caucasian alcoholic patients, both racial groups reported similar ethanol consumption levels. However, the Asian patients had developed their ethanol-related problems earlier and after a shorter drinking period than the Caucasians. It was suggested that this finding might be due to $ALDH_2$ deficiency in the Asians (Clarke, Ahmed, Romaniuk, Marjot & Murray-Lyon, 1990).

In this study, thirteen of the LF subjects (12% of the cohort) experienced brief episodes of memory loss, usually towards the second hour after ethanol. This was almost invariably associated with postural hypotension, tachycardia and syncope.

Wall *et al.* (1994) reported that one male subject in their study with the $ALDH_2$ Homo22 genotype had a severe reaction to ethanol which resembled those described in this study for the Lobster Flushers. The onset and time course of his symptoms were found to parallel his cortisol response to ethanol. Whether or not the severe flushing reactions can be associated with the release of any other pharmacological component, such as histamine or bradykinin, is not known and was not addressed in this study.

In a study on Japanese subjects, non-flushers reportedly drank more frequently and consumed higher strength alcoholic beverages than those who flushed (Suwaki & Ohara, 1985). This was not found in the present study, probably because the subjects did not consume ethanol on a regular basis, if at all.

Ethanol has been shown to decrease subjective alertness in a dose- related manner, especially in sleep-deprived subjects (Peeke, Callaway, Jones, Stone & Doyle, 1980). A characteristic feature of the LF group subjects in this study was their strong desire to sleep after ethanol, despite the fact that they were not sleep-deprived. This may be a partial explanation for the extent of the psychomotor and subjective impairment experienced by these subjects. It is interesting note that the most severely affected subjects were also those who were most likely to say that they would not drive a car in their present condition, which could be an expression of protective deterrence.

4.10.3. ADH_2 genotype and the risk of ethanol dependence

Several reports (Thomasson, Crabb, Edenberg, Li, Hwu, Chen, Yeh & Yin, 1994; Thomasson, Edenberg, Crabb, Mai, Jerome, Li, Wang, Lin, Lu & Yin, 1991; Higuchi, 1995; Chao, Liou, Chung, Tang, Hsu, Li & Yin, 1994; Maezawa, Yamauchi, Toda, Suzuki & Sakurai, 1995; Muramatsu, Wang, Fang, Hu, Heqin, Yamada, Higuchi, Harada & Kono, 1995; Nakamura, Suwaki, Matsuo, Ichikawa, Miyatake & Iwahashi, 1995) have implicated the ADH_2 genotype in affecting the risk of ethanol dependence, at least in Asian populations. The largest study, by Higuchi (1995) in Japan, involved 655 alcoholic and 461 control subjects and it was found that the relative risk for ethanol dependence was eight times greater in ADH_2 Homo11 subjects, and twice as high in the Het group, compared to ADH_2 Homo22 subjects.

If the ADH_2 genotype does influence the risk of developing ethanol dependence, then it may possibly do so *via* the AFR. Other possibilities are that the 'reward' from ethanol consumption and the consequent reinforcement of this

behaviour might relate to the BEC attained after a given ethanol intake, which is greatest in ADH_2 Homo11 subjects and least in those who of the ADH_2 Homo22 genotype.

Although it was not possible to demonstrate any effect of ADH_2 genotype on either flushing or acetaldehyde concentrations, in $ALDH_2$ Homo11 subjects, the influence of the ADH_2 genotype as a risk-factor for ethanol dependence in Asian populations was thought to be not necessarily confined to the $ALDH_2$ deficient groups (Thomasson *et al.*, 1994; Thomasson *et al.*, 1991; Higuchi, 1995; Chao *et al.*, 1994; Maezawa *et al.*, 1995; Muramatsu *et al.*, 1995; Nakamura *et al.*, 1995). Therefore, the putative protective influence of ADH_2 Het or Homo22 genotypes might either depend on variation in acetaldehyde concentrations, below the currently measurable level, or more directly on the ethanol concentrations *per se*.

4.10.4. Other effects

It is most unlikely that group differences might have been due to the effects of food on the absorption and/ or metabolism of ethanol (Rogers *et al.*, 1987) because the subjects were instructed to fast for at least eight hours before the ethanol challenge and their meal diaries were checked to confirm that this had occurred. The first-pass metabolism effect for ethanol has also been reported to be minimal in the fasting state (Caballeria, 1991) and hence its effects were considered to be negligible.

Similarly, the possible influence of drug interactions on the response to ethanol was prevented by the exclusion of subjects who were currently receiving any drugs. Health questionnaires were also checked and subjects who reported any illness were either excluded or their ethanol challenge was postponed.

The cultural/ social differences of the individual subjects, which could theoretically modulate the response to ethanol challenge, did not appear to play an important role, as far as could be ascertained. The cultural/ social differences,

although difficult to characterise by interview, appeared to be approximately equally distributed across the groups.

4.10.5. Possible Directions for Future Investigations

In future studies which are intended to further characterise the incidence and genotype-dependency of the alcohol flush reaction, it will obviously be necessary to greatly refine subject grouping. Groups chosen for their 'Asian' origin are clearly inappropriate because many 'Asians' (e.g. Indians and Pakistanis) are of Caucasian ethnicity. It should also be noted that the term 'Chinese' is very broad and encompasses a large number of other nationalities/ minority groups, some of which would almost certainly display different responses to ethanol. In China today there are over fifty different recognised nationalities and minority groups. However, most Chinese people who live overseas, and who were recruited for this study, are of Han ethnicity and thus the cohort tested in this study is reasonably homogeneous. In future studies, it would thus be appropriate to further classify subjects into Han Chinese, Zhuang etc.

Another interesting focus for future research would be to elucidate the factors which determine why some subjects exhibit flushing of the whole body after ethanol, while others flush only in the face, ears or neck. It has been shown from this study that $ALDH_2$ genotype determines whether a subject will be a Lobster Flusher or just a Facial Flusher, however sub-categories of flushing occur within these two groups and it will be of interest to establish the underlying physiological and biochemical determinants.

ADH_3 genotyping on the DNA samples of subjects in this study could also be conducted in the future to establish whether any ADH_3 effects might have influenced any of the variables which were monitored.

It would also be of interest to unequivocally establish whether the frequency of drinking affects the flush response to ethanol. Many of the subjects in this study reported anecdotally that, in the past, when they drank more often and more regularly, they did not flush as intensely, as when they moderated their intake of alcoholic beverages.

This study has also provided a detailed record of the characteristic chronological sequence of events which occur in Lobster Flushers after ethanol consumption. Such detailed descriptions are lacking in the literature. Future studies should be directed towards establishing the precise biochemical mechanism(s) which underlie this unique and highly unpleasant response.

Previous knowledge suggests that there will be considerable overlap between the factors being tested for their effects, and also between the dependent variables. For example, it is known that $ALDH_2$ genotype, at least, is likely to strongly influence the degree of alcohol flush reaction observed (as discussed previously). Also, the results of individual psychomotor tests and the responses to the subjective questions may well be correlated. More sophisticated modes of data analysis could help to elucidate the relationships between the multiple variables measured in these studies. In particular, it should be possible to construct one or more latent variables from the psychomotor and subjective data and to determine the effects of genotype, flush status or gender on this general measure and on the individual test results. Such approaches to the degree of impairment produced by ethanol, and to the influence this may have on ethanol use, could yield extra insight but the techniques required were beyond the scope of this thesis.

4.11. CONCLUSIONS

In this study, many differences were detected among the ADH_2, $ALDH_2$, 'Degree of Flush', $ALDH_2$/ ADH_2 combined genotype and gender groups in the biochemical, psychomotor, physiological and pharmacokinetic responses of Asian subjects to a low dose of ethanol. Perhaps the most important finding is that the $ALDH_2$ Homo22 group was impaired (both subjectively and objectively) to the greatest extent by ethanol. The ADH_2 genotype also influenced the ethanol response, but to a lesser extent, indicating that the alcohol flush reaction is a more complex process than was initially believed.

The relevance of these findings should be viewed in a wider context. The economies of most of the countries in the Asian region have been developing very rapidly over the last decade. As with the earlier industrialization in the West, motor vehicle numbers have greatly increased. For example, motor vehicle ownership in China has increased over seven-fold from 712,000 units in 1975 to 5,336, 000 units in 1990 (Ellis, 1994). Another consequence of increasing personal affluence is that ethanol consumption has increased very rapidly. A concomitant increase in ethanol consumption and vehicle ownership has an obvious and unwelcome potential for an increase in traffic crashes with an attendant increase in mortality and morbidity. For genetic reasons, a substantial proportion of the Asian population tolerates ethanol poorly, and is likely to experience substantial adverse effects from only small amounts of ethanol. There thus appears to be a pressing need to identify and to specifically educate those who are at greatest risk. These people should be made aware of the fact that even one small drink (in other people's terms) might cause an unexpected and possibly severe reaction.

The Hong Kong Government's 1995 message to ethanol consumers is:

"If you drink, you can't drive - think about it",

a message which is rather oblique and still fails to draw attention to the genetically-determined vulnerability of a large proportion of the individuals within the target population.

Chapter 5.

APPENDICES

APPENDIX A. Example: Questionnaire sent to Subjects
(Page 1)

STUDY SURVEY - Confidential

NAME: .....

ADDRESS: .....

.....

CONTACT TEL: ..... OCCUPATION: .....

AGE: ..... SEX: ..... WEIGHT: ..... DO YOU SMOKE? .....

LANGUAGES SPOKEN AT HOME: .....
(Please specify dialects)

.....

---

a. ***YOUR PLACE*** OF BIRTH: .....

PLACE WHERE SPENT *MOST* OF LIFE: .....

b. ***MOTHER'S*** PLACE OF BIRTH: .....

PLACE WHERE SPENT *MOST* OF LIFE: .....

c. ***FATHER'S*** PLACE OF BIRTH: .....

PLACE WHERE SPENT *MOST* OF LIFE: .....

DO YOU DRINK REGULARLY? .....

AVERAGE NUMBER OF DRINKS EACH TIME? .....

HOW OFTEN DO YOU TAKE ALCOHOLIC DRINKS?
(Please circle answer)

a. More than once a day
b. Once a day
c. 3-4 times per week
d. 1-2 times per week
e. 1-2 times per month
f. Less often
g. Not at all

WHEN DO YOU USUALLY DRINK? a. Weekdays b. Weekends

WHERE DO YOU USUALLY DRINK? a. Friend's place b. Home
c. Bar d. Parties
e. Other .....

APPENDIX A. Example: Questionnaire sent to Subjects
(Page 2)

WHAT TYPE OF BEVERAGE DO YOU MOST OFTEN DRINK? a. Beer c. Spirits
b. Wine d. Other
.....

WHY DO YOU DRINK? a. Special Occasion
b. For taste d. To cope with life
c. To relax e. Other.....

DO YOU EXPERIENCE ANY ADVERSE EFFECTS OF ALCOHOL? (Please describe)

.....

.....

HOW MUCH ALCOHOL IS NEEDED BEFORE EFFECTS ARE SEEN? .....

DO YOU 'GO RED' (FLUSH) AFTER ALCOHOL? .....

HOW LONG (IN MINUTES) DOES IT TAKE FOR YOU TO FLUSH? .....

WHERE DO YOU GO RED? (Please circle) a. Face b. Ears
c. Chest d. Neck
e. Palms f. Arms

DO THESE EFFECTS DETER YOU FROM DRINKING? .....

HAS YOUR FLUSHING/ DRINKING PATTERN CHANGED OVER TIME? .....

(Please Specify) .....

---

a. DO BOTH YOUR PARENTS DRINK? .....

DO THEY FLUSH AFTER ALCOHOL (Present & Past)? .....
(Please specify) .....

b. DO YOUR GRANDPARENTS DRINK? .....

DO THEY FLUSH AFTER ALCOHOL? .....

c. DO YOUR BROTHERS/ SISTERS FLUSH AFTER ALCOHOL? .....
(Please specify) .....

THE END!

APPENDIX B. Example: Informed Consent Form

TELEPHONE: 692 3431 Bosch Building
692 2408 Blackburn Building

The University of Sydney
Department of Pharmacology
N.S.W. 2006

IN REPLY PLEASE QUOTE:

I, ... (name) of
.....(address)
having attained the age of eighteen (18) years freely volunteer to take part in the project outlined hereunder on the understanding that all reasonable care will be exercised by the University of Sydney and others engaged in the project.

PROJECT:
I understand that the purpose of the study is to measure certain perceptual and motor functions of subjects before and after the administration of alcohol (0.3 g/kg).
I also understand that blood samples will be taken via a forearm vein.

I understand that the study will involve attending the laboratory for a duration of approximately four (4) hours.
I undertake not to drive a motor vehicle after ingesting alcohol if my breath alcohol reading exceeds a value of 0.04%.

To the best of my knowledge, no present or past illness prejudices my participation in the study and I agree to abide by the decision of the Investigator as to my acceptibility for inclusion in the trial. I have not knowingly withheld from any person responsible for the project, any information regarding my state of health or current medications.

I agree that if the University of Sydney and others associated with the project exercise all reasonable care, I will in no way hold the University of Sydney or others involved liable in respect of any consequence that might arise from my participation in the project outlined above.

Signed ..... Date .....

Witnessed ..... Date .....

APPENDIX C. Example: Health Diary Form

CONFIDENTIAL

Health questionaire - alcohol study.
* Please answer all questions.

NAME ..... AGE ..... WEIGHT .....

1. Are you currently being treated for any illness or disease ?

..... YES / NO

2. Have you ever had any of the following illnesses: Asthma, diabetes, cancer, heart disease, liver disease, kidney disease, myasthenia gravis.

..... YES / NO

3. Are you currently taking any medications regularly for any reason, including vitamins, bowel pills, tonics, herbal pills, sleeping pills or any prescribed medications ?

..... YES / NO

4. Have you a minor illness, such as a cold or flu, or has any family member developed such an illness in the last few days ?

..... YES / NO

SIGNED ..... DATE .....

APPENDIX D. Example: Meal Diary Form

NAME .....

DAY ..... DATE ..... TIME .....

MEAL DIARY

The following questions relate to your previous meal. Circle the correct answer where appropriate and write clearly. If you are uncertain of exact details, please estimate as accurately as possible.

1. When was your last meal ?

a) Breakfast,
b) Lunch,
c) Dinner,
d) Other (snack etc)

specify .....

2. Approximately how long ago was this ?

HOURS ..... MINUTES .....

3. What did your meal consist of ? (Please specify into fats, carbohydrates or proteins if possible)

.....

.....

.....

.....

.....

.....

.....

.....

4. Did you have anything to drink with your meal ?

(please specify).....

==========

APPENDIX E. Example: Subjective Questionnaire (Page 1)

SUBJECTIVE QUESTIONAIRE - Time:

NAME: .....
DATE: .....

Q1. Would you drive a car now? YES / NO

Q2. If you were drinking with a group of friends, would you accept another alcoholic drink now? YES / NO

On a scale of 0-9 please indicate the way you feel most at the moment.

Q3. Do you feel ...

ALERT DROWSY

0 1 2 3 4 5 6 7 8 9

Q4. Do you feel ...

COORDINATED CLUMSY

0 1 2 3 4 5 6 7 8 9

Q5. Is your level of concentration ...

LOW HIGH

0 1 2 3 4 5 6 7 8 9

APPENDIX E. Example: Subjective Questionnaire (Page 2)

Q6. How intoxicated do you feel ...

NOT AT ALL VERY

0 1 2 3 4 5 6 7 8 9

Q7. Do you feel ...

ATTENTIVE DREAMY

0 1 2 3 4 5 6 7 8 9

Other comments about the way you feel at the present time:

APPENDIX E. Example: Subjective Questionnaire (Page 3)

More specifically, do you...

	Experience a headache?	Yes / No
T=15min	Does your stomach feel uneasy/sick?	Yes / No
	Any Hangover Effects?	Yes / No
	Please describe in more detail:	

More specifically, do you...

	Experience a headache?	Yes / No
T=60min	Does your stomach feel uneasy/ sick?	Yes / No
	Any Hangover Effects?	Yes / No
	Please describe in more detail:	

More specifically, do you...

	Experience a headache?	Yes / No
T=120min	Does your stomach feel uneasy/ sick?	Yes / No
	Any Hangover Effects?	Yes / No
	Please describe in more detail:	

APPENDIX F. Permission to Use Photographs Consent Form

Permission to use Photographs:

I, ... (name) of ..... (address) do give permission for Madeline Yap to use freely, for the purposes of illustrating the effects of ethanol on Asians, photographs taken of me by the Researcher when I was a subject at the Psychopharmacology Research Unit.

Signed ..... Date .....

Witness ..... Date .....

APPENDIX G. ADH PCR method

PCR was performed in a Corbett Research (Sydney, Australia) FTS-1 thermal sequencer.

PCR Conditions:

Cycle	Step	Temperature	Time	TTC
1	1	95	4.00	
	2	55	1.30	
	3	72	1.00	1
2	1	95	0.30	
	2	56	0.20	
	3	72	0.20	35
37	1	72	5.00	1
38	1	25	5.00	1

PCR Reaction tubes:

4µl genomic DNA

Cocktail -
250ng M45.5
250ng He 46 or
250ng B_2C

2µl 10xPCR Buffer
2mM $MgCl_2$
Add H_2O to make up to 16µl/ tube
25mM dNTP
1U Taq DNA Polymerase (Perkin-Elmer, Branchburg, NJ)

Add 16µl of Cocktail
Use capillary tubes, load all 20µl into these reaction tubes.
Run PCR programme.

Running on a 5% polyacryamide gel:

7µl PCR sample + 3µl Loading Buffer (750 ml l^{-1} glycerol, 3.3xTAE, 60 g l^{-1} bromophenol blue).
200ml 1xTBE to make gel buffer.
Run at 120v & 20mA for one hour.
Stain with ethidium bromide for 10min then look under UV light.

MaeIII (Boehringer Mannheim) Restriction Enzyme Reaction -
Ethanol Precipitate:

Use 10µl of sample from the ADH PCR.
Add 40µl H_2O and 5µl 3M NaAcetate.
Mix.
Add 100µl 70% ethanol.
Centrifuge briefly.
Leave in -70°C freezer overnight.

Spin sample in the fridge centrifuge (15min).
Discard ethanol and add 100µl 70% ethanol.
Spin in fridge centrifuge again for 10min.
Carefully discard the supernatant.
Dry the remaining pellet.

Add 31µl H_2O to the pellet and mix.
Remove 15.5µl of sample (for uncut/ future control).
To the remainder (15.5µl) add:
4.0µl MaeIII 5xbuffer
0.5µl MaeIII enzyme (1U)
20µl

Add 2 drops of oil and incubate at 55°C for two hours.

Run 20% polyacrylamide gel (13mA -2hr).
Use 10µl cut sample/ uncut control & add 3µl blue stuff.
Stain for 15min with ethidium bromide.
Destain for 15min.

APPENDIX H: ALDH PCR method

PCR was performed in a Corbett Research (Sydney, Australia) FTS-1 thermal sequencer.

PCR Conditions:

Cycle	Step	Temperature	Time	TTC
1	1	95	4.00	
	2	53	1.30	
	3	70	1.00	1
2	1	95	0.30	
	2	53	0.20	
	3	70	0.20	35
37	1	70	5.00	1
38	1	25	5.00	1

PCR Reaction tubes:
4µl genomic DNA

Cocktail:
250ng Exon12 Clamped Primer
250ng 18mer Primer

3mM $MgCl_2$ (pH9)
25mM dNTP
4µl 5xBuffer (pH8.5-9)
1U Taq DNA Polymerase (Perkin-Elmer, Branchburg, NJ)
H_2O to make up to 16µl/ tube.

Add 16µl of Cocktail to the sample tubes.
Using capillary reaction tubes, load all 20µl into these reaction tubes.
Run PCR programme (1.5hr)

Running on a 5% polyacryamide gel:
7µl PCR sample + 3µl Loading Buffer (750 ml l^{-1} glycerol, 3.3xTAE, 60 g l^{-1} bromophenol blue).
200ml 1xTBE to make gel buffer.
Run at 120v & 20mA (1hr).
Ethidium bromide stain for 10min, then look under UV light.

Making 48% Gels: (overnight one day before use)
In a *glass* beaker add -

3.6ml	100% denaturant solution (7M Urea; 40% v/v formamide; 5g/ 100ml of mixed bed resin AGp 501-X8, 20-50 mesh)
1.41ml	40% Acrylamide (37.5:1)
0.15ml	50x TAE Buffer
2.34ml	dH_20

Total: 7.5ml/ gel.

Degas.

Add 3.75µl Temed (0.05% final)
18.75µl 20% APS

Pour gel and allow at least 3-4hrs to polymerise.

Running DGGE gels:
1 x TAE Buffer level is approx. 8L (7.9L H_20; 160ml 50xTE).
Temperature set to 60°C.

Pre-run for 15min.
80V 20mA/ gel.

Load samples, marker.
Add 5µl loading buffer (20% Ficoll in 1xTAE) to 5-6µl amplified sample.
Run for 1.5hr on a Bio-Rad (Richmond, CA) Mini-protean II gel.

Stain with ethidium bromide and visualize under UV light.

Chapter 6.

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