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Chapter 3

Biochemical and Physiological Research on the Disposition and Fate of Ethanol in the Body

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3.1 Introduction

Few if any drugs have been studied as extensively and over so many years as ethanol or ethyl alcohol (CH_3CH_2OH), the principal psychoactive substance in all alcoholic beverages. Much has already been written and published about the absorption, distribution and metabolism of man's favourite drug, both from the viewpoint of forensic science and legal

medicine as well as substance abuse and biomedical alcohol research. Knowledge about the disposition and fate of ethanol in the body is important in forensic science and toxicology because of the role played by over-consumption of alcohol and drunkenness in many crimes of violence, sudden deaths and particularly in connection with police investigations of drunken driving (Doll et al., 1994; Hume and Fitzgerald, 1983).

Historically, the first scientific efforts to interpret blood alcohol concentration (BAC) in relation to the quantities consumed and the detrimental effects on body functioning can be traced to the pioneer work and publications of Professor Erik MP Widmark (1889-1945) of Sweden (Widmark, 1920). Widmark's research was done during the 1920-1930s and was summarized in his famous German monograph, which first appeared in print in 1932 and was translated into English 50 years later (Widmark, 1932; Widmark, 1981; Andréasson and Jones, 1996).

Information about the absorption, distribution, metabolism and excretion of ethanol can be obtained from various textbooks and periodicals devoted to biomedical alcohol research (Wilkinson, 1980; Von Wartburg, 1989; Batt, 1989; Kalant, 1996b; Lands, 1998; Matsumoto and Fukui, 2002; Ramachandani et al., 2001b). The articles by Kalant (1971; 1996b; 2005) are particularly useful and give a detailed insight into the uptake, distribution and elimination of alcohol in the human body. *Comprehensive Handbook of Alcohol Related Pathology* was the title of a three volume set of books edited by Preedy and Watson (2005). This handbook contained an impressive compilation of information on all aspects of biomedical alcohol research. Journals specializing in clinical pharmacology and toxicology occasionally publish articles dealing with the metabolism and pharmacokinetics of ethanol and adverse drug-alcohol interactions of interest in certain forensic investigations (Holford, 1987; Fraser, 1997; Norberg et al., 2003).

The present chapter combines and updates two separate chapters published in the fourth edition of *"Medical-Legal Aspects of Alcohol."* One chapter dealt with biochemistry and physiology of ethanol metabolism (Jones 2003a) and the other covered the basic principles and concepts underlying studies on the disposition and fate of ethanol in the body (Jones, 2003b).

3.2 Fate of Drugs in the Body

Disposition relates to what happens to a drug after it enters the body and therefore starts with absorption and other presystemic processes followed by the events occurring after the drug enters the blood circulation, particularly its distribution and elimination via metabolism and excretion processes (Benet et al., 1990; Kalant et al., 2006; Waller et al., 2005). Knowledge about the disposition and fate of ethanol in the body predates that of all other drugs and xenobiotics thanks mainly to the early availability of a reliable method for quantitative analysis of ethanol in small volumes of blood (Widmark, 1922). This involved a micro-diffusion method, which utilized only 100 μL of fingertip capillary blood, thus making it possible to take repetitive blood samples and to plot the concentration-time profiles of ethanol (Widmark, 1922; Widmark, 1932).

The word pharmacokinetics was coined in 1953 by a German scientist Friedrich Hartmut Dost (1910-1985) and is concerned with the way that drugs and their metabolites are absorbed, distributed and eliminated from the body and representation of these processes in quantitative terms (Dost, 1953; Gabrielsson and Weiner, 2000; Wagner, 1993). Kinetics comes from the Greek word *kinesis,* which means motion; so pharmacokinetics has to do with the movement or time-course of a drug (Greek=*pharmacon*) in the body (Kalant et al., 2006; Wagner, 1991). Monitoring the concentration (or amount) of a drug in blood or other body fluid and showing how the concentrations change as a function of time forms the basis of clinical pharmacokinetics and therapeutic drug monitoring (Greenblatt and Koch-Weser, 1975a,b; Rowland and Tozer, 1995).

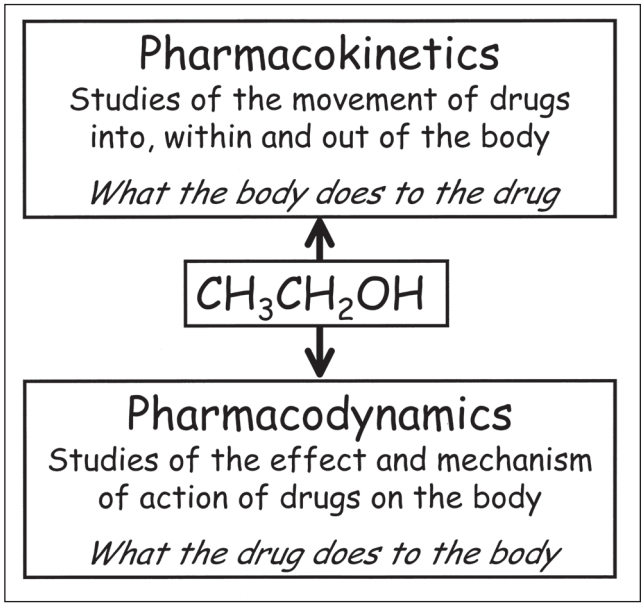


Figure 3.1 *Definitions and distinction between pharmacokinetics and pharmacodynamics, both of which are key concepts in studies of the disposition and fate of drugs in the body.*

The subject of pharmacodynamics is concerned with the physiological effects of drugs and their mechanism of action in the body (Buxton, 2006). This entails investigations of how a drug treatment influences normal body functioning or results in a change in performance and behavior of the patient. Dose-response and concentration-effect relationships, risk of toxicity and overdose and the development of tolerance and dependence also fall under the rubric of pharmacodynamics (Ross, 1990; Buxton, 2006). The terms, pharmacokinetics and pharmacodynamics, are defined and distinguished in Figure 3.1. Detailed knowledge of the dynamics of drug action is of major importance for a proper understanding of the forensic pharmacology of ethanol and other abused drugs.

3.3 Forensic Science Aspects of Alcohol

Medical and legal aspects of alcohol, which are the subjects of this book, are closely related to excessive use of alcohol and the many negative consequences associated with binge drinking and drunkenness (Brewer and Swahn, 2005; MacMillan and Wathen, 2005; Cherpitel, 2007). A person's ability to perform skilled tasks, such as driving a motor vehicle safely, is compromised as blood alcohol concentration increases (Hindmarch et al., 1992). Road traffic crash statistics show that 30-50% of all deaths on the roads in U.S. states and European nations are alcohol-related and involve a drunken driver (Voas et al., 2006). Police investigations of crimes such as sexual assault or bodily harm requiring visits to hospital emergency departments also verify the major role played by over-consumption of alcohol in violence and intentional and unintentional injuries (Cherpitel, 2007).

The analysis of ethanol in body fluids (blood, breath, urine) is the most commonly requested service from forensic science and toxicology laboratories worldwide. Forensic scientists are often required to interpret a person's blood alcohol concentration in relation to the quantities consumed or the effects on a person's behaviour and the ability to form intent (Levine et al., 2005). Such questions commonly arise in forensic investigations of impaired driving, sexual assault, murder, etc. (Garriott, 1996; Jones, 2000c; Langford et al., 1999).

A book by Cooper et al. (1979) gave a detailed appraisal of the types of questions and calculations commonly encountered in forensic casework when drunken drivers are prosecuted. Walls and Brownlie (1985) wrote a book entitled *"Drink, drugs and driving"* which covered the forensic science and legal issues related to driving under the influence of alcohol in Britain. The book by Ley (1997) also concerned drunken driving law and practice in the United Kingdom especially case law and a host of defense arguments raised during the prosecution and defense of drunken drivers (Gullberg, 2004; Jones, 1991b; Breen et al., 1998; Wigmore and Wilkie, 2002).

The science and law of drunken-driving litigation has been extensively covered from the perspective of legislation in the United States (Erwin, 1991; Cohen and Green, 1995; Fitzgerald, 2006; Bartell and Imobersteg, 2007). These books often run into several volumes and include regular updates and supplements dwelling on the interface between science and law. The target readers are mainly lawyers and law firms that specialize in defending drunken drivers and therefore need to be kept abreast of developments in case law as well as the pros and cons of a multitude of science-related defense arguments (Lewis, 1986b; Jones, 1991b; Ferner and Norman, 1996; Langford et al., 1999; Gullberg, 2004). Books by Rockerbie (1999) and Drummer (2001) also focused on the forensic pharmacology of ethanol and other abused drugs.

Forensic alcohol research has developed alongside biomedical alcohol research and is tightly linked to the disposition and fate of alcohol in the body and the analytical methods used to determine ethanol in body fluids such as blood, plasma, breath, saliva and urine (Jones, 2000a; Jones, 2006). Some studies are undertaken for the sole purpose of evaluating the strengths and weaknesses of a plethora of defense arguments raised to cast a doubt on evidence used during prosecution of drunken drivers (Jones and Logan, 2007; Gullberg, 2004; Wigmore and Wilkie, 2002; Langford et al., 1999; Breen et al., 1998; Khanna et al., 1989)

Articles dealing with the disposition and fate of ethanol in the body are published in many different scientific journals, both those specializing in substance abuse as well as forensic science, toxicology and legal medicine. Ever increasing amounts of information is available through the Internet with many websites specializing in information about alcohol and alcohol-related problems, including drunken driving. A good starting point for general and specialist information on virtually any topic is the web-based encyclopedias, such as Wikipedia. The *U.S. National Institute on Alcohol Abuse and Alcoholism (NIAAA)* created a useful database of published articles on alcohol, which was aptly christened ETOH. This source of information is searchable online gratis but, unfortunately, since 2005 this database is no longer continually being updated with new information.

Noteworthy too are many articles dealing with forensic aspects of alcohol written in German journals such as *Blutalkohol* (blood alcohol), which first appeared in 1961 and is still going strong. Unfortunately, much of this work is unknown outside German speaking countries and accordingly the articles are rarely cited in international peer-re-

viewed journals. The book by Cooper et al. (1979) focused on the older German literature, which makes it a useful reference source for people without access to *Blutalkohol* or who don't read German.

3.4 Ethyl Alcohol

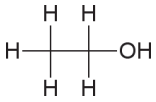
A. Chemistry

In organic chemistry there are many different types of alcohol, all having the general formula R-OH, where R denotes an aliphatic hydrocarbon chain and OH is a hydroxyl group. To a chemist the word alcohol conjures up a family of organic compounds with broadly similar chemical properties, each molecule containing one or more hydroxyl groups (-OH). Examples of the alcohols most often encountered in forensic science and legal medicine are methanol, ethanol, isopropanol, n-propanol and ethylene glycol. The chemical structure and major physico-chemical properties of ethanol, which is the alcohol of prime concern in this book, are summarized in Table 3.1.

Ethanol is a clear, colorless, flammable liquid with a characteristic, agreeable odor and a sharp taste; it burns with a blue flame. The chemical properties of ethanol are largely determined by the presence of a single hydroxyl group and the short length of the hydrocarbon chain. Ethanol is a weak acid (pk_a 15.9 at 25°C) and the propensity to donate a proton is therefore very limited and requires the presence of a strong base, such as metallic sodium to give the ethoxide ion ($CH_3CH_2O^-$) and hydrogen gas. At physiological pH ethanol is unionized and the small size of the molecule means that it easily crosses biological membranes by passive diffusion through aqueous channels. Ethanol distributes into the total body water, which comprises between 50-60% of body weight. The hydroxyl group of ethanol participates in hydrogen bonding and can also be oxidized first to acetaldehyde and then to acetate, and the end products of the reaction are CO_2 and H_2O. Ethanol can form esters by reaction with carboxylic or fatty acids.

Ethanol has a remarkably simple chemical structure considering the wide spectrum of biochemical, behavioral, and pharmacological effects on the body (Deitrich et al., 1989). Together with other organic solvents such as ether and chloroform as well as anesthetic gases, such as flurocarbons and nitrous oxide, ethanol belongs to a class of pharmacological agents known as central nervous system (CNS) depressants (Goldstein, 1994; Krasowski and Harrison, 2000; Chao and Nestler, 2006). The high solubility of ethanol in water and low dissolution in lipids (fatty tissue) means that large quantities must be administered to produce stupor and anesthesia compared with other sedative-hypnotics and anesthetic agents.

Table 3.1
Major Physico-Chemical Properties and Other Characteristics Of Ethanol in the Body

Property	Characteristic of Ethanol
CAS-number[1]	64-17-5
Molecular weight	46.07 g/mol
Empirical formulae	C_2H_6O
Molecular formulae	CH_3CH_2OH (primary alcohol)
Structural formulae	
Common name	Beverage or grain alcohol
Manufacture	Fermentation of starch, sugar or other carbohydrates
Boiling point	78.5°C
Melting point	-114.1°C
Density	0.789 g/mL at 20°C
Dissociation constant (acidity) pK_a	15.9 at 25°C.
Dipole moment (polarity)	1.69 D
Dielectric constant (polarity)	24.3
Water solubility	Mixes completely in all proportions
Endogenous concentration	0.0005-0.0015 g/L in blood
Main metabolites	Acetaldehyde, acetic acid, and ethyl glucuronide
Principal mass fragments	m/z 31 (base peak) m/z 45 and m/z 46 (molecular ion)
Permissible exposure limit (workplace)	1000 ppm or 1.9 g/m^3
Plasma/blood ratio	1.16:1[2]
Blood/breath ratio	2300:1[3]
Fatal blood concentration	Median 3.6 g/L[4]

[1] Chemical abstract service registry number. [2] Depends to some extent on hematocrit of the blood sample. [3] Average for venous blood and end-expired breath in the post-absorptive phase with blood-alcohol > 0.5 g/L. [4] Wide range depending on many factors, especially age, drinking pattern and degree of habituation.

Many of the effects of ethanol on brain chemistry and neurotransmission, such as the alleviation of anxiety, loss of inhibitions and prolongation of reaction time and motor incoordination resemble what happens after medication with barbiturates and benzodiazepines. Indeed, the mechanisms and sites of action of ethanol in the CNS are shared by these depressant drugs. Experiments both in-vivo and in-vitro have shown that the cognitive and behavioral effects of ethanol are mediated via cell membrane receptors for the inhibitory neurotransmitter $GABA_A$. Ethanol enhances $GABA_A$ receptor functioning by binding to a specific site on the ion channel to allow the flow of chloride ions into the cell. Another receptor site for the action of ethanol is the N-methyl-D-asparate (NMDA) glutamate receptor (Tabakoff and Hoffman, 1993; Korpi, 1994; Mehta and Ticku, 1999). Ethanol exerts an antagonistic effect on the NMDA subtype brain receptor for the major excitatory neurotransmitter glutamate (Diamond and Gordon, 1997). This antagonism helps to explain some of the euphoric and dysphoric effects of ethanol intoxication (Krystal et al., 2003).

Small doses of ethanol are perceived as stimulation although in reality ethanol is a depressant of the central nervous system (Tupala and Tuhonen, 2004). The feelings of euphoria experienced after drinking ethanol can be explained by suppression of central inhibitory mechanisms (Källmen and Gustafson, 1998). As larger amounts are consumed and successively higher blood-ethanol concentrations are reached ethanol exerts its negative influences on a person's performance and behavior, such as impaired coordination, lack of good judgment, sedation, ataxia, incoherent speech, loss of motor control, and finally at very high blood-alcohol levels (> 4 g/L) unconsciousness and death (Carni and Farre, 2003).

B. Amounts of Alcohol Consumed

Alcohol might be considered a medicine, a food, a depressant drug or a poison depending on the context in which the drug is being discussed. The metabolism of ethanol liberates energy, actually 7.1 kcal/g (29 kj/g) when completely oxidized in the body to carbon dioxide and water. This can be compared with 9 kcal/g from fats and 4 kcal/g from protein and carbohydrate. However, the calories derived from cellular oxidation of ethanol are usually referred to as "empty" calories because they cannot be stored for use when needed, for example, as glucose is converted into glycogen (Lieber, 1994a). Another reason for alcohol calories being considered empty is the fact that alcoholic beverages lack vitamins and minerals present in conventional foodstuffs. Indeed, malnutrition and a deficiency in certain vitamins, e.g. thiamine, folate, pyroxidine, and vitamin A, represents a common sequel to abuse of alcohol and is a prominent feature in the treatment and rehabilitation of alcoholics (Lieber, 1994b).

Ethanol is a fairly weak pharmacological agent compared with other psychoactive drugs used for recreational purposes. This is explained, at least in part, by the high solubility of ethanol in water and the fact that body-water makes up 50-60% of body weight. This provides a large volume to dilute the ingested ethanol before it reaches the brain to cause inebriation. A person has to drink between 25 g and 50 g of ethanol to experience mild euphoria and alter cognitive functioning. An analgesic effect from opiates, such as morphine and codeine, is achieved after intake of 10 mg or 100 mg, respectively. This compares with 15,000 to 20,000 mg of ethanol, which is necessary to produce a feeling of warmth and well-being (tipsy). These amounts are contained in 1½ ounces of spirits (40% v/v), one glass (150 ml) wine (12% v/v) or one bottle (330 ml) of export beer (5% v/v).

People consume enormous quantities of ethanol compared with other drugs and medication they might take. The total per capita consumption of alcohol in a country is a strong indicator of the overall health hazard as reflected in statistics from hospital emergency departments, arrests for public intoxication, deaths resulting from liver cirrhosis, prevalence of alcohol dependence and drunken driving convictions as well as acute poisonings (Room et al., 2005). Because many people are abstainers the amounts of alcohol consumed by those who actually indulge in drinking vastly exceeds average per capita figures (Williams and DeBakey, 1992).

C. Alcoholic Beverages

The dosage form of a drug represents the physical form in which the substance is administered to patients and the particular delivery system, whether as solution, tablets, capsules, coated tablets, syrups, spray, etc. (Rowland and Tozer, 1995). Characteristics of the type of alcohol beverage consumed (concentration of ethanol, sugar content and other diluents) will influence the rate of absorption after oral intake and pharmacokinetic parameters C_{max} and t_{max} are altered (Rowland and Tozer, 1995; Kalant et al., 2006).

Ethanol is commonly consumed as beer, wine, spirits, or cocktails, which can be considered the dosage form as opposed to drinking pure ethanol solvent diluted with water. Besides ethanol and water, many alcoholic beverages contain trace amounts of other low-molecular weight substances, known as the congeners. These might consist of other alcohols (low concentrations of methanol, n-propanol and higher alcohols), and along with other substances (aldehydes, esters, lactones) the congeners impart the taste and flavor to the drink. Indeed, gas chromatographic analysis of the congener

profile can help to identify the type of beverage consumed. Forensic science aspects of congener analysis to investigate claims of drinking alcohol after involvement in a crash have been reviewed (Bonte, 2001; Iffland and Jones, 2003).

Hard liquor is often diluted with various soft drinks that contain CO_2 and this gaseousness is one factor that might accelerate the rate of absorption of ethanol, probably by facilitating stomach emptying (Ridout et al., 2003; Roberts and Robinson, 2007). Many types of beers as well as the popular "alco-pops" are spiked with generous quantities of carbohydrates, which tend to delay gastric emptying and slow the uptake of alcohol into the bloodstream (Kalant, 1996b; Goldberg et al., 1979).

D. Analysis of Ethanol in Body Fluids

Methods such as gas liquid chromatography with a flame ionization detector have made it an easy task to determine ethanol in body fluids and tissues with a high degree of accuracy, precision and specificity (Tagliaro et al., 1992; Moriya, 2005). More detailed descriptions of the analytical methods currently used for legal purposes to determine ethanol in blood and breath, including the instrumentation and standard operating procedures, are presented elsewhere in this monograph.

The existence of punishable limits of blood alcohol concentration for driving a motor vehicle (e.g., 0.08 g/dL in all U.S. states) puts considerable importance on the analytical results. Legal consequences for a person who might be just above or below a statutory alcohol limit requires strict control of sampling and analysis of body fluids intended for analysis of ethanol. Examples of pre-analytical factors include preparation and positioning of the subject, the technique used to draw blood, the type of evacuated tubes used and the chemical preservative they contain, and the labeling, packaging, transport and registration of specimens at the laboratory (Peng and Chiou, 1990; Narayanan, 2000; Skopp, 2004).

The volume and composition of the specimen submitted for analysis, whether plasma or serum as opposed to whole blood or if the blood specimen was clotted or hemolyzed, should be documented. Sampling is often a neglected part of the toxicological analysis and unless done properly this will compromise the final result making it difficult or impossible to use and interpret in a legal context. This underscores the need to use standardized protocols and procedures for sampling blood or other body fluids intended for toxicological analysis of drugs and metabolites.

Much has been written about accuracy, precision, linearity, and specificity of the methods suitable for forensic work. Replicate determinations of blood-ethanol concentration made by different technicians who work independently should be one prerequisite. The calibration of instruments and the source and traceability of the ethanol standards also needs to be well documented. The aliquots of blood are usually diluted 1:5 or 1:10 with an internal standard (aqueous n-propanol or t-butanol) prior to analysis by automated headspace gas chromatography with a flame ionization detector (FID). Post-analytical factors to consider include making an allowance for uncertainty in the final result of the analysis, participation in external proficiency tests, monitoring daily performance with control charts and knowledge of the stability of ethanol in blood during storage. A recent study showed that the concentration of ethanol in blood samples from drunken drivers decreased by 0.1 g/L after storage for 6-months at 4°C (Jones, 2007a). The chain of custody of specimens and the track-record of the laboratory from peer-inspections and accreditation are other important elements in the overall quality assurance of the analytical work.

E. Reporting Blood Alcohol Concentration

Different countries have their own legal traditions and standards of proof in alcohol and road-traffic legislation, which is reflected in, among other things, the threshold blood-alcohol limits for driving and the way these are reported. The analytical methods, the types of specimen analyzed (serum or blood) and the concentration units have not been standardized internationally. For example, the punishable alcohol limits for driving are reported as g/100 mL or g/dL in USA, mg/100 mL in UK and Ireland, g/L in France, Spain and most of continental Europe. In Germany and the Scandinavian countries, mg/g or g/kg (per mille) appears on the statute books. Forensic medical practice in Germany also mandates that analysis be done on serum and the result then divided by a factor, such as 1.2, to give the corresponding blood alcohol concentration that is considered by the courts.

The different units used to report blood-ethanol concentration sometimes causes confusion when articles appearing in different scientific journals are compared and contrasted. The Widmark method of analysis measured the aliquots of blood by weight using a torsion balance, because this operation could be accomplished more accurately than measuring an exact volume of blood with a pipette (Widmark, 1922). The results were therefore reported as mass/mass units (e.g., mg/g or g/kg), which are not the same as mass/volume units (g/L or g/100 mL) because the density of whole blood is 1.055 g/mL on average (Lentner, 1981).

The question of mass/volume or mass/mass units is not trivial. This warrants consideration in pharmacokinetic studies when blood-alcohol curves are evaluated. The C_{max} and rate of elimination of ethanol from the bloodstream (k_0 or ß) as well as the apparent volume of distribution of ethanol

(V_d or rho) will differ depending on the units. In the seminal work of Widmark a C_{max} of 1.0 mg/g, a ß-slope of 0.15 mg/g/h and a rho factor of 0.68 would correspond to 1.055 g/L (C_{max}), 0.16 g/L/h (ß) and 0.66 L/kg (rho) if mass/volume units had been used. In many biochemistry and physiology journals, the blood alcohol concentrations are given in SI units such as mmol/L or μmol/L. To convert mmol/L to mass/volume requires knowledge of the molecular weight of ethanol, which is 46.07 g/mol (Table 3.1). A blood-ethanol concentration of 21.7 mmol/L is the same as 1.0 g/L or 0.1 g/dL. Examples of the different ways of reporting ethanol concentrations in blood and serum for clinical, research or forensic purposes are presented in Table 3.2.

Ethanol is not evenly distributed between the plasma and erythrocyte (red cell) fractions of whole blood, which calls for caution when results from hospital clinical laboratories are used for legal purposes (Barnhill et al., 2007). Ethanol concentrations are 15-20% higher in serum and plasma compared with whole blood (Iffland et al., 1999). Many articles appearing in general medical or pharmacology journals mention and report blood alcohol concentration but a careful reading of the article reveals that serum or plasma was the specimen actually analyzed (Raufman et al., 1993; Al-lanqawi et al., 1992).

F. Water Content of Biofluids

Ethanol distributes into all body fluids and tissues according to the distribution of water in these fluids and tissues. The distribution of ethanol between plasma or serum and whole blood is predictable from the known distribution of water between red-cells and plasma fractions. The amount of water in body fluids or tissue is easily determined by gravimetric methods involving desiccation or freeze drying. Studies have shown that erythrocytes (red cells) contain ~70% w/w water, plasma ~93% w/w water whole blood ~80% w/w water (Lentner, 1981; Rosenfeld, 1996).

The most comprehensive study of the water content of serum and whole blood involved measurements in 833 volunteers (141 women and 692 men) selected from among laboratory staff, physicians, university students and researchers (Iffland et al., 1999). Analysis of water content was done at three different laboratories and the results showed a remarkably good agreement. The overall mean distribution ratio of water (serum/blood) was 1.16:1 and the minimum and maximum values were 1.08 and 1.21, with a standard deviation (SD) of 0.0163. The large sample size and the involvement of independent laboratories give this study a high degree of credibility and practical usefulness.

The age of subjects providing the specimens of blood and serum were from 16-71 y and most were between 20-30 y. Dividing the concentration of ethanol in serum by 1.16 gives the concentration expected to have existed in whole blood for a healthy individual from this population. Because of variations in water content of blood between different people, owing to gender differences in hematocrit or disease states such as anemia, the mean plus twice the SD is recommended as a conversion factor. If the serum or plasma concentration of ethanol is divided by 1.2 (mean + 2 x 0.0163) this would give the benefit of doubt to the suspect in a criminal trial such as drunken driving.

In connection with the analysis of ethanol in saliva, urine or cerebrospinal fluid, the difference between w/w and w/v concentration units can be ignored because the specific gravity of these liquids is close to unity (Lentner, 1981). Also in medical examiner cases, the water content of blood and its density vary depending on many factors such as postmortem interval, cause of death, sampling site and state of the body, such as degree of putrefaction (Kugelberg and Jones, 2007; Jones, 2000b). Correcting for the differences between w/w and w/v concentration is probably unnecessary when dealing with autopsy specimens owing to the many other variable factors involved.

Table 3.2
Concentrations of Ethanol in Plasma or Serum Compared with Whole Blood Expressed in Different Concentration Units (Modified from Jones, 2003a; Jones, 2003b)

Serum ethanol (plasma) mmol/L	Plasma (serum) mg/mL or g/L[1]	Whole blood, mmol/L[2]	Whole blood, mg/mL or g/L[3]	Whole blood, mg/g or g/kg[4]
5	0.23	4.3	0.20	0.19
10	0.46	8.6	0.39	0.37
20	0.92	17.2	0.79	0.75
30	1.38	25.9	1.19	1.13
50	2.30	43.1	1.98	1.88

[1] (S-ethanol mmol/L x 46.07)/1000. [2] (S-ethanol mmol/L)/1.16. [3] (whole blood mmol/L x 46.07)/1000. [4] (g/L/1.055) where 1.055 is the density of whole blood in g/mL.

Many forensic practitioners based in Germany recommend that the water content of autopsy blood samples be determined as a matter of routine along with the concentration of ethanol. The analytical results can then be adjusted to a water content of 80% w/w, which corresponds to fresh blood from living subjects. However, interpretation of blood-alcohol results in postmortem toxicology depends on many other factors (e.g., sampling site, postmortem diffusion and neoformation), as discussed elsewhere in this book, and in other publications (O'Neal and Poklis, 1996; Jones, 2000b: Kugelberg and Jones, 2007).

3.5 Alcohol in the Body

The disposition and fate of drugs in the body depends on factors influencing uptake from the gut (absorption), transportation to all parts of the body by the blood (distribution), removal from the body (elimination) by excretion through the kidneys, the lungs, and the skin and metabolism in the liver. Each of these physiological processes shows considerable inter- and intra-subject variation depending on drinking pattern, the dose and dosage form of the alcohol, individual characteristics of the subject (gender, age, body composition, and health status) and many physiological, genetic and environmental influences.

The quantity of ethanol consumed and the speed of drinking are the two major determinants of the resulting blood alcohol concentration, although as indicated in Figure 3.2 a host of other parameters warrant consideration.

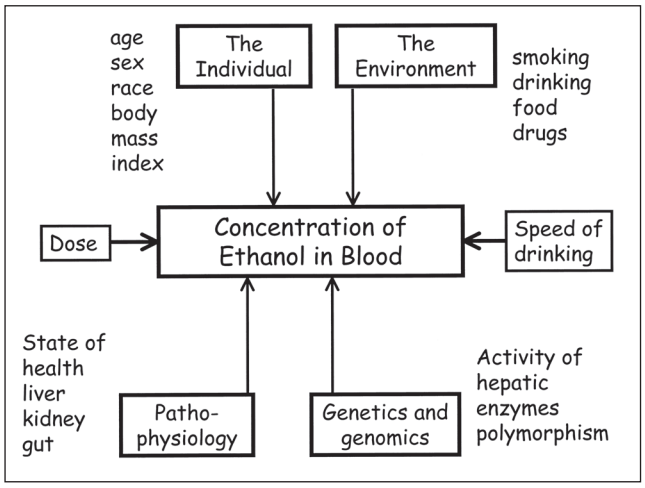


Figure 3.2 *The dose of ethanol and the speed of drinking along with many other factors (individual, genetic, patho-physiological and environmental) influence the pharmacokinetic profiles of ethanol and other drugs.*

The bulk of the dose of ethanol entering the blood stream (95-98%) undergoes metabolism by oxidative enzymes, which are mainly located in the liver (Kalant, 1991). A very small fraction of the ingested ethanol (~0.1%) is cleared by non-oxidative metabolism into conjugated metabolites, ethyl glucuronide and ethyl sulfate, which are water soluble and are excreted in the urine (Schmitt et al., 1995; Bergström et al., 2003). Studies of the disposition kinetics of ethanol usually involve repetitive sampling and analysis of whole blood, although breath, urine and saliva are sometimes used to characterize the time-course of ethanol in the body (Jones, 1993b).

A. Endogenous Ethanol

Trace amounts of ethanol are produced naturally in the body during microbial fermentation of dietary carbohydrates in the gut. In addition, certain metabolic reactions during the end-stages of carbohydrate metabolism can produce low concentrations of acetaldehyde in blood, which is then reduced to ethanol by alcohol dehydrogenase (Jacobsen, 1950; Ostrovsky, 1986). Metabolic precursors of endogenous ethanol (EE) are pyruvic acid and acetaldehyde. Any ethanol produced in the intestines must pass via the portal vein through the liver where enzymes are available to oxidize ethanol. The amounts of EE reaching the systemic circulation are negligible under normal circumstances, owing to an effective first-pass metabolism in the liver (Logan and Jones, 2000; Logan and Jones, 2003).

With use of highly sensitive and specific analytical methods such as gas chromatography, very low concentration of EE are measurable in blood and other body fluids from abstinent subjects. The concentrations in blood or plasma are close to 1 mg/L (0.001 g/L or 0.0001 g/100 mL) or less with a span from 0.5 to 2.0 mg/L (0.00005-0.0002 g/dL). In any individual case the concentration of EE might vary depending on the person's state of health, genetic (racial), dietary, and environmental factors (Sprung et al., 1981, Jones et al., 1983). The work of Sprung et al., (1981), who employed highly reliable gas chromatographic methods of analysis, is particularly convincing finding concentration in blood of less than 0.001 g/L in healthy individuals.

Residents (N=1557) of the United Arab Emirates, a location where use of alcohol is forbidden, provided blood samples for determination of ethanol by gas chromatography-mass spectrometry a highly sensitive and specific method (Al-Awadhi et al., 2004). The volunteers belonged to 13 different nationalities and included both men and women. The median, highest and 25% and 75% percentiles for EE concentrations in blood were 0.0004 g/L, 0.035 g/L and

0.001 and 0.009 g/L, respectively. The authors concluded that these very low concentrations lacked any forensic or social-medical significance (Al-Awadhi et al., 2004).

After an inhibitor of hepatic alcohol dehydrogenase (4-methyl pyrazole) was administered to humans, the concentration of EE in blood increased (Sarkola and Eriksson, 2001). One physiological role of ADH is to protect the organism from inadvertent intake of ethanol from eating overripe fruits and fruit juices or fermented sugars (Krebs and Perkins, 1970). People inheriting a mutant form of aldehyde dehydrogenase (ALDH), which is inactive in metabolizing acetaldehyde, are prone to generate higher concentrations of EE in blood. This was the mechanism suggested to explain that Japanese exhibited higher concentrations of EE in blood compared with Caucasians (Logan and Jones, 2000).

Much higher concentrations of EE were reported in two papers originating from investigators in Brazil (Agapejev et al., 1992a and 1992b). Specimens of blood and cerebrospinal fluid (CSF) from in-patients at a psychiatric hospital were analyzed by a non-specific chemical oxidation method. The concentrations of EE in these ostensibly abstinent patients showed large inter-subject variations sometimes being as high as 40-50 mg/dL (0.05-0.05 g/dL). The selectivity of the analytical method used was strongly criticized and the likelihood that other constituents of the blood were misidentified as ethanol was raised (Jones, 1994). Normal metabolites in blood and cerebrospinal fluid (CSF) that might have originated from the diet or the medication being prescribed to the patients were considered to have introduced an artifact.

Low concentrations of ethanol in blood and breath of patients suffering from various gastrointestinal disorders including obstruction and small intestinal overgrowth syndrome were reported as being of endogenous origin (Spinucci et al., 2006). In a child with short-gut syndrome a dangerously high concentration of EE was reported in plasma and breath (Dahsham and Donovan, 2001). However, this latter report was challenged because details of the analytical methods used to determine ethanol were lacking. Cross reactivity with other substrates produced in high concentration in this clinical condition such as lactate was suggested as an explanation (Logan and Jones, 2003). Another report of a patient suffering from short-bowel syndrome found a blood-ethanol concentration of 15 mmol/L (~0.75 g/L) after a carbohydrate-rich fruit drink was ingested (Jansson-Nettelbladt et al., 2006). Moreover, cultures from gastric fluid and feces showed the presence of *Candida kefyr* a species of yeast capable of fermenting sugars into ethanol.

Availability of a carbohydrate substrate and yeast infection in susceptible individuals is likely to produce measurable concentrations of ethanol and this has been best documented in Japanese subjects (Kaji et al., 1984). The question of EE is sometimes raised as a defense challenge in drunken driving trials although a recent review of such cases considered the allegation to be bogus (Gatt and Matthewman, 2000). The production of EE remains a scientific curiosity and in some individuals, especially in people of East Asian origin, elevated levels are likely to be produced under certain conditions. Such individuals usually have defective enzymes for metabolism of acetaldehyde along with considerable health problems, including yeast infections of the gastrointestinal tract (Logan and Jones, 2000).

B. Absorption

Absorption is the process by which a drug enters the blood circulation after administration by any extra-vascular route e.g., oral, intra-muscular, intra-dermal, sublingual, or rectally (Benet et al., 1990; Rowland and Tozer, 1995; Buxton, 2006). The transfer of a drug from the site of administration into the systemic circulation also belongs to the absorption process (Benet et al., 1990). Ethanol is sometimes given by intravenous infusion and this route of administration is useful to quickly reach a steady-state concentration in blood or to avoid problems with first-pass metabolism of ethanol in the stomach or the liver (Wilkinson et al., 1976; O'Connor et al.1998; Ramchandani and O'Connor, 2006)

1. Uptake from the gut

In forensic science scenarios, for all practical purposes, ethanol is taken by mouth (*per os*), and thus enters the gastrointestinal tract where absorption occurs partly through the stomach wall (~20%), but primarily from the duodenum and small intestine (~80%), owing to the much larger surface area provided by the microvilli. Absorption occurs by a diffusion process in accordance with Fick's law and therefore the concentration gradient across the gastric lumen is a key variable factor for how fast alcohol enters the portal venous blood (Berggren and Goldberg, 1940). After drinking alcoholic beverages, whether in the form of beer, wine or spirits, the alcohol they contain first reaches the stomach where absorption takes place during the drinking period and for some time afterwards (Cooke and Birchall, 1969; Cooke, 1970).

The stomach forms part of the alimentary canal and can be considered to resemble a muscular sack that can hold about 1.5 liters of fluid (Ganong, 1979). Emptying of the stomach occurs when the pressure in the antrum region exceeds that in the duodenum so that liquids and small particles are able to pass through the pylorus valve (Lartigue et al., 1991). Much depends on coordination of gastric and duodenal contractile activity in this region of the stomach (Lartigue et al., 1994). Emptying is facilitated by peristaltic waves in the antrum,

and is opposed by pyloric resistance to the passage of food. Under normal conditions, the pylorus remains nearly totally closed, because of the tonic contraction of the pyloric muscle. However, this closure is weak enough to allow fluids to pass through into the duodenum but the passage of semisolids (chime) is prevented until a strong antral peristaltic wave occurs (Griffiths et al., 1968; Petring and Flachs, 1990).

Figure 3.3 illustrates the sites of ethanol absorption through the stomach wall and the upper part of the small intestine and how this might impact the shape of the BAC curve during the first two hours post-dosing because of different rates of absorption. The anatomical placement of the antrum and pyloric regions are indicated on this graph.

2. Importance of gastric emptying

The physiological mechanisms regulating gastric motility and thereby stomach emptying via the pyloric sphincter are important determinants of the rate of absorption because of the much larger surface area in the duodenum, jejunum and small intestine compared with the stomach. Inter- and intra-individual differences in gastric emptying can account for variability in the absorption profiles of ethanol as reflected in widely different C_{max} and t_{max} of the resulting blood-alcohol curves (Madsen, 1992).

Gastric emptying and rate of ethanol absorption was investigated in volunteers who received a fat emulsion or saline as control treatment directly into the ileum (McFarlane et al., 1986). This study design avoids an interaction between ethanol and food in the stomach, which otherwise would complicate interpretation of the results. Absorption of 0.5 g/kg ethanol was much slower after treatment with lipids, which prevented emptying of the stomach into the duodenum. The resulting C_{max} of the BAC curve was lower and the entire absorption phase was more prolonged, lasting for several hours. Similarly, a delayed absorption of ethanol was reported when lipids were infused into the duodenum.

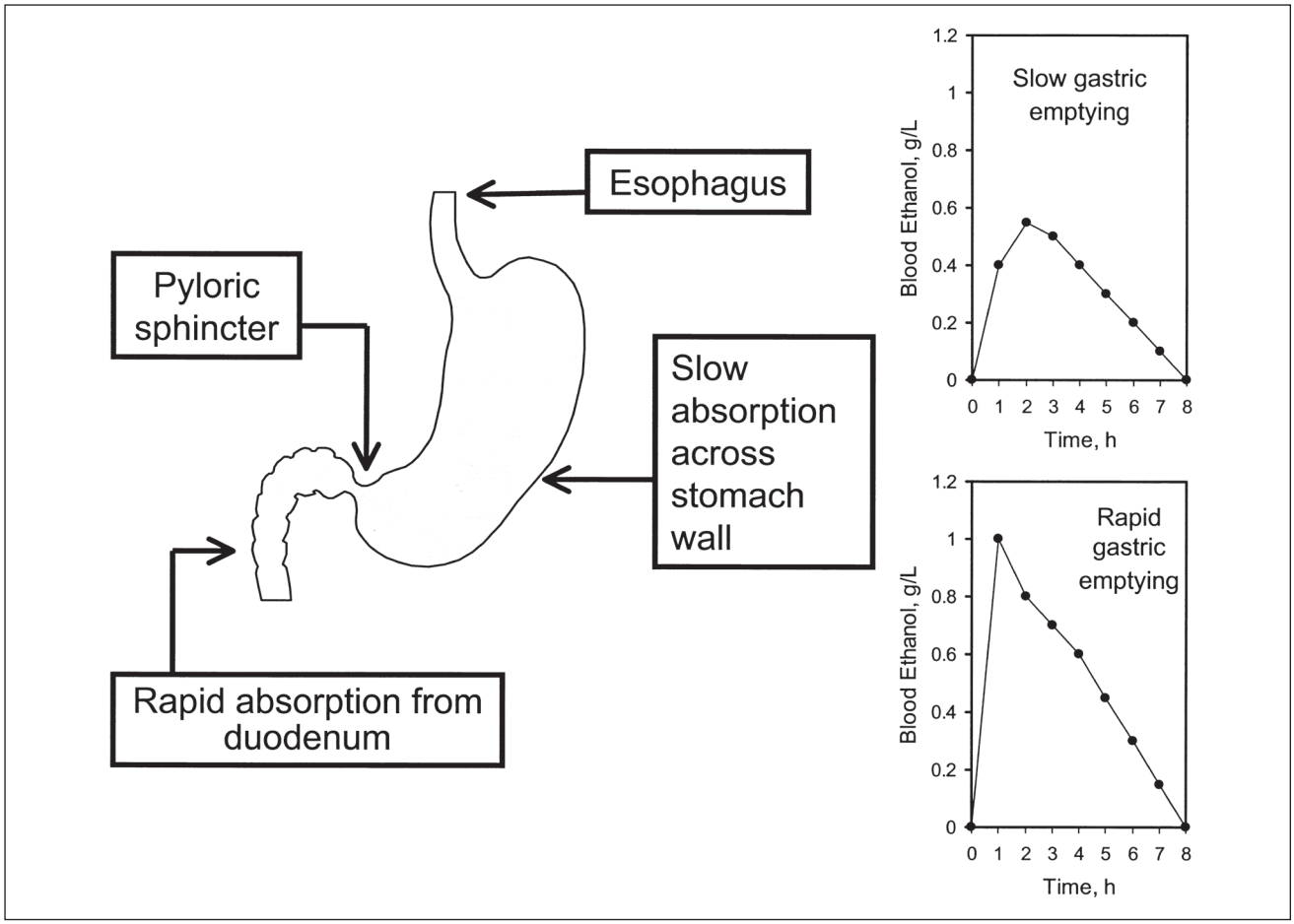


Figure 3.3 *Schematic blood-alcohol profiles illustrating absorption of ethanol from the stomach and duodenum and the effect of rapid or delayed gastric emptying on the resulting peak blood alcohol concentration.*

Table 3.3 contains a list of factors that might cause a fast or slow absorption of ethanol mainly by influencing gastric emptying (Rose, 1979; Vantrappen, 1994; Jonderko et al., 1997; Madsen, 1992). Perhaps the most important variable in this connection is the presence of liquid or solid food in the stomach before alcohol is consumed (Sedman et al., 1976b; Wilkinson et al., 1977a; Jones and Jönsson, 1994b; Watkins and Adler, 1993; Jones and Neri, 1991; Singh, 1999). The effect of food is complicated depending on the amounts ingested and the time of eating the meal in relation to consumption of the alcohol. Furthermore, the composition of the food in terms of its calorific value and the relative proportions of fat, carbohydrate, and protein also play a role in gastric emptying (Ramchandani et al., 2001a; Jones et al., 1997a; Horowitz et al., 1989; Rogers et al., 1987).

Besides an influence of food in the stomach on reducing the rate of gastric emptying and delaying absorption of ethanol there is also a paradoxical effect on the total amount of alcohol that enters the systemic circulation. This apparent loss of alcohol has been difficult to explain but seems to be related to a prolonged absorption phase and an appreciable first-pass metabolism and higher activity of alcohol metabolizing enzymes in the fed state. Some consider that ethanol binds to various constituents of the meal, which prevents direct contact with the absorption surface in the stomach and thus a much slower absorption into the portal venous blood (Cortot et al., 1986; Shultz et al., 1980; Horowitz et al., 1989).

Treatment with certain medicinal drugs can alter gastric motility, such as the prokinetic drugs cisapride and metoclopramide, which speed-up emptying of the stomach and accelerate absorption of ethanol into the bloodstream (Kechagias et al., 1997; Kechagias et al., 1999; Oneta et al., 1998; Greiff et al., 1994; Deponti et al., 1993). Gastric emptying appears to be faster in the morning compared with the evening, although this might depend on diurnal variations in blood-glucose concentration, which is another variable influencing stomach emptying (Lötterle et al., 1989; Schvarcz et al., 1995; Goo et al., 1987). Accordingly, the time-of-day when alcohol is consumed plays a role for the speed of absorption of ethanol. Smoking of cigarettes was shown to delay gastric emptying, resulting in a slower uptake of alcohol into the bloodstream and lower C_{max} and a later occurring t_{max} in venous blood (Hanson and Lilja, 1987; Johnson et al., 1991).

Any abnormalities to the gut whether as a consequence of surgery for morbid obesity or other reasons should be considered when the rate of absorption of ethanol is considered (Gubbins and Berych, 1991; Griffiths et al., 1968; Lam et al., 1980; Mattila and Venho, 1979; Yokoyama et al., 1995). The ingested alcoholic beverages themselves can also influence gastric emptying, depending on whether they were consumed undiluted or mixed with various soft drinks or sweeteners (Franke et al., 2004; Franke et al., 2005; Jian et al., 1986).

3. Inhalation of ethanol vapors

Small amounts of alcohol can enter the bloodstream if a person is continuously exposed to ethanol vapors in the air breathed. In one study, it was shown that concentrations of ethanol in the ambient air necessary to cause an accumulation in the blood (BAC 0.3 g/L, 0.03 g/dL) were so high that they could hardly be tolerated (Lester and Greenberg, 1951). In a well-designed study subjects were exposed to ethanol vapors in an environmental chamber after they consumed a moderate dose of ethanol and had a measurable BAC (Kruhoffer, 1983). Under these conditions, the elimination of ethanol already in the bloodstream was slowed appreciably because the amounts inhaled through the lungs balanced the amounts being metabolized (Kruhoffer, 1983).

Table 3.3
Factors Influencing the Rate of Absorption of Ethanol from the Gut

Slow rate of absorption

1. Food in the stomach
2. Various carbohydrates and amino acids (fructose, glycin)
3. Smoking cigarettes
4. Drugs that delay gastric emptying (anticholinergic agents, propantheline)
5. Beers with high content of carbohydrates
6. Trauma, shock and massive blood loss

Fast rate of absorption

1. Drinking in the morning after an overnight fast
2. Drinks with higher concentrations of ethanol e.g., undiluted spirits
3. Carbonated drinks
4. Drugs that accelerate gastric emptying (e.g., cisapride, metoclopramide, erythromycin)
5. Low blood sugar level
6. Surgery to the gut (e.g., gastrectomy or gastric bypass)

The quantity of ethanol inhaled and absorbed through the lungs will depend on the size of the room, whether open windows exist, the time of exposure, the concentration of ethanol in the ambient air and the degree of lung ventilation during exposure (Lewis, 1985; Mason and Blackmore, 1972). The results from human studies stand in conflict with animal models using mice and rats in which inhalation of alcohol vapor is a standard method to induce tolerance and dependence on the drug (Goldstein and Zaechelein, 1983). It should be noted that small rodents have a higher minute volume (lung ventilation per min) than humans and cannot complain about the disagreeable high concentrations of alcohol vapor they are being exposed to.

4. Absorption through skin

There is no compelling evidence that ethanol penetrates the unbroken skin to any significant extent, which probably stems from its low lipid solubility compared with higher alcohols (Bowers et al., 1942; Kalant, 1971; Goldstein, 1983). However, if there are cuts and abrasions on the exposed surface and open blood vessels, measurable amounts of ethanol might be absorbed (Jones and Rajs, 1997a). It appears that the rate and extent of uptake of ethanol through the lungs or per-cutaneously is always much less than the overall rate of metabolism (~ 6-8 g/h), making it virtually impossible to achieve an increasing BAC by these routes of administration (Anderson et al., 1991; Pendlington et al., 2001).

The question of whether significant amounts of ethanol might enter the bloodstream via the intact skin has recently received attention because of widespread use of ethanol-based products as hand-rubs and disinfection of the skin in hospital workers (Brown et al., 2007; Miller et al., 2006a). Alcohol-based hand rubs chiefly consist of ethanol in concentrations of up to 80 vol% and some products also contain isopropanol (Archer et al., 2007). Intensive use of ethanol-based soaps and hand-rubs by health-care workers (30 times per h) failed to raise the concentration of ethanol in blood by more than 0.02 g/L immediately after use (Brown et al., 2007; Kramer et al., 2007) and submitting to a breath-alcohol test would give a negative result (Miller et al., 2006b).

5. Concentration of ethanol in the beverage consumed

Absorption and distribution of ethanol in the body occurs by simple diffusion across biological membranes in accordance with Fick's law. This means that more concentrated solutions of ethanol are absorbed faster than weaker ones (e.g., spirits compared with beer).

Roine et al., (1991) administered the same dose of ethanol (0.3 g ethanol per kg) as 4 vol%, 16 vol% or 40 vol% solutions in water to healthy subjects as a bolus dose (finished in 10 min). When these drinks were ingested on an empty stomach, the C_{max} and AUC were about the same regardless of the concentration of ethanol. However, when 8 other subjects drank 4% v/v or 40% v/v ethanol 60 min after eating a standardized (rather fatty) breakfast, the C_{max} and AUC were higher after the more dilute alcoholic drink. The difference in BAC profiles was most striking during the absorption period although by 120-150 min post-dosing the two curves met. In the post-prandial state, the absorption rate of ethanol according to the concentration gradient predicted by Fick's law is seemingly not the deciding factor.

In a follow-up study, Roine et al. (1993) compared BAC profiles after 11 men ingested 0.3 g/kg ethanol in the form of either beer (4.3% v/v) or whiskey (40% v/v). When the alcoholic drinks were consumed 1 hour after breakfast or at the same time as eating breakfast, the peak BAC and AUC were higher after beer compared with whiskey. In the fasting state, whiskey gave a higher BAC and AUC although t_{max} was about the same for the two beverages. The authors attempted to interpret these results with reference to first-pass metabolism in the stomach depending on the concentration of ethanol in the drink consumed (Roine et al., 1993). The very small doses of ethanol used in these studies and the short drinking time makes it unwise to extrapolate to real-world conditions where much larger doses are consumed over longer times.

Figure 3.4 compares the shapes of BAC curves after subjects drank either brandy or beer in a crossover design study, with each subject acting as his own control (Takala et al., 1957). The beer curves showed a slower rise and a lower and slightly later occurring peak BAC compared with brandy. But by 120 min post-dosing the curves ran close together and stayed this way for the rest of the time that the measurements were made (Takala et al., 1957). The brandy and beer were consumed on an empty stomach and under these conditions the beverage with the highest concentration of ethanol was absorbed faster according to Fick's law (Berggren and Goldberg, 1940; Springer, 1972). Because beers contain more carbohydrates than brandy this might explain the slower absorption by influencing gastric emptying. It should be noted that the largest difference between the curves in Figure 3.4 was during the absorption phase of the alcohol curves up to 60 min after start of drinking.

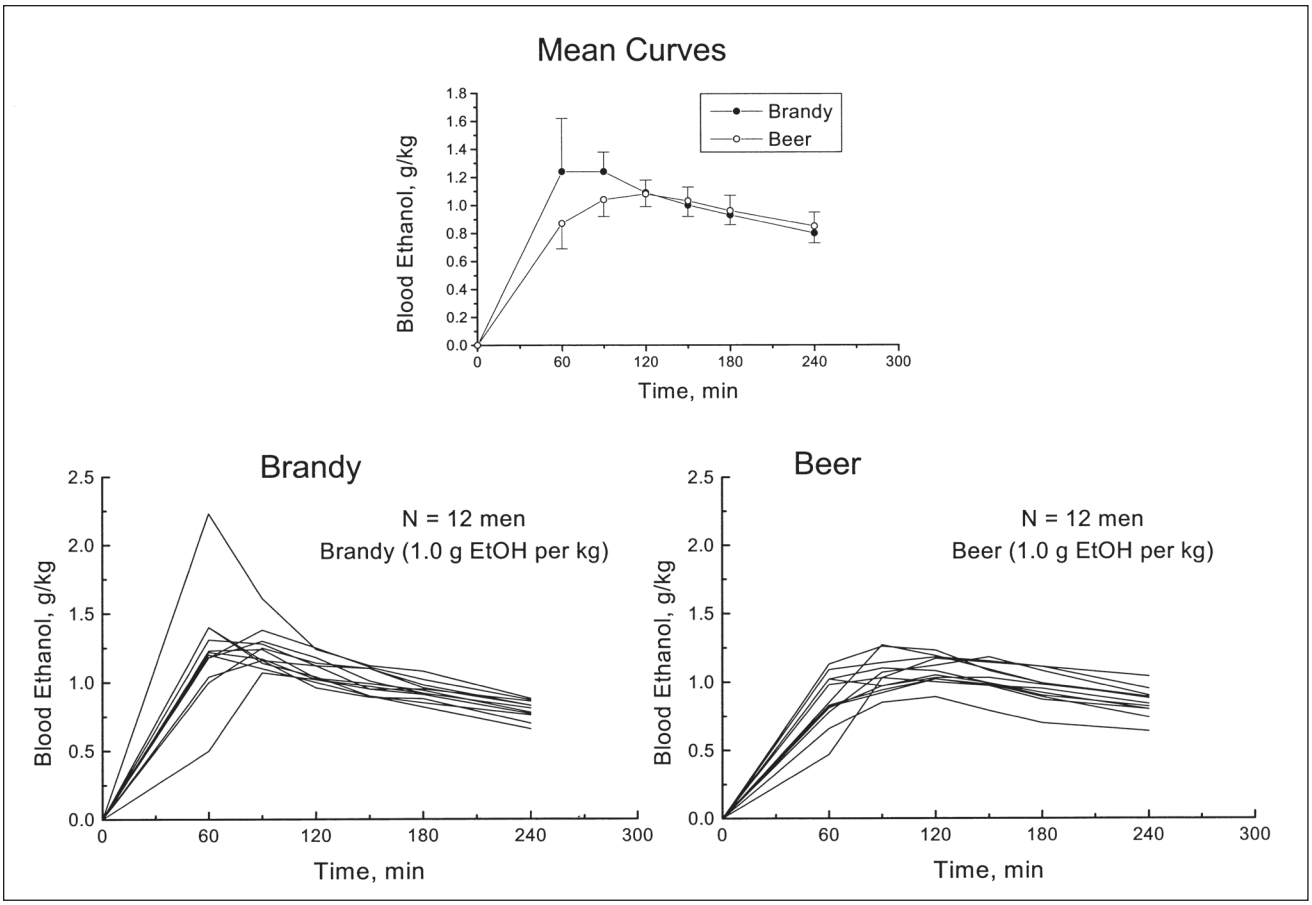


Figure 3.4 *Individual blood-ethanol profiles after the same dose of ethanol was ingested on an empty stomach as brandy (left trace) or beer (right trace). The mean curves (N=16) are also shown redrawn from a paper by Takala et al. (1957).*

Gender differences in rate of absorption of ethanol have not been thoroughly investigated and hormonal influences, phase of menstrual cycle and whether oral contraceptive steroids were used need to be considered (Correa and Oga, 2004; Baraona et al., 2001; Brick et al., 1986; Gill, 1997; Mumenthaler et al., 1999; Mumenthaler et al., 2000; Mumenthaler et al., 2001). To what extent these factors account for gender-related differences in the rate of absorption or elimination of ethanol from the body needs further investigation with larger numbers of subjects (Wang et al., 1992; Lammers et al., 1995).

The major gender difference in ethanol pharmacokinetics concerns the volume of distribution. Women are smaller than men and their body composition is different with less water per kg body weight in the female gender. This gender-related difference in body water is reflected in higher C_{max} and C_0 if alcohol is administered according to body weight (Breslin et al., 1997; Mumenthaler et al., 2000; Thomasson, 1995; Davis and Bowen, 1999; Cowan et al., 1996). Females have a smaller aqueous volume owing to more fat and less body water per kilogram total body weight (Edelman and Leibman, 1959; Van Loan, 1996). The mass of the liver in women represents a larger fraction of lean body mass than in men and this was suggested to account for a faster disappearance rate of alcohol from the bloodstream (steeper ß-slope) in females (Kwo et al., 1998; Thomasson, 2000; Dettling et al., 2007). The drinking habits of women in terms of amounts consumed and frequency of intake are becoming more like that of men (Room et al., 2005). Sex-related differences in the pharmacokinetics and pharmacodynamics of ethanol alone and in combination with prescription drugs require more investigations (Gandhi et al., 2004).

The absorption stage of the blood-alcohol curve is undoubtedly the most variable and unpredictable aspect of ethanol pharmacokinetics and can depend on age, gender and body mass index (Holt 1981; Friel et al., 1995a; Madsen, 1992). More attention needs to be given to the factors influencing gastric emptying and first-pass metabolism when forensic scientists and others engage in theoretical calculations of the BAC expected for a given intake of alcohol. Lack of

attention to factors that alter rate of absorption probably accounts for many conflicting reports in the literature, such as the effects of drugs on the pharmacokinetics of ethanol (Gibbons and Lant, 1975; Jones et al., 1999; Gentry, 2000a and 2000b). It becomes obvious from reading some of these articles that investigators have confused the effects of drugs on absorption rate of ethanol with the effect on distribution and elimination rate of ethanol.

Figure 3.5 illustrates the unpredictable nature of the absorption phase and highlights the effect of an unusually rapid absorption leading to an overshoot C_{max} (right panel). The measured BAC is higher than expected for the dose of ethanol administered although this is followed by a diffusion plunge. By 40-60 min post-dosing the expected BAC is attained. The dashed diagonal lines in Figure 3.5 are the lines of best fit to the BAC-time points determined by linear regression analysis of the concentration-time points in the post-absorptive phase. When this line is extrapolated to time of starting to drink one obtains the y-intercept denoted C_0 and this is an important pharmacokinetic parameter when the distribution volume of ethanol is determined.

A marked retardation in rate of ethanol absorption occurs if there is food in the stomach before drinking starts. This was well documented in a rat model by removing the stomachs and analyzing the contents for ethanol at various times post-dosing (Shultz et al., 1980). Both C_{max} and AUC were vastly different from the unfed control animals and it was suggested that alcohol was bound to components of the food and that this bound-fraction was retained in the stomach for many hours even after the BAC curve had peaked and was in the descending phase. The bound-alcohol could have been metabolized in the stomach or during first passage of blood through the liver. Appreciably lower C_{max} and smaller AUC was observed when the amino acid glycine (2 g/kg) was administered to rats before they received a large dose of ethanol by gavage (Iimuro et al., 1996). Care is needed when results from animal studies are extrapolated to humans, owing to differences in basal metabolic rate, hepatic enzyme activity, blood and body water distribution and stomach emptying mechanisms.

Figure 3.6 compares BAC profiles in four volunteers who drank 0.8 g ethanol per kg body weight either on an empty stomach or after eating a standardized breakfast (Jones and Jönsson, 1994b). In the fed state, the C_{max} is appreciably lower and t_{max} occurs later. Moreover, the areas under the curves are significantly less in the alcohol plus food arm of the study indicating a reduced bioavailability of ethanol. Besides obvious differences in the shapes of these BAC profiles, the food-induced lowering in C_{max} meant that the perceived effects of alcohol on the subjects were also less marked.

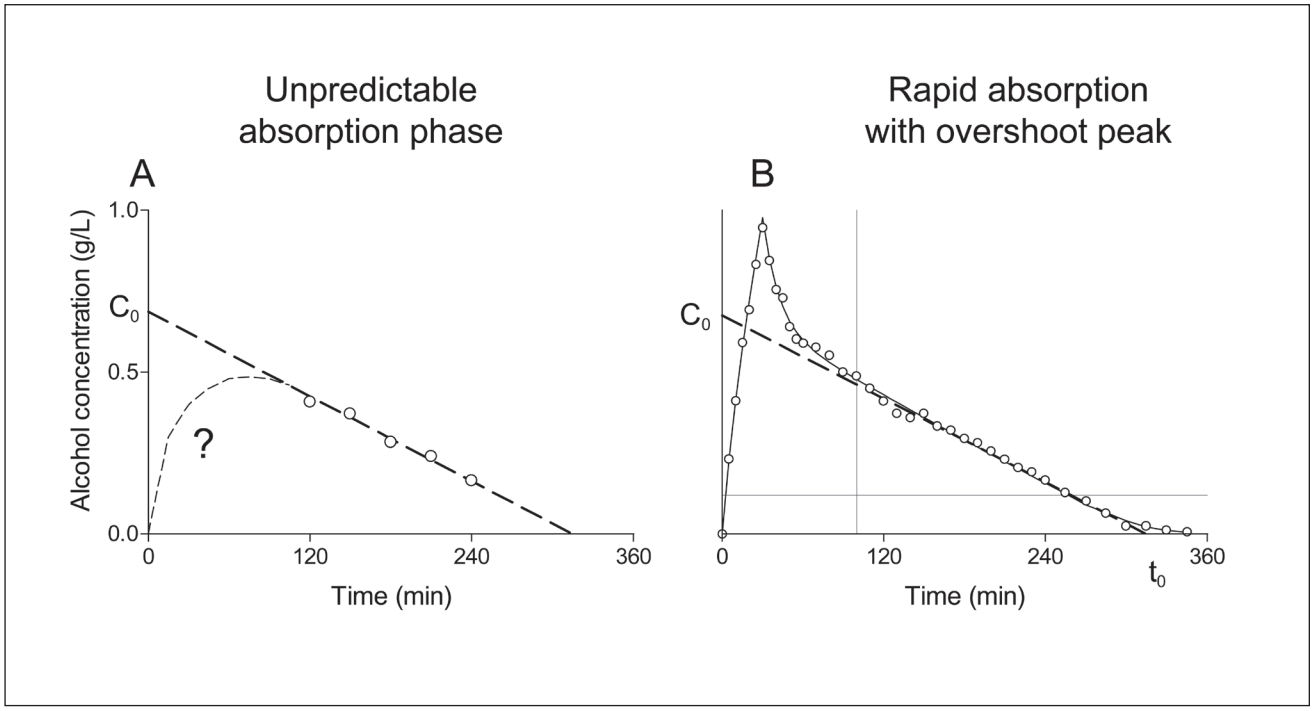


Figure 3.5 *Hypothetical blood alcohol concentration-time profiles illustrating the unpredictable nature of the absorption phase (Curve A) and a curve showing rapid absorption with pronounced overshoot peak followed immediately by a diffusion plunge (Curve B).*

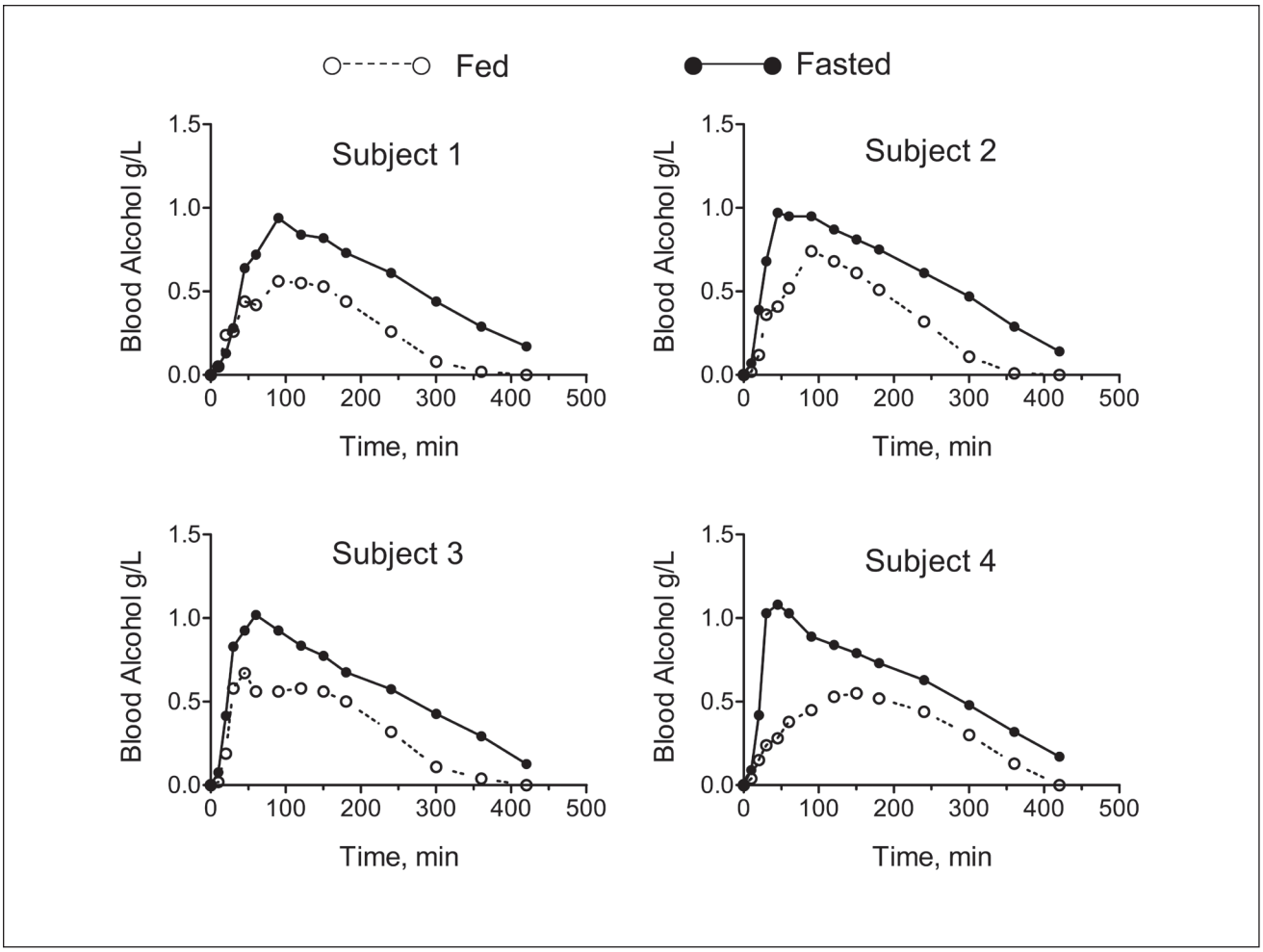


Figure 3.6 *Food-induced lowering of blood-alcohol profiles observed after four subjects participated in a crossover design study and ingested 0.80 g ethanol per kg body weight in the morning on an empty stomach or immediately after eating a standardized breakfast (fed state) (data from Jones and Jönsson, 1994b).*

C. Distribution

After absorption from the gut, ethanol enters the portal venous blood and is transported to the liver then to the heart via the hepatic vein before reaching all parts of the body (Kalant, 1971). Alcohol (ethanol) mixes with water in all proportions and easily passes the blood-brain barrier and therefore starts to exert its untoward effects on the central nervous system immediately after drinking begins.

The distribution of drugs into all body fluids and tissues depends on a number of physiological factors as well as the physico-chemical properties of the drug (Benet et al., 1990). Factors of importance for speed of distribution are cardiac output, tissue mass, regional blood flow, capillary permeability and partitioning of the drug between blood and the particular tissues (Buxton, 2006).

The volume of distribution of a drug often denoted V_d or V, which is expressed in liters or liters per kg body weight, is an important concept in clinical pharmacokinetics. This volume V_d connects the amount of the drug in the body to the concentration measured in a specimen of blood or plasma. One should note that the V_d of a drug does not necessarily reflect any physiological volume or space and values are different for different drugs. One definition of V_d is the volume of fluid required to contain the entire drug in the body at the same concentration as existing in the blood or plasma (Buxton, 2006).

In a healthy male subject (70 kg body weight) plasma has a volume of 3 L, whole blood 5.5 L; extravascular fluid outside the blood amounts to 12 L and total body water is 42 L. Ethanol is an example of a drug that does not bind to plasma proteins and distributes into the aqueous compartment. So V_d for ethanol is close to expectations for total body water, after allowing for the fact that the water content of blood is 80% w/w.

1. Arterial-venous differences

The concentrations of drugs in different sampling compartments in the vascular system have not been well studied, perhaps because of ethical constraints associated with puncturing an artery and the need to take repetitive samples (Chiou, 1989; Jones et al., 2004). For a drug such as ethanol the arterial (A) and venous (V) differences are small or negligible for body organs and tissues with a rich blood supply (e.g., brain and kidney). By contrast, the A-V difference is appreciable across skeletal muscle, owing to a low ratio of blood flow to tissue mass and the large amount of water in the tissue, which acts as a reservoir for ethanol (Guyton, 1986; Mather, 2001; Jones et al., 1997b). This leads to temporal variations between the concentration of ethanol in arterial and venous blood circulation depending on the time after drinking when samples are drawn and completeness of equilibration of ethanol in all body fluids and tissues.

When absorption and equilibration of the dose of ethanol is complete the concentrations in arterial and venous blood will be momentarily the same (Martin et al., 1984; Jones et al., 1997a; Jones et al., 2004). During the entire post-absorptive period the concentration of ethanol in venous blood is slightly higher than in the arterial blood, because some of the alcohol is metabolized in the liver before returning to the skeletal muscles. The magnitude and existence of A-V differences in ethanol concentration is shown in Figure 3.7 for near simultaneous samples of blood taken from a radial artery at the wrist and a cubital vein at the elbow on the same arm (Jones et al., 2004). The graph illustrates experiments in a male volunteer who drank either 0.6 or 0.8 g ethanol per kg body weight on two occasions several weeks apart. Blood specimens were taken from artery and vein by means of indwelling catheters at regular times after end of drinking.

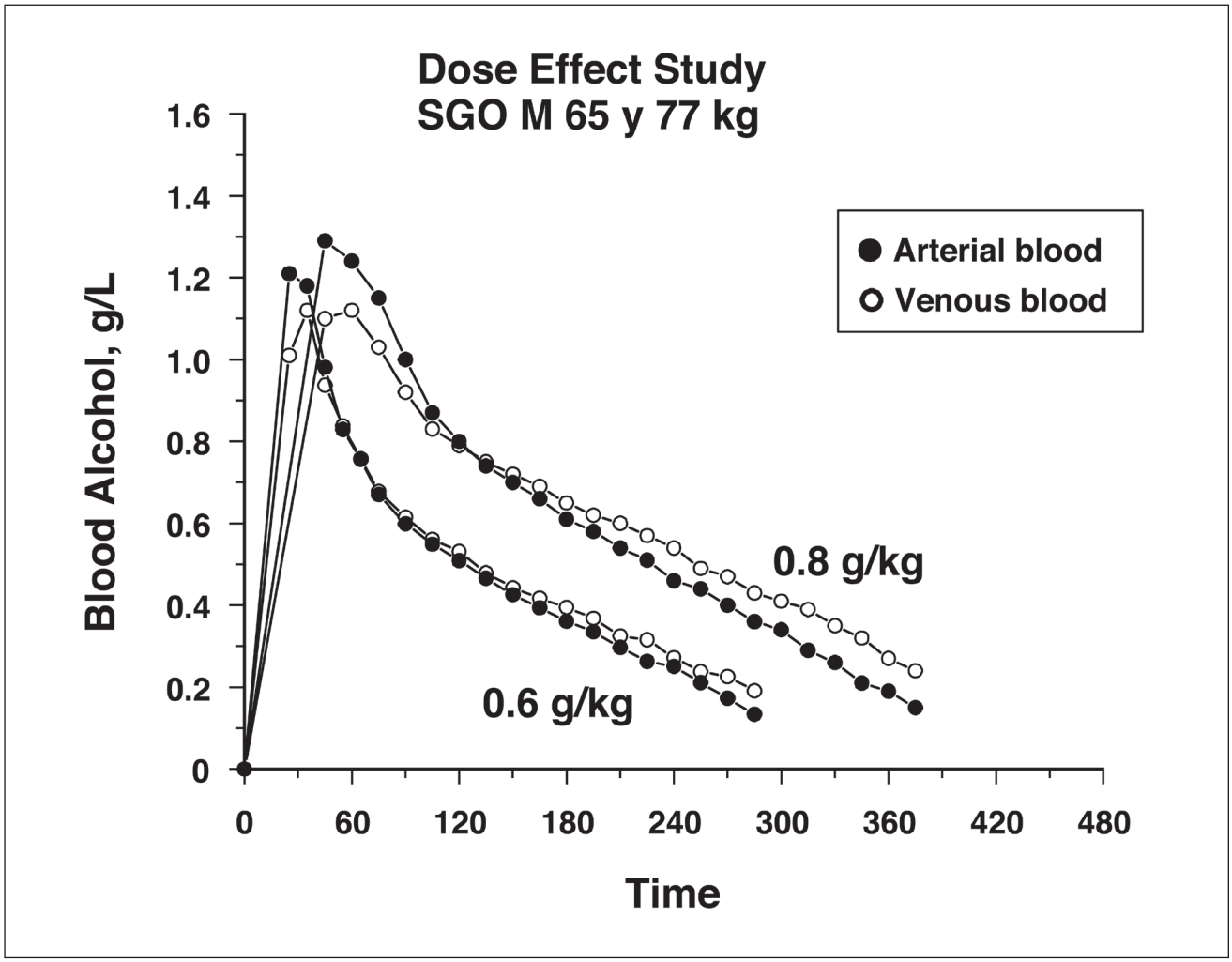


Figure 3.7 *Comparison of the concentrations of ethanol in near simultaneous samples of blood taken from a radial artery (at the wrist) and a cubital vein (at the elbow) on the same arm. The graph depicts experiments in one male subject who had consumed ethanol 0.6 g/kg or 0.8 g/kg in 20 min (Jones et al. unpublished work).*

This graph shows that the arterial (A) and venous (V) differences were positive for the first 90 minutes after end of drinking (A-V difference ~0.2 g/L on average) and then decreased as time after the end of drinking increased (Jones et al., 2004). By about 90 min post-drinking the A-V differences were zero and at all later times the concentration in the venous blood was slightly higher than in the arterial blood (~0.05 g/L). What this means is that blood/body fluid distribution ratios of ethanol depend in part on the blood sampling site in the vascular system and the status of absorption and distribution of alcohol in the body.

The time point at which the arterial and venous curves cross is the moment when ethanol is fully equilibrated in all body fluids and tissues. Thereafter, there is a redistribution of ethanol back from the skeletal muscles into the central compartment where metabolism occurs in the liver. One consequence of the A-V differences in ethanol concentration is that the apparent elimination rate of alcohol (ß-slope) is slightly faster for arterial blood. However, the areas under the alcohol curves showed no significant differences depending on the sampling compartment. Similar BAC differences, albeit less pronounced, were reported when capillary blood was compared with venous blood (Sedman et al., 1976a; Jones et al., 1989). Concentrations of blood gases, glucose and ethanol in capillary blood can be considered midway between those of the venous and arterial blood concentrations (Chiou, 1989).

2. Plasma/blood and serum/blood ratios

Whole blood consists mainly of water (~80%) with red and white cells, proteins, lipids and a host of other endogenous substances. Whole blood contains about 45-55% red cells or erythrocytes and this number is referred to as the blood hematocrit, which is slightly lower in females compared to males (Lentner, 1981). The plasma contains about 92% water and accordingly will contain a higher concentration of ethanol than whole blood by about 15% (92/80=1.15). The red cells contain mainly hemoglobin and the water content is 66-70% so the concentration of ethanol in erythrocytes will be correspondingly lower than that of whole blood and plasma. These expectations were fulfilled when actual determinations of the concentration of ethanol in plasma, serum, whole blood and red cells were reported (Winek and Carfagna, 1987; Jones et al., 1990; Charlebois et al., 1996; Iffland et al., 1999).

Figure 3.8 shows mean concentration-time profiles of ethanol for whole blood and plasma in nine volunteers who drank 0.3 g ethanol per kg body weight in 15 min on an empty stomach. Concentrations of ethanol in plasma specimens were always higher than in whole blood for the duration of the study and during absorption, distribution and elimination phases. This is expected from the difference in water content between the specimens analyzed as discussed above. The differences between plasma-blood are the same as for serum-blood in terms of the concentration of ethanol (Winek and Carfagna, 1987). However, this percentage difference in concentration means that as the alcohol curves approach zero the absolute differences in concentration of ethanol become less and less (see Figure 3.8). The slope of the post-absorptive phase of the alcohol curves will be steeper for plasma and serum compared with whole blood because of the differences in water content.

Iffland et al. (1999) reviewed a large number of published studies of the distribution of ethanol and water between plasma-serum and whole blood. Charlebois et al. (1996) reported a mean serum/blood ethanol ratio of 1.14:1 with a standard deviation of 0.041 (N=235) and the range was from 1.04-1.26. In the same study, the mean distribution ratio of ethanol between erythrocytes and whole blood (N=167) was 0.965 and the range was from 0.66 to 1.00. The water content of red cells is ~70% compared with ~80% for whole blood. Hak et al. (1995) took two near simultaneous blood specimens from 134 volunteers who had consumed alcohol. One of the specimens was centrifuged to give serum and ethanol was determined by gas chromatography in both body fluids. The mean serum/whole blood ratio was 1.15 with standard deviation 0.02 and the range was from 1.10 to 1.25. The concentrations of ethanol in the specimens analyzed were 1.05 g/L (mean) for whole blood (range 0.21 to 1.54 g/L) and 1.20 g/L (mean) for serum (range 0.25-1.83 g/L).

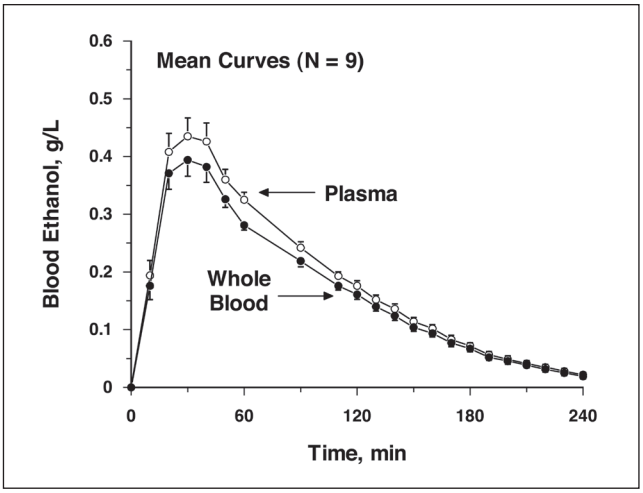


Figure 3.8 *Mean concentration-time profile of ethanol in plasma and whole blood after healthy men (N=9) drank a small dose of ethanol corresponding to 0.3 g/kg body weight in 15 min after an overnight fast (data from Jones et al., 1990).*

Driving under the influence of alcohol laws refer to punishable concentration of ethanol in specimens of whole blood and not in plasma or serum (e.g., that portion of the blood remaining after the erythrocytes are removed by centrifugation). This must be considered whenever analytical results from hospital clinical chemistry laboratories, where plasma or serum are usually analyzed, are used for legal purposes. The plasma or serum concentration of ethanol should be divided by a factor of 1.2 or 1.25 to provide a conservative estimate of the coexisting concentration in whole blood.

3. Volume of distribution

The rate and extent of drug distribution into body tissues from the central blood compartment is an important concept in pharmacokinetic calculations (Benet et al., 1990; Kalant et al., 2006; Rowland and Tozer, 1995). The mathematical relationship between the amount of drug in the body and the concentration in plasma or blood is referred to as the apparent volume of distribution, often denoted V_d. This volume can be calculated from the definition of concentration as amount/volume. Thus volume is given by amount/concentration. The concentration of a drug in the blood or plasma is easily determined so concentration is known. The amount of drug administered to the body or the dose is known (mg or mg/kg) so volume is easily calculated. Knowledge of the V_d enables investigators to calculate the amount of drug absorbed and distributed in the body from the concentration measured in a specimen of blood or plasma.

The extent of drug distribution is generally referred to as apparent volume of distribution (V_d) because V_d does not necessarily reflect any particular anatomical space or volume. Much depends on the physicochemical properties of the drug in question, its molecular structure and size, polarity, and solubility in water and in lipids. The speed of distribution depends on the person's resting state and the ratio of blood flow to tissue mass. Drugs are not evenly distributed into all body compartments and tissues at the same concentration as the blood depending on relative solubility in lipids and protein binding characteristics of the active substance.

Distribution of ethanol between the blood and the rest of the body is easier to understand compared with most other drugs because ethanol enters the total body water compartment and does not bind to plasma proteins. In fact, the total body water (TBW) can be measured by ethanol dilution experiments and gives results that agree well with methods based on isotope dilution (Jones et al., 1992; Endres and Grüner, 1994; Norberg et al., 2001).

Estimation of TBW by ethanol dilution entails administering a know amount of ethanol and waiting a few hours for absorption and distribution to be complete. Ethanol is then determined in a series of blood or plasma specimens during a large segment of the post-absorptive period and the concentration-time points fitted to a straight line by linear regression analysis. Extrapolation back to the time of drug administration gives the y-intercept or C_o, which is the blood or plasma concentration expected if the entire dose was absorbed and eliminated without any metabolism occurring. If the amount of drug in the body (dose) is expressed as mg/kg or g/kg and the concentration in blood is mg/L or g/L, then V_d or the ratio of dose/C_o will have the units of L/kg.

D. Metabolism

Chemical reactions are slow or won't occur at all at body temperature (37°C) without the help of catalysts, which are conveniently contained within cells in the form of complex proteins or enzymes. An enzyme binds to its substrate forming an enzyme-substrate complex or intermediate, which facilitates chemical reaction by lowering the activation energy necessary. Zakhari (2006) has written a very readable overview of the enzymatic pathways involved in the human metabolism of ethanol.

Ethanol is removed from the body primarily by oxidative metabolism (~95-98%) and the remainder is excreted unchanged in breath, sweat and urine (Kalant, 1996b). However, a very small fraction (< 0.1% of dose) undergoes non-oxidative metabolism by Phase II conjugation enzymes to produce water-soluble metabolites ethyl glucuronide (EtG) and ethyl sulfate (Schmitt et al., 1995; Schmitt et al., 1997; Seidl et al., 2001). Ethyl glucuronide and ethyl sulfate are specific metabolites of ethanol, and urinary analysis of these conjugates has found applications in forensic and clinical medicine as a biochemical marker of recent drinking (Droenner et al., 2002; Hoiseth et al., 2007a).

Figure 3.9 illustrates the fate of ethanol in the body showing the relative amounts eliminated unchanged in breath, urine and sweat and the amounts metabolized by oxidation and conjugation.

1. Oxidative metabolism

Human metabolism of ethanol takes place through three enzymatic processes or pathways as illustrated in Figure 3.10, namely alcohol dehydrogenase (ADH), catalase and cytochrome P 450 (CYP2E1). The primary alcohol group (-OH) in ethanol is oxidized to an aldehyde group (-CHO) by class I ADH (Zakhari, 2006). Of the three oxidative enzymes catalase is quantitatively of least importance because of the need for hydrogen peroxide to allow the reaction to proceed and this is lacking in-vivo. The principal catalytic reaction in ethanol oxidation is via Class I ADH, an enzyme abundantly located in the cytosol fraction of the hepatocyte (liver cell).

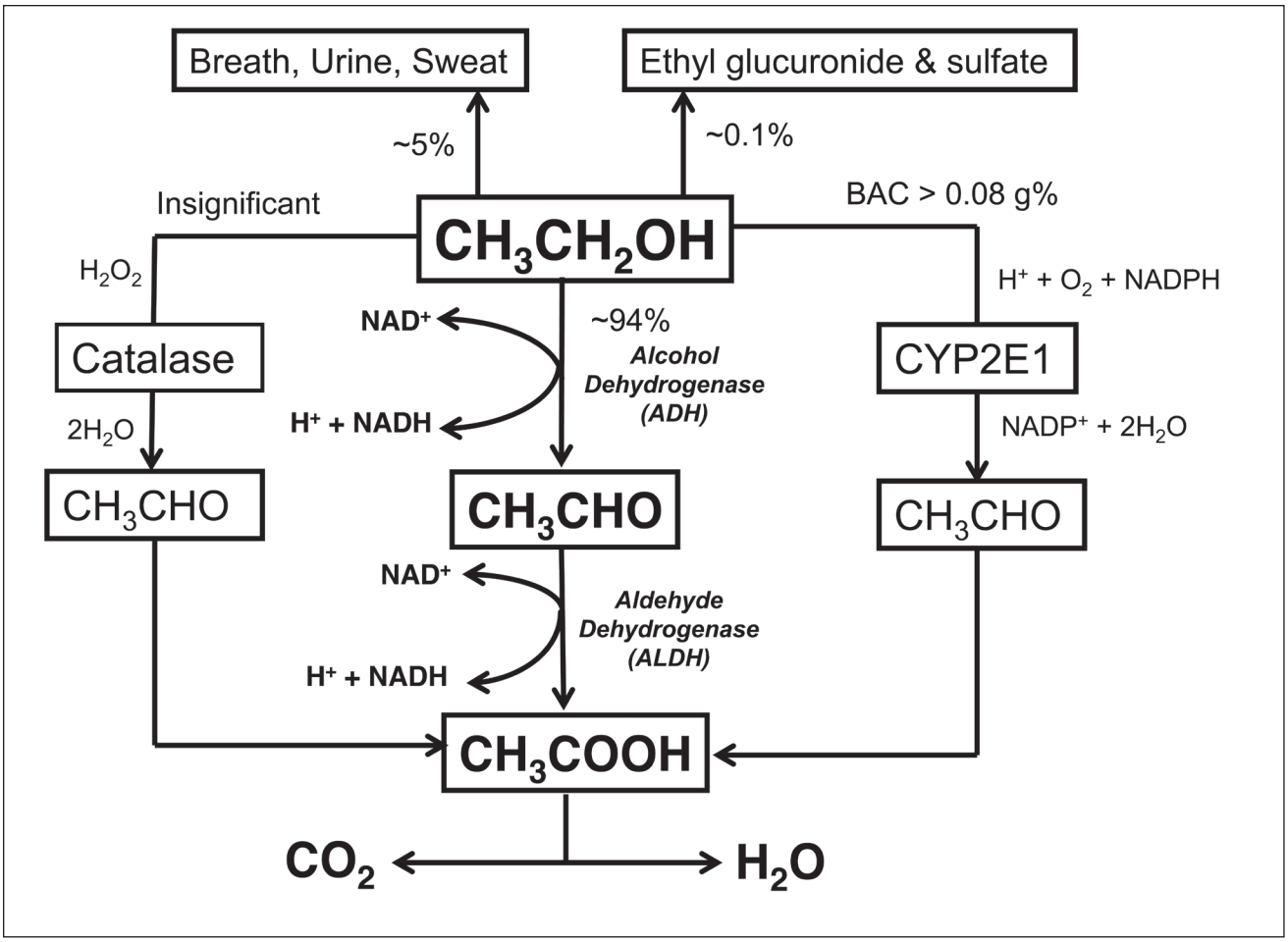


Figure 3.9 *Scheme showing the metabolism of ethanol and the relative amounts oxidized (~94%) and excreted (~6%) and the small fraction (~0.1%) that undergoes phase II conjugation reactions to produce ethyl glucuronide and ethyl sulfate. Note that oxidation via catalase enzyme is considered to play an insignificant role in-vivo owing to the need for hydrogen peroxide.*

The second stage in the metabolism of ethanol is oxidation of acetaldehyde into acetate with the help of aldehyde dehydrogenase (ALDH), an enzyme primarily located in the mitochondria. Both the first and second stage in the oxidation of ethanol require involvement of the coenzyme, nicotinamide adenine dinucleotide (NAD^+), which is converted into its reduced form (NADH) during the reactions. The acetate produced from oxidation of acetaldehyde enters into normal pathways of metabolism and is converted into acetyl coenzyme A. The end products of ethanol metabolism are carbon dioxide and water.

The increased ratio of NADH/NAD^+ that occurs during metabolism of ethanol has important ramifications for other NAD-dependent reactions and normal functioning of the liver (Day and Yeaman, 1994). During ethanol oxidation a number of metabolic disturbances occur including increased synthesis of lipids and reduced oxidation of fatty acids and this leads to an accumulation of fat in the liver. Pyruvate is shifted towards lactate which has consequences for gluconeogenesis (production of glucose) leading to hypoglycemia in heavy drinkers. The ratio of ß-hydroxybutyrate-to-acetoacetate in mitochondria increase appreciably and ketosis results (Lieber, 1990; Lieber, 1994a). The clinical consequences of hepatic oxidation of ethanol include development of fatty liver, hypoglycemia, lactic acidosis, and attacks of gout (Lieber, 1990).

2. Hepatic alcohol dehydrogenase

Alcohol dehydrogenase or ADH (alcohol-NAD^+ oxidoreductase, EC I.I.I.I.) is widely distributed in body organs and tissue such as stomach, kidney, and lungs and especially in the cytosol fraction of the liver cells. Mammalian ADH was first purified from horse liver in the late 1940s by Bonnischsen and Wassén (1948). Its physiological role during evolution was probably to protect the body from the small amounts of

ethanol produced endogenously in the gut or ingested after eating over-ripe fruits or other sugar-containing foodstuffs such as berries or honey that had undergone fermentation (Krebs and Perkins, 1970). Few enzymes have been studied so extensively as mammalian ADH and its three dimensional structure is well established; and with the tools of molecular biology, the genes encoding these peptides have been cloned (Hittle and Crabb, 1988; Agarwal and Goedde, 1990).

Humans are equipped with seven different genes located on a small segment of chromosome 4 that can encode medium chain ADH enzymes (Edenberg, 2007). Mammalian ADH is made up of a polypeptide chain containing 375 amino acid residues which gives a molecular weight of ~40,000 Daltons (Jörnvall and Höög, 1995; Jörnvall et al., 2000). Each active enzyme is a dimeric molecule comprising two ADH subunits, which can combine in various ways to produce isozymes (Von Wartburg and Papenberg, 1966). Each enzyme contains two atoms of zinc and this metal is essential for the catalytic activity (Pettersson, 1987). Based on similarities in amino acid sequence, kinetic properties as well as substrate specificity and sensitivity to chemical inhibitors, the seven ADH types can be divided into five classes (Table 3.4). Mammalian ADH belongs to a family of enzymes organized into different classes (I to V) according to their structure, physicochemical properties such as substrate specificity, Michaelis constant and specificity and sensitivity to inhibitors.

Three genes encode class I enzymes (ADH1A, ADH1B and ADH1C), and these produce subunits referred to as alpha, beta and gamma (Zakhari, 2006). The resulting proteins of class I ADH are the enzymes mainly involved in metabolism of ethanol and their molecular structure and amino acid sequence is very similar. There are three different ADH1B alleles that differ by a single amino acid at the binding site for substrate. Switching the position of a single amino acid dramatically alters the catalytic properties of the enzyme. Indeed, the polymorphism of class I ADH is considered as a possible explanation for inter-individual and inter-ethnic differences in rates of ethanol metabolism (Crabb et al., 1987; Ehrig et al., 1990; Mizoi et al., 1994; Tam et al., 2006).

The nomenclature describing ADH enzymes has changed over the years and the current situation for class I enzymes is summarized in Table 3.4. The main hepatic enzyme the human class I ADH consists of three subunits denoted with the Greek letters alpha (α), beta (ß), and gamma (γ). These can associate randomly to produce six possible active enzymes (dipeptides). Moreover, the genes that encode the beta and gamma peptides are polymorphic which leads to three different ß subunits ($ß_1$, $ß_2$, $ß_3$) and two different γ subunits (γ_1, γ_2).

The various ADH subunits combine to produce dipeptides and examples of both homodimers and heterodimers exist (Crabb et al., 1993; Crabb, 1995). The three ß homodimers exhibit different catalytic properties and are expressed with different frequencies in different racial groups (Borson and Li, 1986). Studies have shown that European Caucasians have a predominance of the homodimer $ß_1ß_1$ isozyme whereas in 85% of Asians the $ß_2ß_2$ variant dominants (known previously as atypical ADH). In about 25% of African-Americans there is an abundance of the $ß_3ß_3$ form. Asian populations inheriting the $ß_2$-ADH variant could therefore have both homodimer $ß_2ß_2$ or heterodimer $ß_2ß_1$ capable of oxidizing ethanol (Harada, 1990; Harada and Okuda, 1993; Wall et al., 1996). Studies have shown that the $ß_2ß_2$ form of the enzyme has a high V_{max} for the oxidation of ethanol and accordingly those equipped with this isozyme should be able to eliminate ethanol faster than those with a preponderance of the $ß_1$-ADH which has a lower V_{max}.

Table 3.4
Polymorphic Forms of Alcohol Dehydrogenase (Class I ADH) and Aldehyde Dehydrogenase (Class II ALDH) Showing the Relative Frequencies of Occurrence of the Various Isozymes in Different Racial Groups (Modified from Hittle and Crabb, 1988; Edenberg 2007; Zakhari, 2006)

Gene nomenclature New	Gene nomenclature Old	Protein Subunit	Human populations with a high frequency of the particular isozyme.
ADH1B*1	ADH2*1	$Beta_1$	Caucasians (90-95%), African-Americans (85%), Asians (35%)
ADH1B*2	ADH2*2	$Beta_2$	Caucasians (<5%), African-Americans (<5%), Asians (65%)
ADH1B*3	ADH2*3	$Beta_3$	Caucasians (<5%), African-Americans (15%), Asians (<5%)
ADH1C*1	ADH3*1	$Gamma_1$	Caucasians (50-60%), African-Americans (85%), Asians (95%)
ADH1C*2	ADH3*2	$Gamma_2$	Caucasians (40-50%), African-Americans (15%), Asians (5%)
ALDH2*1		High activity	Predominant in Caucasians and African-Americans
ALDH2*2		Low or no activity	Predominant in East Asians, mainly Chinese and Japanese

In spite of different patterns of class I ADH isoenzyme the average rate of ethanol metabolism was not much different between Asians and Caucasians (Mizoi et al., 1994; Mizoi et al., 1987; Adachi et al., 1989; Li et al., 2000). This is probably accounted for by large intra-ethnic group variations caused by dietary and environmental factors. Indeed, in well-controlled studies in which the test subjects were carefully matched for age, body composition, use of drugs and their smoking and drinking habits, no statistically significant racial differences in the rate of ethanol disposal have been found (Bennion and Li, 1976, Reed, 1978; Tam et al., 2006).

The class I ADH enzymes also oxidize secondary alcohols to the corresponding ketones instead of aldehydes and so 2-propanol and 2-butanol are converted into acetone and 2-butanone, respectively (Ehrig et al., 1988). Under appropriate conditions, e.g., excess NADH in the liver hepatocytes, ketones can also become reduced to the corresponding secondary alcohols (Jones, 1995c).

3. Inhibition of alcohol dehydrogenase

Much attention has been given to ways of accelerating or retarding the rate of ethanol metabolism by treatment with various drugs. The most successful way to block the metabolism of ethanol is by treatment with 4-methyl pyrazole (fomepizole), which functions as a competitive inhibitor of ADH and competes with ethanol for binding sites on the enzyme. The disappearance rate of ethanol from the bloodstream can therefore be slowed appreciably if 4-methyl pyrazole is administered (Shannon, 1998). Indeed, this drug has been developed into a commercial product called fomepizole (Antisol®) and is widely used in the treatment of patients poisoned with methanol or ethylene glycol (Jacobsen and McMartin, 1997; Henderson and Brubacher, 2002). Intravenous administration of fomepizole has become the antidote of choice for treatment for people poisoned with toxic alcohols such as methanol and ethylene glycol (Velez et al., 2007; Hall, 2002). Blocking the Class I ADH enzyme prevents methanol and ethylene glycol from being converted into their toxic metabolites, formic acid and oxalic acid, respectively (Brent et al., 2001).

The metabolic interaction between ethanol and methanol metabolism is illustrated in Figure 3.10 and this reaction for a long time has had therapeutic applications in the treatment of patients poisoned with methanol (Jacobsen and McMartin, 1986; Barceloux et al., 2001; Haffner et al., 1992). Also shown are the various enzymes and coenzymes involved and the chemical structures of two well-known inhibitors of ADH (4-methyl pyrazole) and ALDH (disulfiram). Administration of ethanol to compete with methanol in treatment of poisoning is less satisfactory if children might have ingested this toxic alcohol or in an alcoholic suffering from liver dysfunction (Eder et al., 1998; Bekka et al., 2001). This has led to the development of 4-methyl pyrazole as an antidote for methanol and ethylene glycol poisoning instead (Hall, 2002; Henderson and Brubacher, 2002).

Abnormally low rates of ethanol metabolism were reported in people who were malnourished or had been on low-protein diets and then consumed alcohol (Bode et al., 1971; Bode, 1978). The underlying mechanism for this finding is probably related to a lowered activity of enzyme protein owing to the poor nutritional condition. Support for this explanation came from animal models (Bosron et al., 1984; Mezey, 1998). The possible influence of liver cirrhosis and rate of ethanol metabolism is covered later in this chapter.

The rate of ethanol metabolism as reflected in the disappearance rate of ethanol from blood is usually relatively slow after an overnight fast. The slope of the post-absorptive elimination phase was 0.11 g/L per h after 0.4 g/kg was given by intravenous infusion to healthy volunteers who had not eaten any breakfast. This rate was boosted to 0.174 g/L per hour (60%) during the time the subjects received a mixture of amino acids intravenously, which are precursors of proteins (Lisander et al., 2006). Intravenous administration of equicaloric glucose failed to bring about any statistically significant increase in the rate of ethanol elimination from blood compared with the fasting state (0.121 g/L per h). The mean BAC curves after treatments with amino-acids, equicaloric glucose and an energy-free control treatment (Ringers acetate) are shown in Figure 3.11. The steeper slope after treatment with amino-acids was evident in each of the six subjects tested.

Ethanol pharmacokinetics was also studied in patients undergoing dialysis because of renal failure, although this condition was not associated with a diminished rate of ethanol elimination from the bloodstream (Jones and Hahn, 1997). Although the kidney has some metabolic activity including ADH enzymes this represents only a small fraction of the total enzyme activity contained in the liver, which is the major site of ethanol oxidation.

Substances capable of boosting the elimination rate of ethanol from blood, so-called sobering-up agents tend to attract a lot of attention from the news media and these drinks are often advertised and offered for sale to the public. The composition of such energy drinks can vary widely but they almost always contain a lot of carbohydrates particularly fructose, the substance that has been studied most often as sober-aid (Rogers et al., 1987). However, in many such studies the results are difficult to interpret because of so many experimental variables, such as dose of ethanol and fructose, the timing and routes of administration relative

to ethanol, etc. (Soterakis and Iber, 1975; Goldberg et al., 1979; Crownover et al., 1986; Jones, 1991a). The effect of intravenous fructose administration was used to investigate whether first-pass metabolism was predominantly gastric or hepatic (Parlesak et al., 2004). The authors concluded that because fructose is metabolized in the liver and not in the stomach, their results support the notion that only a negligible part of first-pass metabolism occurs in the stomach.

In studies of this nature, it is crucial to distinguish the influence of sugars on absorption of ethanol from the gut and a lowering of C_{max} from influences on rate of metabolism, as reflected in a steeper slope of the post-absorptive elimination phase (Mascord et al., 1991). In many published studies into the so-called "fructose effect" this has not always been the case and the wrong conclusions have been drawn. In a recent well-designed study of a commercial sober-up faster drink negative results were found (Musshoff et al., 2007). The elimination rate of ethanol from blood was 0.17 mg/g per h (SD 0.016) after the sober-aid compared with 0.18 mg/g per h (SD 0.029) without the drink acting as a control group.

The rate of metabolism of ethanol is tightly linked to hepatic oxygen consumption and the energy requirements of the body and the person's basal metabolic rate (Lisander et al., 2006). In patients suffering from severe burn trauma ethanol was metabolized faster owing to the hypermetabolic state induced by thermal injuries (Jones et al., 1997b). All-in-all it does not appear possible to boost the rate of ethanol metabolism by drug treatment over and above that seen in a healthy well-nourished individual (Jones, 1991a; Mascord et al., 1988).

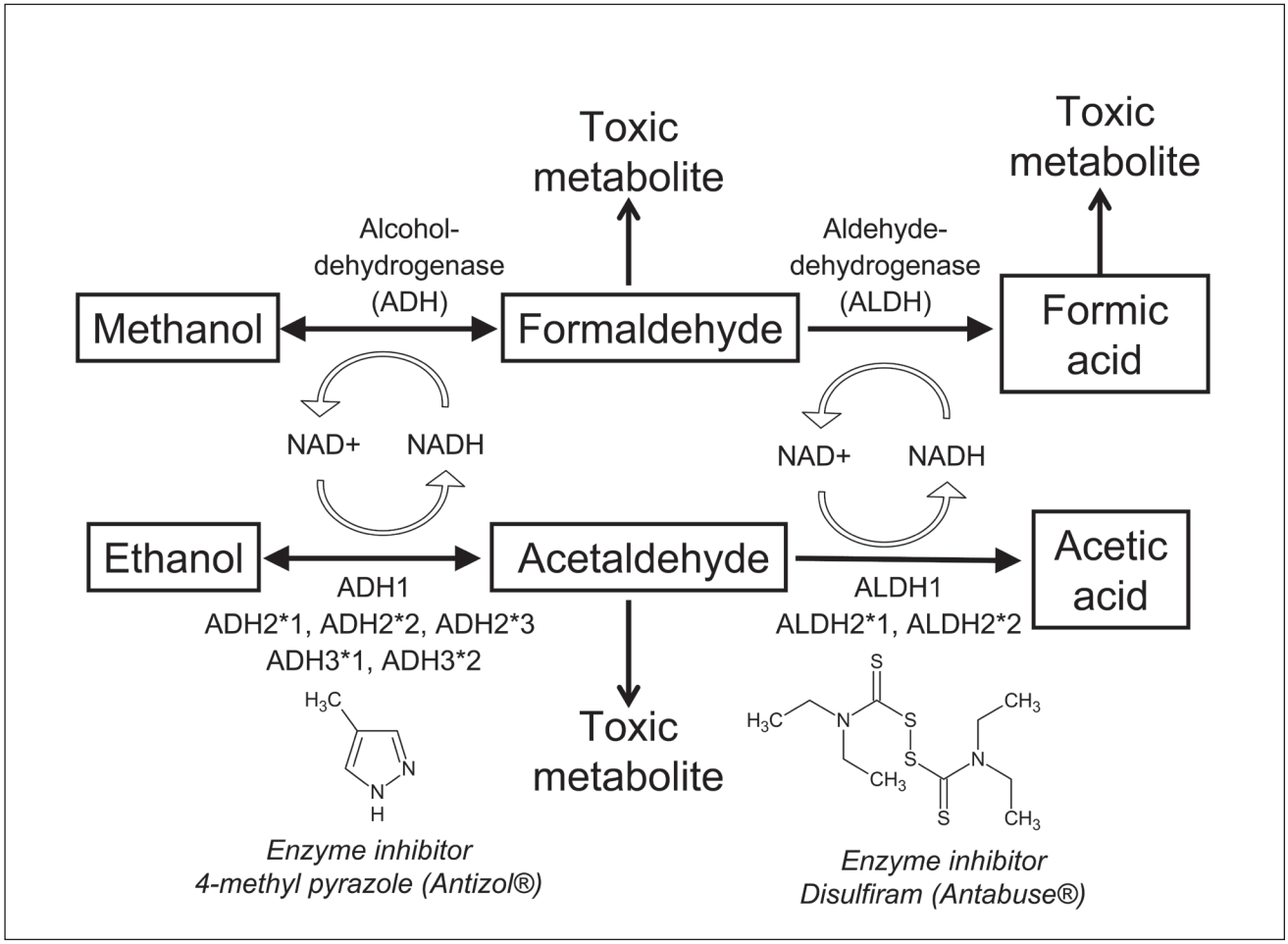


Figure 3.10 *Scheme showing oxidative metabolism of ethanol and methanol into their respective aldehydes and carboxylic acids. The various isozymes of alcohol (ADH) and aldehyde dehydrogenase (ALDH) are shown and the chemical structures of well known enzyme inhibitors, 4-methyl pyrazole (ADH inhibitor) and disulfiram (ALDH inhibitor).*

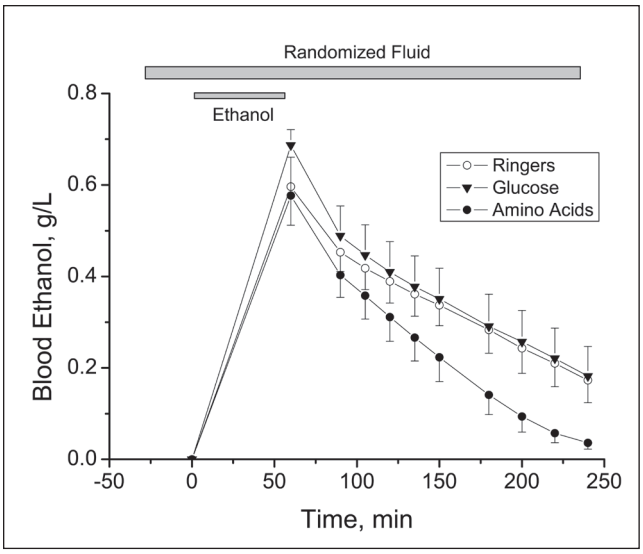


Figure 3.11 *Mean blood alcohol concentration profiles (N=6) after a dose of 0.5 g ethanol per kg body weight was given by intravenous infusion and at the same time subjects received infusions of amino acids, equi-caloric glucose or a calorie-free control solution of Ringers acetate (data from Lisander et al., 2006).*

4. Gastric alcohol dehydrogenase

ADH (class IV) is widely expressed in the stomach of various species, including man, as confirmed by biopsy and histochemical methods that are difficult to fault (Seitz et al., 1993). The class IV stomach ADH shows differences in catalytic activity depending on a person's age, gender, drinking habits and ethnicity (Danielsson et al., 1994). The presence of gastric ADH attracted attention after it was claimed that this enzyme was responsible for an appreciable pre-systemic metabolism of ethanol. This was offered to explain the reduced bioavailability of ethanol when given orally compared with the same dose administered by intravenous infusion.

The gastric ADH had a higher k_m value for ethanol as substrate compared with liver class I ADH (Parés et al., 1994). Some investigators proposed that gastric ADH played a major physiological function by metabolism of ethanol already in the stomach. It has been suggested that inter-subject differences in activity of gut-ADH might be one reason for the inherent differences in ethanol-induced organ toxicity and untoward effects of alcohol on the body (Lieber, 1994a and 1994b). In-vitro experiments have demonstrated the possibility to block the action of gastric ADH by treatment with certain drugs, e.g., aspirin, ranitidine and cimetidine (Palmer et al., 1991). This led to the suggestion that this drug-alcohol interaction might have social-medical consequences (Hernandez-Munoz et al., 1990; Roine et al., 1990). However, alcohol and drug effects under in-vitro conditions are not always verified when in-vivo experiments are conducted, as exemplified by the aspirin-ethanol interaction (Melander et al., 1995). This topic of drug-alcohol interactions and clinical implications was recently reviewed (Jones, 2003d).

In the 1980s considerable interest was aroused in the notion that a substantial part of ingested ethanol underwent metabolism already in the stomach. This process became known as gastric first-pass metabolism (FPM) of ethanol (Roland and Tozer, 1980; Lieber, 1997b). The strongest proponents for gastric FPM of ethanol were Dr. Charles Lieber and his research group from New York. These investigators published scores of articles dealing with the effects of gender, ethnicity, age, alcoholism as well as various commonly prescribed medications, such as aspirin, cimetidine and ranitidine (Roine et al., 1990; Caballeria et al., 1989a; Caballeria, 1989b; DiPadova et al., 1992). Evidence supporting the importance of gastric ADH in FPM of ethanol included the following:

- Identification by histochemical methods of several forms of alcohol dehydrogenase (primarily class IV or sigma ADH) in the human gastric mucosa (Cabelleria et al., 1991; Moreno and Pares, 1991).
- The fact that about 20% of the dose of ethanol is absorbed directly through the stomach wall and therefore gets exposed to the gastric ADH enzyme (Kalant, 1971).
- The area under the BAC time-curve is always less when ethanol is administration by mouth (peroral) compared with the same dose given intravenously, thus indicating a lower systemic availability (Baraona et al., 1994).
- First-pass metabolism is reduced after concomitant use of certain drugs (e.g., aspirin, cimetidine, ranitidine) known to inhibit gastric ADH enzymes in vitro (Roine et al., 1990; DiPadova et al., 1992; Gentry et al., 1999).
- First-pass metabolism is less pronounced in subjects with smaller gastric mass e.g., after gastrectomy (Caballeria et al., 1989b).
- First-pass metabolism is diminished or absent in Japanese subjects many of whom apparently lack gastric ADH enzymes (Baraona et al., 1991)

a. Gastric first-pass metabolism

First-pass metabolism is a term associated with the oral administration of drugs and indicates that a certain amount

of the active substance has been eliminated before reaching the systemic circulation (Pond and Tozer, 1984). Accordingly, molecules of the drug undergo pre-systemic metabolism either in the gut (stomach or intestine), or more commonly as the portal blood flows through the liver. First-pass metabolism is associated with a reduced and variable bioavailability and the pharmacological effect of the drug at its site of action is also diminished.

After oral administration, ethanol is absorbed from the stomach and intestine and transported with the portal vein through the liver then on to the heart via the hepatic vein before the systemic arteries distribute ethanol throughout the total body water. Experiments by several research groups have indicated that the area under the BAC time curve is considerably less after oral compared with intravenous administration of the same dose. The smaller areas under the curves (AUCs) after oral compared with intravenous was taken as an indication of first-pass metabolism of alcohol occurring either in the stomach or during the time blood passes through the liver. However, comparing AUC after oral and intravenous administration of the same dose is only strictly valid as a method to determine systemic availability and first-pass metabolism for drugs that obey first-order kinetics (Rowland and Tozer, 1995).

Presystemic oxidation of ethanol by gastric ADH attracted considerable interest when it was suggested that the stomach mucosa functioned as a protective barrier to the toxic effects of ethanol (Caballeria et al., 1991; Dipadova et al., 1987; Gentry et al., 1994). The magnitude of first-pass oxidation of ethanol by gastric ADH seemed to be influenced by a host of variable factors: age, gender, ethnicity, drinking habits (alcoholism), type of beverage (beer or spirits) and the concomitant intake of various drugs (for reviews see Levitt, 1993; Lieber, 1997b, 1994a, 1994b).

If a significant first-pass metabolism of ethanol actually existed, this would have implications in forensic science and legal medicine when blood-alcohol calculations are made. The Widmark equation, which is widely used for this purpose, rests on the assumption of 100% availability of an oral dose. One study suggested that the activity of gastric ADH was appreciably less in women compared with men and was also decreased in alcoholics compared with moderate drinkers (Frezza et al., 1990). Moreover, a large proportion of Japanese seemingly lacked an active gastric ADH enzyme. First-pass metabolism should therefore be small or negligible in these individuals (Baraona et al., 1991).

A general consensus exists that a small fraction of an oral dose of ethanol fails to reach the systemic circulation, but whether this FPM occurs predominantly in the stomach or the liver is still unsettled (Levitt and Levitt, 1994; Levitt and Levitt, 2000b). As hinted at above, FPM is not so easy to evaluate when AUCs after oral and intravenous administration are compared owing to ethanol's non-linear Michaelis-Menten kinetics. Studies have shown that AUC for ethanol increases more than proportionally with increasing dose (Wagner, 1993). Moreover, many experiments on gastric first-pass metabolism involved very small doses of ethanol (e.g., 0.15-0.3 g/kg) and no consideration was given to the existence of saturation kinetics. The percentage difference in AUC after oral and intravenous administration was greater the smaller the doses of ethanol (0.15 > 0.25 > 0.30 g/kg). The magnitude of FPM was especially marked if the drinking subjects took the alcohol 1 hour after they had eaten a fat-rich meal, which slows absorption and leads to highly variable AUC especially after small ethanol doses (Fraser, 1998).

Whether the site of FPM was primarily the stomach or the liver proved difficult to resolve, and strong differences of opinion were ventilated in the literature (Levitt et al., 1994; Gentry et al., 1994). In one study of the bioavailability of ethanol, investigators used a deuterium labeled analogue (0.3 g/kg), which was given perorally, and unlabeled ethanol (0.3 g/kg) was given at the same time intravenously (Ammon et al., 1996). In other experiments to avoid FPM occurring in the stomach ethanol was also given intraduodenally. From a careful evaluation of the BAC profiles for various routes of administration, the investigators concluded that FPM in the stomach could only account for a few percent of the total dose of 0.6 g/kg administered. They also found that gender-related differences were small or negligible despite earlier work showing that FPM was less in women. All-in-all many published papers from different research groups lead to the conclusion that the liver is the primary site for FPM of ethanol (Fraser, 1998; Levitt, 1994; Levitt and Levitt, 2000b).

Large inter-subject variations in blood-alcohol curves are seen when small amounts of ethanol (< 0.3 g/kg) are consumed after eating a meal. Under these conditions, absorption of ethanol is slow and intermittent and hepatic first-pass metabolism is substantial. This leads to appreciable inter-subject variation in C_{max}, t_{max} and AUC for a given dose as shown in Figure 3.12.

By contrast, when moderate doses of ethanol (0.50-0.80 g/kg) are ingested on an empty stomach, the first-pass metabolism is more or less insignificant or absent (Wagner, 1986; Amir et al., 1996). The absorption of ethanol is so rapid under these conditions that the liver enzymes become saturated with substrate thus preventing any FPM from occurring. Under fasting conditions, ethanol-dilution can be used to estimate total body water giving values in good agreement with isotope dilution methods (Jones et al., 1992; Enders and Grüner, 1994). The saturable nature of ethanol kinetics

means that AUC is highly dependent on the dose and speed of gastric emptying (Wagner, 1986a; Fraser et al., 1992; Levitt and Levitt, 1994). Low concentrations of ethanol in the portal venous blood passing through the liver are cleared very effectively by hepatic enzymes, and AUCs are diminished (Fraser et al., 1995; Oneta et al., 1998). The swifter the absorption and the higher the ethanol concentrations in portal venous blood the less is the hepatic first-pass metabolism. Kalant (2005) gave a balanced and convincing review of food-induced effects on FPM and in this connection the liver was thought to be more important than the stomach.

Levitt and Levitt (2000b) argued convincingly that the liver is the primary site for pre-systemic metabolism of ethanol. They point out, among other things, that the amount of ADH enzyme in the gastric mucosa is only a fraction of that in the liver (Levitt and Levitt, 1994). Another confounding factor is the effect of food on hepatic blood flow, which increases rate of exposure to metabolizing enzymes (Host et al., 1996; Lautt and Macedo, 1997). With a rapid absorption of ethanol the concentrations reaching the liver overwhelm the capacity of ADH so that hepatic FPM is no longer evident. Gastric residence time is therefore a key variable in any discussion of FPM of ethanol and the longer the drug remains in the stomach the more opportunity for oxidation by gastric ADH (Oneta et al., 1998). Clearly the rate of absorption is a key consideration not only for determining C_{max} but also AUC and indirectly FPM (Levitt et al., 1997; Levitt and Levitt, 2000b). Gastric ADH activity and consequently gastric FPM was less pronounced in many Japanese subjects who seemed to lack class IV ADH in the stomach (Baraona et al., 1991); neither were there any diurnal variations in gastric FPM of ethanol (Sharma et al., 1995).

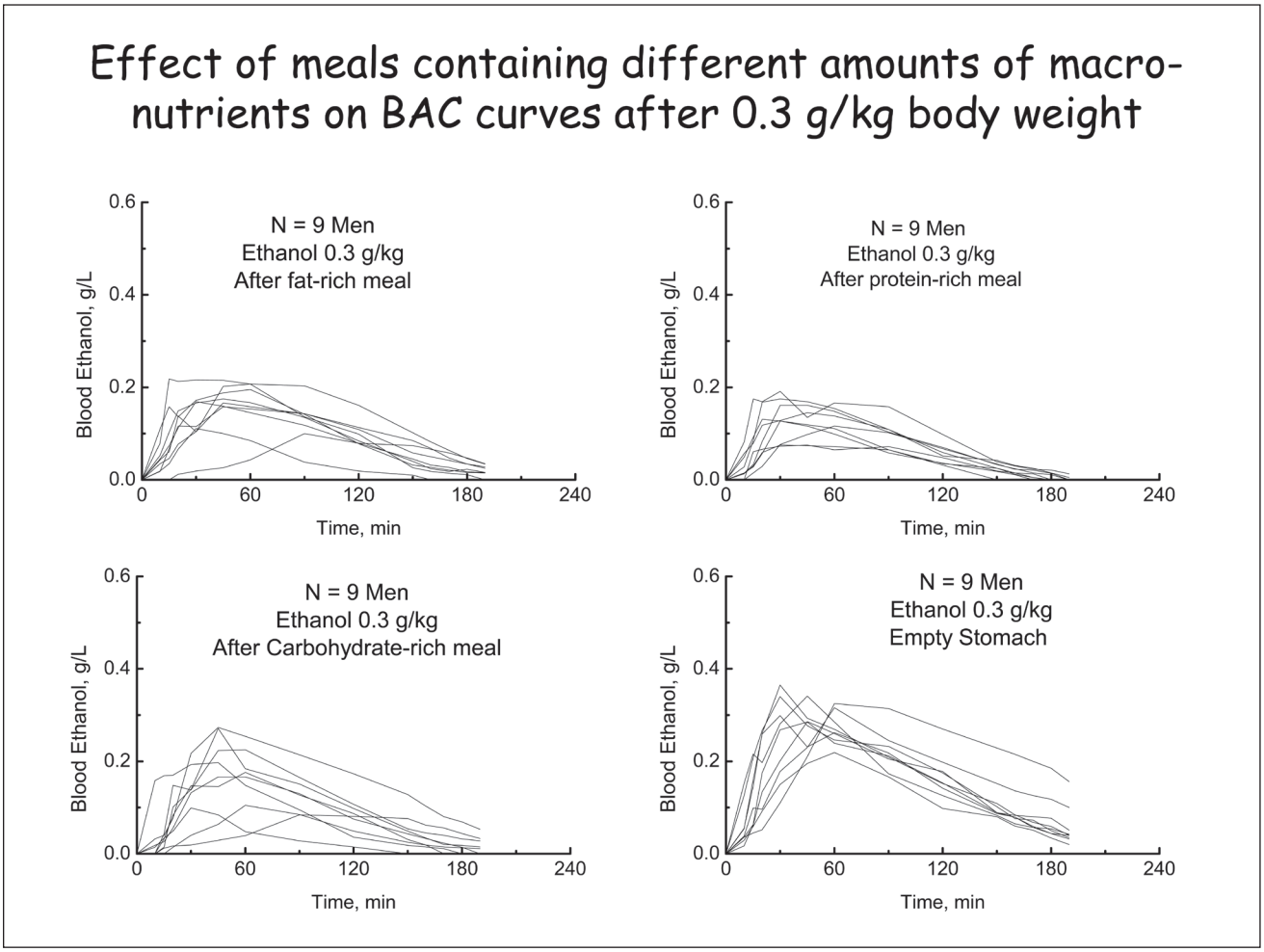


Figure 3.12 *Large inter-subject variation (N=9 subjects) in blood alcohol concentration profiles after a small dose of alcohol (0.3 g/kg) was consumed after eating standardized breakfasts consisting of high-fat, high-protein or high-carbohydrate meals compared with a no-food control condition (data from Jones et al., 1997a).*

The role of the stomach and gastric ADH in first-pass metabolism of ethanol seems to have been much exaggerated and blood-alcohol measurements are not so appropriate to investigate this question (Levitt and Levitt, 2000a). These workers produced convincing evidence from various experiments that the liver is the primary site for FPM, and this has been endorsed by others (Crabb, 1997). After low doses of alcohol (0.15 g/kg), if the first-pass metabolism occurs in the liver or the stomach it is extremely difficult to evaluate because of capacity limited (saturation) metabolism of ethanol (Levitt, 1993; Levitt, 1994; Levitt and Levitt, 1998). The rate of clearance from the blood is much more effective at very low substrate concentrations reaching the liver via the portal venous blood (Rowland and Tozer, 1995).

b. Drug effects on first-pass metabolism

The issue of FPM of ethanol and the role played by gastric ADH became a major concern for pharmaceutical companies when it was shown that commonly prescribed drugs, such as H_2-receptor antagonists (ranitidine and cimetidine) and aspirin, inhibited gastric ADH under in-vitro conditions (Palmer et al., 1991; Dauncey et al., 1993). A number of studies showed that concomitant use of this medication together with ethanol resulted in higher C_{max} compared with a placebo treatment. The mechanism proposed to account for this finding was inhibition of gastric ADH by the drug treatment so a larger fraction of the dose reached the systemic circulation.

The chemical structures of cimetidine and ranitidine are compared in Figure 3.13 with the well-known inhibitors of class I ADH, namely pyrazole and 4-methyl pyrazole. Note that the 5-membered heterocyclic ring is common in cimetidine and pyrazole.

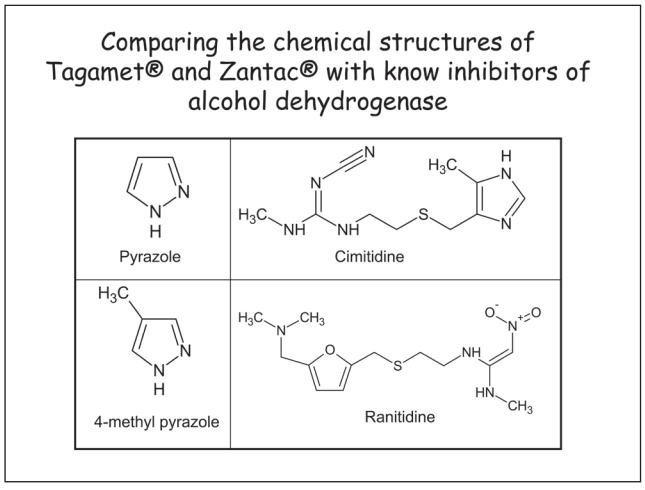


Figure 3.13 *Chemical structures of pyrazole and 4-methyl pyrazole well-known inhibitors of alcohol dehydrogenase (ADH) and cimetidine and ranitidine, suggested inhibitors of gastric ADH.*

Other investigators were less enthusiastic and failed to replicate this drug-alcohol interaction. Indeed, the study by Toon et al. (1994) was particularly convincing because they used a randomized crossover design with an ethanol dose of 0.5 g/kg body weight taken in the morning, at midday and in the evening. In one arm of the study subjects were fasted and in another arm they had eaten a standardized meal. This study failed to disclose any effect of pre-treatment with ranitidine on C_{max}, t_{max} or AUC but significant changes in these parameters was evident after food was consumed prior to drinking alcohol (Toon et al., 1994). Similar negligible effects of ranitidine were reported after 24 men received ethanol doses of 0.15, 0.30 and 0.60 g/kg body weight with or without ranitidine pre-treatment twice daily (Bye et al., 1996). A very small rise in BAC (higher C_{max}) was observed after the smallest dose of ethanol but this might just as well have been caused by the drug treatment altering gastric emptying, independent of any inhibition of gastric ADH. A thorough, well-balanced overview of the effects of H_2-antagonists on gastric ADH and FPM of ethanol was presented by Fraser (1998). He concluded that any effect of these drugs on C_{max} and AUC was small or negligible and only evident after very small ethanol doses (0.15 g/kg) and therefore lacked any clinical or forensic significance (Fraser, 1998).

Supporters of a role of gastric ADH in FPM maintained that bolus dose drinking studies were not appropriate because this differs from most real-world situations. Instead they advocated repetitive intake of smaller doses of alcohol over several hours to test the effect of drug treatment (Gupta et al., 1995; Arora et al., 2000; Baraona, 2000; Lieber, 2000). The C_{max} after pre-treatment with cimetidine became successively higher after four separate doses of ethanol (0.15 g/kg each) were consumed every 15 min for 2 hours (Gupta et al., 1995). Similarly, pre-treatment with ranitidine (150 mg b.i.d for 7 days) and repetitive drinking of alcohol (4 x 0.15 g/kg per dose) also resulted in a higher C_{max} and AUC, which was attributed to drug-induced inhibition of gastric ADH (Arora et al., 2000). The magnitude of FPM was assessed by calculating the difference between the amount of ethanol reaching the blood after i.v. and oral administrations of the same dose using an integrated form of the MM equation to calculate V_{max} and k_m parameters (Arora et al., 2000).

Another aspect of gastric ADH and its inhibition by drugs is that almost all experiments were done in healthy volunteers and not in patient groups suffering from gastritis or other upper GI ailments that require this kind of medication. This was remedied in a study with elderly patients diagnosed with atrophic gastritis who received 0.225 g ethanol per kg both orally and intravenously (Pedrosa et al., 1996). Gastric ADH activity was significantly lower in atrophic gastritis patients compared with controls, but the

main determinant of FPM was the rate of gastric emptying. Neither atrophic gastritis nor treatment with tetracycline had any impact on FPM of ethanol as judged by comparing C_{max} and AUC in male and female patients (Pedrosa et al., 1996). After a 4-h fast and pre-treatment with cimetidine (400 mg twice daily) there was no evidence for any effect of this drug on FPM when two different doses of ethanol (0.15 g/kg and 0.45 g/kg) were compared (Clemmesen et al., 1997). These results tallied with another study in the fasting state in which 0.8 g/kg was administered to 12 volunteers after pre-treatment with cimetidine, ranitidine or omeprazole, which are drugs commonly used for hyperacidity in the stomach (Jönsson et al., 1992).

When evaluating studies dealing with the effect of exogenous factors (e.g., food, drugs, smoking, etc.) on C_{max} and AUC, it is important to recall that the values obtained cannot exceed those predicted for 100% absorption of the dose administered. The only exception is when there is an overshoot peak (Figure 3.5) often the result of rapid gastric emptying and lasting up to 35 min post-dosing (Klockhoff et al., 2002).

A meta-analysis of papers dealing with the effect of H_2-receptor antagonists on C_{max} and AUC concluded that small increases in these parameters were possible after cimetidine and ranitidine but not after famotidine and nizatidine (Weinberg et al., 1998). With larger numbers of participants in the experiments, it was less likely to find such differences in C_{max} and AUC. It was also concluded that relative to accepted legal definitions of intoxication, the effect of these drugs on BAC is unlikely to be clinically relevant (Dauncey et al., 1993).

Opinions differ about the importance of gastric ADH in first-pass metabolism of ethanol and the relative importance of enzymes in the gastric mucosa compared with the liver (Ali et al., 1995). The experiments that involved very low doses of ethanol (0.15-0.3 g/kg body weight) in small numbers of subjects are difficult to interpret. Moreover, the small dose of ethanol was given to the subjects in the morning 1 h after they had eaten a fatty breakfast. This results in a food-induced delay in gastric emptying and a significantly lower C_{max} of the BAC curve (Roine et al., 1990; Caballeria et al., 1991; DiPadova et al., 1992; Frezza et al., 1990; DiPadova et al., 1992). After larger doses of ethanol (0.60-0.80 g/kg) were consumed on an empty stomach (overnight fast) first-pass metabolism of alcohol after pre-treatment with H_2-receptor antagonist drugs was insignificant (Jönsson et al., 1992; Dauncey et al., 1993; Fraser et al., 1992; Fraser et al., 1991).

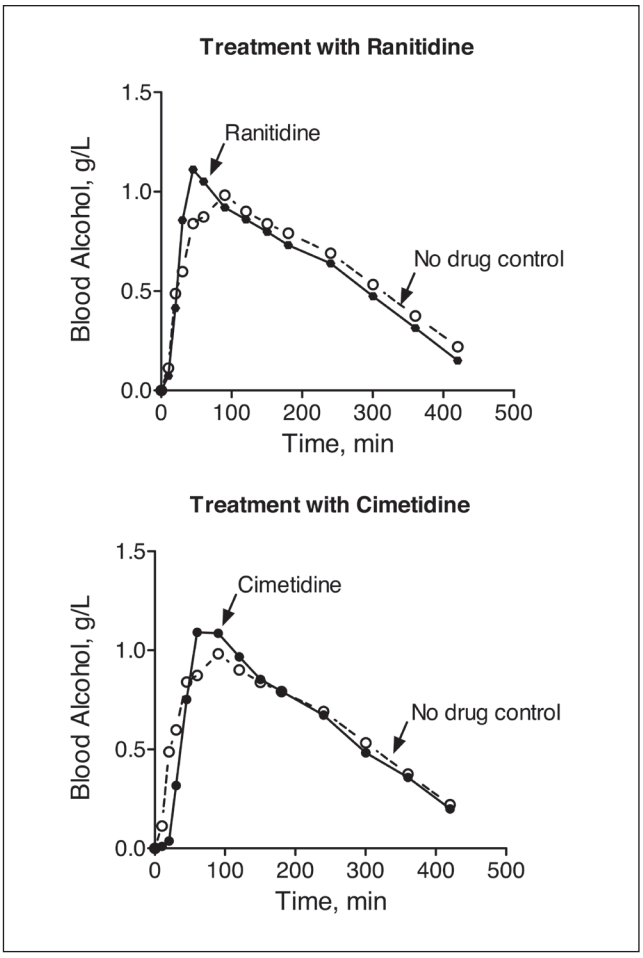


Figure 3.14 *Blood-concentration time profiles of ethanol in two subjects after 0.80 g ethanol/kg body weight were ingested on an empty stomach both before and after medication with cimetidine (Tagamet®) or ranitidine (Zantac®) 400 mg daily for 7 days (from Jönsson et al., 1992).*

Figure 3.14 compares the blood-alcohol profiles in two subjects after they drank 0.80 g ethanol per kg body weight with and without pre-treatment with cimetidine for seven days. The subjects fasted overnight and under these conditions the pharmacokinetic profiles were remarkably similar thus failing to confirm any influence of this kind of drug on the pharmacokinetics of ethanol in the fasting state (Jönsson et al., 1992; Fraser et al., 1992).

Average BAC curves from the same study on ethanol kinetics, which involved a comparison of cimetidine (Tagamet®), ranitidine (Zantac®) and omeprazole (Losec®), is shown in Figure 3.15. The healthy volunteers self-medicated with the drugs for seven days before they drank ethanol

(0.8 g/kg) on an empty stomach. The resulting BAC curves were compared with a no-drug treatment as control. The average BAC profiles (N=12 participants) were similar in all respects, which failed to confirm that drugs prescribed for treating gastric hyper-acidity alter the pharmacokinetics of ethanol or first-pass metabolism.

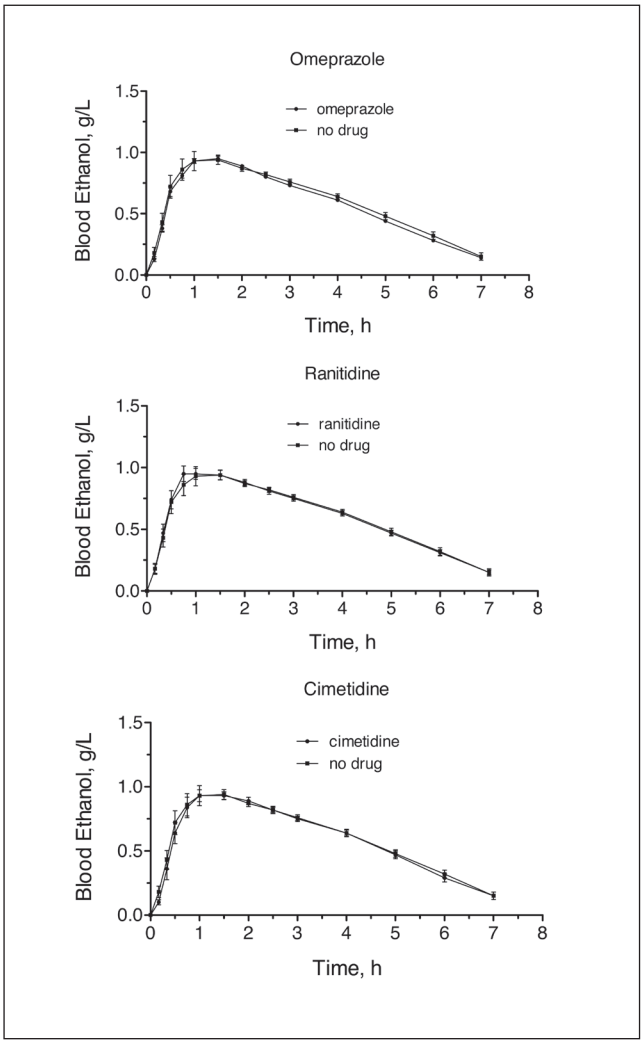


Figure 3.15 *Mean blood-alcohol curves in healthy subjects (N=12) in a randomized study design after they drank 0.80 g per kg body weight on an empty stomach after seven days treatment with cimetidine (Tagamet®), ranitidine (Zantac®) or omeprazole (Losec®) compared with a no-drug control treatment. The mean BAC ± SE are plotted and in the post-absorptive phase the error bars were sometimes smaller than the size of the symbols (data from Jönsson et al. 1992).*

When patients who had undergone a gastrectomy operation consumed ethanol there was a negligible first-pass metabolism, which was thought to support the role of an intact stomach and gastric ADH in pre-systemic metabolism of ethanol (Caballeria et al., 1989b). However, this conclusion was challenged by Levitt (1993) who pointed out that the much faster rate of absorption of alcohol after gastrectomy would tend to saturate the liver enzymes so that hepatic FPM is also negligible (Yokoyama et al., 1995). Accordingly, whether first-pass metabolism of ethanol is predominantly hepatic or gastric still remained unresolved by this experiment with gastrectomy patients.

5. Hepatic aldehyde dehydrogenase

Acetaldehyde is considerably more toxic and chemically reactive than the parent drug ethanol and many of the untoward effects of heavy drinking, including liver damage and dependence, have been attributed to effects of acetaldehyde (Quertemont and Didone, 2006). Acetaldehyde is a pharmacologically active metabolite of ethanol and the body is equipped with highly effective ways to ensure that only low concentrations of acetaldehyde circulate in the bloodstream during ethanol metabolism (Nuutinen et al., 1984).

The second stage in the metabolism of ethanol involves oxidation of acetaldehyde into acetate, which takes place in a non-reversible reaction catalyzed by aldehyde dehydrogenase ALDH (aldehyde: NAD^+ oxidoreductase, EC 1.2.1.3). ALDH is a tetrameric enzyme and comprises four protein subunits; and two major isoenzymes exist, denoted ALDH-1 and ALDH-2 (Goedde and Agarwal, 1990). The ALDH-1 variant is located in the cytosol and the more important ALDH-2 is in the mitochondria. The ALDH-2 isozyme has a low k_m (1 μM or 44 μg/L) and becomes engaged in the catabolism of acetaldehyde when very low concentrations are present in the bloodstream.

The ALDH-1 is a cytosolic enzyme and exhibits a higher k_m for oxidation of acetaldehyde (50-100 μM or 2.2–4.4 mg/L). Besides the presence of ALDH in the liver, this enzyme is widely distributed in other body organs and tissue such as kidney, stomach, intestine, lung, brain, muscle and erythrocytes (Helander, 1993). There is one significant polymorphism of the ALDH2 gene that leads to allelic variants ALDH2*1 and ALDH2*2 (Table 3.4). The latter isozyme is catalytically inactive and fails to metabolize acetaldehyde, which has important clinical consequences in some populations and ethnic groups (Harada et al., 2001). This inactive form of ALDH is present in 40-50% of Japanese, Taiwanese

and Chinese populations as well as other Asian races (Koreans and Vietnamese). Those inheriting one or two copies of ALDH2*2 are sensitive to even small doses of alcohol owing to an accumulation of acetaldehyde in the blood. Indeed, homozygose individuals for ALDH2*2 have a natural protection against heavy drinking and alcoholism (Agarwal and Goedde, 1992; Thacker et al., 1984; Mizoi et al., 1983; Mizoi et al., 1989a). The rate of ethanol elimination was slightly slower in Japanese subjects who were deficient in the low k_m mitochondrial ALDH (Mizoi et al., 1987). In the normal ALDH subjects, ß-slope was 15.8 mg/dL/h compared with 13.6 mg/dL/h in the alcohol-sensitive ALDH deficient group.

a. Toxicity of acetaldehyde

Acetaldehyde (AcH) is the first product of ethanol oxidation by all known pathways and for a long time occupied the center stage in biomedical alcohol research (Brien and Loomis, 1983; Von Wartburg and Bühler, 1984). This toxic metabolite of ethanol has been incriminated in many of the untoward effects of heavy drinking including liver cirrhosis, pancreatitis, and certain forms of cancer (Lindahl, 1992; Lieber, 1994b). For many years the notion that the highly reactive aldehyde group (-CHO) reacted with various neurotransmitters such as dopamine and serotonin to form conjugation products with structures that resembled the opiate narcotics. This hypothesis was thought to be a likely mechanism for ethanol's dependence and reinforcing effects.

Acetaldehyde research entered the spotlight again in the 1970s when it was shown that biogenic amines, such as dopamine and the indolamine serotonin reacted to produce pharmacologically active products (Cohen and Collins, 1970; Davis and Walsh, 1970). Dopamine reacts with acetaldehyde to form salsolinol, and serotonin produces a ß-carboline (Deitrich and Erwin, 1980). These Picket-Spengler condensation products, known as tetrahydroisoquinolines (TIQs), resemble the structure of intermediate products in the biosynthesis of opium alkaloids. Indeed, some experimental work showed that when TIQs were injected directly into the brain of mice or rats, the animals showed a preference for drinking alcohol in free-choice situations (Myers, 1976). These observations were thought to provide a possible molecular basis for alcohol dependence and addiction (McBride et al., 2002; Quertemont and Tambour, 2004). Unfortunately, this research became suspect when it was shown that TIQs could be formed in-vitro during the analytical work-up procedure after blood and tissue were sampled (Eriksson, 1983; Lindros, 1983). Moreover, evidence began to appear suggesting that the concentrations of acetaldehyde determined in blood were abnormally high unless special precautions were taken to avoid artifacts (Eriksson and Fukunaga, 1993; Fukunaga et al., 1993).

The concentrations of AcH in blood and breath after drinking can span a wide range depending on various genetic (racial) and environmental influences such as a person's drinking and smoking habits, the activity of aldehyde dehydrogenase, and whether alcohol-sensitizing drugs are being used (Goedde and Agarwal, 1990; Li et al., 2001). Publications reporting the concentrations of acetaldehyde in the expired air are also reviewed here because this question often arises when breath alcohol analyzers are used in traffic law enforcement to test drinking drivers, namely whether acetaldehyde should be considered an interfering substance (Jones, 1995a).

b. Acetaldehyde-flush reaction

Biomedical research on acetaldehyde gathered momentum in the early 1950s coinciding with the development and testing of a new drug, tetraethylthiuram disulfide (disulfiram) the prototype alcohol-sensitizing drug. The structure of disulfiram is shown in Figure 3.10 and its discovery, as with many other therapeutic agents, was accidentally stumbled upon by investigators in Denmark. The pharmacology and toxicology and the interaction with alcohol was extensively studied before being marketed as Antabuse®, a treatment widely used as aversion therapy in alcoholics (Hald et al., 1948; Christensen et al., 1991). If a person treated with Antabuse® drinks alcohol, he or she experiences a multitude of unpleasant effects, including throbbing headache, giddiness, accelerated pulse, tachycardia, and difficulties in breathing (Asmusson et al., 1948).

Drinking small amounts of alcohol after being treated with Antabuse® causes flushing of the face and neck which sometimes spreads to the shoulders and upper arms and hence the designation alcohol-flush reaction. Hald and Jacobsen (1948) discovered that the concentrations of acetaldehyde in blood and breath were appreciably higher during the alcohol-antabuse reaction, compared with the same dose of alcohol taken alone or after a placebo treatment. The abnormally high concentration of acetaldehyde in blood after drinking appear to be responsible for the adverse effects including facial-flushing commonly seen in Japanese and other Asians and also in Native Americans (Mizoi et al., 1989a 1989b; Mizoi et al., 1979; Gill et al., 1999).

The enzymology and metabolism of acetaldehyde has been studied extensively in Japan a country where about 40-50% of the population experience unpleasant effects, including facial flushing, after drinking small amounts of alcohol (Goedde and Agarwal, 1990; Misoi et al., 1983). Japanese and other East Asians have a mutant form of the

low k_m mitochondrial enzyme of aldehyde dehydrogenase (ALDH-2), owing to a single amino acid defect (Mizoi et al., 1979; Yoshida, 1994). This is a pharmacogenetic trait and the concentrations of acetaldehyde in blood and breath are considerably higher after drinking alcohol. However, large inter-individual variations in the sensitivity to alcohol exist within the same ethnic group depending on the particular genotypes of ALDH inherited (Johnsen et al., 1992; Takeshita et al., 1993). Those with an inactive or deficient ALDH-2 isozyme (homozygous) are afforded a protection against heavy drinking and alcoholism because of their extreme sensitivity to the effects of acetaldehyde (Yin et al., 1992; Higuchi et al., 1994).

c. Analysis of acetaldehyde in blood

Acetaldehyde, which is the primary oxidative metabolite of ethanol, is not easy to analyze in blood samples because of the simultaneous presence of ethanol. Studies have shown that a small fraction of ethanol undergoes oxidation to acetaldehyde in-vitro after sampling the blood (Eriksson, 2001). Special methods are needed to remove the red cells immediately after sampling blood, such as by protein precipitation to minimize this spontaneous formation of acetaldehyde (Eriksson et al., 1982). Many reported blood-acetaldehyde concentrations in the older forensic science literature, such as in medical examiner cases, are artificially too high because they represent formation in the tubes after sampling.

The concentrations of ethanol in body fluids after drinking moderate amounts of alcohol are about 1,000-10,000 times higher than the concentration of acetaldehyde present (Stowell, 1989). So even if trivial quantities of ethanol are oxidized in the tubes in-vitro after sampling blood, the actual in-vivo concentrations of acetaldehyde are grossly overestimated. Besides a spontaneous formation of AcH from ethanol, it was discovered that a rapid disappearance was also occurring but to a lesser extent; so the overall effect was an abnormally high concentration of blood-acetaldehyde (Eriksson, 1983; Nuutinen et al., 1983; Eriksson and Fukunaga, 1993). Large variability in analytical results seemed to depend on the kind of sample pretreatment used prior to quantitative analysis. Scores of publications appeared describing various ways to minimize and correct for this artifact formation e.g., by rapidly (<1 min) removing erythrocytes or by precipitation of plasma proteins with perchloric acid or trapping acetaldehyde as its semicarbizone derivative before removal of proteins (reviewed by Eriksson, 1983). All these various methods as well as numerous modifications have been reviewed in detail elsewhere and will not be covered here (Eriksson, 1980; Eriksson, 1983; Lindros, 1983; Stowell, 1989; Eriksson and Fukunaga, 1993). Because of these difficulties, the appearance of an acetaldehyde peak on the gas chromatogram when blood samples from drunk drivers or autopsy cases are analyzed should not be interpreted as being a reliable reflection of the AcH concentration in-vivo. Reports of high blood-acetaldehyde concentrations in forensic toxicology casework should be considered with caution.

d. Breath acetaldehyde

The problems encountered in measuring acetaldehyde in blood samples, namely artifact formation after sampling, promoted some investigators to turn their attention on the analysis of acetaldehyde in the expired air (Jauhonen et al., 1982; Jones, 1995b). Breath testing furnishes an alternative and indirect way to monitor the concentrations of AcH in pulmonary blood (Stowell et al., 1984; Jones, 1995a). Volatile substances in the pulmonary blood, such as ethanol and acetaldehyde, diffuse across the alveolar-capillary membrane and are exhaled with the breath. Breath-tests for acetaldehyde were therefore considered a more practical and convenient test for clinical purposes because of the non-invasive sampling technique (Jones et al., 1988; Fukunaga et al., 1989).

The plasma/air partition ratio of acetaldehyde at 34°C is about 190:1 compared with 2100:1 for ethanol (Fukunaga et al., 1989; Jones, 1995a). Unfortunately, interpreting results of breath-tests for acetaldehyde is also problematic because of the possibility of this compound being formed locally within the upper-airway and the mouth by micro-organisms (Jauhonen et al., 1982; Kurkivuori, 2007). The concentrations of acetaldehyde actually present in the pulmonary blood are therefore overestimated by analyzing breath samples (Stowell et al., 1984; Eriksson, 2007).

Nevertheless, the analysis of acetaldehyde in the breath provides a fast and non-invasive way to monitor exposure of the lungs and upper-airway to this toxic volatile metabolite of ethanol. The results of analyzing breath AcH should be reported as the concentration in the specimen analyzed without attempting to translate into the presumed blood or plasma concentration. The concentration of AcH in end-expired breath at least in part mirrors the concentration in the pulmonary blood as well as microbial production in the lungs and upper airway (Jones, 1995a).

Table 3.5 presents the concentrations of acetaldehyde measured in human breath from a number of published studies (see Eriksson, 2007 for review). The very low levels of AcH produced endogenously were determined with the aid of a highly sensitive gas chromatographic technique after the breath-AcH content in the specimens was enriched by freeze-trapping (Dannecker et al., 1981). Higher levels of

endogenous AcH were detected in the breath of smokers as might be expected because AcH is a major constituent of tobacco smoke (Hesselbrock and Shaskan, 1985; Talhout and Opperhulzen, 2007). This needs to be considered when breath-testing is done in people who are regular smokers, which is very common in heavy drinkers (Jauhonen et al., 1982).

Concentrations of AcH in exhaled air were higher in alcohol-sensitive Japanese subjects who had inherited a genetically inactive ALDH enzyme compared with Japanese with normal functioning ALDH (Fukunaga et al., 1989). Much higher exhaled AcH was also evident in Caucasians who drank a small dose of ethanol after swallowing a drug that blocks the ALDH enzyme (calcium carbimide) (Jones et al., 1988). Results from a recent review dealing with breath-acetaldehyde concentrations in relation to blood-ethanol is also included in Table 3.5 (Eriksson, 2007).

All-in-all even under extreme conditions, the peak concentrations of AcH in breath are below 50 μg/L and this concentration is insufficient to cause an "apparent ethanol" response when a single wavelength (3.4 μm) infrared breath analyzer was evaluated (Jones, 1986). Accordingly, allegations that elevated concentrations of acetaldehyde, the proximate metabolite of ethanol by all known pathways, are a potential interfering substance when breath-alcohol instruments are used for medicolegal purposes is yet another "red herring" (Jones, 1986; Jones, 1995b). Moreover, individuals with a high concentration of acetaldehyde in breath also show an intense flushing of the face and neck and complain of nausea, tachycardia (rapid beating of the heart), and difficulties in breathing (Jones et al., 1988).

6. Microsomal oxidative enzymes CYP2E1

Besides the alcohol dehydrogenase pathway, enzymes capable of metabolizing ethanol are located in the microsomal fraction of the hepatocytes, within the smooth endoplasmic reticulum. The discovery of this additional pathway of ethanol disposal, originally called the microsomal ethanol oxidizing system (MEOS), opened up exciting new areas of research such as studies of the mechanism of drug-alcohol interactions (Lieber and DeCarli, 1970 and 1972). MEOS belongs to the cytochrome P450 family of enzymes that play such a crucial role in the metabolism of many endogenous and exogenous substances (Teschke and Gellert, 1986; Gonzalez, 1988). The cytochrome P450 enzymes serve as primary defense mechanisms for detoxifying xenobiotics imbibed with the diet or inhaled with the air breathed (Fuhr, 2000; Song, 1996). Cytochrome P450 enzymes constitute a family of proteins having a broad and overlapping substrate specificity crucial for metabolism of drugs and xenobiotics (Koop and Coon, 1986). The P450 enzyme involved in the metabolism of ethanol is designated CYP2E1 and is also responsible for the oxidation of aromatic hydrocarbons such as toluene and benzene as well as chlorinated alkanes (trichloroethylene, chloroform), acetone, 1-butanol, and 2-propanol (Teschke and Gellert, 1986).

Table 3.5
Breath-Acetaldehyde Concentrations In Humans Under Various Conditions With And Without Ethanol Administration

Subjects tested	Conditions	Breath acetaldehyde µg/L
Abstinent alcoholics (Dannecker et al. 1981)[1]	Smokers Non-smokers	0.25 ± 0.002 0.014 ± 0.003
Abstinent non-alcoholics (Hesselbrock and Shaskan, 1985)[1]	Smokers Non-smokers	0.016 ± 0.003 0.004 ± 0.001
Japanese, ethanol 0.4 g/kg (Fukunaga et al. 1989)[2]	ALDH deficient Normal ALDH	10.8 ± 5.5 2.1 ± 1.4
Caucasians, ethanol 0.25 g/kg (Jones et al. 1988)[3]	Placebo Calcium carbamide[3]	Range 0.4–1.5 Range 9.2–56
Review of published studies (Eriksson, 2007)[4]	Blood-ethanol conc. 0.36-1.56 g/L	0.44–2.51

[1] Endogenous acetaldehyde concentrations. [2] Alcohol-sensitive Japanese with inactive form of ALDH2. [3] After pre-treatment with calcium carbimide (50 mg), a potent inhibitor of ALDH2 taken 60 min before subjects drank a small dose of ethanol (0.25 g/kg). [4] Review based on several published studies.

The CYP2E1 enzyme has a higher k_m for oxidation of ethanol (0.6-0.8 g/L) compared to ADH (0.02-0.05 g/L) and therefore blood-alcohol should surpass approximately 1 g/L before the enzyme plays any significant role in elimination of ethanol from the bloodstream (Lieber and DeCarli, 1970). After chronic intake of alcohol, the CYP2E1 enzyme is inducible, that is, it becomes more effective in the oxidation of drugs and xenobiotics owing to increased synthesis of the enzyme (Mezey, 1972). This accounts for the clinical finding that alcoholics and heavy drinkers have an enhanced capacity to metabolize ethanol (Mezey and Tobon, 1971). However, this hyperactivity is rapidly reversible and after a few days of abstinence the ability of an alcoholic to oxidize ethanol faster than a moderate drinker is lost (Mezey, 1972; Keiding et al., 1983). The enhanced activity of CYP2E1 explains metabolic tolerance and accounts for the often faster elimination rate of ethanol 0.3 g/L/h (0.03 g/dL per h) in some cases. A recent study in 22 chronic alcoholics reported that the rate of ethanol disappearance from blood (ß-slope) ranged from 0.13 to 0.36 g/L/h with an average of 0.22 g/L/h (Jones and Sternebring, 1992). Similar enhanced rates of ethanol clearance from blood in alcoholics during detoxification have been reported by other investigators (Bogusz et al., 1977; Adachi et al., 1991; Haffner et al., 1991).

Many common drugs and environmental chemicals are oxidized by cytochrome P450 enzymes, which open the possibility for pharmacodynamic drug-alcohol interactions (Lieber, 1990; Lieber, 1994a; Jones, 2003d). A serious drug interaction resulting in liver failure occurs between ethanol and acetaminophen (paracetamol) owing to induction of CYP2EI enzymes after prolonged heavy drinking (Waller et al., 2005; Roberts and Buckley, 2007). Furthermore, the enhanced activity of the enzymes in alcoholics means they also metabolize a host of environmental chemicals and toxins faster than expected, creating a potential danger from highly reactive metabolites of these substances (Lieber, 1994a). This renders alcoholics more vulnerable to the toxic and carcinogenic effects of organic solvents such as aromatic hydrocarbons (benzene, toluene), trichloroethylene and carbon tetrachloride. The ethanol-inducible cytochrome P450 is also activated by dietary influences such as prolonged fasting and eating low carbohydrate diets (Sato and Nakajima, 1985; Sato and Nakajima, 1987).

7. Non-oxidative metabolism of ethanol

The oxidative pathway of ethanol metabolism via acetaldehyde and then to acetate has been extensively studied, and carbon dioxide and water are the reaction end products. Less than 0.1% of the entire dose of ethanol ingested is metabolized by non-oxidative pathways producing ethyl glucuronide (EtG), ethyl sulfate (EtS), posphatidylethanol (PEth) and fatty acid ethyl esters (FFAA). These non-oxidative metabolites have received considerable interest from researchers and are discussed in more detail in Chapter 4 of this book but for completion will be mentioned briefly below (Helander, 2003; Helander and Jones, 2007; Musshoff and Daldrup, 1998; Musshoff, 2002).

a. Ethyl glucuronide and ethyl sulfate

The short-chain aliphatic alcohols, ethanol, methanol and n-propanol are mainly oxidized in the body to their respective aldehydes and carboxylic acids as discussed in detail above. However, it is also well know that drugs containing hydroxyl groups in the molecules to some extent can participate in enzyme-catalyzed conjugation reactions. Indeed, primary alcohols with longer carbon chains and particularly branched chain secondary and tertiary alcohols undergo fairly extensive conjugation.

The conjugation reactions of ethanol and other drugs were discovered more than a century ago when ethyl glucuronide (EtG) was isolated from urine after experimental animals received ethanol. Much later, the mechanism leading to EtG formation was shown to involve a phase II conjugation reaction with the microsomal enzyme UDP-glucuronosyltransferase. For many years, this non-oxidative pathway of ethanol metabolism attracted little interest, because low concentrations of EtG in body fluids were troublesome and laborious to determine. With the introduction of highly sensitive and specific analytical methods based on technology such as gas- and liquid-chromatography combined with mass spectrometry (GC-MS and LC-MS) a revival of interest in EtG formation and degradation occurred (Schmitt et al., 1995). Another non-oxidative pathway of ethanol metabolism is the conjugation reaction leading to the formation of ethyl sulfate (EtS), which is also excreted in urine after drinking (Helander and Beck, 2004; Halter et al., 2008).

The pharmacokinetic profiles of ethanol and ethyl glucuronide in blood are compared in Figure 3.16, exemplified for four healthy volunteers who drank 0.5 g ethanol per kg body weight on an empty stomach (Hoiseth et al., 2007a). Note that blood-concentrations of EtG are about 1000 times less than those of ethanol and that the EtG is measurable in blood for 6-8 hours longer than that of ethanol. This created a major interest in EtG, namely as a way to disclose recent drinking after ethanol had been metabolized completely.

Figure 3.16 demonstrates a short time-delay in the development of EtG (later occurring t_{max}) and also a slower elimination from blood after C_{max} is reached. This leads to an accumulation of EtG in body fluids after a period of continuous heavy drinking. EtG has found applications as a

biomarker to verify whether a person has consumed alcohol even if ethanol is no longer measurable in blood or urine. This extends the detection window by 5-10 h and this has obvious practical advantages as an acute marker of alcohol consumption. However, care is needed with interpretation because of the high sensitivity of the test and the risk of reaching a false accusation of alcohol consumption. Even casual exposure to ethanol-containing liquids, such as cosmetics, mouthwash or cough medication or even in handrubs containing ethanol might lead to a positive urinary EtG test (Costantino et al., 2006; Rohrig et al., 2006).

Only a small fraction of the dose of ethanol undergoes conjugation (< 0.1%) and the urinary EtG concentrations are sensitive to dilution (e.g., diuresis), although to some extent this can be corrected for by measuring and reporting the ratio EtG/creatinine (Bergström et al., 2003). Furthermore, EtG is sensitive to enzymatic hydrolysis so, if biological samples are contaminated with certain bacteria (e.g., *E. coli)* which is common in urinary tract infections, this might lead to false-positive or negative results (Helander and Dahl, 2005; Helander et al., 2007).

In a case of drunken driving it was alleged that the blood specimen had been contaminated with ethanol from the swab used to clean the skin prior to sampling. This issue was resolved by the analysis of EtG because a positive finding would support the contention that ethanol had undergone metabolism and was therefore ingested. However, if the measured blood-ethanol was partly the result of skin contamination and partly metabolism this would not be possible to resolve. EtG is also finding applications in postmortem toxicology as a way to test whether microbial synthesis of ethanol has occurred after death (Kugelberg and Jones, 2007; Hoiseth et al., 2007b). A recent study showed that there is a risk that EtG might be degraded if the corpse has undergone putrefaction (Hoiseth et al., 2008). Under these conditions, there is a lot of advantage in analysis of EtG in hair strands, a body specimen which is less prone to changes in drug concentrations after death (Pragst and Balikova, 2006).

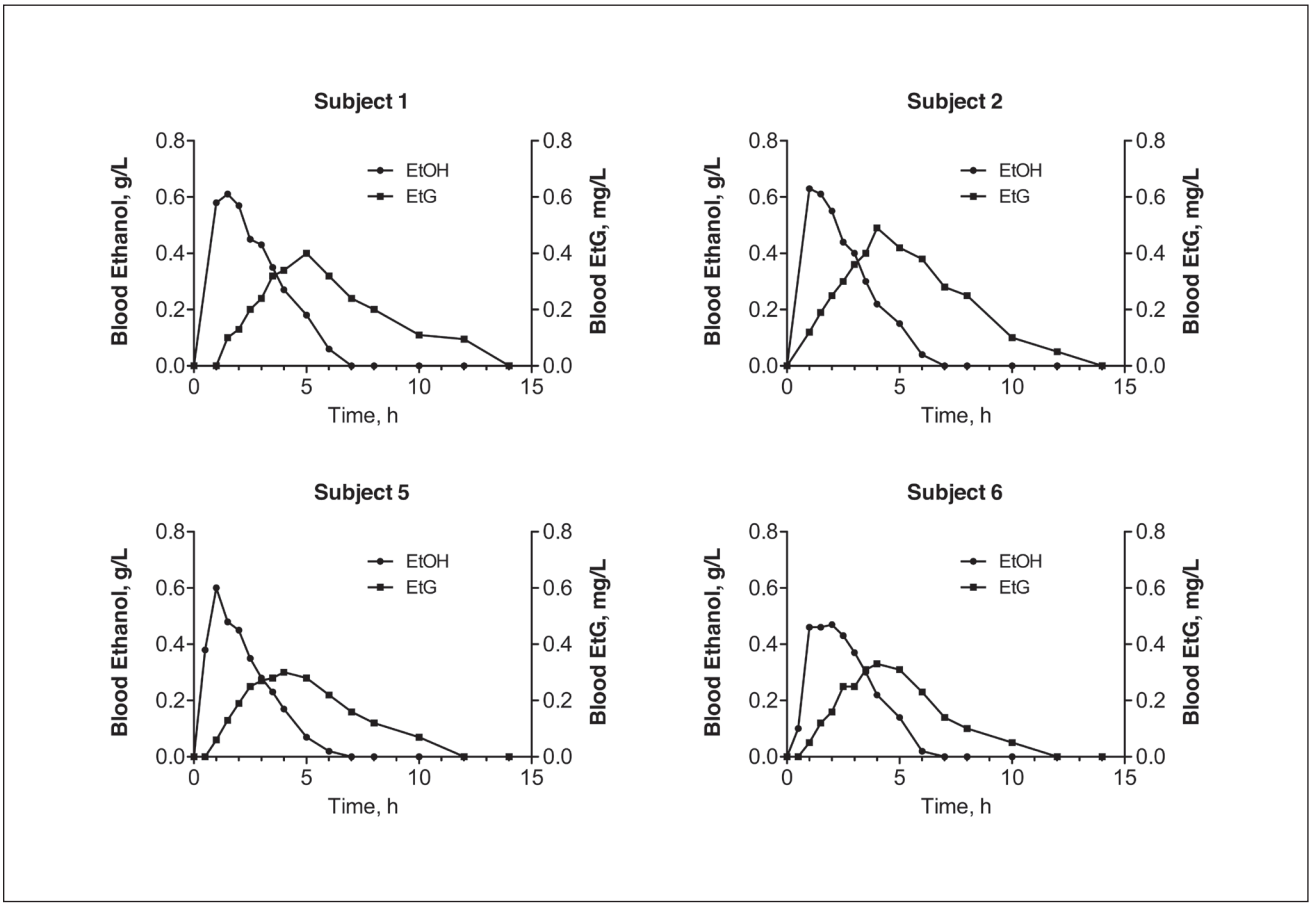


Figure 3.16 *Concentration-time profiles of ethanol and ethyl glucuronide in the blood of four healthy volunteers who drank 0.5 g ethanol per kg body weight in 15 min (graph replotted from a study by Hoiseth et al., 2007a).*

Another non-oxidative metabolite of ethanol that has received attention is ethyl sulfate (EtS), which might offer certain advantages over EtG; both conjugates have similar pharmacokinetics profiles (Helander and Beck, 2005; Halter et al., 2007). There is seemingly a reduced risk that EtS is synthesized by urinary tract bacteria or degraded in-vitro after sampling (Helander and Dahl, 2005). Other minor metabolites of ethanol with potential as biomarkers include the conjugates ethyl nitrite and ethyl phosphate.

b. Fatty acid ethyl esters

Free fatty acids (e.g., palmitate, myristate, stearate and oleate), which are formed during the degradation of triglycerides, can participate in enzymatic reactions with low molecular weight alcohols (e.g., ethanol and methanol) to produce the corresponding esters. These reactions are catalyzed by fatty acid synthase and possibly other enzymes (Laposata, 1999). Identification of these short-chain esters in blood, tissue, or hair, has attracted interest as biomarkers of excessive drinking with potential clinical and forensic applications. Some evidence suggests that FAEE might be the mediators of ethanol-induced tissue damage to organs such as kidney, liver and the pancreas, where highest levels have been detected at autopsy in alcoholics (Laposata and Lange, 1986).

After alcohol intake, the serum concentration of FAEE closely parallels that of blood-ethanol itself but the terminal elimination phase is a lot longer (Laposata, 1997; Doyle et al., 1996). Thus FAEE can be identified in blood for some time after ethanol is no longer measurable and might serve as another biomarker for excessive drinking (Borucki et al., 2007; De Giovanni, 2007). The analytical methods used originally to determine FAEE were rather cumbersome, involving thin-layer chromatography and GC methods, but more recently significant improvements have occurred by use of GC-MS with deuterium labeled internal standards and headspace solid phase micro-extraction (Musshoff, 1998). Care is also needed to include preservatives because FAEE can undergo enzymatic hydrolysis after sampling (Pragst et al., 2001).

c. Phosphatidylethanol

Another non-oxidative metabolite of ethanol, which has attracted interest as a biomarker of excessive drinking, is phosphatidylethanol (PEth) (Alling et al., 1984). This metabolite is formed in cell membranes in the presence of ethanol in a reaction catalyzed by the enzyme phospholipase D (see other chapter in this book for more details). Molecules of ethanol replace water in the usual hydrolysis reaction of phospholipids such as phosphatidylcholine. Because PEth is formed in cell membranes only in the presence of ethanol, this reaction appears to be highly specific as a marker for heavy drinking (Hansson et al., 1997).

Fairly heavy drinking over several weeks is necessary to produce measurable amounts of PEth in blood and this substance is mainly located in the erythrocytes (Hansson et al., 2001). The methodology used to determine PEth in plasma or tissues is continuously being improved upon, which means that this non-oxidative metabolite is starting to find routine clinical applications as a marker for excessive drinking (Varga et al., 1998; Varga et al., 2000). Indeed, in one study PEth was detectable in blood for two weeks after alcohol-dependent patients, with a self-reported daily intake of 60-300 g ethanol per day, had entered a detoxification unit (Hartmann et al., 2007). Compared with traditional biomarkers of heavy drinking, such as liver enzymes and mean corpuscular volume as well as carbohydrate efficient transferrin, the analysis of PEth showed better specificity and sensitivity (Hartmann et al., 2007).

3.6 Pharmacokinetics of Ethanol

The work of Erik M.P. Widmark published during the 1930s is still widely accepted and cited in forensic science practice when the pharmacokinetics of ethanol are discussed (Widmark, 1932). Establishing the concentration-time course of ethanol in blood or other body fluids is a fundamental starting point for any pharmacokinetic evaluation. In the studies of Widmark, he determined the concentrations of ethanol in whole blood and plotted the results against time of sampling measured from the start of drinking. However, in other situations, such as in clinical pharmacology and therapeutic drug monitoring, the biological specimens used for analysis of drugs are mostly serum or plasma. The concentrations of ethanol and many other drugs are different in plasma compared to whole blood, owing to the differences in water content between these biofluids and binding of drugs to plasma proteins.

The pharmacokinetics of ethanol might also be investigated by repetitive sampling of urine voids or saliva (oral fluid) to establish concentration-time profiles. However, the pharmacokinetic parameters C_{max}, t_{max}, C_o and disappearance rate will be dependent on the biofluid analyzed. The pharmacokinetics of ethanol will be highlighted with emphasis on factors that influence the peak BAC reached after drinking and the rate of disappearance of ethanol from the bloodstream, because these parameters are much discussed in forensic casework (Jones, 1993a).

A. First-Order Kinetics

The mathematical foundations used to characterize the time course of drugs in the body have their roots in studies of the

kinetics of chemical reactions, namely zero-order, first-order, second-order and third-order processes (Wagner, 1981; Wagner, 1993). Because most drugs or medications are administered to the body in mg amounts they are eliminated from the bloodstream according to the principles of first order kinetics as shown below (Gabrielsson and Weiner, 2000).

$$dC_t/dt = -k_1 C_t$$

Integration between the limits C_0 and C_t and taking natural logarithms (ln) gives the equation for first-order elimination of a drug from blood or plasma, namely:

$$\ln C_t = \ln C_0 - k_1 t$$

Where k_1 is the first order elimination rate constant, having units of reciprocal time and C_0 is the theoretical concentration extrapolated back to the time of drug administration.

With first order kinetics, the rate of elimination is directly proportional to the concentration or amount of substance participating in the enzymatic reaction. As the time after administration increases and the concentration of the drug in blood or plasma decreases the rate of the reaction slows down appreciably. For drugs that obey first-order kinetics, the concept of half-life ($t_{½}$) is useful to describe the rate of elimination of the drug from plasma. The half-life is defined as the time necessary to reduce the amount of drug in the body by half, which implies that after a time corresponding to five half-lives about 97% of the drug would have been eliminated.

Unlike most drugs ethanol is administered in large amounts and its pharmacokinetics is more complicated because the enzymes involved in metabolism become saturated with substrate. The concept of elimination half-life of a drug as described above is not applicable to ethanol because the half-life is variable and dependent on the prevailing concentration in blood (Rowland and Tozer, 1995). Two widely used models to describe the pharmacokinetics of ethanol, namely zero-order and Michaelis-Menten kinetics, are shown schematically in Figure 3.17.

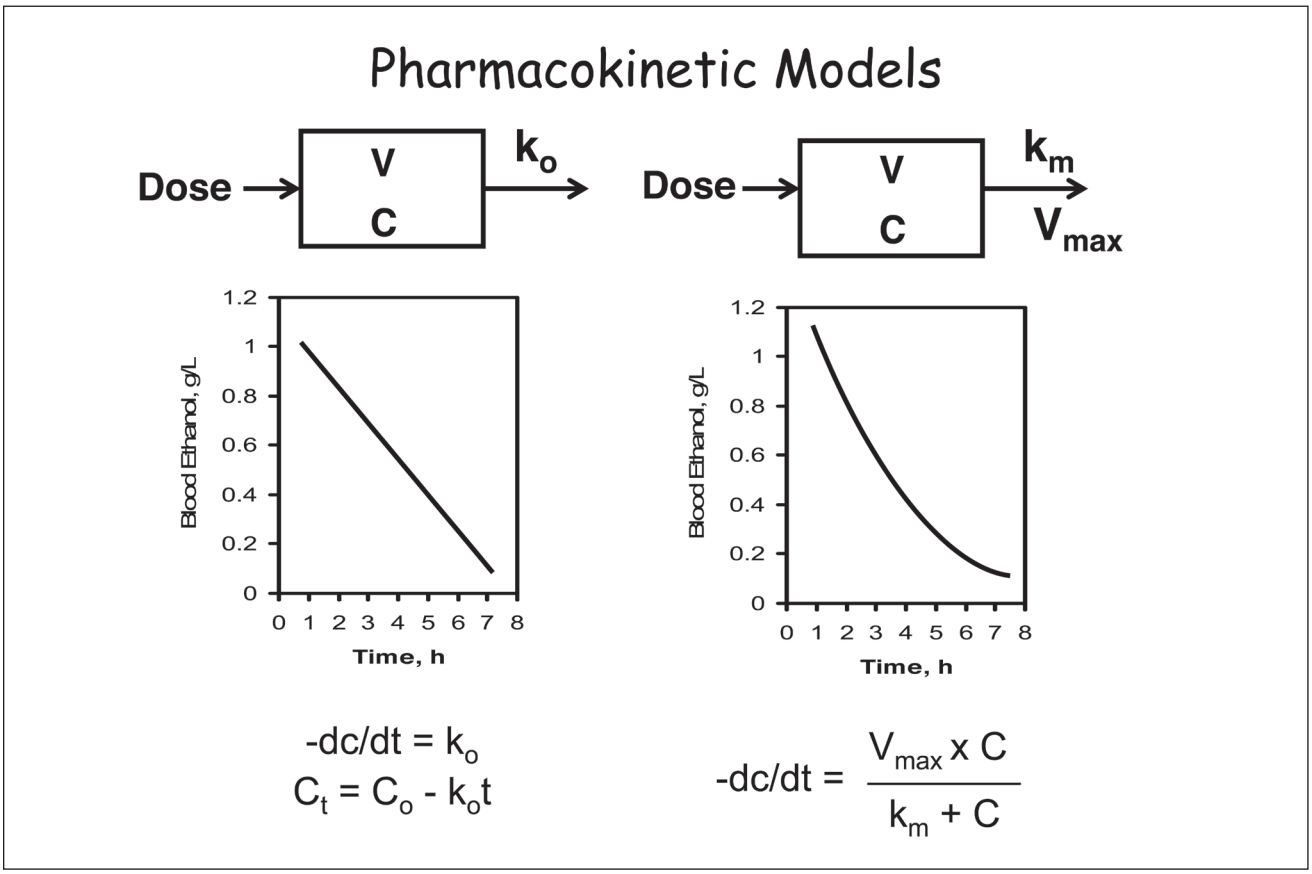


Figure 3.17 *Diagram showing two widely used pharmacokinetic models to describe the elimination kinetics of ethanol, namely zero-order (left panel) and Michaelis-Menten kinetics (right panel). The corresponding reaction rate equations are shown below the plots.*

B. Zero-Order Kinetics

When moderate doses of ethanol are ingested and the blood alcohol concentration exceeds 0.2 g/L (0.02 g/dL), the main metabolizing enzyme ADH (k_m of 0.05-0.1 g/L) is saturated with substrate (Von Wartburg, 1989; Wagner, 1973). This can be interpreted to mean that within the BAC range encountered after social drinking the elimination rate of ethanol from the blood stream occurs at a constant rate per unit time according to the principles of zero-order kinetics (Wagner et al., 1976; Wilkinson, 1980; Rowland and Tozer, 1995).

$$dC_t/dt=-k_0$$

Integration between the limits C_0 and C_t and taking natural logarithms (ln) gives the equation for zero order elimination of ethanol from blood or plasma, namely:

$$C_t=C_0 - k_0t$$

Where k_0 is the zero order elimination rate constant independent of concentration and expressed in units of concentration per unit time and C_0 is the theoretical concentration extrapolated back to the time when ethanol was administered.

The first analytical methods for quantitative determination of ethanol in blood utilized the principle of chemical oxidation, and the limit of quantitation was about 0.1 g/L or 0.01 g/dL; concentrations below this could not be determined with any degree of certainty. Accordingly, the true nature of the post-absorptive part of the concentration-time profile of ethanol (hockey stick shape) was established long after Widmark's classic work in the 1930s when more sensitive analytical methods had become available (Lundqvist and Wolthers, 1958).

Zero-order kinetics apply to the major portion of the post-absorptive limb of the blood-alcohol curve, and the elimination rate constant is best determined by linear regression analysis of concentration-time data. Widmark (1981) determined the pharmacokinetic parameters of ethanol in healthy volunteers under the following strictly controlled conditions:

1. The volunteer subjects were healthy men (n=20) and women (n=10) all of whom were moderate drinkers.
2. They drank a moderate bolus dose of ethanol which was administered in the form of undiluted spirits (30-40 vol%) corresponding to 0.50-0.90 g/kg in a drinking time of 15-20 min.
3. Ethanol was determined in capillary (fingertip) blood samples taken at 30-60 minute intervals and the concentrations of ethanol were determined by a chemical oxidation method.
4. The blood alcohol concentrations were reported in mass/mass units, actually mg ethanol per gram whole blood (mg/g) because the aliquots taken for analysis were weighed on a torsion balance.

A typical blood-alcohol curve obtained under conditions specified by Widmark is shown in Figure 3.18 and the key pharmacokinetic parameters are indicated on the plot.

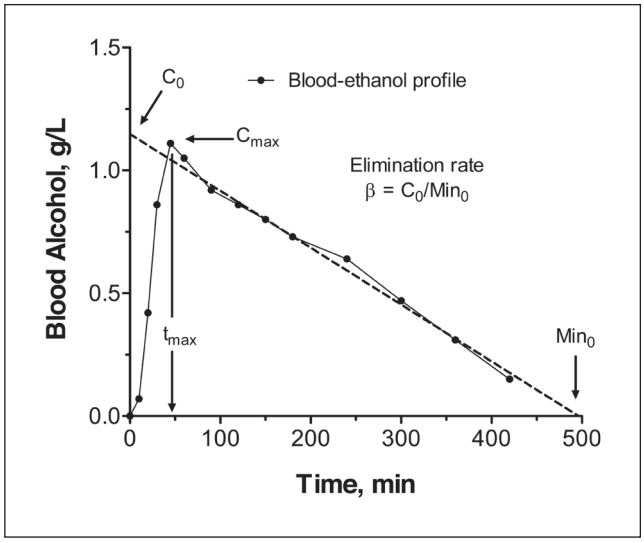


Figure 3.18 *Blood alcohol concentration-time profile with definition of key characteristics (C_{max}, t_{max}, min_o, C_o and beta slope) according to the Widmark principles.*

If the experimental conditions are changed, such as by switching beer or wine for neat spirits or if the alcohol is given together with or after food instead of on an empty stomach, or even if a breath-alcohol analyzer is used instead of taking blood samples, the resulting pharmacokinetic parameters (ß and "r") will not agree with those reported by Widmark. The slope of the post-peak declining phase of the BAC curve (ß-slope) is a measure of the rate of disappearance of ethanol from the blood and this is usually expressed per min or per hour ($ß_{60}$). Widmark found that the average ß-slope was slightly steeper for women compared with men averaging 0.0025 mg/g/min or 0.15 mg/g/h (span 0.10–0.24 mg/g/h).

Note that the concentrations of ethanol in blood were in mass/mass units and as discussed earlier this has implications for the pharmacokinetic parameters, such as C_{max} and C_o. The V_d or rho factor is therefore a dimensionless ratio. The units most commonly used today for "rho" are liters/

kg because BAC is reported in mass/volume units, such as mg/ml, g/dL, and g/L. In the terminology used in pharmacokinetics, the Widmark "rho" factor represents the volume of distribution of ethanol, which is sometimes abbreviated V_d and corresponds well with the ratio of concentration of alcohol in the body to concentration in the blood (Rowland and Tozer, 1995; Jones et al., 1992).

C. Non-Linear Saturation Kinetics

According to Widmark, the decrease in BAC during the post-peak phase occurs at a constant rate per unit time as expected for zero-order elimination kinetics (Widmark, 1932; Widmark, 1981). Such a process can be described mathematically with the simple equation, $C_t=C_o - k_0t$ (Von Wartburg, 1989). However, when the BAC decreases to reach a concentration of about 0.10 or 0.20 g/L (0.01-0.02 g/dL), the time course changes to a curvilinear function. The shape of the entire post-peak elimination phase starting from a moderately high BAC and extending down to very low BAC (<0.1 g/L) looks more like a hockey-stick rather than a straight line (Lundqvist and Wolthers, 1958; Wilkinson, 1980; Wilkinson et al., 1980).

The rectilinear segment and the hockey-stick portion of the BAC curve can be fitted to the Michaelis-Menten (M-M) equation (Wagner, 1973; Wagner et al., 1976). Accordingly, the rate at which the blood alcohol concentration decreases with time (-dC/dt) depends on 3 parameters: V_{max} the maximum velocity of the enzymatic reaction, k_m the Michaelis constant defined as the concentration of substrate (ethanol) at half maximum velocity, and the underlying initial blood alcohol concentration (C).

The M-M equation is usually written as $dC_t/dt=-[V_{max} \times C_t]/[k_m + C_t]$ and this equation can be converted into two limiting forms depending on the concentration (C_t) of ethanol in the blood. If C_t is much greater than k_m, the M-M rate equation simplifies to $dC_t/dt=-V_{max}$, and the elimination occurs at a constant rate (zero-order kinetics). When the concentrations of ethanol in blood are much less than k_m, the rate of reaction becomes proportional to the prevailing substrate concentration $dC_t/dt=-[(V_{max}/k_m) \times C_t]$, which is the same as $dC_t/dt=-[k_1 \times C_t]$, the equation for first-order kinetics.

The concept of saturation kinetics was formulated many years ago based on studies of the reaction between a substrate and a single enzyme. Because of the existence of multiple forms of ADH (Table 3.4), having different k_m and V_{max} values suggests that a series of parallel M-M equations might be more appropriate to describe the elimination kinetics of ethanol (Keiding et al., 1983). Fitting the ethanol concentration-time data to a single M-M function, which assumes involvement of a single enzyme, fails to consider the multiple molecular forms of Class I ADH or the participation of CYP2E1 enzymes, which play an increasingly important role after high doses (Lieber, 1999; Von Wartburg, 1989).

Lundqvist and Wolthers (1958) were first to investigate the elimination kinetics of ethanol in humans at very low substrate concentrations. This was possible thanks to the availability of a more sensitivity analytical method based on enzymatic oxidation (Bonnichsen and Theorell, 1951). When the plasma-concentration time data were plotted the shape of the curve looked more like a hockey stick rather than a straight line and after moderate doses the results agree well with expectations for operation of M-M kinetics.

Strong experimental support for application of the M-M equation to describe the post-peak disappearance phase of the blood-alcohol curve was soon forthcoming from specialists in pharmacokinetics (Wagner et al., 1976; Wilkinson et al., 1977a; Wagner et al., 1989). The advantages and limitations of using the more complicated M-M equation to describe the elimination kinetics of ethanol instead of the simpler one-compartment model and zero-order kinetics proposed by Widmark is open to question (for reviews see Wilkinson, 1980; Holford, 1987; Von Wartburg, 1989; Norberg et al., 2003).

Ethanol exhibits non-linear saturation kinetics and the pharmacokinetic parameters change as the dose administered increases (Wagner, 1973; Ludden, 1991). This capacity-limited metabolism means that the rate of disappearance from blood approaches a limiting value (V_{max}) as the amount of drug administered increases and the enzymes become progressively saturated with substrate (Ludden, 1991). This pharmacokinetic model of capacity-limited saturation kinetics applies to ethanol (Fujimiya et al., 1989) and some other commonly prescribed drugs, such as propranolol, acetylsalicylic acid, and phenytoin (Ludden, 1991; Rowland and Tozer, 1995; Roberts and Buckley, 2007). Overdosing with a drug that obeys capacity-limited metabolism would mean a delayed clearance of the toxic substance and prolonged exposure and heightened risks to the patient (Rowland and Tozer, 1995). The areas under the concentration-time curves for drugs that have capacity-limited metabolism increase disproportionally with increasing dose and this might have serious clinical consequences for the patient (Wilkinson et al., 1980; Norberg et al., 2003; Roberts and Buckley, 2007).

Despite the overwhelming evidence for M-M kinetics in ethanol metabolism the zero-order model still persists in forensic science and toxicology applications. In Table 3.6 the rates of ethanol elimination were calculated using the M-M equation starting at different initial BAC and for two individuals with widely different V_{max} and k_m for hepatic class I alcohol dehydrogenase (Forrest, 1986). One of these

individuals was assumed to be a fairly rapid metabolizer (V_{max} 0.228 g/L per h and k_m 0.095 g/L) and the other a slow metabolizer (V_{max} 0.16 g/L per h and k_m 0.06 g/L). The table shows that the biggest differences in disposal rates of ethanol occur at relatively low BAC (0.10 to 0.50 g/L). At higher blood-ethanol levels (1.0 to 3.0 g/L), which are usually encountered in forensic toxicology, the rates of elimination are not much different within individuals because the ADH enzyme is now fully saturated with substrate.

Table 3.6
Calculated Rates of Metabolism of Ethanol as a Function of the Concentration at the Metabolic Site for Two People with Different Capacity to Oxidize Ethanol

Concentration in blood at site., (g/L)	Fast metabolizer[1] Rate (g/L per h)	Slow metabolizer[2] Rate (g/L per h)
0.10	0.116	0.100
0.20	0.154	0.123
0.50	0.191	0.142
1.0	0.208	0.150
150	0.214	0.153
2.0	0.217	0.155
3.0	0.221	0.156

[1] V_{max} 0.228 g/L per h, k_m=0.095 g/L. [2] V_{max} 0.160 g/L per h, k_m=0.060 g/L

The average BAC in drunken drivers is appreciable (median 1.6-1.8 g/L), which means that the Class I ADH enzymes are probably fully saturated and zero-order elimination kinetics can be used to describe the concentration-time profiles. Nevertheless, more complicated pharmacokinetic analysis has been suggested from time to time including multicompartment models and parallel Michaelis-Menten equations (Rangno et al., 1981; Adachi et al., 1989; Wedel et al., 1991; Smith et al., 1993). The notion of saturation kinetics of ethanol metabolism finds application in situations in which small amounts of ethanol enter the body such as by inhalation or absorption through the skin (Lewis, 1985). Non-compartment kinetic models (Hahn et al., 1994) and population pharmacokinetics have been applied to the evaluation of blood- and breath-alcohol curves to a limited extent (Clardy, 2004).

D. The Widmark Equation

The concept of zero-order kinetics still dominates the way that blood-alcohol calculations are made for legal purposes, including forward and retrograde extrapolations. The BAC expected after a known drinking pattern is almost always calculated using the Widmark equation, which relates the amount of alcohol absorbed and distributed in all body fluids with the prevailing concentration of alcohol in the blood (Barbour 2001; Cowan et al., 1996).

Knowledge about the way alcohol distributes in the body is necessary to understand and appreciate many applications of Widmark's equation in forensic science. Alcohol is poorly soluble in bone and fatty tissues, does not bind to plasma proteins and for all practical purposes can be considered to distribute only in the water fraction of the body. This means that the distribution volume of ethanol or the Widmark rho-factor, which expresses the concentration of ethanol in the body to the concentration in blood, must obviously be less than unity, roughly 60/80 or 0.75 for a person with a total body water of 60% and a blood water content of 80%. Note that the distribution ratio of alcohol between the body and the plasma is lower than for whole blood, being roughly 60/90 or 0.67 because water content of plasma is 90-92% w/w.

Widmark established the BAC profiles in 10 women and 20 men and then back extrapolated the linear post-absorptive limb of the curve to the time of starting to drink alcohol. This y-intercept, which he denoted as C_0, represents the BAC if absorption and distribution of the entire dose had occurred instantaneously without any of the alcohol being metabolized. The value of C_o (g/kg) was always greater than the dose of ethanol administered (g/kg) because the blood contained more water per unit volume than the body as a whole. To be able to calculate the amount of alcohol in the body from the value of C_o, Widmark introduced the "rho factor," which if multiplied by the person's body weight gave the reduced body mass (in German "reduzierte Körpermasse"). With this adjustment, the measured BAC (mg/g) or C_0 was the same as the dose of alcohol administered in g/kg, that is, C_0=g/(kg x r). In this way, Widmark arrived at an equation relating the amount of alcohol absorbed and distributed in the body (A), to the person's body weight in kilograms (p) and the concentration of ethanol measured in a specimen of whole blood (C);

$$A = r \times p \times C$$

A=amount of alcohol equilibrated in all body fluids and tissues at time of blood sampling.
r=reduction factor or Widmark rho factor (the ratio of alcohol in the body to alcohol in the blood), being approximately 0.7 for men and 0.6 for women.
p=the body weight in kg.

C=the concentration of alcohol measured in a specimen of whole blood expressed in units of mg/g or g/kg.

Because total body water is 50-60% of body weight and water content of whole blood is fairly constant at 78-83%, the Widmark factor "r" can only take certain values e.g., 50/83 to 60/78 or 0.60 to 0.77 for most average people (Rosenfeld, 1996). Individual variations in rho will depend on factors influencing body water content and blood water content. The main variable is the proportion of fat-to-lean tissue in the body, which in turn depends on age, gender and obesity as reflected in body mass index (Jones, 2007b). The value of rho for women can be expected to be less than for men for the simple reason that women have less body water and also slightly more water in their blood, owing to a lower hematocrit value (packed cell volume) (Lentner, 1981; Van Loan, 1996; Mirand and Welte, 1994).

Widmark (1932) derived his distribution factor "r" from drinking experiments with 20 men and 10 women, and found the following average values, standard deviation (SD) and coefficients of variation (CV):

Men 0.68 (SD ± 0.085, CV 13%), range 0.55 to 0.86
Women 0.55 (SD ± 0.055, CV 10%), range 0.47 to 0.65

Another early study from Sweden expanded this work to include another 20 women and 10 men who drank 0.5-0.9 g ethanol per kg as brandy or vodka on an empty stomach (Österlind et al., 1944). The values of ß and rho obtained were in good agreement with those reported by Widmark except for finding a slightly larger rho-factor in women of 0.61 ± 0.072 (± SD) with a range from 0.48-0.75 compared with 0.70 ± 0.071 for men with a range from 0.60-0.82.

From these two early studies average rho factors of 0.70 for men and 0.60 for women found wide application in forensic blood-alcohol calculations. Note that the rho-factor is not the same as the proportion of water in the human body because ethanol was determined in blood, which contains about 15% of solid matter. If the concentration had been determined in blood-water, e.g., by protein precipitation and water-concentration time plots constructed, then the rho factor calculated as dose (g/kg)/C_0 would be the same as the fraction of water in the body.

To verify Widmark's rho factors, one should follow his strictly controlled drinking conditions listed above. Moreover, BAC was reported in mass/mass units and the dose of alcohol was in g/kg, which means that the V_d derived as dose/C_o is without dimensions. If the BAC had been reported in units of g/L and dose in g/kg then the ratio dose/C_o will have units of L/kg, being less than the values given by Widmark. This follows because the average density of whole blood is 1.055 being greater than unity, which must be considered when Widmark's rho values are compared with those published by today's investigators (Lentner, 1981).

Breath-alcohol instruments should not be used in pharmacokinetic studies to estimate blood-ethanol concentration. The BAC/BrAC ratio is a moving target and depends on many factors (see later in this chapter), including the person's pre-exhalation breathing pattern, breath sampling characteristics of the breath-analyzer and position of the BAC (rising or declining limbs) at time of sampling (Jones and Andersson, 1996b). In most comparative studies between breath-ethanol and venous blood-ethanol the breath-test results tend to underestimate the BAC by about 10-15% if a BAC/BrAC ratio of 2100:1 is used for calibration and tests are done in the post-absorptive period (Jones and Andersson, 2003). In Widmark's experiments the volunteer subjects had fasted overnight before they drank alcohol, which is different from administering alcohol 2-3 hours after lunch or together with a sandwich or bar snack, as is done in many published studies.

Furthermore, Widmark administered neat spirits e.g., brandy, whiskey or vodka and this was taken as a bolus dose in a drinking time of 5-15 min (Jones, 1984). Deviation from these experimental conditions can often explain failures to verify the values of ß and rho reported by Widmark. An updating of Widmark's work is, however, justified because the body composition of people in the 1920s and 1930s in Sweden is probably not representative of the average person today (Forrest, 1986; Jones, 2007b). Obesity is an acute problem worldwide even among young people and this should be considered in blood-alcohol calculations (Yanovski and Yanovski, 2002; Flegal et al., 2002: Jones, 2007b).

A useful forensic application of the Widmark equation is to estimate the amount of alcohol absorbed and distributed in all body fluids and tissues at the time that blood is sampled (Wagner et al., 1990). If there is ethanol remaining unabsorbed in the stomach from a recent alcoholic drink, this is not within the systemic circulation and therefore does not enter the Widmark calculation so the actual quantity consumed is underestimated. Furthermore, metabolism of ethanol starts immediately upon ethanol reaching the gut during and after drinking, so an appreciable quantity of consumed alcohol is eliminated by the time the blood sample is taken.

If the time of starting to drink is known the amount of alcohol removed by metabolism needs to be added to the amount in the body at the time of sampling blood:

Total amount consumed=(BAC x rho x kg) + (B_{60} x h)

BAC is the concentration of ethanol in blood, rho is Widmark's factor, kg is the person's body weight in kilograms, B_{60} is the rate of elimination of ethanol from the whole body, which can be taken as 0.1 g/kg/h and h is the time in hours elapsed from start of drinking to the time of blood sampling. An alternative approach is to back extrapolate BAC assuming an elimination rate of 0.15 g/L/h to give a theoretical BAC at the time of starting to drink. From this BAC the amount of alcohol in the body is calculated using the Widmark formula [A=BAC x rho x kg]. The grams of ethanol (A) can be translated into the number of drinks consumed.

Widmark's calculations are less reliable in answering questions such as what BAC can be expected at a given time after drinking a known quantity of ethanol? Use of the Widmark equation assumes that absorption and distribution of the entire dose of ethanol was complete and that first-pass metabolism, whether in the stomach or liver or both organs was negligible. Studies have shown that systemic availability of ethanol after social drinking is never 100% and presystemic metabolism is sometimes appreciable (Zink and Reinhardt, 1984; Jones and Neri, 1985; Jones et al., 2006). A lot can be said for trying to standardize the plethora of blood-alcohol calculations used in clinical medicine and forensic science (Brick, 2006).

E. Updating the Widmark Equation

1. Body mass index

Average body composition has changed considerably over the 70 years since Widmark's experiments, and obesity has become a major public health problem (Borkan and Norris, 1977; Yanovski and Yanovski, 2002). The factors determined by Widmark apply to individuals with a normal body composition and for this reason are less appropriate for people who are overweight, obese or emaciated (Jones, 2007b).

Obesity is determined by a simple mathematical formula relating a person's height and body weight, namely weight divided by the person's height in meters squared (kg/m^2), which gives the body mass index (BMI). This calculation can be done by first multiplying height in inches by 0.0254 to give meters; next multiply weight in pounds by 0.45 to give kilograms. A BMI between 25 and 29.9 is considered overweight and BMI over 30 is the first stage of clinical obesity. For a person who is 5 ft, 6 in tall and has a body weight of 180 lb his BMI is shown below to be 29.0;

BMI =
(180 x 0.45) divided by (66 x 0.0254)
x (66 x 0.0254)=29.0

2. Total body water

In 1981 investigators from New Zealand (Watson et al., 1981) published a paper, which subsequently became well cited, in which they suggested updating the Widmark equation. They presented a multiple regression equation relating total body water in liters (dependent variable) to the person's age (y), body weight (kg) and height (meters) as independent variables. These anthropometric values (age, weight and height) came from population studies of TBW in healthy individuals. Two TBW equations were presented—one for men and one for women—as well as the residual standard deviations from the regression analysis as shown below.

For men (N=458) aged 17-86 y, their age (y) and body weight (kg) were found to be the most relevant variables;

TBW (liters)=20.03 – 0.1183 age (y) + 0.3626 weight (kg)

The residual standard deviation on TBW estimated by this equation was ± 3.86 liters.

For women (N=265) aged 17-84 y, their body weight was the most important variable;

TBW (liters)=14.46 + 0.2549 weight (kg)

The residual standard deviation on TBW estimated by this equation was ± 3.72 liters.

Because the Watson et al. (1981) formulae provide subject-specific values for TBW this is a strong motivation for adopting the above equations in blood-alcohol calculations such as those used in drunken driving trials. Information about a person's age, weight and height can be easily verified, which gives added confidence to BAC calculations that include TBW values targeted to the actual person concerned rather than using population average values for the distribution factor. The TBW derived according to the above equations has to be divided by the water content of blood (0.8 g per g or 0.85 g per mL) to give the rho factors comparable with those of Widmark. Gabe (1997) made a re-appraisal of the original Watson et al. (1981) formulae and pointed to some minor deficiencies in the calculations, such as the need to use mass/volume units for blood-water content if the BAC is reported in such units. In a woman with a BMI of 30.6 kg/m^2 the Widmark rho factor was determined experimentally and found to be abnormally low (0.45 L/kg), which underscores the need to consider BMI in blood-alcohol calculations made for legal purposes (Jones, 2007b). In people

who might be grossly obese (BMI > 40) the corresponding Widmark rho factors have not been determined.

Assuming a man with a body weight of 75 kg, height 180 cm and age 35 y, a calculation using the above equation gives a TBW of 43 L, which corresponds to 57% of body weight. Dividing percent TBW (57%) by percent water in whole blood (80% w/w) gives 0.71 as the ratio of water in whole body to water in blood, which is the definition of Widmark's rho factor. This value is slightly higher than the average for men reported by Widmark 0.68 ± 0.085 (± SD).

Seidl et al. (2000) have also proposed an update of the Widmark equation to take into account the so-called absorption deficit. This refers to an apparent loss of alcohol resulting in a lower C_{max} and diminished C_0 for the dose of ethanol administered. Measurements of TBW were made using a non-invasive bioelectrical impedance method and blood-water content was determined by desiccation. The investigators proposed the following equations to give Widmark's rho or r factors for men (r_m) and women (r_w)

For men

$$r_m = 0.31608 - 0.004821 \text{ weight (kg)} + 0.004632 \text{ height (cm)}$$

For women

$$r_w = 0.31223 - 0.006446 \text{ weight (kg)} + 0.004466 \text{ height (cm)}$$

These equations were verified by drinking experiments and taking into consideration age, gender, body weight, and height of the individual and also their TBW. For a 75 kg man, height 180 cm and age 30 years, the above equation estimates r_m of 0.788, which is considerably higher than 0.71 determined by Watson et al. (1981) and 0.68 according to Widmark (1932). Many others' formulae and nomograms are available to compute rho factors from anthropometric data and body mass index for use in blood-alcohol calculations (Forrest, 1986; Barbour, 2001; Gullberg and Jones, 1994).

In criminal cases it is important to give the accused any benefit of the doubt when scientific and technical evidence is proffered. This becomes important when Widmark calculations are made owing to uncertainty in the person's rho and whether bioavailability of ethanol was indeed 100%. During social drinking over several hours and especially when alcohol is ingested along with food or snacks the bioavailability is always less than 100% (Jones et al., 2006; Gullberg, 2007; Welling, 1996). A confidence interval approach was one useful suggestion when BAC calculations are made and the results presented in evidence in drunken driving cases (Arstein-Kerslake, 1986).

F. Pharmacokinetics of Ethanol Metabolites

The kinetics and dynamics of ethanol's metabolites (acetaldehyde, acetate and ethyl glucuronide) have been much less studied compared with the parent drug ethanol (Mascord et al., 1992). The concentrations of these substances are considerably lower than ethanol itself and more sophisticated analytical methods are necessary for reliable quantitative analysis of the metabolites (Droenner et al., 2002; Nuutinen et al., 1984; Fujimiya et al., 1989)

The acetate produced during metabolism of ethanol enters the metabolic pool as acetyl coenzyme A and becomes involved in the general pathways for oxidation of fats (Lundqvist et al., 1962). The concentrations of acetaldehyde circulating in peripheral blood after drinking ethanol are very low, being about 0.04-0.09 mg/L when reliable methods of analysis are used and when the ALDH enzymes are not inactivated by drugs such as disulfiram or genetic influences on the activity of ALDH (Eriksson and Fukunaga, 1993).

The concentration of acetaldehyde and acetate generated during the metabolism of ethanol depends in part on the rate of oxidation of ethanol and therefore the reoxidation of NADH, which is considered the slowest step in the process (Mascord et al., 1992; Korri et al., 1985). Reoxidation of NADH, which occurs in mitochondria, is dependent on the rate of oxidative phosphorylation (Lundquist et al., 1962). This means that the rate of ethanol disposal is linked to hepatic oxygen consumption within the cell and the person's basal metabolic rate. After drinking ethanol, the concentrations of AcH and acetate in blood rise initially and then remain at a more or less steady-state concentration provided that the alcohol metabolizing enzymes are working at full capacity (Korri et al., 1985; Roine et al., 1988).

A swift metabolism of ethanol should give a higher steady state concentration of acetate and acetaldehyde compared with slow metabolism of ethanol (Nuutinen et al., 1985). Indeed, subjects with elevated blood-acetate during the oxidation of ethanol were heavy drinkers with enhanced capacity to oxidize ethanol (Korri et al., 1985). An abnormally high concentration of acetate in blood was taken as an indication of metabolic tolerance often associated with advanced drinking habits and alcoholism (Roine et al., 1988; Nuutinen et al., 1985; Korri et al., 1985).

An animal model (rabbits) and intravenous administration of ethanol verified that blood-acetate reached a steady state during oxidation of ethanol (Fujimiya et al., 2003).

G. Physiological Range of Ethanol Elimination Rates

A large number of published studies dealing with the elimination rate of ethanol from blood in humans (ß-slopes) show that this important pharmacokinetic parameter can vary by about four-fold among different individuals. Some abnormal high or low values can be ignored because of weaknesses in the experimental design or other artifacts. Nevertheless, there is still a large inter-individual variation in the rate of elimination of ethanol from blood in the drinking population.

Table 3.7 presents a range of ethanol elimination rates from blood (ß-slopes) that can be expected to apply to an entire population of drinkers. Admittedly, values beyond a low extreme of 0.1 g/L/h and a high rate of 0.35 g/L/h have occasionally been reported in the literature but a detailed evaluation of the articles reveals flaws in the experimental design or analysis or interpretation of the results. To determine the ß-slope, the dose of ethanol administered and the blood sampling protocol must be optimal to allow fitting a straight line to the post-absorptive concentration-time data.

Calculating alcohol burn-off rates based on just two blood samples taken 60 min apart is not recommended without knowledge of the position on the blood-alcohol curve when sampling occurs. In over 1000 drunk drivers, each of whom provided two samples of blood about 1 hour apart, the average alcohol burn-off rate was calculated and this gives a good estimate for this population of individuals. It is generally accepted that the vast majority of drunken drivers are in the post-absorptive phase when they are arrested but extreme values are still possible because some individuals might have had a delayed gastric emptying and thus not reached peak BAC at the time of sampling. In this population of drinkers, the mean burn-off rate was 0.19 g/L/h (95% range 0.09 to 0.29 g/L/h), being slightly faster in women (0.22 g/L/h) compared with 0.18 g/L/h for men (Jones and Andersson, 1996a). In another large study with drunk drivers when only considering values above 0.1 g/L/h to represent the post-absorptive phase, a median ethanol elimination rate of 0.22 g/L/h was found (Neuteboom and Jones, 1990). The higher average elimination rates of alcohol from blood in this latter study can be explained by the fact that all values below 0.1 g/L/h were omitted when averages were calculated. Many drunken drivers are heavy drinkers and alcoholics with an induced capacity to eliminate ethanol owing to induction of CYP2E1 (Park et al., 1996).

Table 3.7
Ranges of Ethanol Elimination Rates from Blood in Humans that Might be Expected to Apply Under the Conditions or Circumstances Specified (Modified from Jones, 1993a and 2003b)

Elimination rate	Expected values g/L/h	Experimental conditions/treatment
Slow	0.08–0.1	Malnourished individuals and people eating low protein diets. Advanced state of liver cirrhosis with portal hypertension. Administration of the ADH inhibitor drug 4-methyl pyrazole (fomepizole)
Moderate	0.1-0.15	Healthy individuals after overnight (10 h) fast and bolus ingestion of ethanol (< 1 g/kg)
Rapid	0.15-0.25	Regular drinkers after consumption of alcohol in the fed-state and with an appreciable starting BAC. Intravenous administration of nutrients such as fructose or amino acids during the post-absorptive phase.
Ultra-rapid	0.25-0.35	Alcoholics and binge drinkers during detoxification with very high blood-ethanol levels (> 3.5 g/L). Induction of CYP2E1 enzymes by drugs or ethanol. People with a genetic predisposition for ultra rapid metabolism of ethanol. Hypermetabolic state, e.g., induced by drugs or burn trauma.

3.7 Characteristics of Blood-Alcohol Curves

The disposition and fate of alcohol in the body is usually depicted as a plot of the blood alcohol concentration as a function of time after the start of drinking. Figure 3.19 gives examples of BAC profiles derived from experiments in which eight healthy volunteers drank 0.8 grams of ethanol per kg body weight in 30 min after an overnight fast. The alcoholic drink was made from 96 vol% ethanol, which was diluted to 20% v/v with a CO_2 free and a sugar-free orange soft drink. Many variable factors can influence the shapes of blood-alcohol profiles and these will be discussed below (Gustafson and Källmen, 1988).

A. Ingestion of Alcohol on an Empty Stomach

If alcohol is consumed on an empty stomach, such as after an overnight 10 h fast, these conditions favor rapid gastric emptying, which is associated with a much faster absorption of ethanol into the bloodstream (Jones, 1984). The absorption surface area in the duodenum and jejunum is enormous compared to the stomach, owing to the contribution of microvilli covering the mucosa of the intestines. However, some people might experience a pyloric spasm after they drink neat spirits on an empty stomach, probably because of irritation of emptying mechanisms or contractions of the stomach leading to a much slower absorption of ethanol into the bloodstream and consequently a lower C_{max} and a later occurring t_{max}.

The shape of BAC profiles if there is a rapid gastric emptying resembles the curves seen after ethanol is administered by constant rate intravenous infusion (Alha, 1951; Wilkinson et al., 1976; Jones and Hahn, 1997; Davidson et al., 1997). If the rate of absorption of ethanol is unusually rapid, one observes an overshoot peak (see Figure 3.5) in the BAC curve and for a short time C_{max} is higher than expected for the amount (dose) of alcohol consumed. The alcohol has entered the blood stream (~5 L) faster than it can be re-distributed to the rest of the body water (~40 L) and the overshoot is followed by a so-called diffusion plunge. During this time the alcohol in the central compartment (blood) distributes throughout the total body water (Hahn et al., 1997). The diffusion plunge appears to be an exponential function with a half-life of ~7 min, which means that it takes about 35 min (5 x t½) for the absorbed alcohol to equilibrate with the total body water.

Examples of overshoot peaks and C_{max} higher than expected were observed when women who had undergone gastric bypass surgery for morbid obesity drank a small dose of alcohol (0.4 g/kg) (Klockhoff et al., 2002). Another group of women of similar age and body mass index served as the controls. The individual BAC curves for the bypass patients are shown in Figure 3.20 and the presence of an overshoot peak is strikingly obvious and is a consequence of the very rapid absorption of alcohol into the blood stream. As expected the women also felt more inebriated immediately after drinking compared with the non-operated control group. The average BAC curves for the two conditions (operated and non-operated) are also shown in Figure 3.20 and it can be seen that it takes about 35 min for equilibration of the dose of ethanol.

Besides a radically reduced size of the stomach, individuals who had been operated on for obesity no longer have a functional pyloric sphincter, so the ingested alcohol entered the intestines and the bloodstream almost immediately after it was consumed. This deserves consideration when people who have undergone such an operation perform skilled tasks such as driving home after consumption of small doses of alcohol.

Other medical problems with the upper gastrointestinal tract, such as ulcers, dyspepsia, gastritis, *helicobacter pylori* infections, gastroesophageal reflux disease (GERD), might also impact the speed of ethanol absorption but these influences remain to be studied (Salmela et al., 1994; Lieber, 1997b; Yokoyama et al., 1995; Simanowski et al., 1998). Studies have shown gastric emptying is likely to be altered in people suffering from diabetes mellitus, GERD, hyperthyroidism and anorexia nervosa and in those taking certain medications (Nowak et al., 1995; Kong and Horwitz, 1999; Deponti et al., 1993).

Table 3.8 gives the mean and median blood alcohol concentrations at various sampling times after healthy men drank a moderate amount of alcohol (0.68 g/kg) as neat whiskey on an empty stomach (Jones, 1984). Besides mean and median BAC the coefficient of variation and the 95% range of BAC are shown for 48 male subjects. If the coefficient of variation (CV%) is taken as a relative measure of uncertainty in BAC, it is obvious that the errors incurred are greatest early after the end of drinking and also late in the post-absorptive phase six hours later. Least variation (CV=8%) was found at 100 min after end of drinking but even at this time point the BAC might vary between 0.59 to 0.83 g/L in 95 of 100 subjects. This human dosing study highlights the need to allow for uncertainty when blood-alcohol calculations are made for legal purposes.

Certain features of BAC profiles that are of particular interest in forensic science and legal medicine are illustrated in Figure 3.21. The time needed to reach the peak BAC after the end of drinking, the increment in the BAC before reaching the maximum concentration and the slope of the rectilinear post-peak disappearance phase (ß-slope) are indicated.

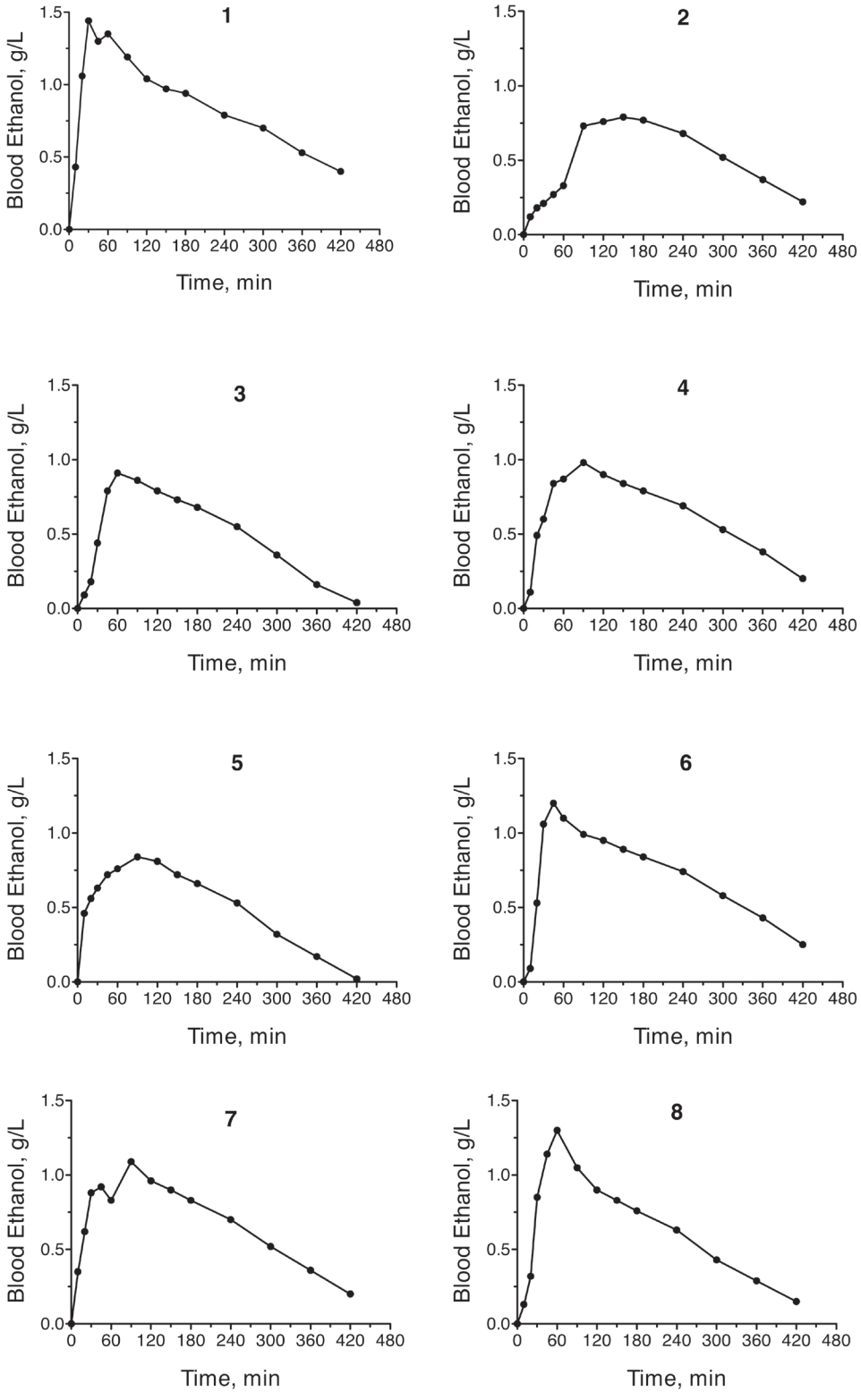


Figure 3.19 *Examples of blood-ethanol profiles after 8 healthy men who drank 0.80 g ethanol/kg body weight in 30 min after an overnight (10 h) fast. The drink was made from ethanol solvent (96% v/v) diluted with orange juice to give a 15-20% v/v solution of ethanol.*

Table 3.8
Magnitude of Inter-Individual Variation in Blood Alcohol Concentration (BAC) at Various Times after Healthy Men (N=48) Drank Neat Whiskey (0.68 g Ethanol per kg Body Weight) on an Empty Stomach (Jones, 1984)

Time, min[1]	Mean BAC g/L ± SD[2] (median BAC)	CV%[3]	95% range of BAC (± 2 x SD)
10	0.80 ± 0.279 (0.88)	35%	0.24–1.35
40	0.82 ± 0.141 (0.85)	17%	0.54–1.10
70	0.77 ± 0.083 (0.78)	11%	0.60–0.94
100	0.71 ± 0.059 (0.72)	8%	0.59–0.83
160	0.61 ± 0.062 (0.61)	10%	0.49–0.73
220	0.50 ± 0.063 (0.54)	13%	0.37–0.63
280	0.39 ± 0.071 (0.40)	18%	0.25–0.53
340	0.24 ± 0.074 (0.25)	30%	0.09–0.39
400	0.10 ± 0.072 (0.08)	72%	0.00–0.24

[1] Time from the end of drinking, which lasted exactly 20 min. [2] Standard deviation. [3] Coefficient of variation (mean/SD x 100).

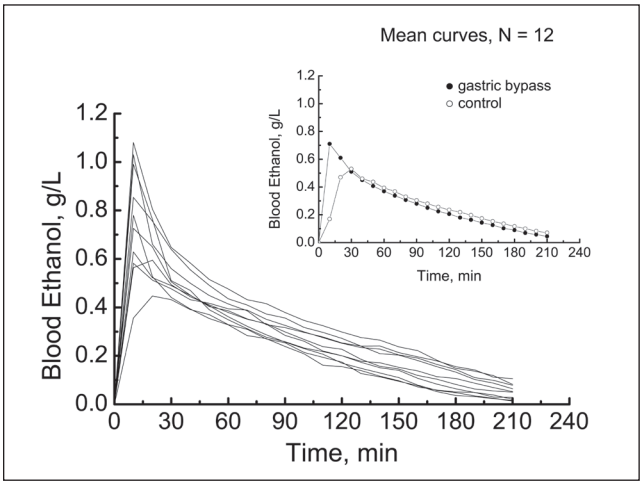


Figure 3.20 *Concentration-time profiles of ethanol in 12 healthy women who had undergone surgery for obesity (gastric bypass) two years earlier. The insert graph (top right) shows average curves for the bypass patients and a control group of non-operated women (data from Klockhoff et al., 2002).*

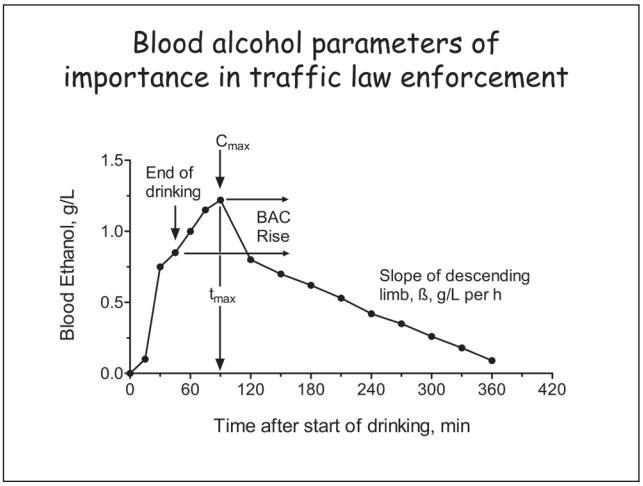


Figure 3.21 *Schematic blood-ethanol concentration-time plot to illustrate aspects of medical-legal interest including C_{max} and t_{max} and the ß-slope of the elimination phase. The alcohol was ingested over 60 min and the peak BAC was reached 30 min after the end of drinking.*

Table 3.9
Rates of Disappearance of Ethanol from Blood (ß-slopes) and Times to Reach the Maximum Blood Alcohol Concentration (t_{max}) in Controlled Experiments for Various Drinking Conditions

Conditions	N[1]	Mean ß-slope	Time to peak (t_{max})[2]
Straight whiskey[3]	150	0.134 (0.09–0.18)	30 (5–105)
EtOH[3] + juice	65	0.141 (0.12–0.17)	60 (0–120)
Drinks + food	15	0.160 (0.13–0.21)	78 (0–230)
Alcohol + food	10	0.174 (0.14–0.20)	90 (15–90)
Alcohol alone[3]	10	0.142 (0.12–0.17)	45 (0–60)
Beer[4] + food	9	0.120 (0.09–0.15)	7 (5–25)
Beer alone	9	0.150 (0.10–0.18)	16 (5–45)
Alcoholics[5]	20	0.230 (0.14–0.36)	##[5]

[1] Number of subjects. [2] Timed from the end of drinking. [3] The alcohol was consumed on empty stomach after an overnight fast. [4] Two bottles of beer. [5] Tests made during detoxification all were in the post-peak phase.

Results from the determination of ß-slope in a large number of controlled drinking experiments are collected together in Table 3.9, which highlights the considerable inter-individual variability in this pharmacokinetic parameter, spanning from 9 to 36 mg/dL/h (0.09–0.36 g/L/h). The lowest values were seen in fasted subjects who ingested a bolus dose of ethanol in the morning and the highest values were observed in alcoholics during detoxification. This four-fold difference emphasizes the dangers associated in assuming an average burn-off rate when retrograde extrapolations of a person's BAC from time of sampling to the time of driving are contemplated; such a practice is sometimes required by law.

The shape of the BAC-time course depends on a host of experimental variables including the dose of alcohol taken, the speed of drinking, the kind of beverage consumed (beer, wine, spirits), the fed or fasting state of the subject as well as the individual's age, sex, and body composition (proportion of fat:lean tissue). Both pre-analytical (sampling, transport, storage, preservation) and analytical sources of variations are important to consider in overall reliability of results.

B. Inter- and Intra-Individual Variations

Human beings show an enormous variation in their response to ethanol as well as other drugs. Besides difference in behavioral response the disposition kinetics also show considerable variation from occasion to occasion in the same individual (Nagoshi and Wilson, 1989; Norberg et al., 2000). Jones and Jönsson (1994b) showed that 42% of the variance in ß-slope was attributable to between-subject variation and 58% was accounted for by variation within subjects. In the same study, other important pharmacokinetic parameters such as peak BAC, r, C_o, and AUC showed significantly more variation between than within subjects. The notion of conducting an alcohol tolerance test to establish a person's rate of alcohol disposal (ß-slope) seems hardly worthwhile considering the large within-subject variance component. Instead, a range of elimination rates such as 0.09 to 0.25 g/L/h can be assumed to apply and in my experience this should include the vast majority of individuals. Indeed, drunken drivers who are also heavy drinkers and often dependent on alcohol, would be more likely to have ß-slopes of 0.20 g/L/h or more rather than 0.10 g/L/h or less. Studies of ethanol metabolism in monozygotic and dizygotic twins have also demonstrated the involvement of both genetic and environmental influences on the pharmacokinetics of ethanol (Martin et al., 1985; Bosron et al., 1993; Kopun and Propping, 1997; Li, 2000; Vesell et al., 1971).

Figure 3.22 gives details from an experiment designed to determine the repeatability of elimination rates of ethanol from blood (ß-slope) in controlled drinking experiments (Schonheyder et al., 1942). Three healthy men drank 40 g ethanol diluted to 200 ml with water on 10 separate occasions. The post-peak parts of the curves between 100 and 240 min were determined by analyzing a series of seven triplicate capillary blood samples. The authors used an analysis of variance and other statistics to show that the ß-slope varied as much within the same subject as between subjects. Note that all results fell within the range published by Widmark, namely 0.11-0.24 mg/g/h (mean 15 mg/g/h).

Inter- and intra-individual variations in the pharmacokinetics of ethanol where investigated in 12 men who ingested the same dose of ethanol under the same strictly controlled conditions on four occasions over one month. The ethanol dose was 0.8 g per kg body weight ingested mixed with orange juice on an empty stomach. Figure 3.23 gives examples

of the concentration-time plots, and the between-subject and within-subject variation in rates of ethanol elimination from blood were evaluated by one-way analysis of variance (ANOVA). The results of this statistical test are given in Table 3.10, showing that the variance ratio F=3.93 was statistically highly significant (p<0.001). However, a breakdown of the total variance showed that, 42% was attributed to between-subject sources of variation and 58% was from within-subject sources.

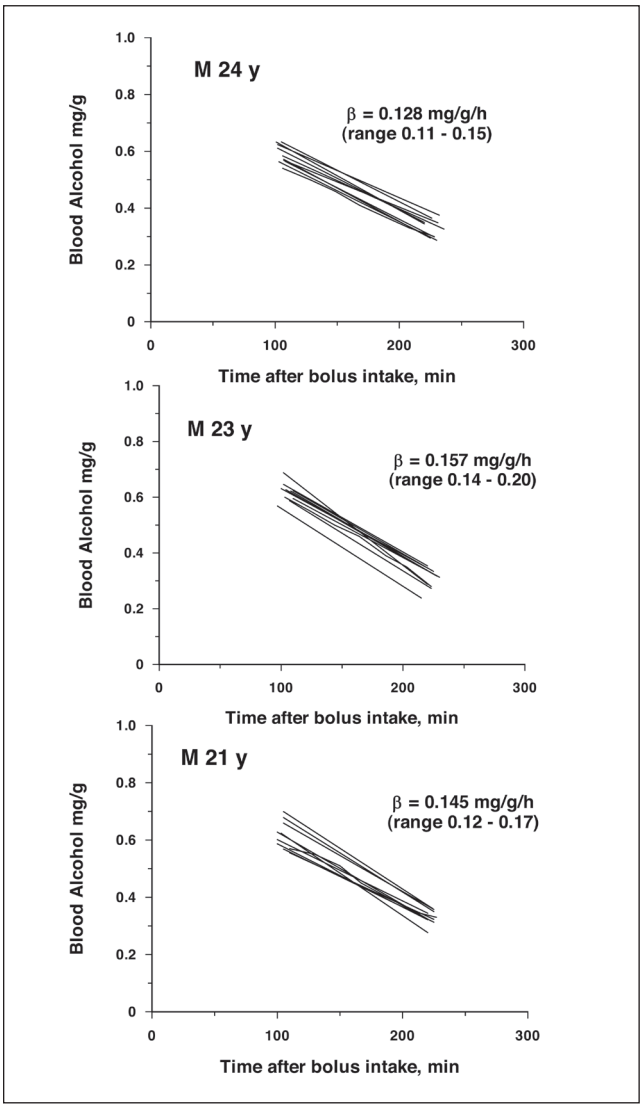


Figure 3.22 *Intra-individual variations in the slopes of the elimination stages of the blood-alcohol curves in three subjects who drank 0.50 g/kg on 10 separate occasions. Plots redrawn from paper by Schonheyder et al. (1942).*

The results of this analysis shows that elimination rates of ethanol from blood vary just as much between individuals as within the same individual from occasion to occasion (Passananti et al., 1990; Jones and Jönsson, 1994a; Li et al., 2000). For the vast majority of people the elimination rate of ethanol spans from 0.1 to 0.25 g/L/h and this range is useful to use in legal proceedings when back extrapolation becomes an issue (Jones, 1993a; Jones and Andersson, 1996a; Piekoszewski and Gubula, 2000; Mishra et al., 1989; Mumenthaler et al., 1999). After development of metabolic tolerance, such as that found in alcoholics, the rate of ethanol elimination after a drinking binge is likely to exceed 0.25 g/L per h (Kalant, 1996a; Jones, 2007b). Another way to report the elimination rate of ethanol is from the body as a whole and results are dependent on body weight. A good rule of thumb is that a person eliminates 0.10 g ethanol per kg of body weight per hour and this figure seems to be independent of gender (Fransson et al., 2005).

In Figure 3.24, the elimination rates of alcohol from the blood were rank ordered for 48 healthy men aged 20-60 years who drank 0.68 g ethanol per kg as neat whiskey after an overnight fast. In this study the results are all between 0.1 and 0.2 g/L/h for these strictly controlled conditions (Jones, 1984). Age-related differences in the rates of metabolism of ethanol were investigated by several research groups because the activity of CYP450 enzymes is known to decrease with aging (Vestal et al., 1977; Jones and Neri, 1985). However, the kinetic parameter of ethanol that changed the most during aging was the Widmark factor rho (volume of distribution), probably because body water decreases in the elderly (Schoeller, 1989; Van Loan, 1996). The rate of alcohol elimination from blood (ß-slope) was barely influenced by aging after moderate amounts of alcohol were consumed and when the ADH pathway is mainly responsible for oxidation of ethanol (Lieber, 2000).

C. Factors Influencing C_{max} and t_{max}

The dose and speed of drinking are important considerations when the peak BAC, the time of its occurrence, and variability in areas under the BAC profiles are discussed in forensic situations (O'Neill et al., 1983; Jones et al., 1991). In general, the larger the dose of alcohol and the faster the rate of drinking the higher is the BAC, and impairment of the individual is more pronounced. Small doses of ethanol are absorbed faster than larger doses as might be expected. Moreover, after low doses (< 0.3 g/kg) some of the alcohol is metabolized during the first passage of blood through the

liver, which is known as first-pass metabolism (FPM). This pre-systemic clearance of ethanol is especially marked when small doses of alcohol are ingested after eating food (Wagner, 1986; Oneta et al., 1998; Welling et al., 1977). Variability in absorption profiles and C_{max} are less if the dose of alcohol is administered per kg body water instead of per kg body weight. This follows because of the different proportions of adipose tissue in different individuals with the same body weight (Wang et al., 1992; Graham et al., 1998).

The factors influencing C_{max} and t_{max} and elimination rate from blood are examples of questions that often arise in traffic law enforcement when drunken drivers are prosecuted. The peak BAC (C_{max}) and the time of reaching the peak (t_{max}) after drinking depend primarily on dose, rate of drinking and gastric emptying. Various medications influence gastric emptying such as the antibiotic erythromycin (Edelbroek et al., 1993; Jones et al., 1999; Greiff and Rowbotham, 1994). Treatment with the prokinetic drug cisapride accelerated gastric emptying and caused a faster absorption of ethanol and an earlier occurring peak concentration in blood (Kechagias et al., 1999). The area under the concentration-time curve (AUC) is another useful parameter of exposure of the body organs and tissue to alcohol for the particular dose administered. The rate of elimination of alcohol from the whole body, which is sometimes denoted as B_{60} is the product of ß and volume of distribution V_D or can be derived as the ratio of dose/time$_0$.

In a recent survey of the literature dealing with time to reach peak BAC after end of drinking, Iffland and Jones (2002) produced the data shown in Table 3.11. The results illustrate the large inter-subject and inter-study differences with the peak BAC being reached between 0-30 min as well as >90 min after end of drinking. It is important to note that the doses of alcohol and duration of drinking in the various studies cited are much smaller than for the typical drunk driver. Nevertheless, the table illustrates that two blood samples taken 30-60 min apart is not sufficient to allow making an unequivocal statement of whether the person was in the post-peak phase at the time of driving.

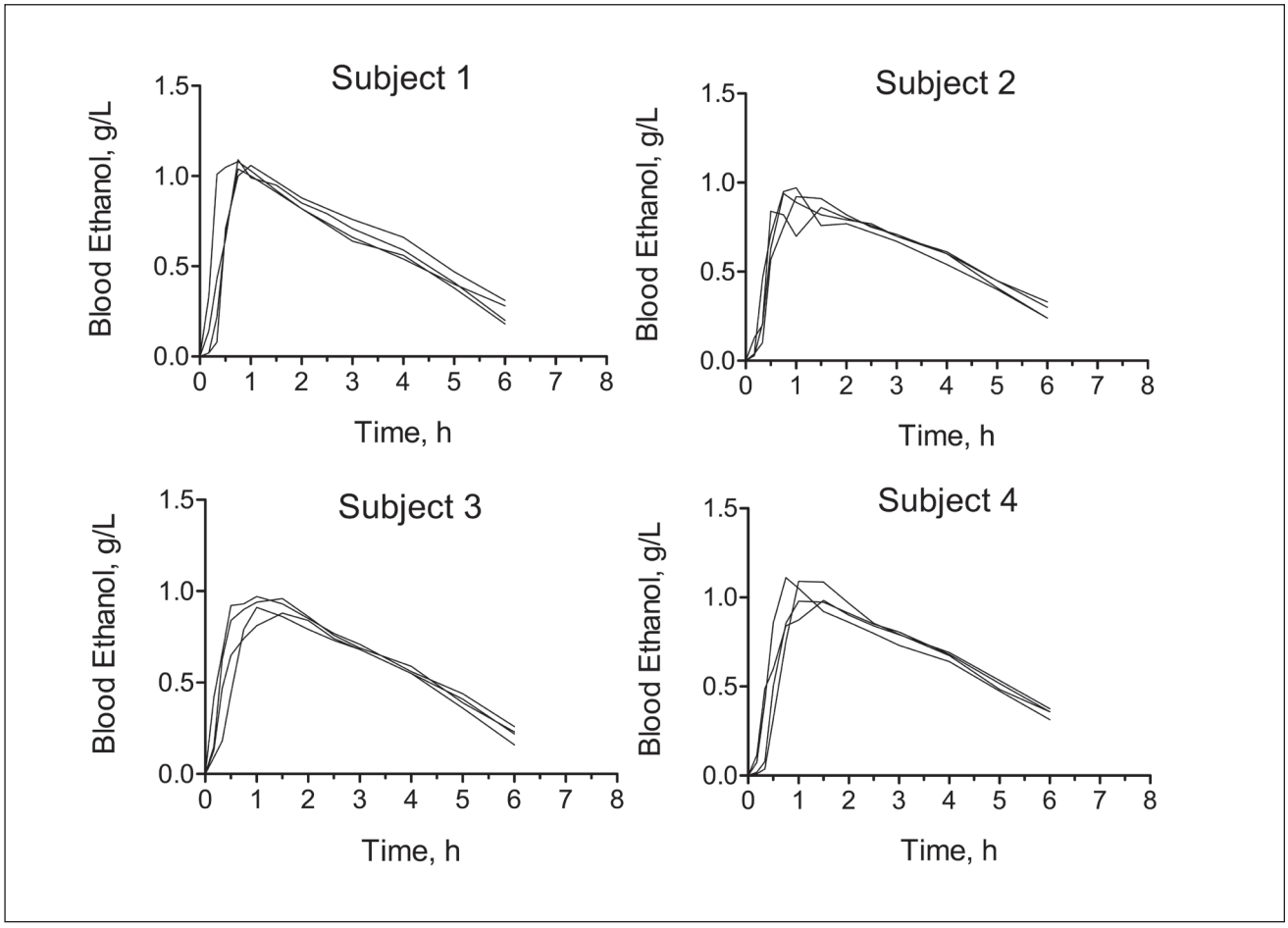


Figure 3.23 *Intra-individual variations in the concentration-time profiles of ethanol during absorption, distribution and elimination phases in four subjects who drank 0.8 g/kg body weight on four separate occasions over four weeks (data from Jones and Jönsson, 1994a).*

Table 3.10
Analysis of Variance Establishing Variations Between and Within Subjects for the Elimination Rate of Ethanol from Blood. Each Subject Was Tested on Four Separate Occasions after a Dose of 0.80 G/kg Body Weight after an Overnight Fast (Data from Jones and Jönsson, 1994a)

Source of variation	DF[1]	Sums of squares (SS)	Mean Square (MS)	Variance ratio (F)
Between subjects	11	56.32	5.12[2]	3.93 ($p<0.01$)
Within subjects	36	46.94	1.30[3]	
Total	17	103.26		

[1] DF=degrees of freedom.
[2] Between subject $SD_b=\sqrt{(MS_b - MS_w)}/4$. Percent variation between subjects=42%.
[3] Within subject $SD_w=\sqrt{1.3}$. Percent variation within subjects=58%.

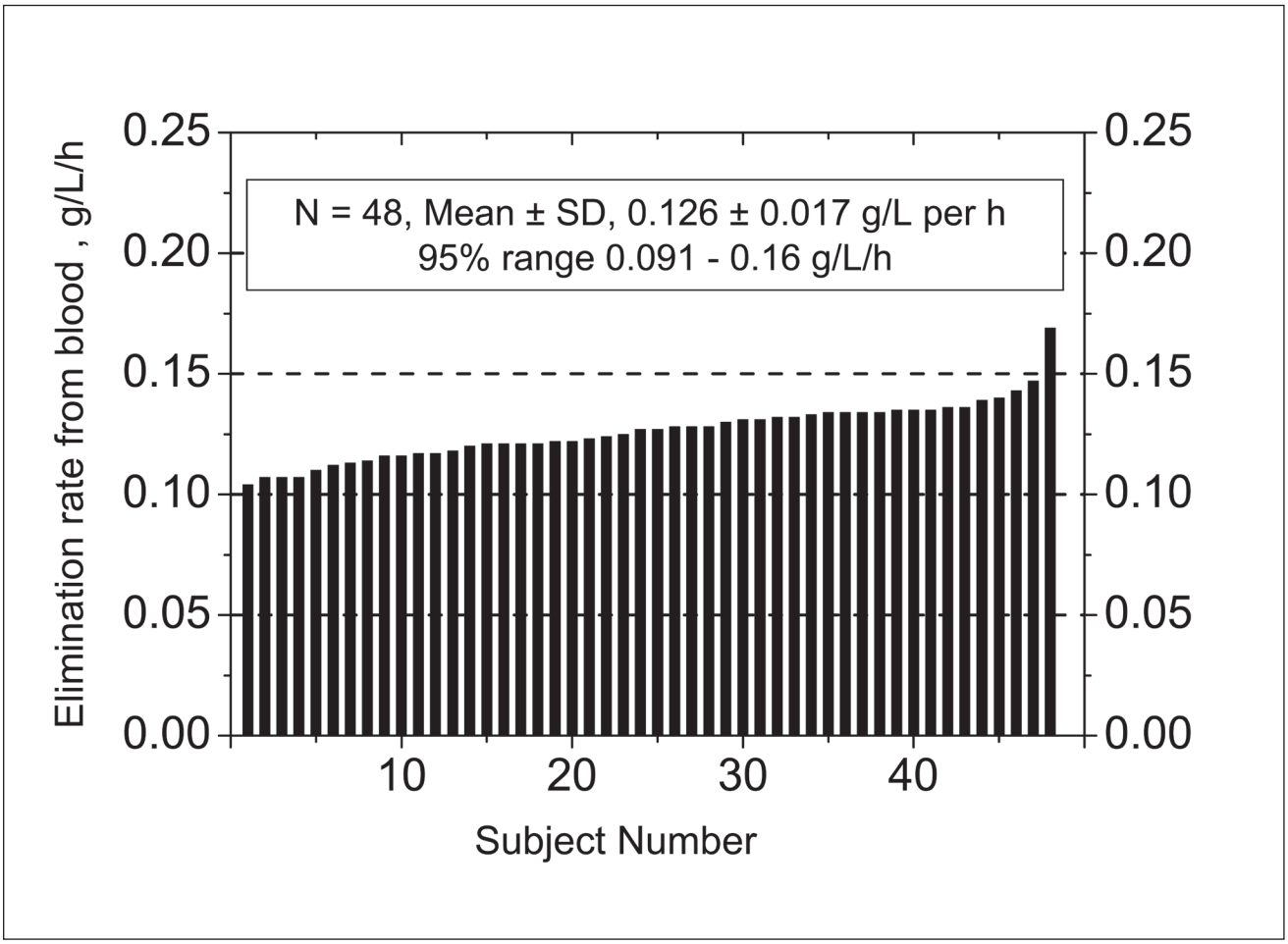


Figure 3.24 *Rank ordering of the rates of disappearance of ethanol from blood (ß-slopes) in 48 healthy men after they drank 0.68 g/kg on an empty stomach as neat whiskey (Jones, 1984).*

Table 3.11
Times in Minutes Needed to Reach Maximum Blood Alcohol Concentration After End of Drinking According to Various Published Studies (Reproduced from Iffland and Jones, 2002)

Dose g/kg	Drinking Time (min)	Hours since last meal	N[1]	Number of subjects reaching peak blood ethanol concentration within				Reference
				0–30	31–60	61–90	> 90	
0.30	60	not given	12	12	-	-	-	Heifer (1976)
0.30	30	> 4	10	9	1	-	-	Kühnholz et al. (1993)
0.34	15	> 8	6	5	1	-	-	Jones et al. (1991)
0.50	5	> 8	20	-	19	1	-	Alha (1951)
0.50	60	not given	12	11	1	-	-	Heifer (1976)
0.50	30	> 4	10	9	1	-	-	Kühnholz et al. (1993)
0.51	15	> 8	16	11	3	1	1	Jones et al. (1991)
0.68	20	> 8	83	33	26	21	3	Jones et al. (1991)
0.75	10	0	23	8	9	5	1	Krauland et al.(1966)
0.75	10	1	15	-	1	6	8	Krauland et al. (1966)
0.75	10	3	30	-	10	14	6	Krauland et al. (1966)
0.75	10	6	55	7	21	13	14	Krauland et al. (1966)
0.75	5	> 8	31	-	20	4	7	Alha (1951)
0.80	60	not given	12	9	2	1	-	Heifer (1976)
0.80	50-55	3	19	2	10	4	3	Gerchow and Steigleder (1961)
0.80	30	> 4	10	6	2	1	1	Kühnholz et al. (1993)
0.80	30	> 8	65	40	19	3	1	Jones (unpublished work)
0.85	25	> 8	44	13	24	7	-	Jones et al. (1991)
1.00	5	> 8	41	1	21	13	6	Alha (1951)
1.02	25	> 8	3	-	1	1	1	Jones et al. (1991)
1.20	50-55	3	17	2	5	5	5	Gerchow and Steigleder (1961)
1.25	5	>8	38	1	14	14	9	Alha (1951)
1.60	50-55	3	19	5	8	6	-	Gerchow and Steigleder (1961)

[1] N=number of subjects participating in the studies.

Zink and Reinhardt (1984) published a classic study in which volunteer subjects consumed massive doses of ethanol continuously for up to 10 h under real-world conditions. The peak BAC reached was between 3-4 g/L. The BAC increased gradually during the drinking period and by the end of drinking the BAC was sometimes close to its maximum concentration even before the last drink was finished. It seems that during a period of heavy drinking much of the alcohol becomes absorbed into the bloodstream and distributed throughout the body compartments during the drinking period and that the amount contained in the last drink is insufficient to raise the BAC further. In reality however much depends on the frequency of drinking and the amount of alcohol contained in the last drink.

Hundreds of human dosing studies have been published documenting the shapes of blood-alcohol curves after different drinking conditions. In studies of this kind it is important to ensure that the blood-sampling schedule is optimal to allow an unequivocal determination of the post-absorptive elimination phase. Without this information it is simply not feasible to arrive at a reliable estimate of the rate of elimination of alcohol from the bloodstream.

D. Effects of Food in the Stomach

Stomach emptying and factors influencing this process are important determinants of the speed of absorption of alcohol (see Table 3.3). It has been known since the 1920s that the systemic availability of ethanol is appreciably less when drinking occurs together with or after a meal compared with drinking on an empty stomach (Widmark, 1932). Several mechanisms were suggested to account for this finding: (i) binding of ethanol molecules to dietary components particularly amino acids, (ii) a more effective enzymatic oxidation when alcohol flux through the liver is reduced, (iii) formation of ethyl esters in the stomach and intestines, (iv) oxidation of ethanol by food components, (v) oxidation in the stomach by gastric alcohol dehydrogenase present in the mucous surfaces (Schmidt and Oehmichen, 1984).

By comparing blood-alcohol profiles in the fed and fasting states, Widmark estimated that between 10-12 g from a moderate dose of 60-70 g "disappeared" when volunteers drank alcohol after a meal compared with drinking on an empty stomach (Widmark, 1934). He proposed that alcohol might be chemically bound to amino acids in the diet such as glycine and thus prevented from being absorbed into the bloodstream. The effect of food in lowering the bioavailability of ethanol has been confirmed many times by different investigators (Goldberg, 1943; Sedman et al., 1976b, Lin et al., 1976; Jones and Jönsson, 1994b; Wilkinson, 1977b), although the mechanism of this effect was more difficult to explain.

The areas under the BAC time profiles are appreciably less when the same dose of ethanol is given by oral administration compared with the intravenous route. This was taken to indicate that a significant pre-systemic metabolism was occurring and that gastric ADH was therefore incriminated in this process. The proponents for a biological role of gastric ADH have been quick to suggest that because alcohol is retained in the stomach for a longer time when taken together with food, this gives more opportunity for metabolic breakdown to occur.

Factors influencing gastric emptying are complex and a lot depends on whether the food was liquid or solid (Wilkinson et al., 1977b; Welling, 1977). Different constituents of a meal are emptied separately from the stomach depending on the relative proportions of liquids and solids. The composition of the food eaten plays a role in the absorption rate of ethanol (Jones et al., 1997a; Pikaar et al., 1988).

In one study it was shown that eating a meal increased the clearance rate of ethanol from the blood even when the alcohol was administered intravenously (Hahn et al., 1994). This suggests a food-induced increase in activity of alcohol-metabolizing enzymes and/or enhanced liver blood flow after eating food leading to a more effective hepatic clearance of ethanol (Bode, 1978; Host et al., 1996; Dedrick and Forrester, 1973). Furthermore, eating a meal during the post-absorptive phase of the alcohol curve at about three hours post-drinking also boosted the rate of ethanol metabolism as reflected in a steeper slope for a few hours after the meal (Jones and Jönsson, 1994).

Hepatic ADH activity is appreciably less in the fasting state compared with the well-nourished organism, which might explain the slower rate of disposal of ethanol after an overnight fast (Borson et al., 1980). Adequate intake of proteins was especially important in this respect because a protein-deficient diet dramatically lowered the rate of ethanol disappearance from blood (Bode, 1978). When a protein-rich meal was reinstated the ß-slope recovered and even surpassed the original values after a few days. Furthermore, the liver blood-flow is enhanced by feeding and this mechanism may help to facilitate the clearance rate of ethanol from blood (Svensson et al., 1983; Schmidt and Oehmichen, 1984).

Figure 3.25 shows mean blood-alcohol profiles obtained in a cross-over design study aimed at comparing the effect of food on the pharmacokinetics of ethanol. Average curves are shown for N=10 men after they drank a dose of 0.8 g per kg body weight on an empty stomach (fast) or after eating a standardized breakfast (fed). The major influence of food in the stomach before drinking is a lowering in C_{max} and a smaller area under the curve. The slope of the post-peak elimination phase is not much different for the two conditions of drinking.

In the fed state the BAC curve ran below the fasting curve for the entire blood sampling period of eight hours post drinking, giving the impression that a smaller dose of ethanol had been administered under fed conditions. The values of C_{max} and t_{max} are read directly from these curves and the equation used to calculate the ß-slope and rho factors are also shown. By extrapolating the rectilinear elimination portions back to the time of starting to drink one obtains the y-intercept (C_0), which represents the BAC expected if absorption and distribution were instantaneous and without any metabolism taking place. The C_o in the fed sate is significantly lower than in the fasting state and this has implications when the distribution volume of ethanol is calculated as the ratio of dose/C_o.

Table 3.12
Mean Pharmacokinetic Parameters[1] of Ethanol in Healthy Men (N=10) After They Ingested a Dose of 0.8 g Ethanol per kg Body Weight According to a Crossover Design Experiment Either After an Overnight Fast or Immediately After a Standardized Breakfast (Data from Jones and Jönsson, 1994b)

Conditions	C_{max} g/L	t_{max} min	C_O g/L	Rho (V_d) L/kg	Min_0 min	ß-slope (ko) g/L/h	B_{60} g/kg/h
Fed	0.62	120	0.97	0.82	393	0.15	0.123
Fasted	0.96	45	1.16	0.69	495	0.14	0.097

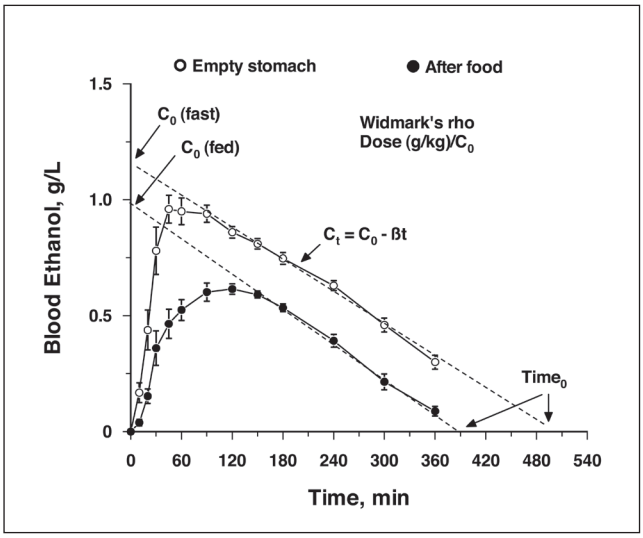


Figure 3.25 *Mean blood-ethanol profiles after N=10 subjects drank 0.8 g/kg after an overnight fast or after they had eaten a standardized breakfast. The pharmacokinetic parameters of ethanol are indicated on the curve for fasting conditions. Note the lower C_0 parameter in the fed state indicating an absorption deficit and apparent loss of ethanol (Jones and Jönsson, 1994b).*

Numerous publications including studies by Widmark found that C_{max} was appreciably lower and t_{max} often prolonged when alcohol was consumed together with or after a meal. In addition to a smaller area under the BAC curve in the fed state, later studies showed that the rate of metabolism was faster in a well nourished organism (Jones and Jönsson, 1994a).

The major pharmacokinetic parameters of ethanol with and without food are compared in Table 3.12 based on the curves drawn in Figure 3.25. The C_{max} was lowered by about 0.30 g/L when the subjects had eaten a meal before drinking and t_{max} occurred considerably later, 120 min vs. 45 min from start of drinking. A comparison of C_0 values under fed and fasting conditions illustrates the notion of an absorption deficit because the value of C_0 (fed) was 0.97 g/L compared with 1.16 g/L (fasted), a difference in alcohol concentration of 0.19 g/L. This difference in C_o leads to differences in the rho factor, which are therefore abnormally high for men in the fed state (Table 3.12). The slopes of the post-absorptive elimination phase did not depend on the fed or fasting state because the ß-slope was about the same (0.14 g/L/h). However it was obvious that the extrapolated time to reach zero BAC ($time_0$) was shorter in the fed state by 102 min or 1.7 h. This is approximately the time needed to metabolise completely the absorption deficit of 0.19 g/L or the observed difference in C_0. The absorption deficit might be different for different drinking conditions, e.g., fast vs. slow absorbers, beer vs. liquor, and large vs. small meal before drinking.

The disappearance rate of ethanol from the bloodstream did not depend on the fed or fasting state of the subjects as verified by comparing ß-slopes in Table 3.12. However, when the rate of alcohol elimination per unit body weight was calculated (B_{60}), by dividing the dose (g/kg) by the extrapolated time to zero BAC (min_0), the overall rate of metabolism was significantly faster after eating a meal. This dichotomy can only be explained if a pre-systemic metabolism of ethanol occurs during the absorption stage of the blood-alcohol curve, that is, during the first two hours post dosing. This is generally referred to as first-pass metabolism (FPM). The major influence of food in lowering the BAC profile was noticed already by Widmark and he referred to it as a "loss of alcohol" much later becoming known as an absorption deficit (Widmark, 1932; Seidl et al., 2000). The B_{60} for elimination rate of ethanol from the whole body can also be derived as the product of ß and rho (Jones, 1984).

It is important to realize that if ethanol had been determined in samples of serum or plasma or even the water fraction of the blood, the parameters such as C_{max}, rho, ß-slope would have been slightly different. This follows because the concentrations of ethanol depend on the water content of the specimens and plasma=serum > whole blood. The slope in the post-absorptive phase is steeper for plasma resulting in a higher C_0 and a correspondingly smaller rho

factor. However, the extrapolated time to zero BAC (min_0) and the rate of elimination from the whole body dose/min_0 would be the same regardless of whether plasma or whole were the specimens analysed. This observation is not generally realized and many believe that the Widmark rho factor is the same as the percent body water, which is not the case because whole blood contains about 20% solids (lipids, proteins, etc.).

E. Gender Differences

Women and men are different when it comes to how they react to alcohol and other drugs, and sex influences several pharmacokinetic parameters (Schwartz, 1996). Women are generally smaller than men (lower body weight) with more fat and less water per kg than men. Age, menstrual cycle, and menopause in women are associated with hormonal changes that might be important for activity of drug-metabolizing enzymes and also influence gastric emptying as well as mood and behavior after consumption of alcohol (Schwartz, 1996; Gill, 1997). Some investigators showed that the activity of gastric ADH was reduced in women compared to men, and suggested that this is an important consideration for C_{max} and toxicity of ethanol (Frezza et al., 1990) A lower proportion of body water per kg body weight in women might also account for them reaching a higher blood alcohol concentration after the same dose per kg and similar pattern of drinking as a man (Thomasson, 1995; Davis and Bowen, 1999; Lucey et al., 1999).

The metabolism and pharmacokinetics of ethanol in women is not as thoroughly investigated as in men. However, one early study with 20 women found values of ß and "r" in close agreement with those reported by Widmark (Österlind et al., 1944). Some studies in women have focused on the influence of oral contraceptive steroids and also hormonal changes associated with the menstrual cycle (Jones and Jones, 1984; Hobbes et al., 1985; Marshall et al., 1983; Brick et al., 1986). The results of this research are conflicting and definite conclusions cannot be drawn because increases, decreases, and no changes in ß-slope have been reported in various studies of the interaction between ethanol and contraceptive steroid drugs (Sutker et al., 1987).

The finding is robust and verified in many studies that women have a slightly steeper slope in the post-absorptive segment of the alcohol curve than men assuming zero-order kinetics. This implies a faster rate of elimination of ethanol from blood compared with men (Dubowski, 1976; Marshall et al., 1983; Cole-Harding et al., 1987). However, a steeper ß-slope in combination with a smaller volume of distribution in females (lower "r" factor) means that the overall rate of clearance of ethanol from the body expressed as g/kg/h and defined as the product of ß and r was not statistically significant between the sexes (Jones, 1989). As a rule of thumb, the rate of body clearance of alcohol is about 0.10 g/kg/h and therefore a person weighing 70 kg can eliminate 7 g of pure ethanol per hour or roughly the amount contained in one bottle of light beer.

The major gender difference in ethanol pharmacokinetics concerns the value of the distribution factor "r" (Whitfield et al., 1990; Dubowski, 1976). This factor strongly depends on body composition, especially the amount of water per kg of body weight and therefore the proportions of fat to lean tissue in the organism (Watson et al., 1981; Wang et al., 1992). Body water decreases in the elderly and "r" factors for men aged 20-29 y, 30-39 y, 40-49 y and 50-59 y were 0.72 ± 0.042, 0.71 ± 0.036, 0.68 ± 0.026, and 0.66 ± 0.041 L/kg (mean ± SD), respectively (Jones and Neri, 1985). Because women have less water per unit body mass compared with men, the female sex can be expected to have a smaller volume of distribution—lower "r" factor (Goist and Sutker, 1985). Widmark reported an average "r" value of 0.68 for men (span 0.51–0.85) and 0.55 for women (span 0.44–0.66). Similar "r" values for men have been confirmed in several recent controlled studies of the pharmacokinetics of ethanol (Jones, 1984; Jones and Neri, 1985).

Attention was also given to possible hormonal influences on pharmacokinetics of ethanol in men and women and also in women during different phases of the menstrual cycle (Haddad et al., 1998; Correa and Oga, 2004). Rates of ethanol metabolism were studied after use of oral contraceptive steroids, a treatment that might alter the activity of alcohol metabolizing enzymes (Marshall et al., 1983; Jones and Jones, 1984; Mumenthaler et al., 2001).

Strong support for hormonal influences came from a study of ethanol metabolism in men who had been castrated owing to testicular cancer (Mezey et al., 1988). The finding of an enhanced elimination rate implicates testosterone as an endogenous factor modulating the activity of alcohol-metabolizing enzymes (Mezey et al., 1989). Although it is fairly well established that alcohol alters behavioral changes including aggression during various stages of the menstrual cycle (Brick et al., 1986; Jones and Jones, 1976), it was more difficult to prove an effect on the rate of ethanol metabolism (Haddad et al., 1998). Large numbers of subjects are needed in the studies as well as biochemical analysis of female sex hormones in serum (estrogens—estrone, estradiol, estrol) to unequivocally document the particular phase of the menstrual cycle (follicular, preovulatory, luteal, premenstrual).

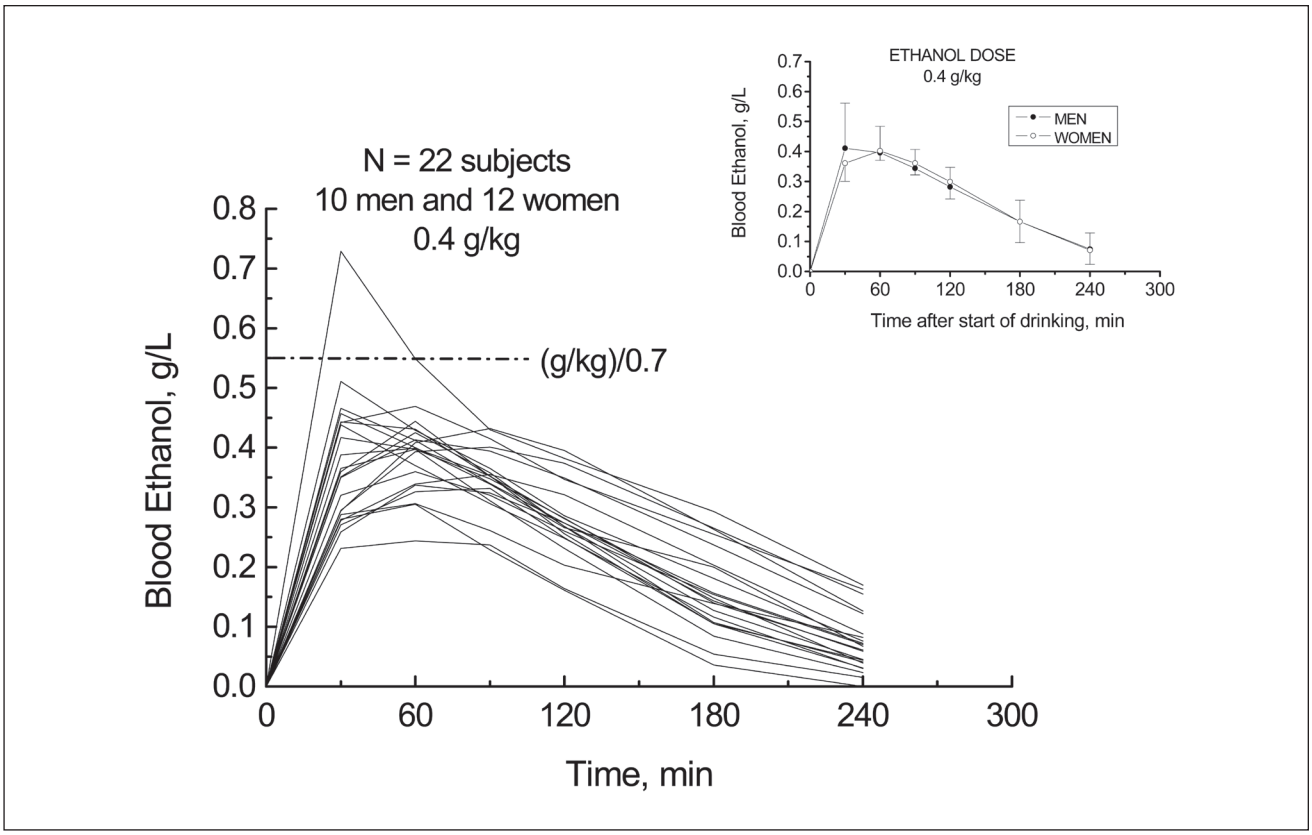


Figure 3.26 *Concentration-time profiles of ethanol in venous blood from 10 men and 12 women after they consumed 0.4 g ethanol per kg body weight about two hours after their last meal. The mean curves are shown as an insert (top right). The dotted horizontal line is the BAC expected for the dose of ethanol assuming a rho factor of 0.7 independent of gender (Fransson et al., 2005).*

Table 3.13
Pharmacokinetic Parameters of Ethanol in 10 Men and 12 Women Who Drank 0.4 g Ethanol Per kg Body Weight in 15 Min 2-3 Hours After Eating Lunch. Values Shown are Mean ± SD (Fransson et al., 2005)

Parameter[1]	Men (n=10)	Women (n=12)
C_{max} mg/g	0.426 ± 0.136	0.417 ± 0.027
t_{max} min	48.00 ± 15.49	60.00 ± 24.49
C_0 mg/g	0.510 ± 0.064	0.577 ± 0.038[2]
ß-slope or k_0 mg/g/h	0.115 ± 0.016	0.138 ± 0.019[2]
V_d or rho	0.796 ± 0.103	0.696 ± 0.047[2]
AUC mg x h/g	56.82 ± 17.67	58.66 ± 7.56
B_{60} g/kg/h	0.090 ± 0.016	0.096 ± 0.014

[1] For definitions of parameters see main text.
[2] Statistically significant difference between the sexes ($p<0.01$).

Figure 3.26 shows BAC profiles for 22 subjects (12 women and 10 men) who drank 0.4 g/kg ethanol in 15 min at 2-3 hours after they had eaten lunch. The drink was made from ethanol solvent (95% v/v) after dilution with a soft drink to 20% v/v. Large variation in the shapes of these individual profiles is obvious. The horizontal dotted line represents the theoretical maximum BAC arising for 100% availability of the 0.4 g/kg dose and assuming a distribution volume of 0.7 L/kg for both men and women. Only one male subject exceeded this BAC and evidently he had not eaten any lunch. In all the other subjects, the actual BAC curves were considerably lower than those expected theoretically, which suggests a reduced bioavailability probably being caused by first-pass metabolism or some other mechanism. The average curves (insert top right) agreed well with no marked gender-related differences in patterns of absorption, distribution or elimination.

Statistical summaries of the pharmacokinetic parameters for men and women and differences between the sexes are shown in Table 3.13. The rates of disappearance of ethanol from blood (ß-slope or k_0) were determined, as described

by Widmark, by fitting a straight line to the concentration-time points on the post-absorptive portions, normally starting 90 minutes after end of drinking (Fransson et al., 2005). The Widmark rho factors were larger than expected, which probably reflects the fact that the subjects had not fasted overnight before they drank the alcohol. The ethanol was diluted to ~20% v/v with a soft drink instead of consumption of neat spirits as recommended by Widmark (1932). Nevertheless, the gender-related differences in C_o and rho were statistically significant as expected from differences in total body water between men and women. The ß-slope was also slightly steeper in women compared with men ($p<0.01$) but the overall rate of ethanol elimination per kg body weight (B_{60}) was independent of gender.

Dettling et al. (2007) determined the elimination rates of ethanol and other pharmacokinetic parameters in 64 women and 68 men under controlled experimental conditions. The aim was to establish gender differences in elimination rates of ethanol and to test if this depended on size of the liver, which was estimated according to a nomogram based on age, body weight and gender. The comparison between the men and women is shown in Table 3.14.

The authors concluded that the elimination rate of ethanol was slightly faster in women because liver weight represented a large proportion of lean body mass than in males.

F. Repetitive Drinking

Figure 3.27 is a rare example of an experiment in which the alcohol was given in divided doses (Jones, unpublished work). The two male subjects drank a moderate dose of neat whiskey in 15 min after an overnight fast and the same dose of alcohol was ingested again about 2 hours later. After the first dose the peak BAC was reached 15 min after end of drinking compared with 55-60 min after the second dose of whiskey was consumed. More studies are needed that involve repetitive drinking, which is closer in keeping with real-world drinking scenarios.

G. Effect of Age on Widmark Parameters

Aging can have a major influence on absorption, distribution and metabolism of drugs and this deserves consideration when physicians prescribe medication to the elderly. Both hepatic function and kidney function deteriorates in later life, and this impacts on metabolism and urinary excretion of drugs and their metabolites. Moreover, the percentage of water in the body decreases in the elderly (Schoeller, 1989), which is important for drugs such as ethanol that distribute into the total body water. The blood alcohol concentrations after a dose of ethanol administered per kg body weight are expected to be higher in the elderly, especially in men (Jones and Neri, 1985; Mirand and Welye, 1994).

Table 3.14
Gender-Related Differences in Pharmacokinetic Parameters of Ethanol According to a Study by Dettling et al. (2007). Values Reported are Mean ± Standard Deviation

Blood-alcohol parameter	Women (N=66)	Men (N=68)
Dose of ethanol, g/kg	0.79-0.88	0.85-0.95
Maximum BAC (C_{max}) g/L	0.819 ± 0.184	0.841± 0.155
BAC y-intercept (C_o) g/L	1.119 ± 0.229	1.161 ± 0.159
ß-slope g/L/h	0.179 ± 0.030	0.159 ± 0.028
B_{60} elimination rate from body, g/h	9.623 ± 1.54	11.87 ± 1.65
Distribution factor rho, L/kg	0.721 ± 0.109	0.788 ± 0.090
Distribution volume, L	43.19 ± 7.21	60.13 ± 9.142
Body mass index kg/m^2	21.4 ± 1.76	22.8 ± 1.56
Liver weight, kg	1.562 ± 0.133	1.99 ± 0.160
Elimination rate, g/kg liver/h	5.01 ± 0.678	4.85 ± 0.659

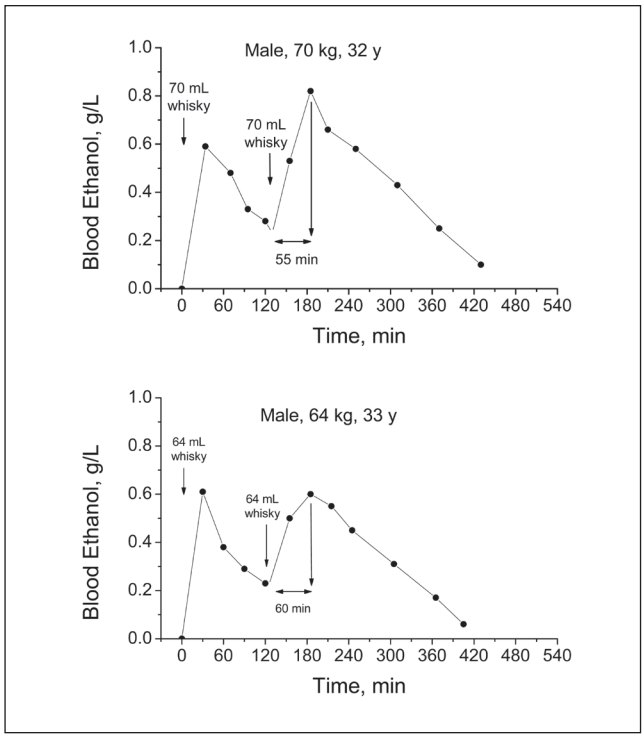


Figure 3.27 *Blood-ethanol profiles after ethanol was administered as neat whiskey in divided doses after an overnight fast (Jones, unpublished data).*

In a little known German study, 20 men over 60 y of age drank ethanol (0.68 g/kg) either as wine or spirits during 60 min and the resulting BAC profiles and parameters of ethanol pharmacokinetics were compared with published data for younger men (Hein and Vock, 1989). The absorption of alcohol was slightly slower after drinking the wine compared with spirits, although C_{max} occurred 30 min after end of drinking for both beverages. There were no beverage-related differences (spirits vs. wine) in ß-slope (0.137 ± 0.013 vs. 0.138 ± 0.015), C_o (1.09 ± 0.094 vs. 1.06 ± 0.076), and rho-factor (0.627 ± 0.053 vs. 0.645 ± 0.045). However, the men over 60 y had lower V_d (Widmark's rho) and a higher C_o compared with younger men, as expected because of less body water per kg body weight in the elderly.

The pharmacokinetics and effects of alcohol were investigated in men aged 20-60 y (Jones and Neri, 1985; Jones and Neri, 1994). Widmark rho factors (V_d) decreased with each decade of increasing age, being 0.720 ± 0.042 L/kg (20-29 y), 0.707 ± 0.036 L/kg (30-39 y), 0.678 ± 0.026 L/kg (40-49 y), 0.661 ± 0.041 L/kg (50-59 y). The C_o values showed a corresponding increase with advancing age as expected. However, C_{max} showed a tendency to increase with age but this parameter is also very much dependent on the speed of gastric emptying, which varied widely within each age group.

Age and bioavailability of ethanol was studied by Oneta et al. (2001) who found that first-pass metabolism was increased in the elderly (mean age 71 y), which was attributed to a slower gastric emptying and therefore more time for pre-systemic metabolism by gastric ADH. When the same dose of ethanol (0.225 g/kg) was given intravenously, the serum-alcohol curves (C_{max} and AUC) were significantly higher in the elderly compared with younger individuals (mean age 37). This was explained by a reduction in the available water space for dilution of ethanol in the elderly (Oneta et al., 2001). The dose of ethanol administered in this study (0.225 g/kg) was too low to enable a proper evaluation of other important pharmacokinetic parameters of ethanol such as V_d and C_0 and the influence of age on the elimination rate from blood.

H. Blood-Alcohol Profiles after Drinking Beer

More studies are needed to characterize the BAC or BrAC profiles of ethanol under real-world conditions after consumption of beer to complement the many bolus dose drinking studies with neat spirits (Schwartz et al., 1996).

In one study with low-strength beer (2.25% v/v), eight women (body weight 47-80 kg) and four men (body weight 67-100 kg) drank 500 mL (8.8 g ethanol) within 15 min after an overnight fast. The resulting C_{max} and t_{max} showed wide variations with the lightest women reaching a peak BAC of 0.28 g/L and the heaviest man 0.09 mg/L; and t_{max} occurred at between 10 and 30 min after end of drinking (Magnisdottir and Johannesson, 2000).

The complicated nature of ethanol pharmacokinetics and pharmacodynamics are illustrated by the title of an article by Holford (1997), namely "Complex PK/PD models—an alcoholic experience." The experiment involved a comparison of the BAC profiles after drinking beer containing different concentrations of ethanol, either 2.75% v/v or 4.0% v/v. The low-alcohol beer (2.75 vol%) was compared with a medium brew (4.0 vol%) and each subject drank 2.5 L of each beer over a two-hour period in a careful double blind study. When results were adjusted for the different amounts of alcohol in the drinks, the area under the BAC time curve after drinking the weaker beer was only 52% of the AUC for the normal strength beer and C_{max} was halved. The reason for the lower apparent bioavailability of alcohol in the weaker drink was not investigated further but it was felt that differences in absorption rate, first-pass metabolism and changes in hepatic blood flow might have contributed (Holford, 1997). Springer (1972) reported a study in 10 men who drank 0.75 g ethanol per kg as either 4%, 8%, 20% and 44% v/v in water on an empty stomach and the BAC profiles were plotted for six hours post-dosing. No statistically significant differences in BAC profiles or kinetic parameters of ethanol were noted, al-

though AUC for the 44% ethanol was less than for the weaker solutions. It seems that other constituents of the alcoholic beverages (sugar, CO_2, etc.) can probably explain differences noted in some studies (Pfeiffer et al., 1992).

So-called non-alcoholic beers have gained in popularity in some quarters, although these drinks often contain between 0.5-1% v/v ethanol. In a study with 20 men who drank 3000 mL of low-alcoholic beer over three hours, their blood-ethanol concentration was not measurable after beer containing 0.5% v/v ethanol. The BAC was always less than 0.1 g/L after drinking the beer with 0.9% v/v ethanol (Neuteboom and Vis, 1991). Drinking 3000 mL (100 oz) of 0.9% v/v beer corresponds to an intake of 21 grams of ethanol, which is almost exactly the amount the liver can metabolise over a three hour drinking time for a 70 kg male person (0.1 g/kg/h). The bioavailability of alcohol is considerably reduced when taken in such a dilute form as 0.5-1% v/v so Widmark calculations are not appropriate under these conditions.

In another study involving consumption of other types of beer, 16 students (8 men and 8 women) drank 3 pints of lager beer (4.2% v/v ethanol) over 30 min, which must be considered forced drinking for such a large volume (Ward et al., 1991). The resulting C_{max} was 0.74 ± 0.07 g/L (± SD) and t_{max} was 60 min after end of drinking in the men compared with C_{max} of 1.13 ± 0.17 g/L and t_{max} of 90 min in the women. Intake of 3 pints of low-alcohol beer (1% v/v) under the same conditions gave a peak BAC of 0.13 g/L whereas no measurable BAC was evident after drinking 3 pints of non-alcoholic lager (< 0.05% v/v).

In a study with people classified as light (N=13), moderate (N=12), heavy (N=12) and very heavy (N=14) drinkers depending on a survey of their previous drinking habits, both C_{max} and t_{max} were measured after lager beer (4% v/v) was consumed corresponding to an ethanol dose of 0.79 g per kg body weight. This required drinking about 2 litters of beer (3.5 pints) for a man weighing 80 kg and the speed of drinking was staggered over the one hour. The C_{max} ranged from 0.39 to 0.44 g/L and the t_{max} from 10 to 25 min in the different groups of subjects timed from end of drinking which was done at five hours after the last meal. Even after drinking beer, the overall range of t_{max} was 5-95 min after end of drinking (Wright and Cameron, 1998).

I. Retrograde Extrapolation

The rate of disappearance of ethanol from the bloodstream (ß-slope) needs to be carefully considered when requests are made to make forward or backward extrapolations of BAC based on a single measurement at one time point (Lewis, 1986a; Stowell and Stowell, 1998). This question arises from time to time in connection with road-traffic offenses when one needs to know a person's blood alcohol concentration at some time before the time of sampling blood or breath, such as at the time of a crash. This might be 1-2 or more hours before the blood was sampled; and the complexity of the problem is understood when one considers that nothing is known about the position of the BAC curve at the particular time. Only with the help of other relevant information is it feasible to make such a back extrapolation in a criminal case. Information about time of first and last drink, the types of drinks and their alcohol content is often not verifiable and is provided by the suspect.

Back-tracking calculations are often a contentious area of forensic alcohol research owing to the many assumptions that must be made about the pharmacokinetic profile of ethanol, not least of which is whether the C_{max} had been reached by the time of driving. Testifying as an expert witness in driving under the influence trials requires a thorough knowledge of the many factors that impact concentration-time profile of ethanol. Another critical element in retrograde extrapolation is the position of the BAC curve at the time of driving and at the time of obtaining the blood sample, that is, whether the blood alcohol concentration increases, remains unchanged, or decreases after the actual driving incident.

The median and range of times to reach peak BAC after the end of drinking for various experimental protocols and investigators was presented already in Table 3.11. Most of these studies involved rapid drinking of a moderate dose of alcohol on an empty stomach and these conditions are not very comparable with real-life situations and drinking practices. Moreover, time to peak as a measure of the rate of absorption of ethanol tends to be skewed so the median or mode rather than the arithmetic mean are better statistics to indicate central tendency. Most subjects reached peak BAC within 60 minutes after the end of drinking but some required 120 minutes or more. A more prolonged absorption phase and a later occurring peak were frequently observed when alcohol was taken together with or after a meal compared with drinking on an empty stomach. But even when an exceptionally long time was necessary to reach a maximum BAC much of the alcohol was absorbed by the time drinking ended. Thereafter, the BAC increased further at a variable rate before reaching the maximum level.

Sometimes, a very gradual increase was seen or no change at all in BAC for 30-90 min before the curve started to decrease in the post-peak phase. Occasionally, a flat-topped BAC time-course is seen when the rate of absorption balances the rate of elimination and the BAC tends to remain at a constant level. But even during the absorption phase and when the BAC is at a plateau, ethanol is still being cleared from the body by enzymatic oxidation in the liver at

a rate of about 0.1 g/kg body weight per hour. Although the BAC might be decreasing, which gives the impression of a post-absorptive phase, absorption from the gut can still be taking place but more slowly than the rate of clearance of ethanol from the bloodstream of approximately 0.15 g/L/h.

J. Massive Ingestion of Alcohol under Real-World Conditions

Controlled studies of blood-alcohol curves after administration of massive amounts of ethanol under real-world conditions are very limited. One exception was the study by Zink and Reinhardt (1984) in which massive amounts of alcohol were consumed for up to 10 hours of continuous drinking. The study has recently been re-evaluated.

Figure 3.28 gives examples of the blood-ethanol concentration plots for four men accustomed to heavy drinking. In this experiment they drank beer or spirits according to choice at regular intervals for about 6-10 hours (Zink and Reinhardt, 1984). Blood samples were drawn from an indwelling catheter every 15 min during and after the drinking so the time of reaching the peak BAC was carefully noted. These plots show that with this kind of drinking pattern the highest BAC was sometimes reached even before the end of drinking. During prolonged heavy drinking, it seems that so much alcohol has become equilibrated with body fluids and tissues that the quantity of alcohol contained in the last gulp of alcoholic beverage is insufficient to raise the BAC any further. Obviously, this conclusion depends on how much alcohol was contained in the last drink and this information was not made clear in the article (Zink and Reinhardt, 1984). However, six of the volunteer subjects had reached their maximum BAC before the end of drinking, two subjects at the same time as they finished the drink and six subjects at various times after the last drink was taken.

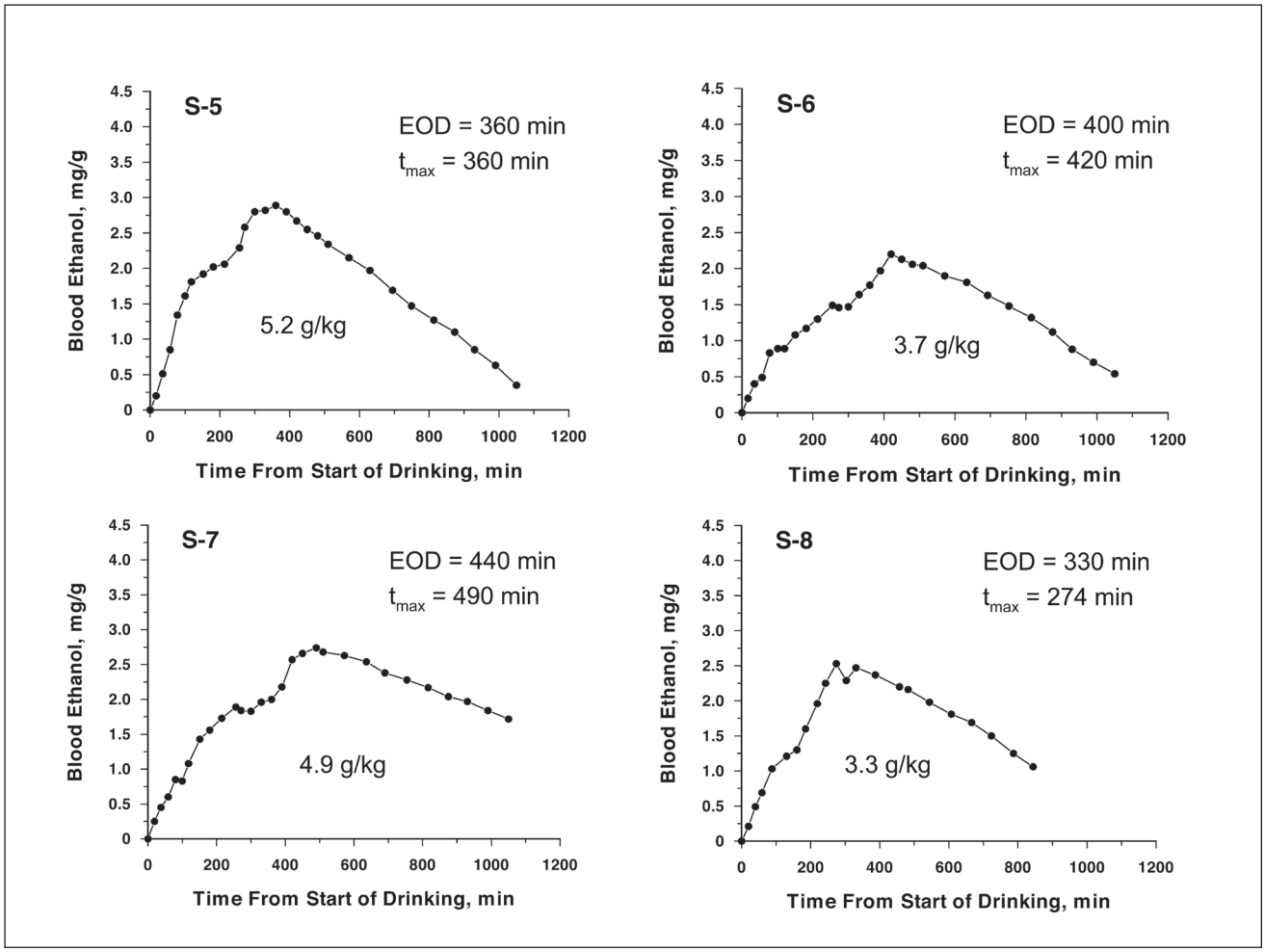


Figure 3.28 *Blood-alcohol curves in four subjects (S-5, S-6, S-7 and S-8) after massive amounts of ethanol were consumed (g/kg=dose per kg body weight) under real-world conditions. The drinking occurred in the company of a spouse or girlfriend and blood samples were taken at 15 min intervals during the 10 hour drinking spree and for several hours post-peak. EOD=end of drinking and t_{max} is time to maximum BAC. Redrawn from data published by Zink and Reinhardt (1984).*

Another interesting aspect of this same study arose when the peak BAC, which ranged from 0.22–0.38 mg/g (220-380 mg/100 g or 232-400 mg/dL), was compared with the theoretical expected values based on the amount of alcohol consumed, the drinking time frame, and the subject's body weight. The BAC calculated according to Widmark's equation vastly exceeded the actual BAC sometimes by as much as 50% or more. The reason for this discrepancy is not fully understood but it seems likely that the rate of ethanol disposal was much faster than expected during the 6-10 hour drinking spree. Whether this can be explained by the induction of P4502E1 enzymes in these regular heavy drinkers or an effective first-pass metabolism remains unsettled. The more effective clearance of ethanol seemed to be occurring during the absorption (drinking) period because in the post-peak phase, the rate of disappearance of ethanol from blood was not abnormally high, the ß-slopes ranging from 0.11-0.27 mg/g/h (0.12 – 0.28 g/L per h).

K. Effects of Drugs on Metabolism of Ethanol

Inhibition of the enzymes responsible for oxidative metabolism of ethanol can slow down the rate of elimination from the blood stream. In-vitro studies showed that pyrazole and its 4-methyl derivative were competitive inhibitors of class I alcohol dehydrogenase (Shannon, 1998). This example of enzyme inhibition was verified under in-vivo conditions and 4-methyl pyrazole (fomepizole) was subsequently developed into a therapeutic agent (Weintraub and Standish, 1988). The drug is registered as Antizol® and is used for treatment of patients poisoned with methanol or ethylene glycol thus preventing these more toxic alcohols being converted into their metabolites (Brent et al., 2001; Paasma et al., 2007). One situation in which abnormally slow rates of ethanol elimination were observed was in people malnourished or who had eaten a low protein diet (Bode, 1978). The underlying mechanism is probably related to a reduced activity of enzyme protein caused by malnutrition and, indeed, some support for this can be found in animal models (Bosron et al., 1986; Mezey, 1998; Hoyumpa and Schenker, 1982). The pharmacokinetics of ethanol was also studied in patients undergoing dialysis owing to renal failure, but as might be expected this condition did not decrease the rate of ethanol elimination from the body (Jones and Hahn, 1997). Although the kidney has some metabolic activity including ADH enzymes, this represents only a small fraction of the hepatic ADH activity.

Well-known species differences exist in the rate of ethanol metabolism and rats and mice oxidize ethanol 3-5 times faster than dogs or humans (Derr, 1993; Wallgren, 1970). Studies have shown that the rate of ethanol combustion is closely linked with the basal energy requirements of the body including oxygen consumption by the tissues. No effective means have been devised to increase the rate of ethanol metabolism above that found in the well-nourished individual (Bode, 1978). However, in trauma patients suffering from severe burns, the rate of ethanol metabolism was boosted considerably compared with normal values of 0.1-0.2 g/L/h. These patients are in a hypermetabolic state owing to their injuries and under these circumstances all energy processes are boosted including consumption of glucose and amino acids (Jones et al., 1997b).

Increasing the elimination rate of ethanol from the bloodstream might be advantageous in some clinical situations, such as in the treatment and detoxification of a poisoned patient. In theory, this could be achieved by enhancing the reoxidation of coenzyme NADH to NAD^+ which is considered to be the rate limiting step in the oxidative metabolism of ethanol. But because human metabolism of ethanol is tightly linked to hepatic oxygen consumption and energy requirements of the body the person's basal metabolic rate would have to be boosted. Speeding up the rate of ethanol metabolism has not proved easy because activity of hepatic enzymes, oxygen consumption and basal metabolism are all important controlling factors.

Drugs capable of boosting the elimination of ethanol, so called sobering-up agents, have also attracted attention but none seem to be especially effective compared with oxidative capacity of the liver in healthy well-nourished individuals (Jones, 1991a; Mascord et al., 1988). Carbohydrates such as fructose and sucrose have probably attracted most attention as sobering-up agents. However, results are conflicting and depend to a great extent on the experimental conditions, such as the dose, the timing and the route of administration relative to ethanol (Soterakis and Iber, 1975; Goldberg et al., 1979; Crownover et al., 1986; Jones, 1991a).

As mentioned earlier in this chapter, drugs widely used for treatment of stomach ailments involving too much acidity, namely H_2-receptor antagonists, received considerable attention because they were found to inhibit the action of ADH in-vitro (Palmer et al., 1991; Caballeria et al., 1991). The chemical structures of cimetidine and ranitidine are shown in Figure 3.13 in comparison with the classic inhibitors of class I ADH, namely pyrazole and 4-methyl pyrazole. The pyrazole-like ring in the cimetidine molecule was thought to account for its inhibitory effect on ADH enzymes. Warnings were felt necessary for people prescribed H_2-receptor antagonists if they increased the oral availability of ethanol and resulted in higher peak BAC after drinking (DiPadova, 1992; Palmer et al., 1991). It was pointed out that people taking these drugs together with alcohol might unknowingly

develop higher peak BAC and this might have implications in connection with accidents at work and on the highway (DiPadova et al., 1992).

The combined influences of drugs prescribed for treating gastric hyperacidity and ethanol has occasionally been raised as a defense argument during prosecution of drunken drivers. The suspects or their lawyers maintained that the BAC was above the legal limit because of inadvertent use of alcohol after medication with Tagamet® or Zantac®. These individuals therefore had a punishable BAC but without intent and the case against them should therefore be dropped. These drugs, which are antagonists of the histamine receptor (H_2-receptor) were also alleged to inhibit the action of gastric ADH. This meant that more of the ingested alcohol entered the systemic circulation in people taking this kind of antacid medication resulting in a higher BAC than otherwise would have been the case (Westenbrink, 1995). This argument depends on the relevance of gastric ADH and its role in first-pass metabolism of ethanol (Levitt et al., 1994; Gentry et al., 1994; Lim et al., 1993). This argument lacks merit because even within the same individual without treatment with these drugs the absorption rate and C_{max} can vary widely and C_{max} cannot be higher than that obtained after 100% availability of the dose of alcohol, such as observed after drinking on an empty stomach. However the C_{max} might be higher or lower than expected depending on the rate of gastric emptying as influenced by the medication being used to counteract hyperacidity (Levitt and Levitt, 1994).

The notion that gastric metabolism of ethanol was responsible for the reduced bioavailability (smaller AUC) of ethanol was challenged by Levitt (1994) in part because of the extremely small amounts of ADH enzyme in the stomach compared with the liver. He maintained that if a first-pass metabolism occurred it was more likely to take place in the liver than the stomach and convincing arguments were developed to support this notion. Moreover, a critical and hitherto unrecognized element in first-pass metabolism of ethanol was the rate of absorption (Levitt and Levitt, 1994). The smaller the dose of ethanol and the slower and more prolonged the absorption the greater the likelihood of a significant first-pass metabolism. With a rapid absorption of alcohol, which is often observed after drinking on an empty stomach, the first-pass metabolism was virtually non-existent (Dipadova et al., 1987). Indeed, in the fasting state, total body water can be reliably estimated by the ethanol dilution technique even after oral administration, which speaks against first-pass elimination taking place (Jones et al., 1992).

Because of the widespread use of cimetidine and ranitidine in clinical practice, research groups from several countries embarked on studies in attempts to verify this adverse drug-alcohol interaction. Use of different experimental designs such as the dose of alcohol and many more volunteer subjects in the test and control groups, the bioavailability of ethanol was not significantly altered after therapeutic doses of ranitidine and cimetidine (Lewis et al., 1993). This conclusion held when alcohol was ingested by healthy volunteers as well as patients who suffered from stomach ulcers (Casini et al., 1994). Ranitidine, a widely prescribed H_2-receptor antagonist, lacked any effect on the pharmacokinetics of ethanol. Whether the dose of alcohol was ingested in the morning, at midday, or in the evening made no difference, the concomitant use of rantitidine (Zantac®) failed to influence C_{max} and AUC compared with a control treatment (Toon et al., 1994; Raufman et al., 1993; Kendall et al., 1994). This conflicting information from different groups of investigators underscores the dangers of drawing conclusions about drug-alcohol interactions on the basis of experiments with too few human subjects and small doses of ethanol (Caballeria et al., 1991).

Summing-up the results from many studies of this example of a drug-alcohol interaction leads to the conclusion that the widely used H_2-receptor antagonists, cimetidine and ranitidine, have no significant influence on the pharmacokinetics and bioavailability of ethanol after small (0.15-0.3 g/kg) or moderate (0.5-0.9 g/kg) doses of ethanol. By contrast, a significant first-pass metabolism of ethanol occurs especially when small doses (<0.30 g/kg) are taken together with or after a meal. The rate of absorption of ethanol exerts a critical influence on peak BAC and area under the curve (Levitt and Levitt, 1994; Winek et al., 1996). Moreover, pronounced variability in the pharmacokinetics of ethanol both between and within individuals means that large numbers of subjects must be investigated to achieve sufficient statistical power to demonstrate effects of a particular drug treatment. With a slow and prolonged absorption of ethanol for whatever reason (food in the stomach or pylorospasm) there seems to be a more effective clearance of ethanol taking place during the absorption phase as the portal vein blood flows through the liver.

L. Elimination Rates of Ethanol in Alcoholics During Detoxification

Alcohol-dependent people drink daily and often reach very high BAC and a continuous exposure of liver enzymes to alcohol leads to the development of both cellular and metabolic tolerance to the drug (Kalant, 1996a). A metabolic tolerance means that chronic drinkers develop a higher capacity to metabolize the alcohol and thus eliminate the drug at a faster rate compared with moderate or first-time drinkers (Lieber, 1997a; Bogusz et al., 1977; Haffner et al., 1991)

Alcoholics and binge drinkers who had been admitted to a hospital clinic for detoxification provided a series of blood samples every few hours for analysis by gas chromatography. Studies of this nature have reported elimination rates of ethanol of up to 0.35 g/L/h (Bogusz et al., 1977; Jones and Sternebring, 1992). In another study alcoholics were allowed to sober-up for a couple of days and then given a moderate dose of ethanol, but now the rate of elimination from blood was in the expected range for moderate drinkers 0.10 to 0.20 g/L/h (Keiding et al., 1983). The faster rate of alcohol elimination in alcoholics is accounted for by induction of the CYP2E1 enzymes by the continuous exposure to high concentrations of ethanol during the drinking binge (Lieber, 1999; Klotz and Ammon, 1998; Fuhr, 2000). The ability to dispose of ethanol faster is lost when P450 enzymes are no longer engaged in metabolism of ethanol, e.g., during abstinence and withdrawal from the drug (Gonzalez et al., 1991; Oneta et al., 2002). At faster rates of ethanol metabolism, higher concentrations of acetaldehyde are produced and hence a higher risk of toxicity in alcoholics (Panes et al., 1993; Nuutinen et al., 1983).

Diurnal variations in activity of alcohol metabolizing enzymes and the rate of ethanol metabolism has been investigated, e.g., by serving alcohol (0.75 g/kg) at 9 A.M., 3 P.M., 9 P.M. and 3 A.M. in random order (Yap et al., 1993). Apart from a higher C_{max} after ethanol in the 9 A.M. session, the other time-of-day differences in pharmacokinetic parameters of ethanol did not reach statistical significance. The higher C_{max} at 9 A.M. is probably explained by faster gastric emptying secondary to low blood glucose (Schvarcz et al., 1995; Jones et al., 1999; Lötterle et al., 1989). Variations in the enzymatic activity of metabolizing enzymes as a function of time of day might be more relevant for drugs other than ethanol (Reinberg, 1992).

M. Ethanol Metabolism in Pathological States

The bulk of the dose of ethanol administered is metabolized in the liver as discussed earlier in this chapter. Accordingly, liver diseases such as hepatitis or cirrhosis might be expected to influence the rate of ethanol metabolism (Bode, 1978; Panes et al., 1989). For ethical reasons, conducting drinking experiments with hospitalized patients suffering from cirrhosis is not feasible today. However, some older literature on this subject is available and the work of Jokipii (1951) deserves mention. His studies were done with both male and female volunteers and hospital patients that were well characterized clinically. They suffered from hepatitis, cirrhosis, diabetes mellitus, hyperthyroidism and dystonia (impaired functioning of the muscles). After an overnight fast, the subjects were challenged with a bolus dose of alcohol (0.50 g/kg as spirits), under strictly controlled conditions advocated by Widmark (1932).

Table 3.15 gives the mean and range of ß-slopes for the different patient groups, according to gender, in comparison with a control group of healthy individuals. The rates of ethanol elimination from blood (ß-slopes) of the alcohol curves were rather similar for the subjects with the various diseases and the healthy control group, apart from a tendency towards lower values in the patients. The lowest ß-slope in the patient groups was 0.07 g/L per h or 0.007 g/dL per h. The distribution of ethanol in the body as reflected in the value of Widmark's "r" was not much different between the patient groups and the control group of healthy subjects (Jokipii, 1951).

In cirrhotic patients suffering from portal hypertension the rate of clearance of ethanol from blood was decreased below 0.10 g/L/h but the reason was attributed to restriction in hepatic blood flow rather than cirrhosis of the liver (Leube and Mallach, 1980). Seemingly, there is so much ADH present in the liver that even with advanced necrosis of the tissue the available enzyme is sufficient for combustion of ethanol. Jokipii (1951) also investigated obesity, diabetes mellitus, acute hepatitis and hyperthyrosis but failed to find an altered rate of ethanol elimination from blood in these conditions compared with a control group.

Table 3.15
Elimination Rates of Ethanol from Blood in Healthy Volunteers and in People Suffering from Various Metabolic Diseases and Pathological States. Ethanol was Given as a Bolus Dose (0.50 g/kg Body Weight) as Neat Spirits (30-40% v/v) After an Overnight Fast (Data from Jokipii, 1951)

Subjects/ conditions	Gender	N	Ethanol elimination rate (ß-slope)[1]
Healthy volunteers	Men	19	0.12 ± 0.019
	Women	23	0.13 ± 0.020
Dystonia[2]	Men	11	0.09 ± 0.016
	Women	6	0.11 ± 0.020
Acute Hepatitis	Men	8	0.09 ± 0.022
	Women	11	0.11 ± 0.016
Liver cirrhosis	Men	5	0.11 ± 0.011
	Women	1	0.10
Hyperthyreosis	Men	3	0.15 ± 0.006
	Women	12	0.13 ± 0.030
Diabetes Mellitus	Men	13	0.11 ± 0.036
	Women	8	0.11 ± 0.031

[1] ß=rate of disappearance of ethanol from blood. [2] Dystonia is a disordered tonicity of muscles.

Psychological stress caused by fear of drawing blood and penetration with a needle was thought to account for an abnormal BAC profile (Hahn et al., 1992). It seems that despite suffering from various metabolic diseases and liver cirrhosis people are still capable of clearing ethanol from the bloodstream (Mezey and Tobon, 1971; Lieberman, 1961). Years ago the role played by the liver in ethanol metabolism was demonstrated in experiments with hepatectomized or eviscerated animals (Clark et al., 1941). Even after removal of the liver these animals were capable of metabolizing ethanol albeit at a slower rate. In a hepatectomized dog the ethanol elimination rate was 0.055 g/L per h compared with 0.14 g/L per h in control animals.

Articles citing an unusually wide range of ß-slopes and especially the existence of extremely low rates of alcohol elimination below 8 mg/dL/h (0.08 g/L/h) should be considered suspect. Some of these abnormal values can be accounted for by making statistical projections from distributions (e.g., mean ± 2 x SD or mean ± 3 x SD) with large standard deviations. This might occur, for example, when the mean and variance are calculated for a non-homogenous experimental group of subjects, e.g., by including moderate drinkers and alcoholics, or ß-slopes for men and women, or fed and fasting conditions prior to drinking. Taking too few blood samples so that the post-absorptive phase is not well characterized or when the residual SD is large can lead to unreliable estimates of ß-slope. A classic example of this can be found in the paper by Winek and Murphy (1984) where a person with a ß-value of 0.01 g/L per h was reported in a peer-review journal without a comment. This erroneous result should have been immediately apparent because in another experiment with the same individual and a higher dose of alcohol the ß-slope was 0.14 g/L per h.

The practice of calculating a person's elimination rate of ethanol from just two blood samples taken at two time points about one hour apart is not recommended (Neuteboom and Jones, 1990). This follows because the results obtained might be influenced by ongoing absorption and distribution of alcohol during the sampling interval. Reliable information about the phase of alcohol metabolism cannot be ascertained from analyzing just two blood samples (Jones and Andersson, 1996a; Simic and Tasic, 2007).

N. Short-Term Fluctuations in Blood-Alcohol Profiles

The notion of short-term fluctuations or a zig-zag effect in the blood or breath-alcohol time course was raised by some investigators although the experimental evidence was rather flimsy. Such a zig-zag phenomenon would have implications in forensic science practice, such as when making back-estimation or forward projection of BAC, as is often requested in criminal proceedings.

Figure 3.29 shows blood-ethanol concentration-time data from an experiment described by Teige et al. (1974). Samples of venous blood were taken every 1-2 minutes through an indwelling catheter and ethanol was determined by gas chromatography. When plotted as a function of time an irregular zig-zag pattern is clearly seen. But this zig-zag trend can be accounted for by a combination of pre-analytical and analytical variations inherent in the methods of sampling blood and determination of alcohol. The insert shows the blood-ethanol time course for samples taken every 20-30 min. The zig-zag effect is now obliterated which underscores the importance of sampling protocol when studying this curious steepling phenomenon.

An attempt to show the existence of spiking in ethanol concentration in blood samples taken at short time intervals is shown in Figure 3.30. This graph depicts a single individual who received ethanol by intravenous infusion over 30 min and blood samples (5 mL) were taken from an indwelling catheter every five minutes for four hours and then every 10-15 minutes for another three hours. Note the lack of any marked irregularity or zig-zag pattern in the post-absorptive part of this plot compared with Figure 3.29. The linear decay profile can be seen to change into a hockey-stick shape at low BAC indicating that the metabolizing enzymes are no longer saturated. The values of ß-slope and "r" agreed with values expected and the standard deviation of the residuals around the regression line had a coefficient of variation of only 3%.

The blood samples were analyzed with a well-proven gas chromatographic method so that analytical sources of variation were negligible compared with sampling variation. Under these conditions the spiking in the BAC curve in the post-absorptive period was virtually non-existent. The irregularities in ethanol concentration between consecutive samples 1-2 min apart were most apparent when a breath-alcohol analyzer was used to analyze ethanol (Dubowski, 1985). The additional physiological sources of variation, such as characteristics of the breath analyzer, breathing pattern, exhalation time, rate of flow of breath into the instrument, body and breath temperature, etc. might have accounted for the exaggerated spiking effect seen in the breath-alcohol profiles (Jones et al., 1989).

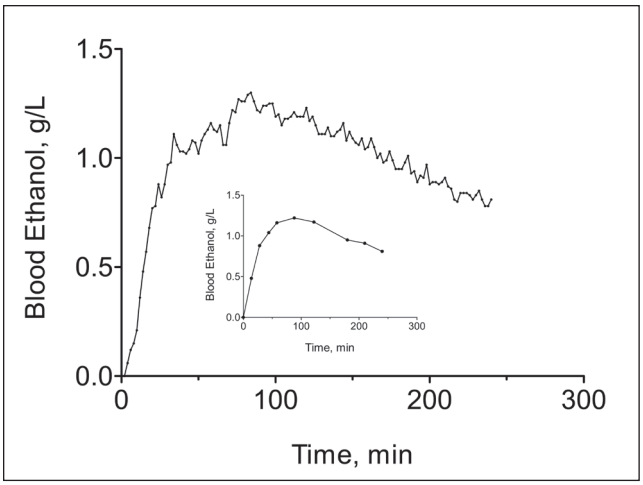


Figure 3.29 *Concentration-time profiles of ethanol when specimens of blood were taken at 1-2 min intervals illustrating irregularities in the curve exhibiting a type of zig-zag or spiking pattern. The dose of ethanol was 1.0 g/kg and was ingested as wine (data from Teige et al., 1974). The insert graph shows the blood-alcohol curve in concentrations every 15-30 min.*

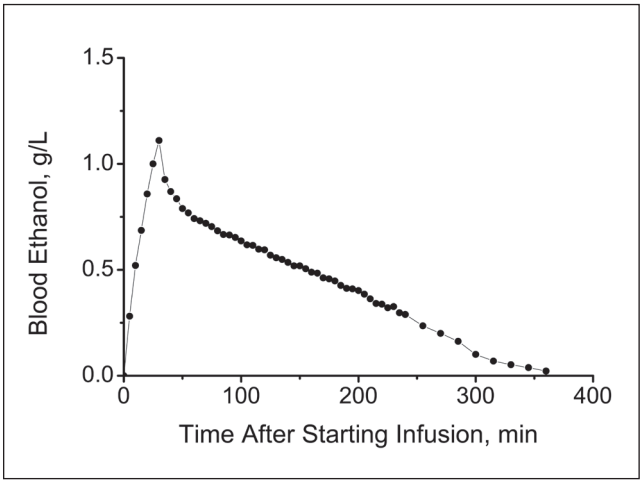


Figure 3.30 *Blood-concentration time profiles of ethanol in one individual after an ethanol dose of 0.60 g per kg body weight was administered by intravenous infusion over 30 min (Jones, unpublished data). Vacutainer tubes (5 ml) were filled with blood from an indwelling catheter every five min for four hours and then every 15-30 min for a further three hours and blood ethanol concentrations were determined by headspace gas chromatography.*

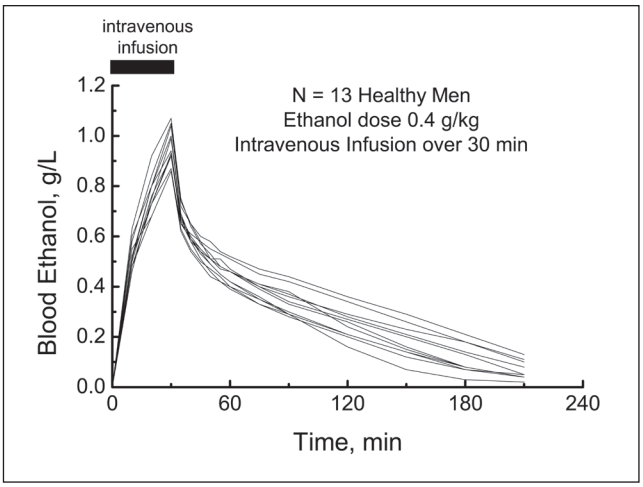


Figure 3.31 *Concentration-time profiles of ethanol in 12 subjects when ethanol (0.3 g/kg) was given by a constant rate intravenous infusion lasting for 30 min (Jones et al., unpublished data).*

In conclusion, it seems that the zig-zag patterns in blood- and breath-alcohol profiles are mainly a consequence of pre-analytical and analytical variations associated with sampling and analysis of ethanol in blood and breath. During the absorption of ethanol from the stomach a spiking effect is more likely because of sudden and unpredictable opening and closing of the pyloric sphincter, a muscle that controls emptying of the stomach contents into the duodenum. This is likely to produce a series of short bursts in absorption rate of alcohol into the portal venous circulation and is reflected in rapid fluctuations in the concentration of ethanol in breath and peripheral blood.

O. Intravenous vs. Oral Route of Ethanol Administration

Drugs can be administered to the body in numerous ways including by mouth, intravenously, subcutaneously, transdermally, sublingually, by inhalation and per rectum. The route of administration is one important consideration for the therapeutic efficacy of prescription drugs and pharmacokinetic parameters such as C_{max} , t_{max} and AUC depend to some extent on the route of administration.

The intravenous route of administration is exemplified in Figure 3.31 which shows typical BAC profiles of ethanol when a 10% v/v solution in saline was given as a constant rate intravenous infusion over 30 min. When ethanol is administered intravenously, the C_{max} occurs at the time the infusion ends and thereafter there occurs an abrupt drop, which

is commonly referred to as a diffusion plunge. The ethanol enters the bloodstream faster than it can be distributed to all body organs and tissues. The process of equilibration of ethanol between the central blood compartment and the rest of the total body water takes about 30-40 min for completion.

In the vast majority of forensic situations ethanol is taken by mouth, but for research purposes and in clinical medicine (e.g., treatment of poisoning) intravenous (i.v.) administration is used. Knowledge of the pharmacokinetics of ethanol by this route of administration is therefore of interest. For example, one advantage of i.v. administration is that first-pass metabolism of ethanol is avoided because the entire dose reaches the systemic circulation (100% bioavailability). The BAC curves when a small dose of ethanol (0.3 g/kg) was administered orally compared with intravenously are shown in Figure 3.32. The curves show results for four test subjects who received the same dose of ethanol (0.3 g/kg) by a constant rate i.v. infusion over 30 min or by oral ingestion in random order one week apart. The bioavailability of ethanol was appreciably lower when the same dose of ethanol was given by the oral compared to the intravenous route of administration, based on monitoring the serum alcohol profiles (Cobaugh et al., 1999).

Although absorption of ethanol starts already in the stomach, the rate of uptake is considerably faster from the upper part of the small intestine, because of the larger surface area. Indeed, after drinking neat spirits on an empty stomach, the rate of absorption is sometimes so fast that the BAC is higher than expected for the dose of ethanol administered (overshoot peak). Under these conditions, ethanol enters the bloodstream before it has time to be redistributed and diluted with the total body water. The temporarily higher C_{max} is followed by a diffusion plunge and the $t_{½}$ of this re-distribution phase was found to be about seven min (Hahn et al., 1995). Accordingly, 35 min (5 x $t_{½}$) are required for the dose of ethanol administrated to equilibrate between blood and other body fluids.

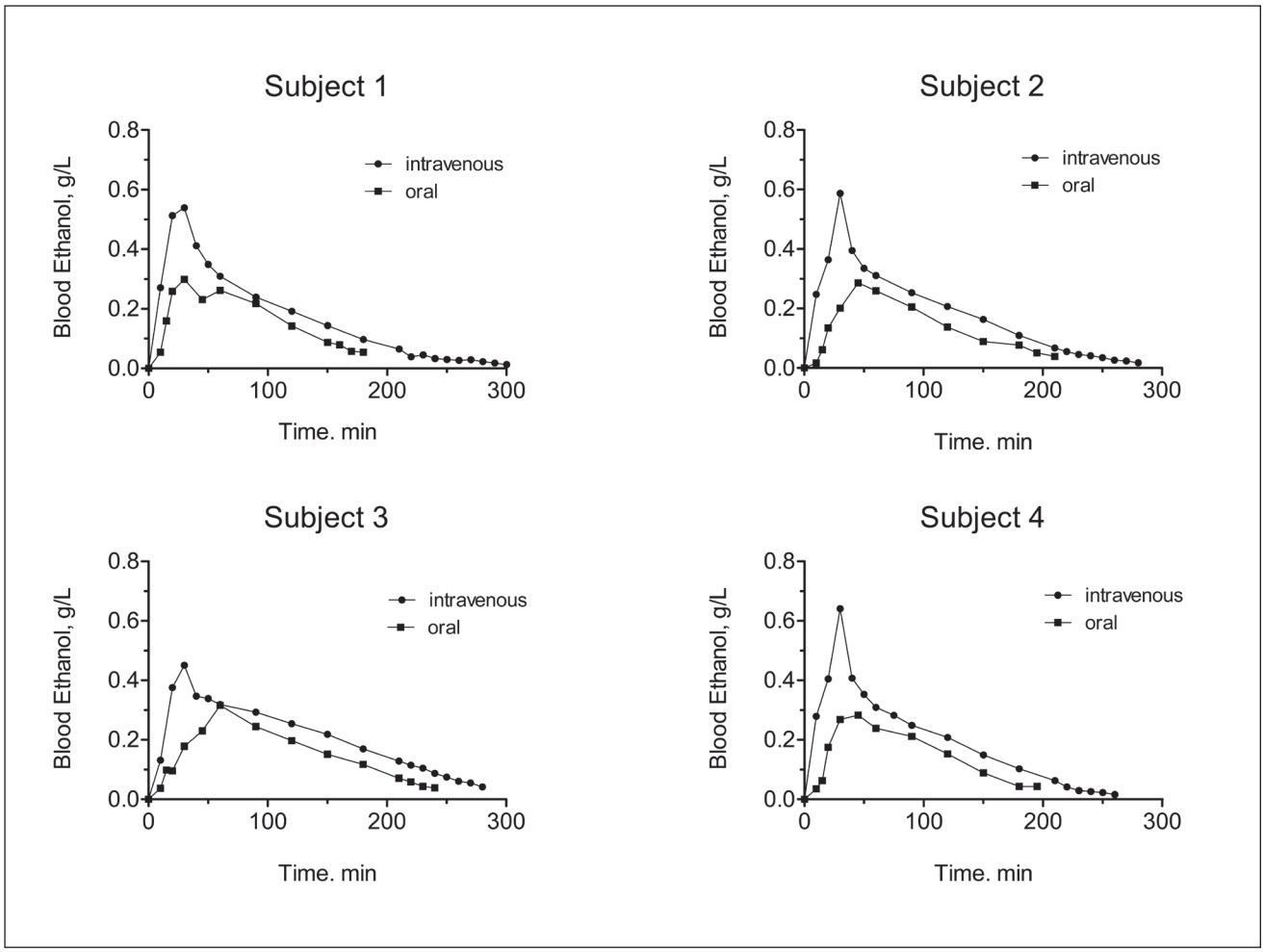


Figure 3.32 *Examples of concentration-time profiles of ethanol observed in four healthy men after the same small dose of ethanol (0.30 g/kg) was given by oral and intravenous routes of administration one week apart (Jones et al., unpublished work).*

3.8 Ethanol in Body Fluids and Tissues

Excretion of ethanol takes place by passive diffusion through the skin, the lungs and the kidney although in quantitative terms these routes of elimination represent only a small fraction (2-5%) of the total dose administered (Kalant, 1971a; Wallgren, 1970). Attempts to enhance the rate of ethanol removal from the body, e.g., by physical exertion thus increasing lung ventilation, by drinking water to increase production of urine or a sauna (steam) bath to increase sweating, are not very effective ways to speed-up the elimination of alcohol from the body (Wallgren, 1970; Kalant, 1971; Kalant, 1996b).

Forensic toxicologists are interested in the analysis of ethanol and other drugs of abuse in a wide range of biological fluids and matrices (e.g., saliva, tears, hair, sweat, nails, cerebrospinal fluid, etc.). Blood is the most important fluid for purposes of interpretation (see Inoue and Seta, 1992; Pichini et al., 1996 for reviews). Alternative specimens have found applications in therapeutic drug monitoring, postmortem toxicology, workplace drug testing, and sports medicine (Rivier, 2000; Cone, 2001).

Ethanol is a small neutral molecule, unionized at physiological pH; it does not bind to plasma proteins and easily penetrates all cell membranes by diffusion through aqueous channels. Ethanol mixes completely with all the water in the body and enters both extracellular and intracellular fluid compartments (Rosenfeld, 1996). Body fluids and tissues containing the most water will contain the most ethanol at equilibrium. The time required for equilibration depends on the blood flow to the various organs and tissues. This means that heart, brain, lungs and kidneys with a high minute-blood volume quickly reach equilibrium with the concentrations of alcohol in the arterial blood. By contrast the skeletal muscle, where the bulk of the water resides, takes a longer time to reach equilibrium with arterial blood ethanol because the ratio of blood flow to tissue mass is smaller for the resting muscles (Chiou, 1989).

A. Water Content of Specimens

Plasma, serum, saliva, urine, and cerebrospinal fluid contain more water than equivalent volumes of whole blood and therefore can be expected to contain more alcohol when equilibrium is reached. However, besides differences in water content, the sampling time after drinking and hence the stage of ethanol metabolism also need to be considered when blood/body fluid ratios are evaluated.

The blood/body fluid ratios of alcohol have been reported in many publications, but often scant attention is given to the stage of ethanol metabolism when the samples are taken. The average values of ethanol distribution ratios are useful, but extreme values are evident, some of which are physiologically impossible, such as a plasma/whole blood ratio of less than unity. This would imply more water in whole blood than in plasma. These abnormal values are best explained by errors in the sampling and/or analysis of ethanol in the blood, the plasma or both media.

Concentration ratios of ethanol should not be calculated after subjects consume low doses of ethanol because this is another explanation for unrealistic blood/body fluid ratios. There are small gender-related differences in blood/body fluid ratios of ethanol because of the lower hematocrit and thus more water in whole blood from females. People with anemia would have more water per unit volume of blood, owing to the deficiency in erythrocytes thus resulting in atypical blood/body fluid ratios of ethanol.

B. Urine

Urine was the first body fluid used for determination of ethanol in clinical and forensic science, probably because large volumes are available without invasion of the body (Widmark, 1914; Biasotti and Valentine, 1985). The main function of the kidney is to filter the blood and thereby remove waste products of metabolism (e.g., urea, creatinine and ureates) as well as any noxious substances and/or their metabolites that might have entered the body from food eaten or the environment. Recreational drugs and prescription medication are excreted to a greater or lesser extent via the kidney and can be determined in the urine (Jones, 2006).

Ethanol diffuses from the renal artery blood into the glomerular filtrate and the UAC (primary or ureter urine) depends on the concentration of ethanol in the water fraction of the arterial blood (Iffland and Jones, 2002). However, the concentration of ethanol in bladder urine depends on other factors besides the arterial blood concentration as illustrated in Figure 3.33. The time after drinking when the bladder was emptied and if there was any alcohol-free urine present before starting to drink will dilute the concentration of ethanol in freshly produced urine (Jones, 2002). The production rate of urine (diuresis) is greatest during the absorption phase and close to the peak concentration in blood. Several hours post-dosing diuresis is no longer evident and difficulties in voiding might occur. The problem of renal failure or use of medication that causes urine production or retention are other confounding factors that have not yet been investigated in controlled studies.

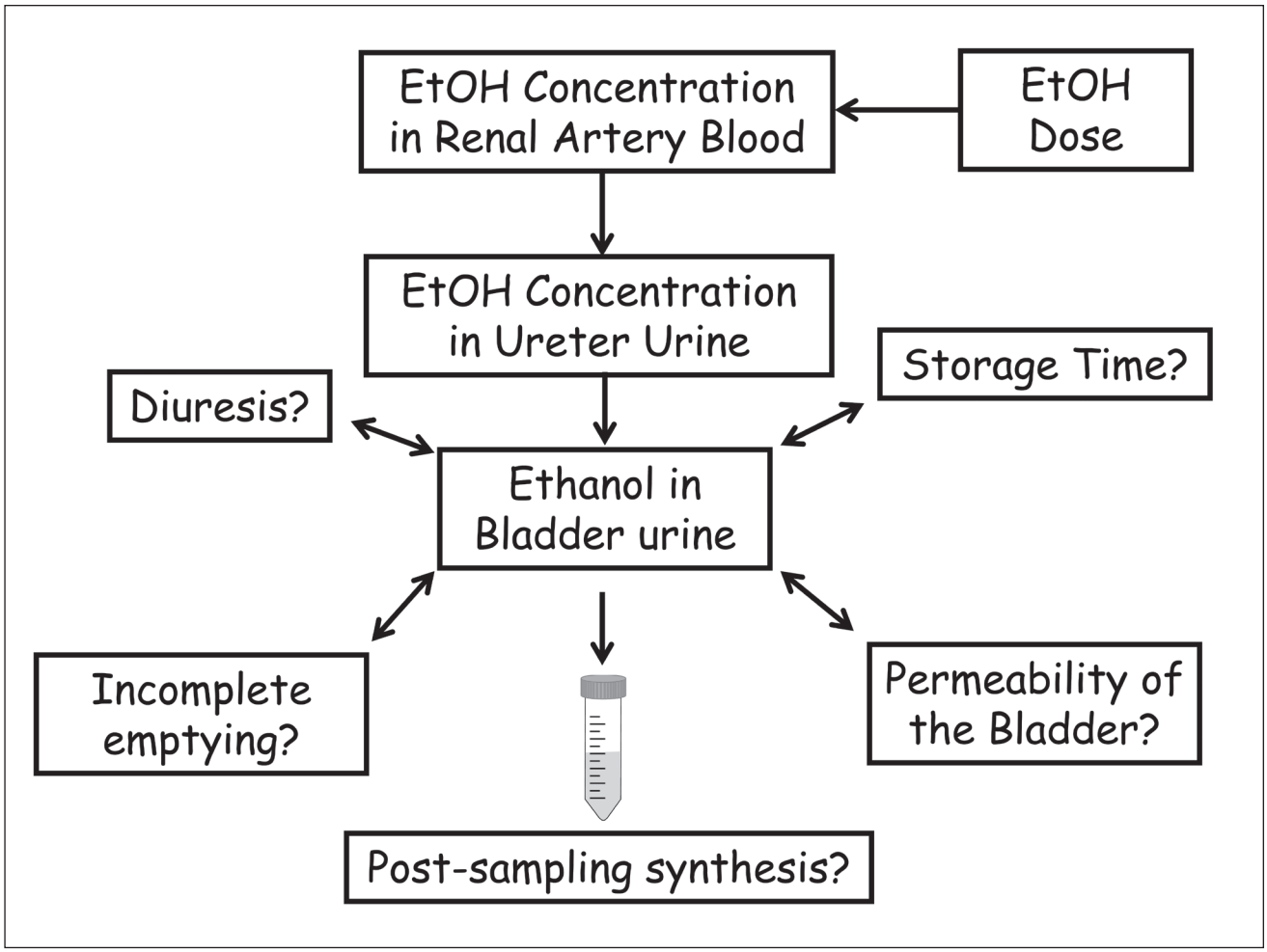


Figure 3.33 *Summary of the factors considered to influence the concentration of ethanol in a specimen of urine (Jones, 2006).*

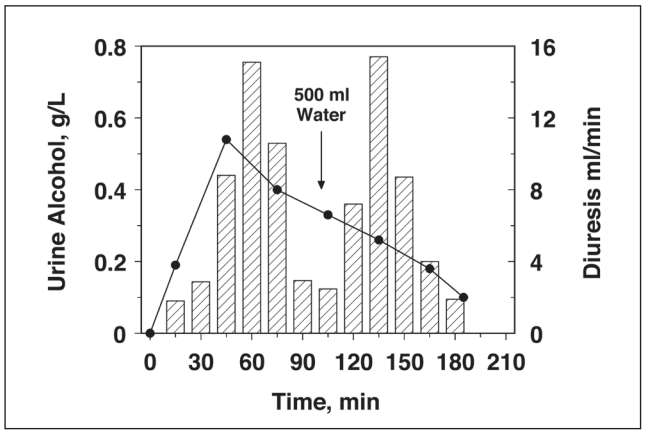


Figure 3.34 *Concentration-time profile of ethanol in urine, ethanol-induced diuresis and lack of effect of drinking water on the concentration of ethanol in urine (redrawn from Widmark, 1914).*

1. Diuresis

It must be a very old observation that consumption of alcohol increases the urge to urinate more frequently and in larger volumes. Figure 3.34 is redrawn from a seminal article by Widmark (1914) that dealt with the analysis of ethanol in urine, diuresis and the influence of drinking water on the concentration of ethanol in subsequent voids. Ingestion of ethanol increased the production rate of urine appreciably as shown by the vertical bars, although by 90 min post-dosing this output of urine subsided. After drinking 500 mL of water, the production of urine increased again for about 45 min before returning to a normal rate of 1-2 mL per min. The total amount of ethanol excreted, which is the product of volume and concentration, is higher after drinking water. However, the concentration of ethanol in successive voids before and after drinking the water maintained the expected time course and trend line in the post-absorptive state.

Ethanol enters the primary urine by passive diffusion depending on the concentration in arterial blood water. To dilute the concentration of ethanol in urine by drinking water would require lowering the blood-ethanol concentration and this is not possible under normal conditions of hydration. These early results reported by Widmark were verified in a recent paper by Bendtsen and Jones (1999), who also measured creatinine and osmolality of urine as biochemical markers for highly dilute specimens. Both biomarkers of urinary dilution decreased appreciably after drinking ethanol and also after drinking 500 mL of water. Despite producing more dilute urine, the concentrations of ethanol in the successive urine samples collected in the post-absorptive phase remained unchanged.

Measuring ethanol in a person's urine gives evidence to support any clinical signs and symptoms of drunkenness; the first drunk-driving laws were founded on use of urine specimens as an indirect way to estimate a person's BAC. Care is needed in the collection of urine specimens to avoid any tampering or attempts to adulterate the specimens, e.g., by dilution with water or other liquids. The population average UAC/BAC ratio of 1.3:1 was adopted in law enforcement to estimate BAC indirectly from analysis of UAC (Walls and Brownlie, 1985). After collection, it is important that the urine specimen contains a preservative such as 1-2% NaF to prevent fermentation of glucose (if present) into ethanol after sampling (Jones et al., 1999). Urine from diabetics might be loaded with glucose and if the person has urinary tract infections, such as with the yeast *Candida albicans,* ethanol is formed if the specimens are allowed to stand at room temperature for 24 h or longer without NaF (Jones et al., 2000).

A recommended sampling strategy is to obtain two successive voids from drunken drivers about 30-60 min apart. The aim of the first void is to empty the bladder of old urine produced during an unknown time when the BAC might have changed appreciably (Cooper et al., 1979; Walls and Brownlie, 1985). The concentration of ethanol in the second void provides information about the BAC existing during the sampling interval, which is usually 30-60 min after the bladder was last emptied. Dividing the UAC in the second void by 1.3 gives an estimate of BAC midway between the two voids (Cooper et al., 1979). However, the UAC/BAC ratio varies widely both between individuals and within individuals depending on the stage of ethanol metabolism, diuresis and the completeness of emptying the bladder from residual urine.

2. Urine-blood ratios

Urine is virtually all water, and whole blood contains 80% w/w water so one can expect a UAC/BAC ratio at equilibrium of about 1.25:1 (100/80=1.25). However, urine is voided in batches and during storage in the bladder the BAC is likely to change, sometimes appreciably, such as during the absorption stage of the alcohol curve (Walls and Brownlie, 1985). On the rising limb of the BAC curve, some of the urine entering the bladder was produced at a lower BAC than actually existed when the urine was voided (Biasotti and Valentine, 1985; Jones, 2006). This makes the UAC/BAC ratios abnormally low if collected during absorption, and ratios would be even lower if there had been an alcohol-free pool of urine in the bladder before drinking started.

In the post-absorptive phase, when the BAC is decreasing at a constant rate of about ~0.15 g/L/h, the concentration of ethanol in urine reflects the BAC that existed some time earlier since the bladder was last emptied (Iffland and Jones, 2002). The UAC/BAC ratios in the post-absorptive phase of the alcohol curve might be abnormally high if the bladder had not been emptied for several hours when BAC was decreasing at a constant rate (Biasotti and Valentine, 1985). In postmortem toxicology, higher UAC/BAC ratios are likely and the urine might contain ethanol even though the BAC was reported as negative. This happens, for example, if the person had consumed a large quantity of alcohol but survived for many hours before death occurred (Jones and Holmgren, 2003b). There is no metabolism of ethanol occurring in the urinary bladder whereas BAC decreases until circulation stops.

The UAC/BAC ratios in drunken drivers for both first and second voids were recently evaluated in a large study (Jones, 2002). An initial void was made soon after arrest followed by a second void at the time a venous blood samples was taken about 60 min later (Jones, 2002; Jones, 2003). The resulting UAC/BAC ratios for each void are shown in Table 3.16 and the ratio for the first void was higher than for the second void ($p < 0.001$) as expected for post-absorptive specimens. The UAC/BAC ratios are also shown after adjusting the BAC, to the same time as urine, was voided based on the assumption that all individuals are in the post-absorptive state and eliminate alcohol from blood at a constant rate of 0.20 g/L/h (Jones and Andersson, 1996a). The time-corrected UAC/BAC ratios for both voids agreed well (mean ratio 1.3:1), suggesting that even the first random void from a drunken driver is a good reflection of fully equilibrated alcohol in the body (Jones, 2003).

Table 3.16
Relationships Between Urine-Alcohol Concentration (UAC) and Blood Alcohol Concentration (BAC) in First and Second Voids from Drunken Drivers (Data from Jones, 2002; Jones, 2003c)

Urine sample	Mean (SD) UAC/BAC	95% range	Mean (SD) UAC/BAC[1]	95% range
First void	1.34 (0.192)	0.94-1.79	1.30 (0.122)	1.11-1.60
Second void	1.22 (0.119)	0.99-1.46	1.32 (0.131)	1.10-1.67

[1] Values adjusted for the metabolism of ethanol at a rate of 0.20 g/L/h between times of sampling blood and urine.

Intentional or unintentional retention of urine, which might occur in conditions such as prostrate enlargement, is likely to skew the UAC/BAC ratios. As an example of this scenario, consider a person who drinks alcohol during the evening, and voids before bedtime when BAC is 1.0 g/L. In the morning 10 hours later the BAC would be zero, owing to metabolism of ethanol during the night. However, the UAC in the first morning void reflects the average BAC existing since the previous void the night before and might be appreciable (~0.5 g/L). This should not cause any major problem in practice because the first thing people do in the morning is visit the bathroom, especially if they have been drinking the night before. Accordingly, the UAC/BAC ratio furnishes useful information about the position of the BAC curve when the samples are taken and also the BAC during the collecting interval (UAC/1.3). If the person was in the absorption phase and the BAC was rising, this calculation UAC/1.3 would underestimate the persons coexisting BAC because the actual UAC/BAC ratio is less than or close to unit shortly after drinking.

Quantitative relationships between the concentrations of ethanol in blood and urine have been established and these are useful whenever BAC is estimated from UAC for whatever reason (Jones, 2006; Payne et al., 1967; Biasotti and Valentine, 1985). When such a prediction is made it is important to consider the uncertainty inherent in the estimated BAC. Nevertheless, a lot more is known about the UAC and BAC relationship and interpreting urinary concentrations of ethanol compared with all other drugs of abuse. Even the first urinary void collected from drunken drivers has a concentration of ethanol highly correlated with BAC as illustrated in Figure 3.35. It seems that during a period of heavy drinking to reach the high average BAC in traffic delinquents (mean 1.6 g/L), the individuals have visited the bathroom to urinate several times. This means that even a random void reflects a freshly secreted batch of urine and hence a close relationship to the BAC. Nevertheless, when urine specimens are analyzed from drunken drivers and the evidence used for prosecution, such as in the UK, where the legal limit for urine is 107 mg/100 mL (1.07 g/L), the police collect and analyze a second void (Biasotti and Valentine, 1985; Jones 2002). Furthermore, with UAC defined by statute, there is no need to translate the analytical result into the expected BAC, which eliminates any concerns about inter- and intra-subject variation in UAC/BAC ratios.

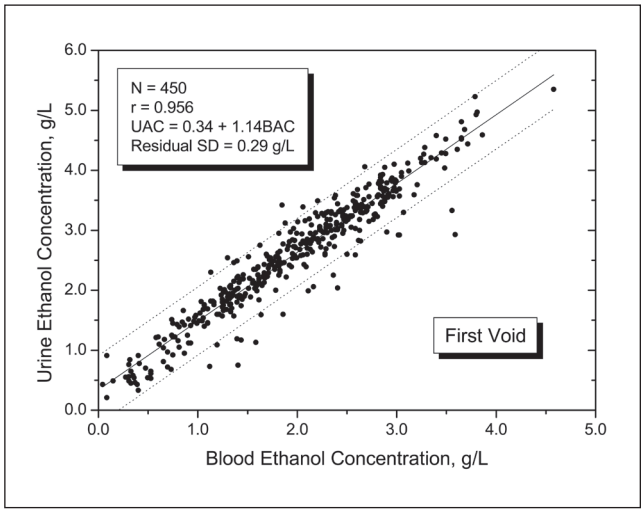


Figure 3.35 *Scatter plot and correlation-regression analysis showing the relationship between ethanol concentrations in urine (random void) and venous blood in specimens collected from apprehended drivers (from Jones, 2006).*

3. Concentration-time profiles

Figure 3.36 shows individual concentration-time curves for ethanol analyzed in blood and urine collected from 30 men who had consumed 0.68 g/kg ethanol on an empty stomach (Jones, 1992a). Note that the bladder was emptied before drinking started so an alcohol-free pool of urine could not dilute the ethanol in freshly produced urine. These curves show that the UAC is less than the BAC during the absorption phase; it then crosses the BAC curve and continues to rise above BAC reaching a higher C_{max} and a later occurring t_{max}. In the post-peak phase, and provided no more alcohol is taken, the UAC is always above the BAC and the UAC remains measurable for about one hour longer than the BAC (Jones, 1992a).

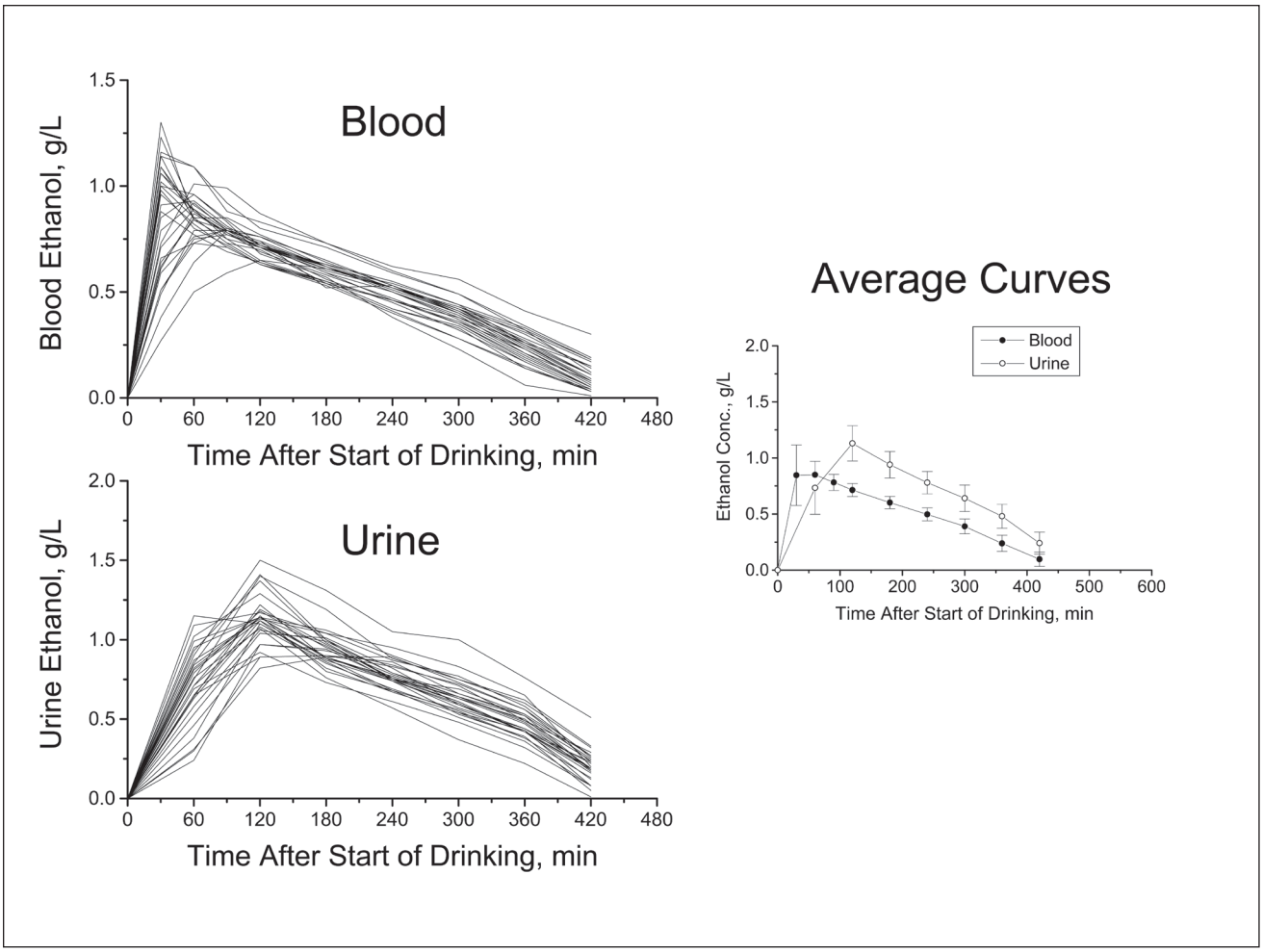


Figure 3.36 *Individual concentration-time profiles of ethanol in blood and urine in healthy men who drank 0.68 g/kg as neat whiskey on an empty stomach. Note that the bladder was emptied before drinking. The average blood and urine curves are also shown to highlight the displacement in the urine curve relative to the blood-alcohol curve (Jones 1992a).*

Individual variations in BAC and UAC profiles are considerable despite the standardized drinking conditions and sample collection protocol. The UAC is shifted in time compared with the BAC and these temporal variations provide useful information about the time after drinking when the urine sample was voided (Iffland and Jones, 2002). Collecting two consecutive urine samples about one hour apart can be useful in evaluating when the person last consumed alcohol. If the UAC-1 < UAC-2 this makes it likely that BAC was still rising and the person had probably just finished drinking. When UAC-1=UAC-2, the peak BAC was probably just passed and a longer time of 1-2 hours had probably elapsed after the end of drinking. Finally, if UAC decreases by 0.1-0.15 g/L between the time of making two voids, this suggests that the post-absorptive phase of the ethanol curve existed and that more than 1-2 hours must have passed after the last drink (Jones, 1990; Jones, 2002; Iffland, 1999).

C. Breath

Recognizing the smell of alcohol on the breath of a drinker is probably the oldest biomarker of over-consumption of alcohol and drunkenness. The first scientific studies to establish a quantitative relationship between ethanol in breath and blood date back to the work of Anstie (1874), who showed convincingly that only a very small fraction of the total amount of ethanol consumed could be recovered in the breath collected over several hours post-drinking. Another important early paper was that of Liljestrand and Linde (1930) who established the physiological principles of breath-alcohol testing. They determined the blood/air distribution ratios of ethanol under both in-vivo and in-vitro conditions. The concentrations of ethanol in blood and breath were highly correlated although the concentration in end-expired air was approximately 2000 times less than in an equal volume of blood. They concluded

that the blood-to-breath ratio of alcohol was approximately 2000:1 and exhibited both inter- and intra-individual variations depending on, among other things, the time after drinking when breath was sampled and the person's breathing pattern (Liljestrand and Linde, 1930).

Technological developments in methods for the capture and analysis of ethanol in breath accelerated in the 1970s with the use of physiochemical methods such as gas chromatography, infra-red analysis and electrochemical oxidation instead of chemical oxidation as with the Borkenstein Breathalyzer (Jones, 1996). The instruments available today for breath alcohol testing are highly reliable and "fit for purposes" as a means of providing evidence for prosecuting drunken drivers (Gullberg, 2000).

1. Breath alcohol physiology

The physiological principles of ethanol excretion in breath and the theory of gas exchange in the lungs have been reviewed elsewhere (Jones, 1990; Hlastala, 1998; Hlastala, 2002). Ethanol equilibrates between the pulmonary blood and the air by diffusion across the alveolar-capillary membrane, and this occurs in a fraction of a second. However, during a prolonged exhalation the alcohol in the alveolar air participates in re-equilibration with ethanol contained in the watery mucous surfaces covering the upper airways (Schimmel, 2004). Moreover, during exhalation the alveolar air cools from about 37°C to 34°C so that the concentration of ethanol in the breath leaving the mouth is appreciably lower than it was when at the alveolar capillary membrane. How much lower will depend on many factors, such as body temperature, breathing pattern and the temperature and humidity of the ambient air breathed (Jones, 1990; Jones, 1982a; 1982b).

Ethanol in the gas phase equilibrates with the mucous surfaces covering the upper airway, nose and mouth and this process should be considered when breath-alcohol tests are interpreted (Anderson and Hlastala, 2007; Anderson et al., 2003). The final breath-alcohol concentration depends on the completeness of equilibration of ethanol in the airways, body temperature, lung size and the temperature and humidity of ambient air breathed (Jones, 1982a; Jones, 1982b; Hlastala and Anderson, 2007).

According to Hlastala (2002) the early proponents of breath-alcohol testing did not fully appreciate the importance of equilibration of ethanol in the airways. Such interactions probably account for much of the variability in blood/breath ratios of ethanol observed in practice (Hlastala, 1998; Hlastala and Andersson, 2007a; Hlastala and Andersson, 2007b). Hypo-ventilation and rebreathing elevate the expired BrAC whereas hyper-ventilation lowers the exhaled BrAC compared with a normal inhalation and exhalation (Jones et al., 1982a). These changes in BrAC depend very much on disruption of the liquid-vapor exchanges of ethanol caused by the abnormal breathing pattern and also an altered temperature in the airways. The temperature coefficient of alcohol solubility is ± 6.5% per degree change in temperature, which makes this a key variable to consider in any quantitative evaluation of breath-ethanol concentration (Jones, 1983).

Despite these many variables the concentrations of ethanol in blood and breath are highly correlated as shown by the results in Figure 3.37, representing tests in drunken drivers. The breath analyzer was the Intoxilyzer® 5000 and there is clearly a strong functional relationship with BrAC increasing as BAC increases (r=0.98) from zero to 3.5 g/L. However, there is considerable scatter of individual data points around the regression line as indicated by the residual standard deviation. This speaks against trying to make a quantitative evaluation of BAC indirectly by analysis of alcohol in the breath, and this practice is not recommended.

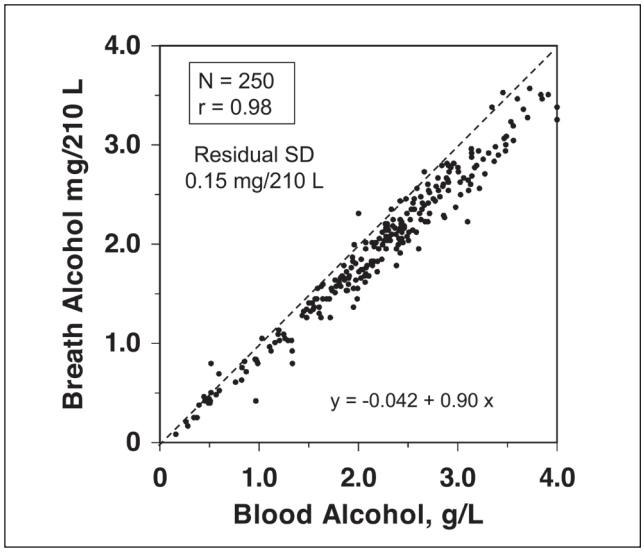


Figure 3.37 *Correlation between the concentrations of ethanol in venous blood and breath of apprehended drunken drivers tested with an Intoxilyzer® 5000 instrument. The dashed diagonal line has a slope of unit showing that breath-test results tend to underestimate venous blood-alcohol in the vast majority of cases (Jones and Andersson, 1996b).*

The claim that diseases of the lungs, such as asthma as reflected by inflammation in the airways or chronic obstructive pulmonary disease (COPD), impact the blood-breath alcohol relationship deserves more attention. In a study with COPD patients the BAC/BrAC ratios were consistently higher than those reported for an age-matched control group, suggesting overall lower concentration of alcohol

in the exhaled breath (Hahn et al., 1991). Some evidential breath-alcohol instruments require that the tested subject provide a minimum breath volume, such as 1.5 liters in one continuous exhalation for six seconds or at a minimum pressure. These sampling characteristics are simply not possible for some people especially those with lung dysfunction. In these instances, options need to be available to sample blood, saliva or urine for determination of ethanol instead.

The quantity of ethanol excreted unchanged in the breath is only a small fraction of the total amount of alcohol consumed. If BAC is 1.0 g/L and the blood-to-breath ratio is 1800:1 at 37°C in the alveolar-capillary interface, 1 liter of alveolar air contains 0.55 mg ethanol. The amount of air inspired and expired per minute, known as the respiratory minute volume, is 6 L (500 mL per breath x 12 breaths per min). Approximately 150 mL of air does not participate in gas exchange and this represents the dead space air (Guyton, 1986). Thus alveolar ventilation at a respiratory minute volume of 6 L/min is only 4.2 L/min or 252 L per hour. A simple calculation shows that 138 mg of ethanol (0.55 x 252) is eliminated from the body via the breath every hour. Because 6-8 grams of ethanol is eliminated from the body per hour by a combination of metabolism and excretion, the 138 mg lost in the exhaled air is only 1.6-2.3% of the total amount eliminated. These amounts will depend on the underlying blood-ethanol concentration and the actual lung ventilation rate of the subject.

2. Blood-breath ratios

Much has been written about blood/breath ratios of alcohol and their variability in connection with breath-alcohol testing for legal purposes (Jones, 1996; Jones, 2000a). A recurring question relates to whether the coexisting blood alcohol concentration was overestimated or underestimated if a breath-alcohol test was made instead of taking a blood sample. This was a common challenge in traffic law enforcement when statutory alcohol limits for driving were defined in terms of blood alcohol concentration (Jones, 1996; Jones, 2000a). Most countries have since abandoned this practice and instead the threshold alcohol limits for driving are defined in terms of breath-alcohol concentration. These limits were derived by assuming a population average blood/breath ratio; Great Britain and Holland opted for 2300:1 whereas most other countries in Europe accepted 2000:1 although the U.S. and Canada endorse a 2100:1 ratio.

In reality, the concept of a constant blood-breath ratio of alcohol is flawed for several reasons (Hlastala, 1998). First, the concentration of ethanol in breath runs closer to the concentration in arterial blood and not the venous blood, which is the specimen most often analyzed in a legal context (Martin et al., 1984). The magnitude and sign of arterial-venous differences in ethanol concentration change during absorption, distribution and elimination stages of the BAC curve (Jones et al., 2004). This makes the venous-blood to breath ratio of ethanol a moving target from about 1800:1 during the absorption limb of the alcohol curve to 2100:1 at 90 min post-drinking, increasing to 2300:1 by 120 min post-drinking and gradually increasing further as the BAC decreases towards zero. When BAC is in the range 0.1 to 0.2 g/L the BAC/BrAC ratio might exceed 3000:1.

Figure 3.38 is a plot of BAC/BrAC ratios as a function of the blood alcohol concentration in drinking drivers (Jones and Andersson, 1996b). Most of the results are between 2000:1 and 3000:1 over the range of ethanol concentrations in the breath in these individuals. The mean and standard deviation of the BAC/BrAC ratio was 2411 ± 205 (coefficient of variation=8.5%) and the range was from 1422 to 3512. The abnormally low values might be explained by interfering substances in the drivers' breath because they had consumed denatured alcohol containing ketones (Jones et al., 1996). There were no gender-related differences in BAC/BrAC ratio in this study: males 2428 ± 261 (± standard deviation, N=369) and females 2444 ± 232 (N=39). The mean BAC/BrAC ratio was higher than 2100:1 by 14%, which gives a definite advantage to the subject who provides a breath sample as opposed to blood for determination of alcohol (Gainsford et al., 2006).

Table 3.17 lists various factors or conditions that might explain unusually high or low BrAC when compared with venous BAC and accordingly high or low BAC/BrAC ratios. The concept of a constant BAC/BrAC ratio is flawed because of the well-known arterial-venous differences in pharmacokinetics of ethanol (Jones et al., 2004). A recently published article compared BAC/BrAC ratios for near simultaneous specimens of blood taken from a cubital vein and a radial artery and verified the close agreement between the time course of ethanol in arterial blood and breath (Lindberg et al., 2007). The mean blood/breath ratio of alcohol for radial artery blood was 2251:1 and the standard deviation was 46 and coefficient of variation 2% (N=15 subjects, 9 men and 6 women).

The major advantage of breath-alcohol analysis over blood samples is the non-invasive nature of the sampling and availability of on-the-spot results of the test (Moynham et al., 1990; Jones, 1996). Most countries have adopted breath-alcohol analysis as roadside screening tests for alcohol as well as for evidential purposes as evidence for prosecution (Emerson et al., 1980; Jones, 1996; Jones, 2000a) Breath-tests for alcohol are also being widely used in alcohol research and clinical and emergency medicine to detect drunkenness. Such a test should not be conducted sooner than 15 min after the last drink to avoid contamination of the exhaled breath with a higher concentration of alcohol that resides in the oral cavity mixed with the mucous surfaces of the mouth (Buczek and Wigmore, 2002).

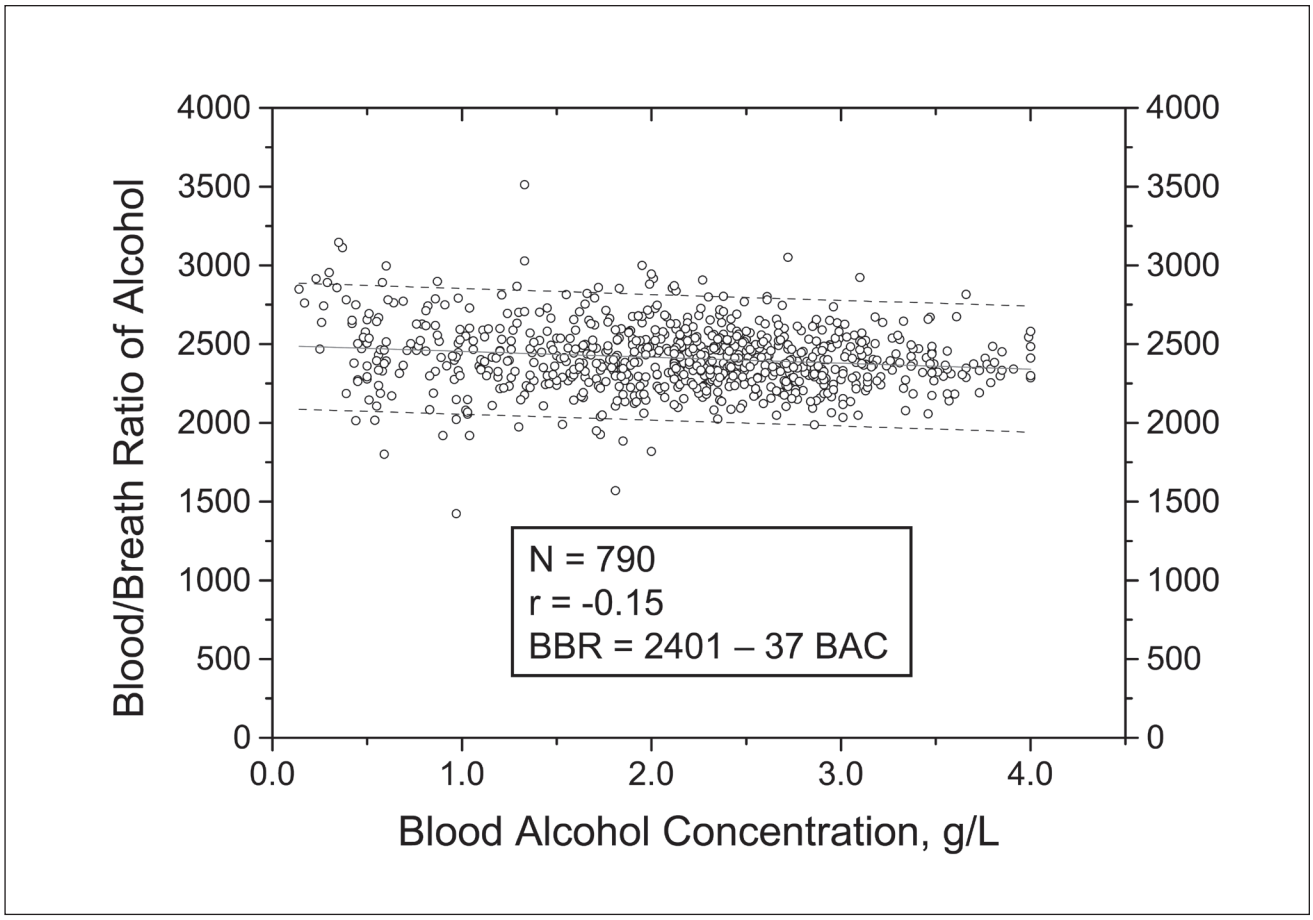


Figure 3.38 *Variation in blood-breath ratios of ethanol in drunken drivers plotted as a function of the blood alcohol concentration and the solid regression line ± 2 times residual standard deviation is also plotted as broken lines (Jones and Andersson, 1996b).*

The potential problem of mouth alcohol is well recognized and its significance can be diminished by washing the mouth with warm water after the end of drinking before a breath-test is made. Many of the latest generation of breath-alcohol instruments also incorporate software to monitor the rise in BrAC during a continuous forced exhalation. The instruments are programmed with algorithms to identify any irregularities (waviness) in the shapes of the alcohol exhalation curves and these give a strong indication that mouth alcohol was present. In reality, the risk of elevating a breath-test result by alcohol retained in the mouth from a recent drink is much exaggerated as an artifact, except when people suffer from gastroesophageal reflux disease (GERD). The risk of obtaining a false high breath-alcohol concentration in people suffering from GERD deserves more investigation (Jones, 2005).

3. Concentration-time profiles

Breath-alcohol instruments and breath-alcohol concentrations are being increasingly used to investigate aspects of ethanol pharmacokinetics (Dettling et al., 2006a; 2006b; Jachau et al., 2004; Pavlic et al., 2007). Figure 3.39 shows a plot of the concentration-time profiles of ethanol in venous blood and end-exhaled breath in four subjects who consumed a moderate dose of alcohol under controlled laboratory conditions. The breath-analysis was done with an Intoxilyzer® 5000 infrared analyzer, and venous blood alcohol concentration was determined by headspace gas chromatography (Jones and Andersson, 2003). To compare the BAC and BrAC curves the latter values were multiplied by 2000 and expressed as mg/2 L breath, The BrAC curves were higher than BAC for up to 60 min post-dosing, but at all later times the BrAC curve ran below the corresponding venous BAC curve. These differences between BAC and BrAC can be explained, at least in part, by the existence of arterial-venous differences in the concentrations of ethanol.

Table 3.17
Factors Influencing Breath Alcohol Concentration (BrAC) and Blood/Breath Ratios of Ethanol

High BrAC and low blood/breath ratios

- Long exhalation time into the breath-analyzer before analysis.
- Tests made during the absorption phase of the blood-alcohol curve when arterial-venous difference in concentration of ethanol is greatest.
- Presence of mouth alcohol when breath was sampled (e.g., within 15-20 min after end of drinking).
- Rebreathing the first exhalation a number of times before analysis of ethanol.
- Breath-holding (hypoventilation) before sampling breath.
- Elevated body temperature (hyperthermia).
- Non-specific method of analysis and presence of an interfering substance.

Low BrAC and high blood/breath ratios

- Hyperventilation immediately before sampling breath.
- Chronic obstructive pulmonary disease (COPD).
- Analyzing breath too soon after the start of exhalation, before an alveolar plateau.
- Tests in people suffering form hypothermia.
- Calculating BAC/BrAC ratios at low BrAC (< 0.2 g/L).
- Use of a low BAC/BrAC calibration factor (e.g., 2000:1 or 2100:1 instead of 2300:1 or 2400:1).

If the blood/breath factor used for calibration had been mg/2.3 L instead of mg/2 L, this would have brought the BAC and BrAC values closer in the post-absorptive phase but would have exaggerated the difference during the rising limb of the curves. With the critical BrAC defined by statute, inter-conversion between BAC and BrAC is not necessary, because both measures are equally objective ways to demonstrate over-consumption of alcohol.

In another controlled study involving nine men and nine women, the observed mean (± SD) BAC/BrAC ratio was 2553 ± 576 and 2417 ± 494, respectively, and the gender difference was not statistically significant ($p > 0.05$) The mean rate of ethanol elimination from venous blood was 0.157 mg/g/h, which was very close to the rate of elimination from breath of 0.161 mg/2 L/h (Jones and Andersson, 2003).

D. Saliva

Although blood, breath and urine are the traditional biofluids for determination of ethanol in clinical and forensic applications, there is increasing interest in alternative specimens, especially saliva or oral fluid as it is commonly referred to (Haeckel and Bucklitsch, 1987; Cone, 2001). Saliva receives considerable attention for analysis of drugs of abuse thanks to the development of better procedures for sampling and more specific and precise analytical methods (Drummer, 2006; Cone and Huestis, 2007). Saliva testing for drugs is actively investigated for use at the roadside in apprehended impaired drivers to help verify observations made by arresting police officers. Newer technological advances, such as immunoassay, have been made enabling on-the-spot sampling and analysis of abused drugs in saliva (Vestraete, 2005). Also in the field of therapeutic drug monitoring a switch to use of saliva instead of plasma or blood is useful, not least because obtaining saliva is more non-intrusive and can be used to monitor patient compliance with their medication (Kintz and Samyn, 2002; Langman, 2007).

1. Saliva production

Saliva is a watery fluid produced in the mouth by submaxillary, sublingual and parotid glands. Over a 24-h period, an adult person produces approximately 500-1500 mL of saliva but most of this is swallowed so the alcohol or drugs it might contain are reabsorbed from the gut. Mixed whole saliva is the specimen generally obtained for analysis of ethanol and other drugs and this matrix is mainly composed of water (99%) (Lentner, 1981).

The first reports of measuring ethanol in saliva and comparing this with blood-ethanol concentration were published in the 1930s. One of the best early studies was a solo-authored paper by Linde (1932), who investigated the relationship between ethanol in capillary blood and in paratoid saliva. He obtained samples of saliva by means of a special silver cannula, thus avoiding contamination of the specimen with other mucous secretions from the mouth. The absorption, distribution and elimination stages of ethanol disposition could be monitored by analysis of saliva, and the time-lag between ethanol entering the blood and appearing in the paratoid saliva was negligible. According to this seminal article, the mean saliva/blood ratio of alcohol was 1.21:1 and the concentration-time profile in saliva was on a slightly higher elevation than blood owing to the more water in saliva (Linde, 1932).

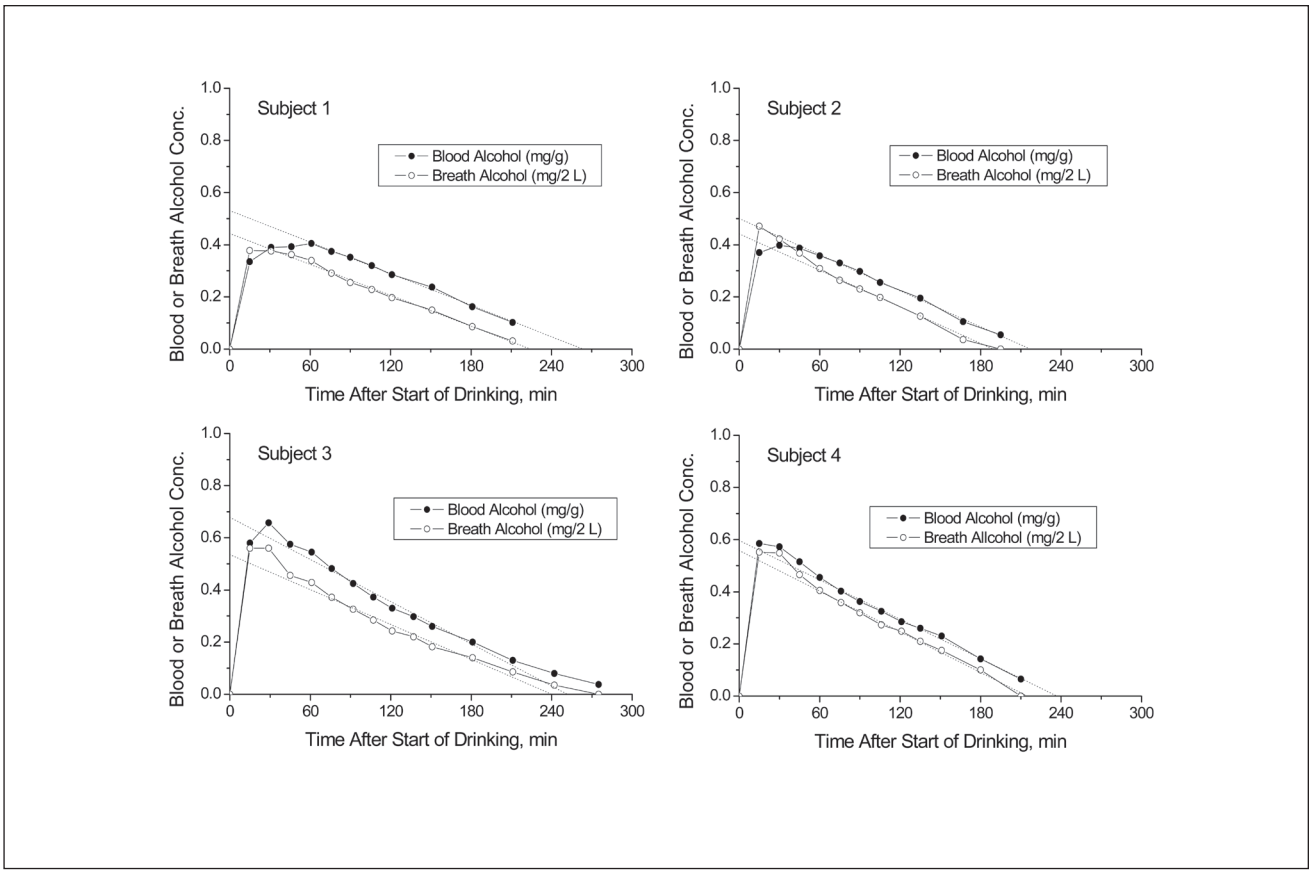


Figure 3.39 *Examples of concentration-time profiles of ethanol in venous blood analyzed by headspace gas chromatography and in end-expired breath determined with a quantitative infrared analyzer Intoxilyzer® 5000. The concentration units are those used for legal purposes in Sweden, namely mg/g for blood and mg/L for breath plotted here as mg/2 L for clarity and use of the same scale (Jones and Andersson, 2003a).*

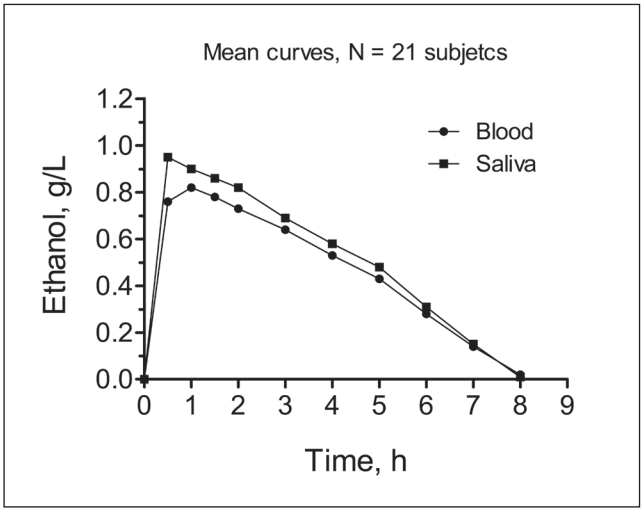


Figure 3.40 *Mean saliva-alcohol and blood-alcohol curves in 21 subjects who drank 0.68 g/kg as neat whiskey on an empty stomach in 20 min after an overnight fast. Standard errors are not shown because these were often smaller than the dimensions of the symbols (Jones, 1993b).*

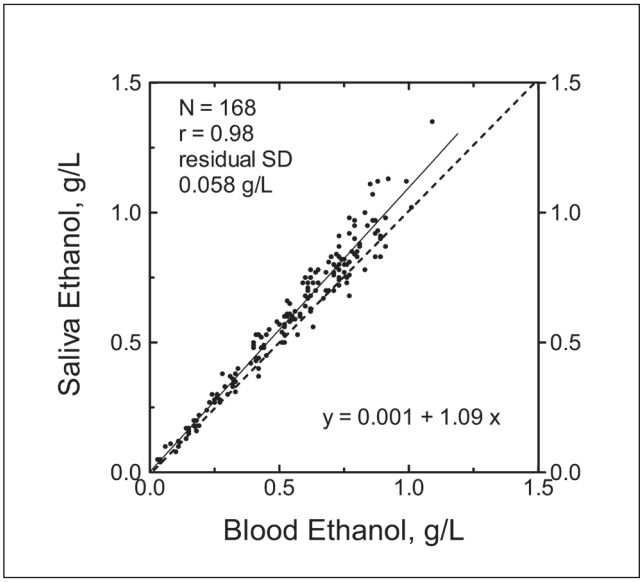


Figure 3.41 *Scatter plot and correlation-regression analysis of the concentrations of ethanol in mixed whole saliva (oral fluid) and fingertip capillary blood (Jones, 1993b).*

The vast majority of studies dealing with ethanol analysis in blood and saliva make use of mixed saliva obtained by making tongue and lip movements and then spitting the specimen into a small tube. The concentration-time profiles of ethanol in saliva and blood are compared in Figure 3.40 showing the generally higher values for saliva as expected from differences in water content.

Problems are sometimes encountered in obtaining a sufficient volume of saliva specimen and some investigators try to stimulate production by allowing the subject to chew on parafilm or some other inert material or by dropping lemon juice onto the tongue, which also enhances the flow rate. More recently, special saliva sampling devices called salivetts have been developed for obtaining the required specimen and these are particularly suited for analysis of drugs of abuse. The subject chews on a cotton wool pad for about one minute and this is later removed from the mouth, placed into a special plastic tube and centrifuged to obtain a clear salivary fluid for analysis of drugs of abuse (Haeckel and Peiffer, 1992; Samyn et al., 1999).

Only the free plasma fraction of a drug is available for passage into the saliva, which makes ethanol a good candidate substance because ethanol is not bound to plasma proteins, which explains the strong associations found between BAC and SAC (Haeckel, 1993). In an experiment involving 48 healthy men who drank 0.68 g ethanol per kg body weight, the mean saliva/blood ratio of alcohol was 1.08 to 1 and this did not depend on the sampling time after the start of drinking (Jones, 1979). In a later study, using the same set of data, the pharmacokinetic profiles of ethanol were compared for saliva and capillary whole blood. The results showed that serial samples of saliva were just as valid as blood for use in pharmacokinetic studies and investigations of the disposition and fate of ethanol on the body (Jones, 1993b). The high correlation of ethanol in near simultaneous samples of saliva and capillary blood is shown in Figure 3.41.

2. Saliva-blood ratios

The most recent investigation of saliva/blood ratios of ethanol (Gubala and Zuba, 2002) reported a mean value of 1.02 when mixed saliva was taken for analysis. This was a large study involving 38 subjects (26 men and 12 women) with multiple samples being taken to give a total of 1152 saliva-blood pairs for statistical evaluation. The saliva-blood values were highly correlated r=0.94 and 50% of them were within ± 0.05 g/L of each other. The authors recommend saliva as a useful medium for testing driver sobriety (Gubala and Zuba, 2002) supporting earlier work in hospital casualty patients (McColl et al., 1979).

3. Concentration-time profiles

Figure 3.42 compares average concentration-time profiles of ethanol in mixed whole saliva (SAC) and capillary blood after three different doses of alcohol were administered (Jones, unpublished work). The nature of these saliva-alcohol curves show a strong dose-response relationship making it feasible to use repetitive samples of saliva to investigate the pharmacokinetics of ethanol.

Most of the current interest in determination of ethanol in saliva relates to on-site alcohol testing, such as in the workplace or in clinical settings (Dubowski, 2002). Several on-the-spot devices for analysis of saliva are available and yield either a qualitative response (alcohol present or not) or a semi-quantitative response (0.2, 0.3, 0.4 g/L, etc.) (Bates and Martin, 1997; Biwasaka et al., 2001). The analytical principle involves enzymatic (ADH-NAD^+) oxidation with a color endpoint, which can be read from a linear scale or with a simple photometer device. Several evaluation studies of these on-site saliva kits show their usefulness for the intended purpose. With the QED devices, there was a high correlation between SAC and near simultaneous measurements of venous blood and breath-alcohol concentrations (Jones, 1995a; Bates et al., 1993). This on-the-spot test for alcohol has found applications in clinical settings such as for monitoring those attending outpatient clinics or emergency medicine departments for alcohol influence (Bendtsen et al., 1999; Biwasaka et al., 2001). Saliva-alcohol testing devices have also been approved for use in the workplace including pre-employment, post-accident and probable cause situations (Dubowski, 2002).

Saliva specimens are being increasingly used for therapeutic drug monitoring, and for some drugs good correlations are obtained with the coexisting levels in plasma (Drobitch and Svensson, 1992; Malamund and Tobak, 1993). The main advantage of saliva is that it can be obtained by non-invasive methods and has become the preferred specimen for drugs of abuse testing (Schramm et al., 1992; Cone, 2001). A disadvantage of saliva is that with those drugs tightly bound to plasma proteins (e.g., benzodiazepines) the concentrations passing over into the saliva are very low and not measurable by conventional methods of analysis (Haeckel and Hänecke, 1993; Lentner, 1981). Obtaining appropriate samples of saliva for drugs of abuse testing still remains a problem area (Crouch, 2005).

Driving under the influence of drugs other than alcohol is a growing concern in many countries, and objective ways are needed to test whether motorists apprehended by the police have used a banned substance (Drummer, 2005; Drummer, 2006). To these ends saliva testing for drugs of abuse has advanced considerably (Drummer et al., 2007; Cone and

Huestis, 2007). Studies have shown that the pharmacokinetics of some drugs in saliva agree reasonably well with plasma concentration-time profiles (Cone and Huestis, 2007). Several devices are now available for collection and analysis of a wide range of illicit drugs in saliva specimens collected at the roadside thus serving as a preliminary screening test (Walsh et al., 2003). All positive results at the roadside should be followed up by verification analysis of blood or saliva by more sophisticated GC-MS or LC-MS technology.

E. Cerebrospinal Fluid (CSF)

1. Production and origin of CSF

The liquid surrounding the brain and spinal cord is the cerebrospinal fluid (CSF) and is a clear watery liquid (mainly water) similar to plasma and interstitial fluid. CSF is formed in specialized structures within the brain called ventricles, which form a part of the choroid plexus. Other constituents of CSF include various proteins and glucose which equilibrate between blood and adjacent brain tissue. The biochemical profile of CSF reflects, to some extent, the chemical reactions taking place within the brain. Sampling and analysis of CSF has diagnostic potential in various neurological and psychiatric disorders as an indirect way to monitor brain chemistry (Guyton, 1986). Lumbar puncture (spinal tap) is the usual way to obtain a sample of CSF from living subjects.

In neurology and other medical disciplines CSF is collected from the subarachnoid space (lumbar CSF) and less frequently from the cisterna magna (cisterna CSF), which is the usual practice during autopsy. The volume of CSF in adults is about 135 mL with 35 mL being found in the ventricles, 25 mL in the subarachnoid space and cisterna and 75 mL in the spinal canal. Because CSF is mostly water and its relative density is very close to 1.0 the ratio of concentrations of ethanol in CSF and blood should be 1.2:1 as expected from differences in water-content.

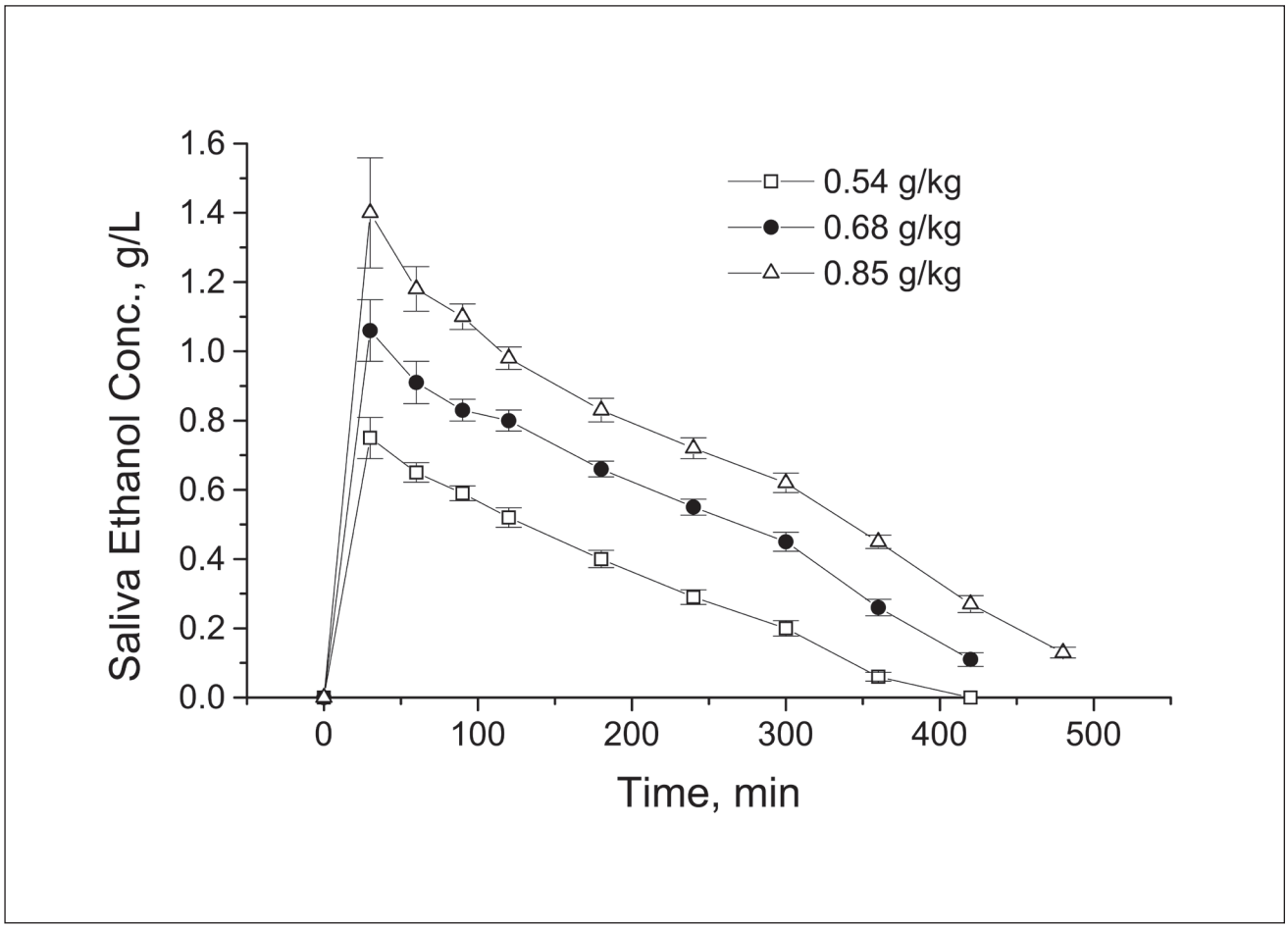


Figure 3.42 *Average pharmacokinetic profiles of ethanol in mixed saliva from healthy men (N=16 per dose) after they drank 0.54, 0.68 and 0.85 g/kg body weight as neat whiskey (Jones, unpublished).*

Because a dead body cannot be bent or positioned so easily, cisternal CSF and not lumbar CSF is the fluid taken during an autopsy. CSF contains enzymes, proteins and other endogenous substances, some of which are useful for clinical diagnosis of neurological disorders. Analysis of hemoglobin, lactate, and proteins (e.g., albumin), neuropeptides, as well as catecholamines such as dopamine, norepinephrine, and serotonin have been reported in CSF for research and clinical purposes.

Alcohol easily crosses the blood-brain barrier and enters the brain and CSF. In a number of older studies, repetitive specimens of lumbar fluid and blood were obtained from patients after they had consumed alcohol. This allowed monitoring the rate of uptake and elimination of ethanol in lumbar CSF samples, and the concentration-time course could be compared with blood-ethanol profiles. Although ethanol reaches the brain almost immediately after it is absorbed into the blood, CSF is produced gradually over a period of time. The time-course of ethanol concentrations in blood and CSF are displaced in time depending on rates of absorption, distribution and elimination of alcohol in the body. Moreover, an alcohol-free pool of CSF before the person begins to drink alcohol will dilute the ethanol content of freshly produced CSF. The combined influence of the difference in water content and the time-lag means that the concentration-time course of ethanol in CSF and BAC are comparable to those described earlier for urine and BAC when the bladder is voided sequentially over a period of time.

An increasing number of articles describe the use of CSF as an alternative specimen in postmortem toxicology for analysis of drugs other than alcohol (Engelhart and Jenkins, 2007). In postmortem toxicology, many classes of drugs can be determined in CSF and the concentrations present are almost always lower than those in blood samples. Interpretation of results from analysis of CSF is, however, a difficult task and should not be used as an indirect way to estimate blood levels.

2. Concentration-time profiles

Figure 3.43 compares blood-ethanol with lumbar CSF ethanol in two subjects from an article by Abramson and Linde (1930), who investigated the concentration-time profiles of ethanol in these body fluids. The specimens were lumbar CSF, which were taken at regular intervals after the volunteers had consumed a moderate dose of alcohol. The traces for CSF show a pronounced time-lag, which reflects in part the water content difference for CSF (99%) and whole blood (80%) and also the longer time required for the alcohol to enter the brain and reach the lumbar region. If cisternal CSF had been sampled, as is done in autopsy work, this would have shown a higher concentration and earlier occurring C_{max} and less of a time lag (Cooper et al., 1979).

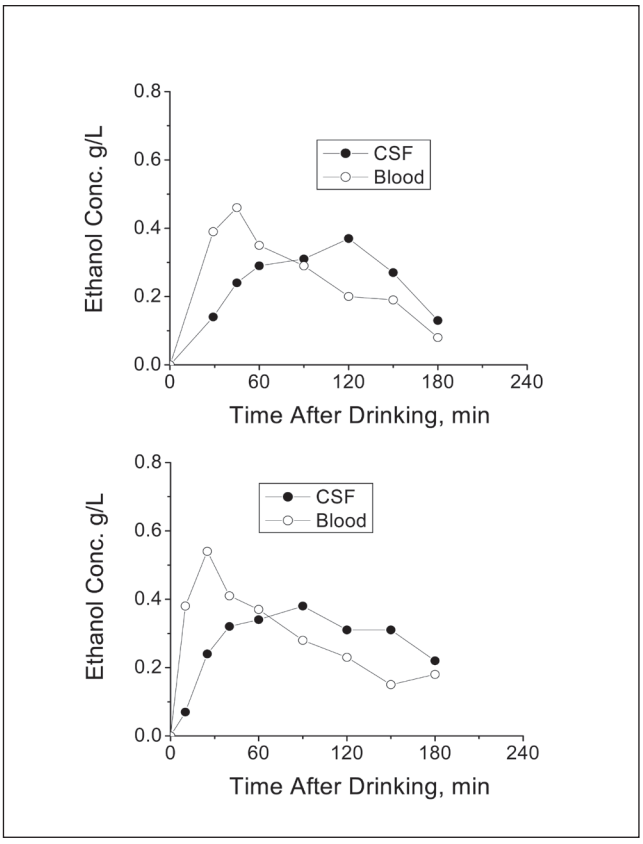


Figure 3.43 *Concentration-time profiles of ethanol in blood and cerebrospinal lumbar fluid in two volunteer subjects (plot redrawn from Abramson and Linde, 1930).*

F. Tears

All body fluids and tissues containing water will take up alcohol. A rather unusual body fluid seldom used for determination of ethanol is tear fluid (Lund, 1984). In one study, the concentrations of ethanol in blood and tears were highly correlated over the absorption, distribution and elimination stages of metabolism. The experiment involved 12 healthy subjects who drank 75 g ethanol with food before serial samples of venous blood and tear fluid were taken for analysis of ethanol enzymatically by the ADH method. Several methods were used to enhance the production of tears including direct irritation of the mucous membranes of the eye with cigarette smoke or chemical irritation of the nose with ammonia vapor or by tickling the nostril with a horse hair. At the appearance of tears in the eyes, the fluid was collected in capillary tubes ready for analysis. The distribution ratio of ethanol between tear fluid and blood was 1.14:1 (SD 0.037) with a range from 1.08 to 1.20. These results were in good agreement with values expected for the difference in water content of tears (100%) and whole blood (80%) (Lentner, 1981).

G. Sweat

Sweat or perspiration is produced by glands in the skin in response to heating as a result of stress, emotion, exercise or a change in the environmental temperature. The composition of sweat resembles the extracellular fluid and is therefore mainly composed of water and salt. The function of sweating is to cool the body so if there is ethanol in the bloodstream some will cross the sweat gland epithelium by passive diffusion from the blood supply to the gland.

The concentration relationship between ethanol in blood and sweat was first investigated as early as 1936 in a study involving healthy men exposed to steam in a hot room (Nyman and Palmlöv, 1936). The ethanol concentration in sweat was only 81% of the blood-ethanol concentration, although the authors warned about the risk of losses during the collection of sweat for chemical analysis. By means of a special device for collecting sweat to avoid such evaporative losses, Buono (1999) found a high correlation between sweat-ethanol and blood-ethanol concentration with the sweat containing about 20% more ethanol than an equal volume of whole blood. This difference in concentration was explained by the higher water content of sweat compared with whole blood and it was recommended that ethanol concentrations should be expressed in terms of the water content of the materials analyzed, e.g., mg ethanol per liter water.

Use of sweat as body fluid for analysis of ethanol has received attention in connection with treatment and rehabilitation of alcoholics as a way to verify that they remain abstinent (Phillips and McAloon, 1980; Phillips, 1984). A tamper-proof sweat-patch was designed to collect and retain alcohol that might pass by diffusion through the skin. Periodically removing the patch and analyzing its ethanol content furnishes evidence to verify or challenge claims of abstinence (Phillips and McAloon, 1980). Some studies showed the feasibility of monitoring absorption, distribution and elimination stages of ethanol metabolism by serial sampling sweat using a wearable sensor designed for transdermal diffusion of alcohol (Swift, 2000; Swift et al., 1992). The pharmacokinetics of ethanol has been investigated in perspiration from humans, although the sampling procedure was not easy to control and the results of the study seem equivocal (Brown, 1985).

Applications of transdermal ethanol testing (through the skin) has advanced considerably, especially in connection with technology for use in the criminal justice system and probation (Hawthorne and Wojcik, 2006). Continuous transdermal alcohol monitoring is used to control the drinking behavior of offenders by sampling insensible perspiration over a period of time. The SCRAM device has emerged as a non-invasive sampling technology in the form of an ankle or arm bracelet. SCRAM is an acronym for Secure Continuous Remote Alcohol Monitoring and utilizes analysis of ethanol in sweat as evidence to support or challenge abstinence. The principles and application of sweat testing for alcohol as well as kinetics of transdermal ethanol exchange have been studied (Anderson and Hlastala, 2006; Sakai et al., 2006; Webster and Gabler, 2007).

H. Mother's Milk

Alcohol penetrates into all body fluids and tissues that contain water including the milk produced by lactating women (Olow, 1923). This raises the question of whether a neonate being breast fed runs the risk of dangerous exposure to ethanol if the mother drinks alcohol before the baby is fed (Mennela and Beauchamp, 1991). However, experiments have shown that the concentration of ethanol in the milk of lactating women runs very close to the concentration in the bloodstream (Olow, 1923). The pharmacokinetic profiles are very similar, reaching zero at roughly the same time (Lawton, 1985). Some investigators made calculations on how long a nursing mother needs to wait after consumption of alcohol before feeding the baby (Ho et al., 2001; Chung et al., 2005).

As an example, assume that an infant drank 300 mL of milk containing a concentration of 1 g/L ethanol. This corresponds to an intake of only 0.3 grams ethanol (Jones, 1992b). This alcohol passes through the liver before it reaches the systemic circulation and is therefore subjected to hepatic first-pass metabolism. Studies with human fetal livers taken in connection with legal abortions and from children undergoing abdominal surgery showed the presence of alcohol dehydrogenase in all specimens. ADH activity was detectable in two-month old fetuses, albeit lower than in children and adults; so even at birth babies are capably of metabolizing ethanol (Pikkarainen and Räihä, 1974).

If the above 0.3 grams of ethanol was distributed in the total body water of an infant weighing 5 kg this would produce a peak BAC of only 0.07 g/L. Because body water in neonates represents a larger proportion of body weight than in adults, a higher Widmark factor, such as 0.85 L/kg should be used in blood-alcohol calculations. Moreover, the concentration of ethanol in mother's milk decreases in parallel with the decrease in blood alcohol concentration as ethanol is metabolized in the liver. Accordingly, if a woman consumed two glasses of wine to reach a BAC (C_{max}) of 0.5 g/L and fed an infant a few hours later, this would not represent any risk because ethanol in blood and milk at this time would be close to zero (Ho et al., 2001).

Other psychoactive recreational drugs and prescription medication represent a much more serious problem for normal development of the neonate, and several fatalities have

been reported (Abdel-Latif et al., 2006; Usher and Foster, 2006). The lactational state is also thought to modify both stimulant effects of ethanol and pharmacokinetics in fed and fasting states (Pepino et al., 2007). The systemic availability of ethanol was diminished in lactating women as reflected in lower BAC at each time point and smaller areas under the blood-alcohol curves compared with control groups of women (Pepino et al., 2007).

I. Brain and Body Organs

Knowledge about the concentrations of ethanol in body organs and tissues comes from medical examiner cases and animal studies. The available evidence seems to suggest that the concentrations of ethanol in body tissues bear a close relationship to values expected from the water content of these tissues (Garriott, 1991). However, after death some body organs (e.g., kidney and liver) might retain metabolic activity as the body cools, and concentrations of ethanol can sometimes decrease by up to 10%, owing to oxidative metabolism (Jenkins et al., 1995). The rate of uptake of ethanol depends on blood flow to the various organs and tissue so skeletal muscles take a longer time to equilibrate with ethanol in blood than does the brain. Ethanol is not evenly distributed within the brain, and gray matter seems to contain a slightly higher concentration compared with white matter, probably because of regional differences in water content and vascularity (Budd, 1993). The question of distribution of ethanol within different regions of the brain and the best choice of specimen for forensic analysis of ethanol was discussed by Moore et al. (1997).

Some recent studies have monitored the penetration of ethanol into human brain tissue non-invasively by use of spectroscopic and magnetic resonance methods (Fein and Meyerhoff, 2000; Hetherington et al., 1999). Use of a homogenate of whole brain tissue is probably the best approach to determine the average concentrations of ethanol in this organ.

J. Vitreous Humor

In postmortem toxicology vitreous humor (VH) or the watery fluid from within the eye is a useful specimen for the analysis of ethanol and other drugs. Moreover, a suitable specimen of VH can be obtained with syringe and needle without the need to perform a complete autopsy (Jones, 2000b; Pounder and Jones, 2007). VH is primarily composed of water, and the concentration of ethanol in VH after equilibration is expected to be higher than in an equal volume of whole blood by a factor of approximately 1.2:1. Indeed, a mean VH/blood ratio of 1.19:1 and a standard deviation of 0.285 with 95% range of 0.63 to 1.75 was found in a recent study by Jones and Holmgren (2001), based on 706 forensic autopsies. The average and range of values agreed well with those reported by other investigators such as Pounder and Kuroda (1994). A scatter plot showing the concentrations of ethanol in femoral venous blood and VH is shown in Figure 3.44

Although BAC and VH were highly correlated (r=0.98) the large scatter of the data points around the regression line, as reflected in the residual standard deviation, introduces considerable uncertainty if BAC is predicted from analysis of ethanol in VH in any individual case (Jones and Holmgren, 2001; Pounder and Kuroda, 1994).

The time necessary for ethanol to become absorbed into the bloodstream and enter the fluids of the eye is relatively short as expected from the rich blood supply to the central nervous system and visual centers. The major advantage of VH over other body fluids for ethanol analysis is that the eyeball is well protected from environmental contamination and is distant from the gut, protecting it from bacteria that might have spread into the blood during autolysis after death (O'Neal and Poklis, 1996).

Analysis of ethanol in both VH and blood is therefore useful in verifying the reported blood-ethanol concentration. Postmortem synthesis of ethanol is less likely to occur in VH and this specimen is highly recommended if the corpse has undergone decomposition (Kugelberg and Jones, 2007). Finding a close agreement between the concentrations of ethanol measured in VH and blood gives confidence to the forensic toxicology results and speaks against any postmortem synthesis of ethanol by putrefaction processes. VH should always be the specimen of choice for analysis of alcohol in cases of trauma, e.g., in explosion deaths and casualties on the road or air crashes (Jones, 2000b).

K. Hair

Analysis of drugs of abuse in hair has emerged as a relatively new and exciting discipline within the field of analytical toxicology (Kintz, 2007; Villain et al., 2004). Because of ethanol's high water solubility and volatility it is impractical to determine this drug directly in hair strands. However, there is increasing interest in the analysis of non-oxidative metabolites of ethanol in hair, such as ethyl glucuronide and FAEE (Pragst and Balikova, 2006). Hair grows at a fairly even rate and as a biological specimen has several advantages over the traditional blood and urine samples. Among other things, the analysis of drugs in hair segments extends considerably the window of detection by weeks or months compared with blood and urine. Segmental analysis of hair along the shaft also permits studying the timeline for incorporation and therefore the pattern of use and abuse of drugs and narcotics (Kintz et al., 2006).

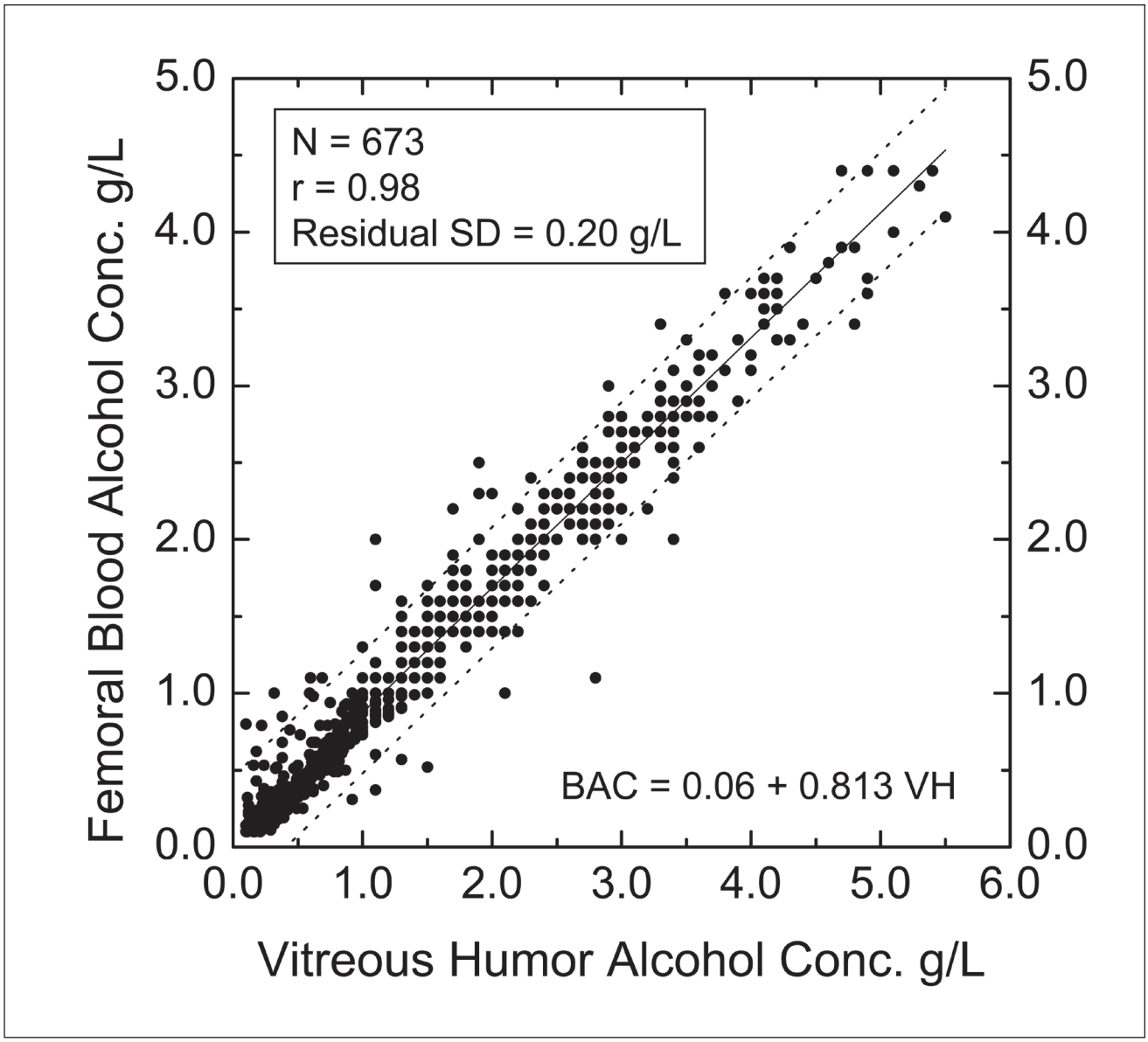


Figure 3.44 *Scatter plot and correlation-regression analysis (N=673) of the relationship between concentrations of ethanol in vitreous humor and femoral venous blood samples taken at autopsy (Jones and Holmgren, 2001).*

The analysis of drugs in hair also has several limitations, such as problems with contamination from the environment if recreational drugs are smoked (e.g., marijuana), use of and from the cosmetics and shampoo as well as bleach, coloring agents and treatments for baldness (Thorspecken et al., 2004). Incorporation of drugs into dark hair differs from grey hair, and differences also exist between different racial and ethnic groups. Drug concentrations are also likely to differ in hair samples taken from different parts of the body, as recently found for ethyl glucuronide in head hair compared with pubic hair (Kintz et al., 2008).

Hair is less prone to decomposition changes as time after death increases and as the body undergoes autolysis, which is a big advantage in postmortem toxicology. Drugs and metabolites are therefore likely to be more stable in hair after death compared with analysis of other body fluids and tissues. However, the concentration of drugs determined in hair does not allow drawing conclusions about the amount taken or the antemortem concentration in blood.

3.9 Concluding Remarks

Much is known about the absorption, distribution, excretion and metabolism of ethanol and the concentrations of this drug in various body fluids, such as saliva, breath, urine, plasma, etc. Most of the information about pharmacokinetics of ethanol has been gleaned from controlled dosing studies in healthy volunteers. However, for ethical considerations, the dose of ethanol is limited and the peak BAC rarely exceeds 1.0-1.2 g/L to avoid problems with nausea and vomiting, which would ruin the experiment. These drinking conditions are far removed from real-world situations in which large doses of ethanol are consumed over several hours in the form of beer, wine and/or spirits with or without concomitant intake of various types of food. For those who testify in court about alcohol-related offenses, such as drunken driving or sexual assault, more studies are needed along the lines described by Kalant et al. (1975) and more recently Ganert and Bowthorpe (2000) and Zink and Reinhardt (1984). These studies involve drinking alcohol under real-world conditions (Winek et al., 1996).

The widely used Widmark factors (ß and rho) are in urgent need of being updated to take into consideration the changing body composition in today's society. Obesity has become a major public health problem in most nations and a person's body mass index (BMI) deserves consideration when blood-alcohol calculations are made. The increasing use of breath-alcohol testing in alcohol research and in law enforcement has led to pharmacokinetic parameters of ethanol being established from breath-alcohol concentration-time plots (Jachau et al., 2004; Dettling et al., 2006a; 2006b).

The non-invasive nature of breath-testing compared with sampling blood and the possibility for on-the-spot results has meant that most countries use breath-alcohol instruments to gather evidence for prosecution of drunken drivers. It is not advisable to testify in court about the pharmacokinetics of ethanol (e.g., retrograde extrapolation) and perform Widmark calculations based on blood-alcohol curves if the result of a breath-alcohol test was used in evidence. Neither should the measured BrAC be converted to a presumed BAC, because the BAC/BrAC ratio is not known in the individual case and as discussed earlier depends on many factors. Knowledge of breath-alcohol parameters and pharmacokinetics derived from breath-alcohol curves are necessary (Dettling et al., 2006a; 2006b). Studies have shown that the breath-alcohol concentration runs closer to the arterial BAC rather than the venous BAC. This makes the venous BAC/BrAC ratio a moving target depending on the time after drinking and whether the person was on the rising or declining limb of the alcohol curve (Jones and Andersson, 2003).

A large amount of information has been obtained about first-pass metabolism (FPM) of ethanol and whether this is predominantly gastric or hepatic (Kalant, 2005). The general consensus seems to be that hepatic ADH is the major enzyme involved in FPM and not gastric ADH. Many of these studies have involved low doses of alcohol (0.15-0.3 g/kg) and revealed the existence of large inter-subject variations in BAC curves and pharmacokinetic parameters, especially when alcohol was ingested after a meal (see Figure 3.12 for examples). If moderate doses of ethanol are ingested (> 0.5 g/kg) after an overnight fast, the pharmacokinetic profile of ethanol can be used to estimate TBW, which speaks against any significant FPM under these conditions. Questions such as the time necessary to reach peak BAC (t_{max}) after end of drinking as well as the increment in BAC after the last drink before reaching the peak (see Figure 3.21) are common in drunken driving litigation (Gullberg, 1982). The rate of absorption of alcohol and the time required to reach C_{max} after the last drink is highly variable and might range from 5 min to 120 min in any individual case (Iffland and Jones, 2002 and Table 3.11).

Requests to back extrapolate a suspect's BAC from the time of the sampling to the time of the driving are often made, but this remains a dubious practice, owing to the many variables and unknowns involved (Jones, 1993a). The pharmacokinetic profile of ethanol is highly variable depending on the pattern of drinking and physiological characteristics of the subjects. In a typical impaired driving trial, only a single measurement of BAC or BrAC is usually available making it very difficult to engage in retrograde calculations with sufficient certainty for a criminal prosecution (Lewis, 1986a; Stowell and Stowell, 1998; Al-Lanqawi et al., 1992). All this speaks in favor of adopting time of test laws rather than time of driving laws when drunken drivers are prosecuted.

Several computer programs of varying sophistication have become available to perform various types of blood-alcohol calculation, such as retrograde extrapolations (Rockerbie and Rockerbie, 1995; Posey and Mozayani, 2007). Although these programs can plot graphs from input variables (BAC and time), they seem to offer no real advantage in terms of reliability over pen and paper or use of an electronic calculator. When blood-alcohol calculations are used it is crucial to appreciate the degree of uncertainty in the results and to allow for this when the reports are written and expert testimony given in court (Montgomery and Reasor, 1992; Jackson et al., 1991; Davies and Bowen, 2000; Gullberg, 2007). When using blood-alcohol charts it was suggested that a mean result should be presented along with a 95% or 99% confidence interval (Arstein-Kerslake, 1986).

There is misleading information in some published papers concerning the pharmacokinetic parameters of ethanol, such as ß-slope and distribution volume (V_d). Very often this can be accounted for by the experimental design used, such as dose of ethanol being too small or that the subjects were not fasted overnight before drinking. If the C_{max} is fairly low (~0.02 g/dL or 0.2 g/L) it is virtually impossible to obtain a reliable elimination rate constant for ethanol by assuming zero-order kinetics, because the alcohol-metabolizing enzymes are not fully saturated. A failure to establish the entire post-absorptive portion of the BAC curve can explain some of the bizarre elimination rates of ethanol reported in the literature (Winek and Murphy, 1984).

Administration of alcohol too soon after eating a meal results in abnormally high volumes of distribution, and these might exceed unity and are therefore impossible. If V_d for ethanol is calculated as the ratio of dose/C_0 it is imperative to ensure that alcohol was consumed on an empty stomach or otherwise administered by intravenous infusion to guarantee 100% bioavailability. The best way to determine the elimination rate of ethanol from blood is to follow the entire post-peak elimination phase by taking repetitive samples at short intervals and then performing a linear regression analysis from which the slope of the declining phase of the curve is given by the regression coefficient (see Figures 3.18 and 3.30).

Taking two blood samples about one hour apart is not the best way to determine the elimination rate of ethanol in any individual case. However, in traffic law enforcement two blood samples one hour apart is all that is available (Jones, 2008). The elimination rate derived from ($BAC_2 - BAC_1$)/t_{diff} gives a reliable average value for this population but the tails of the distribution cannot be trusted (Jones, 2008). In a recent study of double blood samples in drunken drivers a relationship was established between the rate of elimination (g/L per h) and the underlying BAC, with higher rates reported at higher starting BAC (Simic and Tasic, 2007).

This review has hopefully managed to combine the two separate chapters from the previous edition of this book and also gives an update of current knowledge about the disposition and fate of alcohol in the human body. Many of the topics covered reflect to a large extent my own research interests and publications spanning over the past 30 years. This work has involved hundreds of alcohol dosing studies with healthy volunteers (mainly men) or patients admitted to hospitals for various medical conditions. Dose-response relationships and drug-alcohol interactions studies are needed to answer questions of current interest to forensic scientists.

An extensive bibliography of published articles derived from forensic science and legal medicine journals as well as those specializing in clinical and forensic toxicology and alcohol and substance abuse is included. When testifying as an expert witness in criminal cases it is important to ensure that the work cited has undergone peer-review and publication, a fact widely recognized and appreciated by forensic scientists worldwide (Saviers, 2002; Bohan and Heels, 1995; Kassirer and Cecil, 2002). Finally, it is important to recall that science does not exist until it has undergone peer-review and publication (Jones, 2007c).

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