ANTEMORTEM

5.2 MEASURING BLOOD-ALCOHOL CONCENTRATION FOR CLINICAL AND FORENSIC PURPOSES

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Measuring the concentration of alcohol in biological specimens (whole blood, serum, plasma, urine, saliva, and breath) involves the use of relatively simple analytical procedures. The first qualitative methods, albeit primitive, were used in forensic toxicology more than a century ago when deaths from gross intoxication were investigated.[1] Over the years, the methods available for measuring alcohol in blood and other body fluids have become increasingly refined.[2,3]

Unlike the results from other laboratory methods of analysis, the concentration of ethanol in body fluids has deep-rooted social-medical and forensic implications. This follows because of the commonly accepted association between a person's blood-alcohol concentration (BAC) and the degree of impairment.[4,5] Indeed, heavy drinking represents a major cause of accidents on the roads, in the workplace, and within the home, and alcohol abuse and drunkenness are contributing factor in many suicides, trauma deaths, violent crimes, and other kinds of deviant behavior.[6-9] The analysis of alcohol in body fluids has, therefore, emerged as the most frequently requested procedure in forensic science and toxicology.[10]

Furthermore, fast and reliable methods of alcohol analysis are needed in clinical and emergency medicine whenever a patient is admitted unconscious smelling of alcohol, because in these acute situations it is imperative to decide whether gross intoxication or head trauma or both are involved.[11,12] The presence of intracranial blood clots or hemorrhage resulting from head injuries requires swift diagnosis and treatment.[13-15] Moreover, overconsumption of ethanol needs to be quickly distinguished from intoxication caused by drinking a more dangerous alcohol such as methanol or ethylene glycol[16] so that a decision can be made to use invasive therapy including hemodialysis to clear from the blood the toxic metabolites of methanol (formic acid) and ethylene glycol (glycolate and oxalate).[17,18]

The role of alcohol intoxication in traffic accidents is well recognized[19-21] and threshold concentration limits have been defined by statute known as per se alcohol limits.[22] This legal framework means that a certain concentration of alcohol in a specimen of blood, breath, or urine is sufficient to decide a person's guilt or innocence and places extremely high demands on accuracy, precision, and selectivity of the methods of analysis used.[23] Besides threshold blood-alcohol concentration limits for driving, testing for alcohol in the workplace is now regulated by statutes in the U.S. (1991 Omnibus Transportation Employee Testing Act), and similar legislation can be expected to follow in other countries. The act permits conducting pre-employment testing as well as testing individuals engaged with safety-sensitive duties. including random testing, post-accident testing, probable cause testing, return-to-duty, and follow-up testing.[24] Two important concentration limits exist in connection with workplace alcohol testing and these are currently fixed at 20 mg/dL in blood (0.02 g/210 L breath), below which no action is taken. However, drinking on duty or having a blood-alcohol concentration

above 40 mg/dL (0.04 g/210 L breath) are prohibited and the offending individual will be removed from participating in safety-sensitive work.[24]

Punishment and sanctions for driving under the influence of alcohol (DUI) are becoming increasingly severe and include fines, suspension of the driving license, and sometimes a period of mandatory imprisonment or even dismissal and loss of salary in connection with workplace alcohol testing. Moreover, the validity of accident and insurance claims might be null and void if a person's blood-alcohol concentration exceeds some critical threshold limit. The connection between ingestion of alcohol, the person's BAC, and various penalties for over-consumption emphasizes the need to use highly reliable methods for measuring alcohol in blood and other body fluids.

This review deals with clinical and forensic aspects of measuring alcohol when the blood samples are obtained from living subjects. In post-mortem work, the choice of specimens, the method of collection, and, in particular, the interpretation of the analytical results require special considerations. These autopsy issues are covered in more detail in Section 5.3.

5.2.1 UNITS OF CONCENTRATION — PLASMA/SERUM VS. WHOLE BLOOD

One difference between reporting results of alcohol measurements made for clinical purposes and those made for forensic science purposes concerns the units of concentration used. In clinical chemistry laboratories, the SI system is the norm where the mole is the unit of mass and the liter is the unit of volume.[25] The concentration of alcohol in clinical biochemistry is therefore reported as mmol/L or mol/L. By contrast, in forensic science laboratories, the concentrations of ethanol in body fluids are reported in terms of mass per unit volume (mg/dL, g/L, g/dL or mg/mL) or mass per unit mass (g/kg or mg/g). The mass/mass unit of concentration is numerically less than the mass/volume unit by 5.5% because the specific weight of whole blood is 1.055 on the average (1 mL whole blood weighs 1.055 g), so a blood-alcohol concentration of 100 mg/dL is the same as 95 mg/100 g or 21.7 mmoL/L.

When blood samples are analyzed for forensic purposes, a standard procedure is to make duplicate determinations because close agreement between the two results gives the added assurance that a mishap has not occurred when the first determination was made. Besides reporting the mean concentration of alcohol, an allowance should be made to compensate for uncertainty in the method of analysis. This is easily done by making a deduction from the mean BAC to allow for analytical errors and thereby reporting a confidence limit such as 95%, 99%, or 99.9% depending on requirements.[26]

In Sweden, for example, a triplicate blood-alcohol determination is made in all DUI investigations and the lower 99.9% confidence limit on the mean is the value used for prosecution. The amount deducted is given by $3.09\left(SD/3^{1/2}\right)$ where SD is the standard deviation of a single determination at the prevailing BAC for an analytical process under statistical control. Although the mean result gives the best estimate of a person's BAC at the time of sampling, the value that remains after making the deduction is not more than the true BAC with a probability of 99.9%. This means that the risk of reporting a false high result is 1 in 1000 and any remaining uncertainty has relevance only for those individuals with a BAC very close to the critical legal limit for driving.

This practice of reporting analytical results together with confidence intervals for the individual blood-sample analyzed is uncommon in clinical laboratories where single determinations of an analyte are made. Instead, in clinical chemistry laboratories, variability or imprecision of an analytical method is monitored by calculating a coefficient of variation (CV%) derived from analyzing calibration standards or spiked biological specimens along with the unknowns.[27]

Another difference between the analytical work of forensic laboratories as opposed to clinical laboratories concerns the condition of the specimens submitted for analysis. Forensic

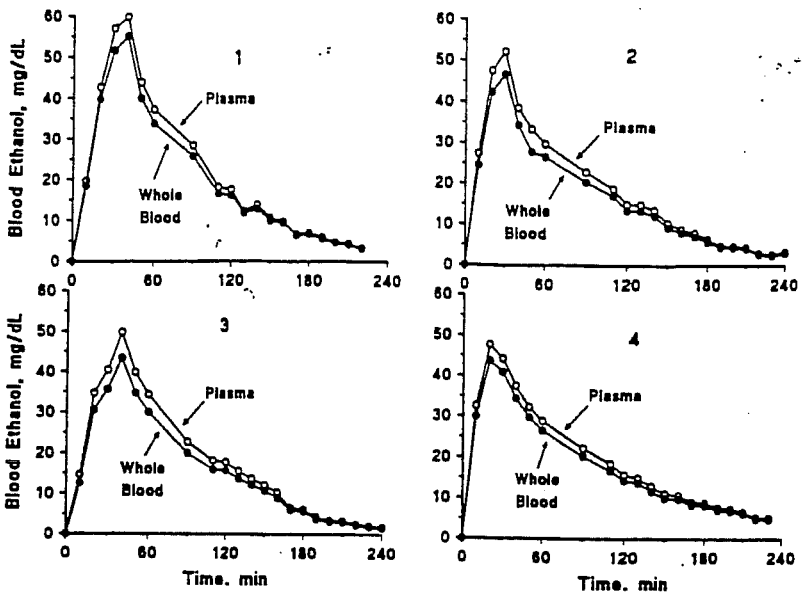

Figure 5.2.1a Concentration-time profiles of ethanol in specimens of whole blood and plasma from four healthy men who drank a bolus dose of ethanol 0.30 g/kg mixed with orange juice in 5 min after an overnight fast.

laboratories normally receive samples of whole blood and these are often hemolyzed and sometimes contain clots,[28] whereas clinical laboratories receive samples of plasma or serum.[29] The amounts of water in these specimens are not the same, with mean values of 91.8 % w/w (SD 0.49) for plasma/serum and 80.1 % w/w (SD 1.03) for whole blood.[30] The results of analyzing alcohol at a clinical laboratory should not be cited in legal proceedings concerning DUI or work-related accidents without an appropriate correction being made or seeking expert help with interpretation of the results.[31,32]

Figure 5.2.1a shows examples of the concentration-time profiles of ethanol in plasma and whole blood. Four healthy men fasted overnight before drinking, within 5 min, ethanol diluted with orange juice in a dose of 0.30 g ethanol per kg body weight. Note that the plasma curves run systematically higher than the whole-blood curves as expected from the water-content of the specimens analyzed. In these 4 subjects, the mean plasma/whole blood ratio of alcohol was 1.10:1 with a range from 1.08 to 1.13. In drinking drivers, the distribution of ethanol between plasma and whole blood had a mean of 1.14:1 (standard deviation 0.041) according to a recent study.[33] Table 5.2.1b compares the concentrations of alcohol in plasma with values expected to exist in whole blood based on a mean plasma/whole blood concentration ratio of 1.14:1. The results are reported in different units of concentration including SI units.

As discussed by Rainey,[29] if the concentration of alcohol in plasma or serum is used to estimate the concentration in whole blood for law enforcement purposes, it is advisable to consider inherent analytical and biological sources of variations in the plasma/blood relationship. A plasma/blood ratio of 1.22:1 corresponds to the mean + 2SD, and this higher conversion factor should be used in forensic work instead of a mean value of 1.14:1. This gives a more conservative estimate of BAC for use in criminal litigation if and when drunk drivers are prosecuted on the basis of the concentration of alcohol determined in plasma or serum. In criminal law, a beyond a reasonable doubt standard is necessary to obtain a verdict of guilty, whereas in civil litigation a preponderance of the evidence is sufficient to determine the outcome.[29]

Table 5.2.1b Comparison of the Concentrations of Ethanol in Whole Blood with Values Expected in Plasma or Serum when Expressed in Different Units of Concentration[a]

Concentration units mg/ml or g/L		Concentration units mg/dL or mg/100 ml		Concentration units g/% w/v or g/100mL		Concentration units mmol/l	
Blood	Plasma/ serum	Blood	Plasma/ serum	Blood	Plasma/ serum	Blood	Plasma/ serum
0.10	0.114	10	11.4	0.010	0.114	2.17	2.47
0.50	0.57	50	57	0.050	0.057	10.7	12.2
1.00	1.14	100	114	0.100	0.114	21.7	24.7
2.00	2.28	200	228	0.200	0.228	43	49.0
5.00	5.70	500	570	0.50	0.570	107	122

[a]*The concentrations in whole blood were derived from the concentration in plasma by dividing by 1.14. If the results are intended for use in forensic casework where a threshold limit in whole blood operates, then a more conservative conversion factor such as 1.22:1 should be used.*

5.2.2 METHODS OF MEASURING ALCOHOL IN BODY FLUIDS

5.2.2.1 Chemical Oxidation Methods

The first quantitative method of blood-alcohol analysis to gain general acceptance in forensic science and toxicology was published in 1922[34] and was known as Widmark's micromethod (developed by Erik MP Widmark of Sweden). A specimen of capillary blood (100 mg) was sufficient for making a single determination and this could be obtained by pricking a fingertip or earlobe. Ethanol was determined by wet-chemistry oxidation with a mixture of potassium dichromate and sulphuric acid in excess. The amount of oxidizing agent remaining after the reaction was determined by iodometric titration. Specially designed diffusion flasks allowed extraction of ethanol from the biological matrix by heating to 50°C before making the final titrimetric analysis. The Widmark method was not specific for measuring blood-ethanol because if other volatiles were present, such as acetone, methanol, or ether, these were oxidized and falsely reported as being ethanol. Evidence for the presence or absence of potential interfering substances was sometimes obtained by qualitative screening tests such as observation of various color changes after adding reagents to test for urinary ketones, methanol, formaldehyde, or acetaldehyde.[35]

By the 1950s, chemical methods were modified in various ways such as by the use of photometry to determine the endpoint of the oxidation reaction instead of volumetric titration. However, analytical procedures based on wet-chemistry oxidation are now virtually obsolete in clinical and forensic laboratories for measuring the concentration of alcohol in body fluids. The history, development, and application of chemical oxidation methods of alcohol analysis have been well covered in several review articles.[2,35-38]

5.2.2.2 Enzymatic Methods

Shortly after the enzyme alcohol dehydrogenase (ADH) was purified from horse liver and yeast in the late 1940s, the way was clear for developing biochemical methods for measuring alcohol in body fluids.[39-42] These became known as ADH methods and the milder conditions for oxidation of ethanol gave enhanced selectivity compared with wet-chemistry oxidation methods.[40,42] However, other primary alcohols such as isopropanol or n-propanol, if these are present in the blood samples, are also oxidized by ADH and this leads to false high concentrations of ethanol being reported.[43] By optimizing the reaction conditions in terms of pH, time, and temperature, methanol was not oxidized by yeast ADH and this source of the enzyme became widely used for clinical and forensic alcohol analysis.[42] In a typical manual ADH

method, blood-proteins were precipitated with $HClO_4$ or CCl_3COOH and after adjusting pH of the supernatant to 9.6 with semicarbazide buffer, the enzyme and coenzyme (NAD^+) were added to start the reaction. The NAD^+ is reduced to NADH in direct proportion to the concentration of ethanol present in the sample being analyzed and after about 1 h the amount of reduced coenzyme is monitored by its absorption of UV radiation at 340 nm.

Later developments in ADH methods meant that proteins in the blood could be separated from the aqueous phase on-line, either by dialysis or micro-distillation.[44] With this modification and the use of an AutoAnalyzer instrument, several hundred blood samples could be analyzed daily. Scores of publications were produced describing various modifications and improvements to the original ADH method and dedicated reagent kits soon became commercially available. These kits were ideal for use at hospital laboratories and elsewhere where the throughput of samples was relatively low. Otherwise, most efforts were directed towards automating the sample preparation and the dispensing of reagents to increase sample throughput and several batch analyzers appeared including a micro-centrifugal analyzer making use of fluorescence light scattering for quantitative analysis.[45,46]

Interest in the use of ADH methods for measuring alcohol in blood and urine have expanded greatly over the past decade owing to the development of methods for drug-abuse testing. In these new procedures, a technique known as enzyme multiplied immunoassay (EMIT) is used whereby an enzyme-labeled antigen reacts with ethanol and the color change of an added substrate is measured by spectrophotometry and the result translated into the concentration of ethanol. Fluorescence polarization immunoassay (FPIA) and the spin-off method known as radiative energy attenuation (REA) detection are other examples of analytical technologies developed to meet the increasing demand for drugs of abuse testing in urine and also for therapeutic drug monitoring. These analytical systems include options for measuring ethanol in biological specimens and results reported in several publications show good agreement in terms of accuracy and precision compared with those obtained by gas chromatography.[47-50] The principles and practice of various immunoassay systems suitable for clinical laboratory analysis were recently reviewed.[51]

Despite these new developments in analytical technology for blood-alcohol testing, particularly EMIT, FPIA, and REA methods, gas chromatography still is the instrument of choice at forensic laboratories owing to its superior selectivity. Indeed, some recent work has shown that serum lactate and lactate dehydrogenase might interfere with the analysis of alcohol by ADH methods.[52] This problem was traced to various side-reactions in which the coenzyme NAD^+ was reduced to NADH which could not be distinguished from the NADH produced during the oxidation of ethanol. This resulted in undesirable false positive results when plasma specimens from alcohol-free patients were analyzed.

5.2.2.3 Gas Chromatographic Methods

In the early 1960s, physical-chemical methods were applied to the analysis of alcohol in body fluids such as infrared spectrometry, electrochemical oxidation, and gas-liquid chromatography (GLC).[2] For the analysis of biological liquids, GLC has become the method of choice in clinical and forensic laboratories whereas electrochemistry and infrared methods are used for analyzing breath alcohol.[2,3] The first GLC methods required that ethanol was extracted from blood by use of a solvent (n-propyl acetate) or by distillation, but later the blood sample was simply diluted (1:5 or 1:10) with an aqueous solution of an internal standard (n-propanol or t-butanol).[53-57] This initial dilution step eliminated matrix effects so that aqueous alcohol standards could be used for calibration and standardization of the detector response. The use of an internal standard meant that any unexpected variations in the GC operating conditions during an analysis influenced the response of ethanol and the standard alike so the ratio of peak heights or peak areas (ethanol/standard) remained constant.[57]

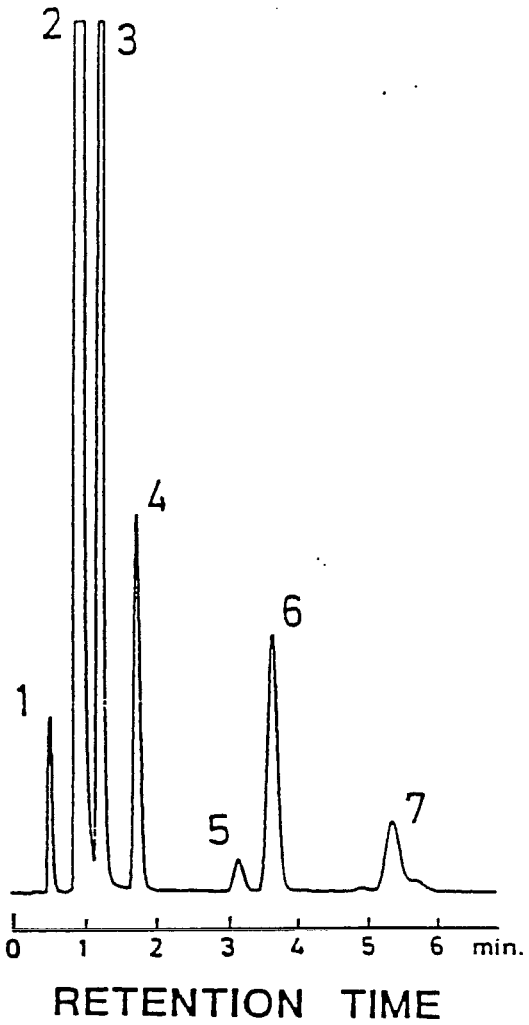

Figure 5.2 2.3a Gas chromatographic trace obtained from analysis of a blood sample containing eight volatile substances. The analysis was done by headspace gas chromatography using a packed column (2 m x 3 mm id.) containing Carbopack C (0.2% Carbowax 1500 on Carbopack 80-100 mesh) as the stationary phase. The components of the mixture were identified as follows: 1 = methanol, 2 = Ethanol, 3 = acetone, 4 = isopropanol, 5 = t-butanol, 6 = methyl ethyl ketone, 7 = 2-butanol.

About 1 to 5 µl of the diluted blood specimen was vapourized in a stream of nitrogen, as a carrier gas (mobile phase), which flowed through a glass or metal column having the dimensions 2 m long by 0.3 mm i.d. and which contained the liquid stationary phase spread as a thin film over an inert solid support material to provide a large surface area. The volatile components of a mixture distribute between the moving phase (carrier gas) and the liquid phase and depending on physicochemical properties such as the boiling point, the functional groups present, and the relative solubility in the liquid phase, either partial or complete separation occurs during passage through the column. Polar stationary phases were an obvious choice for the analysis of alcohols and polyethylene glycol with average molecular weights of 400, 600, 1500, etc. becoming widely available and known as Carbowax phases.[57] Otherwise, porous polymer materials such as Poropak Q and S served as packing materials for the GC columns when low-molecular weight alcohols were analyzed.

The effluent from the column was monitored continuously as a function of time with a thermal conductivity (TC) detector, but this was later replaced by a flame ionization (FI) detector, which was more sensitive and gave only a very small response to water vapor present in body fluids. The concentration of ethanol in blood was determined by comparing the detector response (peak height or peak area) obtained by analyzing identically treated known strength aqueous alcohol standards and making a calibration plot. Methodological details of many of the older GC methods of blood-alcohol analysis have been reviewed elsewhere.[58,59] A manual prepared by Dubowski[60] as a report to the U.S. Government is particularly detailed and makes a useful reference source for more information about GC methods of blood-alcohol analysis.

Gas chromatographic headspace (GC-HS) analysis (Figure 5.2 2.3a) is now the method of choice for the analysis of alcohol and other volatile substances in blood and tissue in forensic science and toxicology laboratories.[61-63] HS-GC requires that the blood samples and aqueous standards are first diluted (1:5 or 1:10) with an aqueous solution of an internal standard and the mixture is then allowed to equilibrate at 50 or 60°C in glass vials kept airtight with crimped-on rubber septums. Prolonged heating of the blood specimen at 60°C converts some of the ethanol into acetaldehyde by a non-enzymatic oxidation reaction involving oxyhemoglobin. This undesirable effect can be avoided by pretreating the blood specimen with sodium

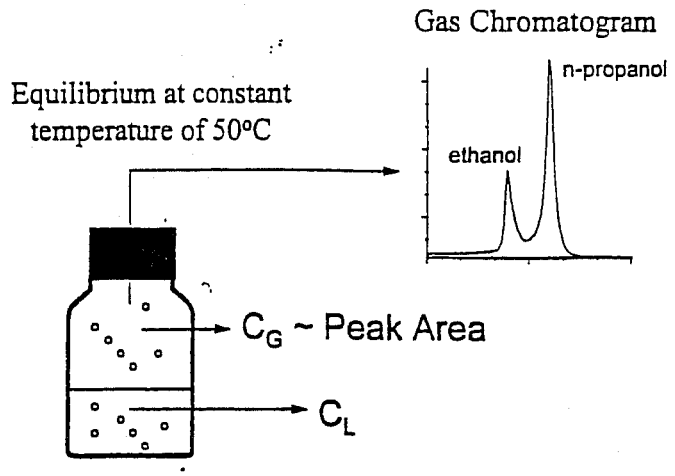

Figure 5.2.2.3b Schematic diagram of the headspace sampling procedure for gas chromatographic analysis. The biological sample is first diluted 1:10 with an aqueous solution of an internal standard such as n-propanol or t-butanol and then made air-tight in a small glass flask with crimped on rubber membrane. After reaching equilibration at 50°C for 15 to 20 min, a sample of the vapor above the diluted blood specimen is removed with a gas-tight syringe or by an automated sampling arrangement and injected into the carrier gas (N_2) for transport through the chromatographic column containing the stationary phase. The resulting trace on a strip-chart recorder shows a peak for ethanol followed by a peak for the internal standard (n-propanol).

azide or sodium dithionite[64,65] or using a lower equilibrium temperature (40 or 50°C) also hinders this oxidation reaction.

The headspace sampling procedure is shown schematically in Figure 5.2.2.3b where a portion of the vapor in equilibrium with the diluted blood sample is removed with the aid of a gas-tight syringe or by an automated headspace sampling device and a highly reproducible injection made onto the chromatographic column. Several manufacturers offer dedicated equipment for headspace analysis and the Perkin-Elmer company has dominated this market since the early 1960s. Various GC headspace instruments have been produced and, in chronological order, these were denoted Multifract HS-40, HS-42, HS-45, and more recently HS-100 mounted on a Sigma 2000 gas chromatograph. This latter combination allows batch analysis of up to 100 samples in a single run but unfortunately this option is no longer available because the HS-GC units currently marketed have the capacity to hold only 40 specimens. This seriously limits the number of blood specimens that can be analyzed in a single run because several calibration control standards and blanks must also be included.

Packed, wide-bore, and capillary columns are feasible[66] for use with headspace gas chromatography. For high resolution work such as when complex mixtures are being analyzed, capillary columns are essential. Traditional packed columns are, however, more robust and those made of glass or stainless steel with dimensions such as 2 m long x 3 mm i.d. are still widely used for routine blood-alcohol analysis because the number of volatile components that might be present is fairly limited.[2,3] Figure 5.2.2.3a shows an example of a gas chromatogram obtained by headspace analysis of an eight-component mixture of low-molecular volatile substances including ethanol with a glass column packed with Carbopak C (0.2% Carbowax 1500 on graphitized carbon black 80 to 100 mesh).

Because HS-GC involves sampling the vapor in equilibrium with the blood specimen, the non- volatile constituents of the biological matrix do not accumulate and clog the GC column or contaminate the packing material. Sensitivity of the assay can be enhanced and matrix effects eliminated in another way, namely by saturating the blood samples and aqueous ethanol

standards with an inorganic salt such as NaCl or K_2CO_3, e.g., 0.5 ml blood + 1 g salt.[67,68] This salting-out technique has proven useful for analyzing trace concentrations of volatiles in blood such as the endogenous alcohols.[69] The advantages of salting-out methods have been discussed in many publications including the comprehensive review by Dubowski.[60,64]

Analysis of blood by headspace GC was combined with a cryofocusing technique, or freeze trap, to concentrate the specimen prior to chromatographic separation of the volatile components with a capillary column. This modification was applied to the assay of complex mixtures of volatiles, for example, the congeners present in alcoholic beverages to determine whether these might be identified in blood samples after drinking.[70]

Gas chromatographic methods of analysis offer the unique advantage of combining a qualitative screening analysis of the components of a mixture based on the time after injection to the appearance of the peak (retention time) with simultaneous quantitative analysis by measuring the detector response as reflected in the height or area under the peak. Several recent reviews have dealt with the use of gas chromatography in forensic science including applications for blood- alcohol analysis.[71,72] Another good review looked at more general applications of headspace analysis when applied to biological specimens for analysis of organic volatile substances, including alcohols.[73]

In forensic work, it is advisable for duplicate determinations to be made on two different column packing materials, thus furnishing different retention times for ethanol. This is important if the blood or tissue samples are putrefied and therefore might contain interfering substances having the same retention times as ethanol when a single stationary phase is used. The risk of obtaining coincident retention times on two or more stationary phases is reduced considerably. Otherwise, two different methodologies such as GC and chemical oxidation or GC and enzymatic oxidation could be used to analyze duplicate aliquots from the same blood sample.[74] HS-GC with two different detectors (flame ionization and electron capture) has been used to screen biological fluids for a large number of volatiles. This dual-detector system was recommended for use in clinical toxicology to aid in the diagnosis of acute poisoning when a host of unknown substances might be responsible for the patients condition.[75]

5.2.2.4 Other Methods

A multitude of other analytical methods has been described for blood-alcohol analysis but none of these can match HS-GC which has become the gold standard in forensic and clinical toxicology laboratories. Methods of measuring blood-alcohol concentration by headspace analysis with electrochemical sensing[76] or a metal oxide semiconductor device[77] were reported, but these are not sufficiently selective when interfering substances might be present. These procedures could be useful to rapidly screen biological samples and thus eliminate specimens that do not contain any alcohol. A modified headspace procedure with a fuel-cell sensor was described for measuring the strength of alcoholic beverages and the results obtained compared favorably with a standard gas chromatographic method.[78]

Several novel methods for analysis of alcohol make use of biosensors prepared from immobilized enzymes. These constructions, called bioelectrodes, have found several applications in clinical laboratory analysis.[79] The end-point of the enzymatic reactions can be monitored either by amperometry, colorimetry, or spectrophotometry.[80-82] The enzyme alcohol oxidase has attracted attention for analysis of alcohol in body fluids and gives reasonably good semi-quantitative results.[83-85] These systems are similar in principle to measuring blood glucose with a glucose oxidase electrode and open-up the possibility for self-testing applications such as the glucose dipstick technology.[86] Fourier transform infrared spectrometry (FTIR) was recently applied to the determination of alcohol in beer[87] and when a purge-and-trap capillary GC separation stage was included, FTIR could also be adopted to measure a wide range of low-molecular weight volatiles including ethanol.[88] A method based on proton nuclear magnetic

resonance spectroscopy proved suitable in pharmacokinetic studies to analyze ethanol, acetone, and isopropanol in plasma samples.[89,90]

In clinical and emergency medicine, freezing point depression osmometry has a long history as a screening test for certain pathological states.[91] Diabetes mellitus and uraemia, often associated with abnormally high concentrations of plasma-glucose and plasma-urea, respectively, cause discrepancies between the osmolality expected from the inorganic ions Na+ and K+ and the values measured by depression of the freezing point. Dedicated equipment is available for measuring freezing-point depression and the test can be done on as little as 0.2 ml plasma. Moreover, the method is non-destructive which means that the same specimen of plasma can be used later for making a toxicological confirmatory analysis if necessary.

The most common cause of finding an increased osmolal gap in plasma samples from emergency service patients is a high concentration of ethanol.[91] Ethanol carries an appreciable osmotic effect because of its low molecular weight (46.05), high solubility in water, and the fact that large quantities are ingested to produce intoxication.[92] Finding a normal osmolal gap speaks against the presence of high concentrations of plasma-ethanol but the reverse conclusion does not hold because ingestion of other solvents such as acetone, methanol, isopropanol, or ethylene glycol, also lowers the freezing point resulting in increased serum or plasma osmolality. The principal limitation of freezing point osmometry as a rapid screening test for plasma-ethanol is obviously the lack of selectivity because other non-electrolytes could be falsely reported as ethanol. Nevertheless, articles continue to be published dealing with the principles and practice of freezing point osmometry in emergency toxicology.[93]

Considerable interest has developed in point-of-care or near patient testing and this treatment paradigm speaks in favor of noninvasive methods of biochemical analysis. Near-infrared spectrometry is a technique with huge prospects and possibilities for the future because drawing blood or puncturing the skin is not necessary.[94] Infrared light is beamed through a subject's fingertip or earlobe and after processing the absorption bands of the emitted light into specific wavelengths, various constituents in the bloodstream can be identified and for some substances a quantitative analysis is possible. However, disentangling the signals of interest from the background noise generated by other biological molecules has proven a challenging problem but progress is rapidly being made with the help of sophisticated computer-aided pattern recognition techniques. Near-infrared spectroscopy has already been successfully applied to the analysis of blood glucose[95] and it will not be long before attention is given to the analysis of drugs of abuse including ethanol.

The feasibility of combining gas chromatography (GC) to separate the volatile components in a mixture and mass spectrometry (MS) as the detector was demonstrated many years ago.[96] GC-MS provides an unequivocal qualitative analysis of ethanol from its three major mass fragments m/z 31 (base peak), m/z 45, and m/z 46 (molecular ion).[96] Selected ion monitoring and deuterium labelled ethanol was used to distinguish between ethanol formed post-mortem by the action of bacteria on blood glucose.[97,98] Also, in clinical pharmacokinetics, ordinary ethanol was analyzed in blood along with its deuterium-labeled analogue to investigate the bioavailability of ethanol and the role of first-pass metabolism in the gut.[99]

5.2.3 BREATH ALCOHOL ANALYSIS

A small amount of the alcohol a person drinks is expelled unchanged in the breath and breath-alcohol measurements provide a fast and non-invasive way to monitor alcohol in the body. A large literature base exists describing the principles and practice of breath-alcohol analysis and the design and evaluation of a multitude of breath-testing instruments for research, clinical, and forensic purposes.[100-102] Analysis of the expired air is also an indirect way to monitor the concentration of other volatile endogenous substances in the pulmonary blood and this

approach has found many interesting applications in clinical and diagnostic medicine.[103,104] However, the main application of breath-alcohol analysis is in the field of traffic law enforcement for testing drunk drivers and more recently also for workplace alcohol testing.[2,24,102] Two categories of instruments for breath-alcohol analysis are available depending on whether the results are intended as a qualitative screening test for alcohol or for quantitative evidential purposes.

Various hand-held devices are being used for roadside pre-arrest screening of drinking drivers to indicate whether their BAC or BrAC exceeds a certain threshold statutory concentration limit for driving.[2,102,105,106] The instruments used for evidential purposes provide a quantitative analysis of BrAC and the result is used as binding evidence for prosecuting drunk drivers.[107-109] Breath-alcohol instruments have also found applications in clinical pharmacokinetic studies of ethanol and drug-alcohol interactions.[110] Hand-held breath-alcohol analyzers are very practical for use in emergency medicine as a quick and easy way to monitor whether patients have been drinking and to estimate the alcohol load in the body.[111-113]

Most of the hand-held screening devices incorporate electrochemical fuel-cell sensors that oxidize ethanol to acetaldehyde and in the process produce free electrons. The electric current generated is directly proportional to the amount of ethanol consumed by the cell. Acetone, which is the most abundant endogenous volatile exhaled in breath, is not oxidized at the electrode surface so this ketone does not give false-positive responses.[114,115] However, if high concentrations of methanol or isopropanol are present in exhaled breath, these are oxidized in the same electrochemical reaction but at different rates compared with ethanol.[113] Care is sometimes needed when the results are interpreted because isopropanol might be produced naturally in the body by the reduction of endogenous acetone.[115] The concentration of acetone in blood reaches abnormally high levels during food deprivation, prolonged fasting (dieting), or during diabetic ketoacidosis.[114]

Most of the evidential breath-testing instruments used today identify and measure the concentration of alcohol by its absorption of infrared energy at wavelengths of 3.4 or 9.5 microns, which correspond to the C-H and C-O vibrational stretching in the ethanol molecules, respectively.[2,100] Selectivity for identifying ethanol can be enhanced by combining infrared absorption at 9.5 microns and electrochemical oxidation within the same unit. The Alcotest 7410 features this dual-sensor technique. Another example from this new generation of breath-test instruments is the Intoxilyzer 6000, which makes use of five different IR wavelengths for identification of ethanol. This reduces considerably the risk of interfering substances being incorrectly reported as ethanol.

Later chapters give additional details of pre-arrest screening instruments as well as more sophisticated units for evidential testing with microprocessor control of the entire breath-test sequence including the volume of breath exhaled, the temperature of breath, and the actual BrAC concentration-time profile. A 15-min observation period before conducting an evidential breath-alcohol test is an important part of the testing protocol to avoid problems with mouth alcohol disturbing the results.[116,117] The presence of mouth alcohol can sometimes be disclosed by looking at the slope of the breath-alcohol concentration profile during a prolonged exhalation.[100] Otherwise, the BrAC measured after the first few seconds of exhalation can be compared with the BrAC reached after an end-exhalation as a way to disclose mouth alcohol. If the result in the first test exceeds the final result, this suggests the presence of mouth alcohol, either caused by recent ingestion or by regurgitation of stomach contents or spontaneous gastro-esophageal reflux. The results of the breath-alcohol test as well as demographic details about the suspect can be printed out directly on-the-spot or stored in the computer memory and down-loaded later to a central computer network for producing summary statistics and quality control charts.

Reporting results of breath-alcohol analysis is a bit confusing and this depends on whether these are intended for use in clinical and emergency medicine or traffic law enforcement. To

Table 5.2.4.4 Inter-Laboratory Proficiency Test of Blood-Alcohol Analysis Performed at Specialist Forensic Toxicology Laboratories in the Nordic Countries[a]

Laboratory	Blood-1	Blood-2	Blood-3	Blood-4	Blood-5	Blood-6
1	0.46	1.01	2.15	1.62	0.74	1.75
2	0.47	1.01	2.27	1.70	0.78	1.83
3	0.46	1.01	2.26	1.67	0.77	1.81
4	0.47	1.00	2.17	1.66	0.78	1.81
5	0.48	1.01	2.15	1.66	0.78	1.79
Mean	0.47	1.01	2.20	1.66	0.77	1.80
SD	0.008	0.005	0.060	0.029	0.017	0.030
CV%	1.7%	0.49%	2.7%	1.7%	2.2%	1.7%

[a]*The blood samples were obtained from apprehended drinking drivers and collected into tubes containing fluoride and oxalate and small portions removed for sending to the participating laboratories. The between-laboratory CVs were always less than 3% and the corresponding within-laboratory CVs were mostly less than 1% (data not shown).*

test if a patient is under the influence of alcohol for clinical purposes, the BrAC is generally converted into the presumed concentration in venous blood. This requires the use of a calibration factor called the blood/breath ratio so that BrAC × factor = BAC. This blood/breath factor is assumed to be a constant for all individuals and 2100:1 has traditionally been accepted for legal purposes.[2,3,102] In many U.S. states a concentration of 0.10 g% in blood is equated with 0.10 g/210 L breath and this 2100:1 relationship is therefore affirmed by statute.[100]

However, many empirical studies have shown that calibration with a factor of 2100:1 tends to give results that underestimate the venous BAC by about 10% when near simultaneous samples are taken 1 to 2 h after the end of drinking.[108] A closer agreement between blood and breath-alcohol is obtained when a 2300:1 factor is used for calibration.[108] Analytical precision improves considerably if BrAC is reported directly instead of estimating the coexisting venous BAC. In experiments with an evidential breath-analyser (DataMaster), the BAC was estimated with a 95% confidence interval of 15 mg/dl compared with 5 mg/230 L when BrAC was reported directly.[118]

Note that breath-alcohol instruments are calibrated to estimate the concentrations of alcohol in whole blood and not the concentration in plasma or serum and this is often overlooked by many clinicians who seem to consider blood and plasma concentrations of alcohol as being the same. To derive the concentration of alcohol in plasma or serum indirectly by the analysis of breath, the breath-test instrument should be calibrated with a plasma/breath factor of about 2600:1 because whole blood contains about 14% less alcohol than the same volume of plasma or serum (Table 5.2.1). Nowadays, when breath-alcohol testing is used for traffic law enforcement the results are almost always reported as the concentration of alcohol in the breath without considering the persons BAC. This avoids making any assumptions about the blood/breath ratio and its variability between and within individuals. Statutory limits for driving in many countries are therefore written in terms of threshold blood and breath-alcohol concentration depending on the specimen analyzed.

5.2.4 QUALITY ASSURANCE ASPECTS OF ALCOHOL ANALYSIS

Much has been written about quality assurance of clinical laboratory measurements and concepts such as precision, accuracy, linearity, recovery, sensitivity, and limits of detection and

quantitation have been discussed in detail elsewhere.[119] In addition, when the results are used as evidence in criminal and civil litigation, the chain of custody record of the specimens is extremely important to document. This chain must be kept intact from the moment of sampling to the moment the results are reported and each person involved in the handling, transport, analysis, and storage of the specimen must be traceable from these written records. The entire procedure including the actual chromatographic traces as well as evidence of correct calibration on the day the specimens were analyzed and also internal and external control tests might need to be documented several months or years later. Important details concerning pre-analytical, analytical, and post-analytical aspects of blood-alcohol analysis are presented below.

5.2.4.1 Pre-Analytical Factors

Information should be given to the subject about the reason for taking a blood-sample and, if necessary, informed written consent should be obtained. The equipment used for drawing blood is normally an evacuated tube (5- or 10-ml Vacutainer tubes) and sterile needle attachment. The blood sampling site is usually an antecubital vein and if necessary a tourniquet can be used to dilate a suitable superficial vein. Sufficient blood should be taken to allow making several determinations of the blood-alcohol concentration and any re-testing that might be necessary as well as the assay of drugs of abuse. The specimen tubes should be gently inverted a few times immediately after collection to facilitate mixing and dissolution of the chemical preservatives; sodium fluoride (10 mg/mL) to inhibit the activity of various enzymes, micro-organisms, and yeasts, and potassium oxalate (5 mg/mL) as an anticoagulant. The tubes of blood should be labeled with the person's name, the date and time of sampling, and the name of the person who took the sample. The Vacutainer tubes containing blood should be sealed in such a way as to prevent unauthorized handling or tampering and special adhesive paper strips are available for this purpose. The blood samples and other relevant paper-work should be secured with tape so that any deliberate manipulating or adulteration is easily detected by laboratory personnel after shipment. After taking the samples, the tubes of blood should be stored in a refrigerated room before being sent to the laboratory by express mail service.

Although pre-analytical factors are more important to consider and control when endogenous substances are analyzed, such as in clinical chemistry, a standardized sampling protocol is also important for forensic blood-alcohol analysis. Two tubes of blood should be drawn in rapid succession and the skin cleaned with soap and water and not with an organic solvent such as ether, isopropanol, or ethanol. Obviously, the blood samples should not be taken from veins into which intravenous fluids are being administered.[120] This kind of emergency treatment is often given as a first-aid to treat patients suffering from shock or trauma as a result of involvement in traffic accidents. The blood samples should be taken only by trained personnel such as a phlebotomist, registered nurse, or physician.

5.2.4.2 Analytical Factors

The blood specimens must be carefully inspected when they arrive at the laboratory, making a note whether or not the seals on the package as well as the individual tubes of blood are intact, if there are any blood-clots, and if the blood seems to have been diluted with other liquids. Details of any mishaps during transport (breakage, leakage of blood), as well as the date and time of arrival should be recorded. The information written on the Vacutainer tubes should be compared with other documentation to ensure the suspect's name and the date and time of sampling are correct. The same unique identification number or barcode should be added to all paperwork and biological specimens received and this number used to monitor passage of the specimens through the laboratory. Ensure that the erythrocytes and plasma fractions are adequately mixed before removing aliquots of whole blood for analysis. Replicate determina-

tions can be made with different chromatographic systems and preferably by different technicians working independently.[26] Any unidentified peaks on the gas chromatograms should be noted because these might indicate the presence of other volatiles in the blood sample.

5.2.4.3 Post-Analytical Factors

Quality assurance of individual results can be controlled by looking at critical differences (range) between replicate determinations. The size of the difference will be larger the higher the concentration of ethanol in the blood specimen because precision tends to decrease with an increase in the concentration of ethanol. Control charts offer a useful way to monitor day-to-day performance in the laboratory; one chart being used to depict precision and another the accuracy of the method by analyzing known strength standards together with unknowns. These charts make it easy to detect the presence of outliers.[26, 121,122] The rate of loss of alcohol during storage needs to be established under refrigerated conditions (+ 4°C) and also when specimens are kept deeply frozen.[123,124] If necessary, corrections can then be made if the blood specimens are reanalyzed after prolonged periods of storage. The chromatographic traces and other evidence corroborating the analytical results, such as calibration plots or response factors, should be carefully labeled and stored safely in fire-proof cabinets. It might be worthwhile to ensure that the laboratory is accredited for making forensic toxicological analyses and the results of external proficiency trials should also be made available for scrutiny.

5.2.4.4 Inter-Laboratory Proficiency

Two papers recently looked at the results from inter-laboratory proficiency tests of blood-alcohol analysis at clinical chemistry laboratories.[125,126] In one study, originating from Sweden, all participating laboratories used gas chromatography for analysis of plasma-ethanol and the coefficients of variation between laboratories were within the range 10 to 17%.[125] In a similar study among UK laboratories, the corresponding CVs depended in part on the kind of methodology used for the analysis of alcohol. Immunoassays generally performed worse than gas chromatographic methods (liquid injection and headspace technique) and the CVs ranged from 8% to 20%.[126]

Table 5.2.4.4 presents results from a recent inter-laboratory comparison of blood-alcohol analysis made at specialist forensic toxicology laboratories in the Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden). All participants used headspace gas chromatography to analyze ethanol and the blood samples were obtained from apprehended drinking drivers. The coefficient of variation between laboratories was always less than 3% regardless of the concentration of alcohol, which testifies to highly standardized analytical work. The corresponding CVs within laboratories were mostly 1% or less based on 3 to 6 determinations per sample. If the overall mean BAC in each sample is taken as the target value, then all the laboratories showed an accuracy to within 5% of that attributed.

5.2.5 FATE OF ALCOHOL IN THE BODY

Ethanol is a small polar molecule and its low molecular weight and weak charge allow it to penetrate easily through biological membranes. After ingestion, ethanol is absorbed readily from the stomach but absorption occurs much faster from the upper part of the small intestine where the available surface area is much larger owing to the presence of microvilli on the mucosal cells. Both the rate and extent of absorption is delayed if there is food in the stomach before drinking.[127] Blood draining from the gastrointestinal tract collects in the portal vein where the alcohol is transported through the liver, and then on to the heart and the systemic circulation. The metabolism of alcohol occurs mainly by the action of enzymes located in the liver although recent research suggests that small amounts of alcohol might be metabolized in

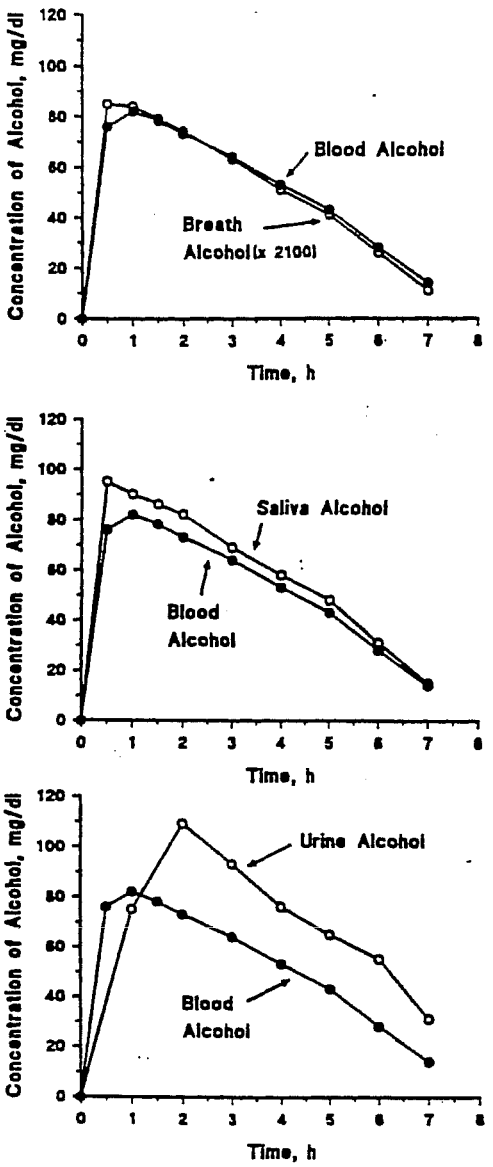

Figure 5.2.5 Mean concentration-time profiles of ethanol in blood vs. breath (x 2100), blood vs. saliva, and blood vs. urine from experiments with healthy men who ingested 0.68 g ethanol per kg body weight as neat whisky in the morning after an overnight fast.

the mucosa of the stomach.[128] After absorption, ethanol distributes uniformly throughout all body fluids and tissue without binding to plasma proteins. Provided that the systemic availability of the dose is 100%, such as when administered by i.v. infusion, it is possible to determine total body water by the ethanol dilution method.[129]

The peak blood-alcohol concentration after drinking, as well as the time of reaching the peak, varies widely from person to person and depends on many factors. After 48 healthy male volunteers drank 0.68 g ethanol/kg body weight as neat whisky on an empty stomach, the peak concentration in capillary (fingertip) blood was reached at exactly 10, 40, 70, and 100 min after the end of drinking for 23, 14, 8, and 3 subjects, respectively.[130] The quantities consumed, the rate of drinking, the dosage form (beer, wine, spirits, cocktails) and most importantly the rate of gastric emptying impacts on the speed of absorption.[131] The concentrations of ethanol in body fluids and tissues after reaching equilibration will depend on the water contents, the ratio of blood-flow to tissue perfusion, as well as various time elements.[110] Figure 5.2.5 shows the mean concentration- time profiles of ethanol in blood, breath, urine, and saliva obtained from experiments with healthy male volunteers who drank 0.68 g/kg as neat whisky in 20 min after an overnight fast.[132]

The bulk of the dose of alcohol entering the bloodstream (95 to 98%) is eliminated from the body by metabolism which takes place mainly in the liver by the action of class I enzymes of alcohol dehydrogenase (ADH).[127] Between 2 to 5% of the dose is excreted unchanged in breath, urine, and sweat, and a very small fraction is conjugated with glucuronic acid.[133] Small amounts of alcohol might undergo pre-systemic oxidation by ADH located in the gastric mucosa or the liver or both, but the quantitative significance of first-pass metabolism (FPM) is still an unsettled question.[134]

At moderate BAC (>60 mg/dL), the microsomal enzymes (P450IIE1), which have a higher km (60 to 80 mg/dL) compared with ADH (km 2 to 5 mg/dL), become engaged in the metabolism of ethanol.[135,136] The P450 enzymes are also involved in the metabolism of many other drugs and environmental chemicals which increases the risk of drug-alcohol interactions and this might account for the toxicity of ethanol in heavy drinkers and alcoholics.[137,138] Moreover, the activity of P4502E1 enzymes increase after a period of continu-

ous heavy drinking owing to a faster *de novo* synthesis of the enzyme, and metabolic tolerance develops as reflected in two- to threefold faster rates of elimination of alcohol from the bloodstream in alcoholics.[139-141]

The effects of ethanol on performance and behavior are complex and involve an interaction with membrane receptors in the brain associated with the inhibitory neurotransmitters glutamate and gamma aminobutyric acid (GABA).[142,143] The behavioral effects of ethanol are dose-dependent and drinking small amounts initially produces feelings of euphoria followed by depression and stupor after large doses are ingested. Ethanol's depressant effects are related to an altered flux of ions through the chloride channel activated by GABA.[144] The fact that ethanol modifies neuro-transmission at the GABA receptor also helps to explain the cross-tolerance observed with other classes of drugs such as the benzodiazepines and barbiturates, which also bind to the GABA receptor complex to produce their effects on brain functioning.[143]

Although ethanol-induced impairment shows a reasonably good overall correlation with the coexisting blood-alcohol concentration, there are large variations in response for different individuals who drink the same amount of alcohol within the same time frame. The reasons for this are twofold; first, larger people tend to have more body water so the dose of alcohol enters a larger volume resulting in lower BACs compared with lighter people with less body water. This phenomenon is known as consumption tolerance and is caused primarily by variations in body weights and the relative amount of adipose tissue which is influenced by age, race, and gender. The second reason for the inter-individual differences in ethanol-induced effects on performance and behavior is called concentration tolerance caused by gradual habituation of brain cells to the presence of alcohol during exposure to the drug over long periods. Besides the development of acute tolerance, which appears after a single exposure (see Chapter 1), a chronic tolerance develops after a period of continuous heavy drinking. The mechanisms accounting for chronic tolerance seem to depend on changes in the composition of cell membranes, particularly the cholesterol content, the structure of the fatty acids, and also the arrangement of the phospholipids in the membranes.[142-144]

In occasional drinkers, the impairment effects at specific blood-alcohol concentrations are often classified according to various stages of intoxication as evidenced by the clinical signs and symptoms observed. This notion was first proposed by Bogen[145] but has been subsequently developed further and improved by others. At a BAC of 10 to 30 mg/dL slight changes in performance and behavior can be demonstrated with highly specialized tests such as divided attention. Between 30 to 60 mg/dL, most people experience euphoria, becoming more talkative and sociable. At a BAC between 60 to 100 mg/dl, a marked euphoria and excitement is often reported with partial or complete loss of inhibitions and in some individuals judgement and control are seriously impaired. When the BAC is between 100 and 150 mg/dL, which are concentrations of ethanol seldom reached during moderate social drinking, psychomotor performance deteriorates markedly and articulation and speech become obviously impaired. Between 150 and 200 mg/dl, ataxia is pronounced and drowsiness and confusion are evident in most people. Controlled studies at high BAC with moderate drinkers are lacking because of the gross intoxication produced, but anecdotal information exists from the field of emergency medicine. The relationship between BAC and clinical impairment is well documented in drunk drivers who often attain very high BACs of 350 mg/dL or more but most of these individuals are obviously chronic alcoholics.[146]

It is important to note that the magnitude and appearance of various signs and symptoms of inebriation depend to a great extent on the rate of drinking and whether the person starts from zero BAC, that is after a period of abstinence or with a residual BAC from a recent drinking spree.[147,148] In the latter case, the severity of intoxication is less pronounced because previous consumption of alcohol means that the individual has acquired some degree of tolerance. Drinking too much too fast is dangerous and if gastric emptying occurs rapidly, the

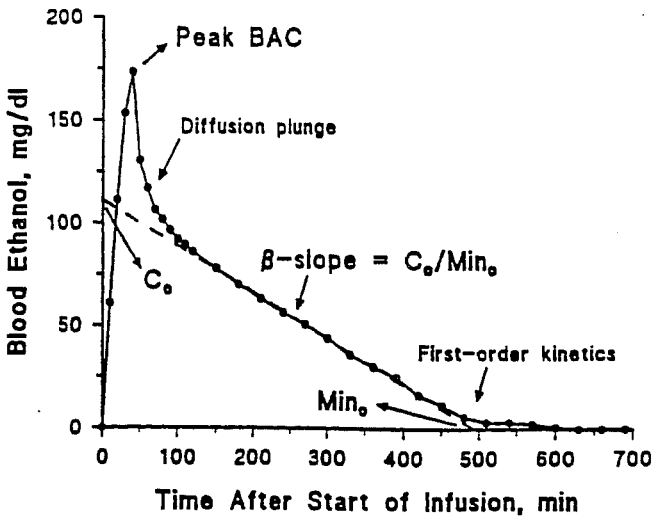

Figure 5.2.6.1 Individual concentration-time profile of ethanol in venous blood after an intravenous administration of 0.8 g/kg at a constant rate over 30 min. Key pharmacokinetic parameters are defined on this trace.

BAC increases with such a velocity that the vomit reflex in the brain is triggered. This physiological response to acute alcohol ingestion has probably saved many lives.

5.2.6 CLINICAL PHARMACOKINETICS OF ETHANOL

The discipline known as pharmacokinetics deals with the way that drugs and their metabolites are absorbed, distributed, and metabolized in the body and how these processes can be described in quantitative terms.[149,150]

5.2.6.1 Widmark Model

The clinical pharmacokinetics of ethanol have been investigated extensively since the 1930s thanks to the early availability of a reliable method of analysis in small volumes of blood.[151] Figure 5.2.6.1 shows a typical blood-alcohol concentration-time profile after intravenous infusion of 0.80 g ethanol/kg body weight at a constant rate for 30 min. The key pharmacokinetic parameters are defined on this trace in accordance with the model developed by Widmark.[151] The peak BAC coincides with the end of the infusion period being followed by a diffusion plunge during which time the ethanol equilibrates between well perfused and poorly perfused organs and tissues. At about 90 min post-infusion, the BAC starts to decrease at a constant rate per unit time according to zero-order kinetics and the slope of this rectilinear disappearance phase is commonly referred to as the alcohol burn-off rate or β-slope. However, specialist texts in pharmacokinetics refer to the zero-order elimination slope as k_o instead of β.[152] When the BAC decreases below about 10 mg/dL, shown in Figure 5.2.6.1, after a time of 450 min, a curvilinear disappearance phase starts to develop and this lasts for as long as alcohol is still measurable in the blood. The elimination of alcohol now follows first-order kinetics and the rate constant is denoted k_1 and clearance from the bloodstream during this terminal phase has a half-life of about 15 min.[153]

The first person to make a comprehensive mathematical analysis of BAC profiles was Erik MP Widmark and details of his life and work were recently published.[154] Widmark introduced the following equation to represent the elimination kinetics of alcohol from blood:

$$C_t = C_o - \beta t \quad (1)$$

where

C_t = blood alcohol concentration at some time t on the post-absorptive part of the curve.
C_o = blood alcohol concentration extrapolated to the time of starting to drink.
β = rate of elimination of alcohol from blood.
t = time in minutes.

The rate of elimination of alcohol from the blood in moderate drinkers falls within the range of 10 to 20 mg/dL/h with a mean value of about 15 mg/dL/h.[129-131] Higher values are seen in drinking drivers (mean 19 mg/dL/h)[155] and in alcoholics undergoing detoxification (mean 22 mg/dL/h).[156] The faster burn-off rates seen in heavy drinkers are probably a consequence of boosting the microsomal enzymes (P450IIE1) owing to prolonged exposure to high concentrations of ethanol. The P450IIEI enzymes have a higher K_m (60 to 80 mg/dL) compared with ADH (2 to 5 mg/dL) and the slope of the elimination phase tends to be steeper starting from a higher initial BAC as in alcoholics compared with moderate social drinkers.[157,158] In a controlled study with alcoholics undergoing detoxification, the mean slope was 22 mg/dL/h with a range from 13 to 36 mg/dL/h.[156] Liver disorders such as alcoholic hepatitis and cirrhosis did not seem to influence the rate of disposal of alcohol in these individuals.[127,156]

The rate of elimination of alcohol from the blood was not much influenced by the time of day when 0.75 g/kg was administered at 9 AM, 3 PM, 9 PM, and 3 AM, according to a recent investigation into chronopharmacokientics of ethanol.[159] However, gastric emptying seems to occur faster in the morning as reflected in a 32% higher peak BAC and an earlier time of its occurrence when 1.1 g/kg alcohol was consumed between 7.15 and 7.45 AM, compared with the same time in the evening.[160] Smoking cigarettes slows gastric emptying and as a consequence delays the absorption of a moderate dose (0.50 g/kg) of ethanol resulting in a lower peak BAC in smokers.[161]

By extrapolating the rectilinear elimination phase back to the time of starting to drink gives the y- intercept (C_o), which corresponds to the theoretical BAC if the entire dose was absorbed and distributed without any metabolism occurring (Figure 5.2.6.1). The empirically determined value of C_o will always be greater than the ratio of dose/body weight because whole blood is 80% w/w water compared with the body as a whole which is 60% w/w on average for men and 50% w/w for women. The apparent volume of distribution (V_d) of alcohol is obtained from the ratio of dose (g/kg) divided by C_o and in clinical pharmacology textbooks the V_d has units of liters/kg.[152] However, because BAC in Widmark's studies was reported in units of mg/g or g/kg, the ratio dose/C_o known as the Widmark r factor is a ratio without any dimensions and should be 5.5% greater than Vd reported in units of L/kg.[154,162]

Values of the distribution factor r differ between individuals depending on age and body composition particularly on the proportion of fat to lean tissue. Obviously, the value of r will also depend on whether whole blood or plasma specimens were used to plot the concentration-time profile and extrapolating to determine C_o.[129] As shown in Figure 5.2.1, the plasma-alcohol curves run on a higher level compared with whole blood-alcohol curves because of the different amounts of water in these specimens. According to Widmark, the relationship between alcohol in the body and alcohol in the blood at equilibrium can be represented by the following equations.

$$A/(p \times r) = C_o \quad (2)$$

$$A = C_o \times (p \times r) \quad (3)$$

where

A = amount of alcohol in grams absorbed and distributed in the body.
p = body weight of the person in kg.
r = Widmarks r factor.
C_o = y-intercept (Figure 5.2.6.1)

These equations make it easy to calculate the amount of alcohol in the body from the concentration determined in a sample of blood provided that the value of r is known and that absorption and distribution of alcohol are complete at the time of sampling blood.

In the fasting state, the factor r will depend on age, gender, and body composition and Widmark reported mean values of 0.68 for 20 men (range 0.51 to 0.85) and 0.55 for 10 women (range 0.49 to 0.76).[151] However, in many later studies with more volunteer subjects, it was found that average values of r were 0.70 L/kg for men and 0.60 L/kg for women with 95% confidence limits of about ±20%.[127] Widmark's equations for β and r can be easily combined by eliminating C_o to give the following equation:

$$A = pr(C_t + \beta t) \quad (4)$$

The above equation can be used to estimate the total amount of alcohol absorbed from the gastrointestinal tract since the beginning of drinking. By rearrangement, the blood alcohol concentration (C_t) expected after intake of a known amount of alcohol is calculated with the help of the following equation.

$$C_t = (A/pr) - \beta t \quad (5)$$

When calculating BAC from the dose administered or vice versa, it is necessary to assume that the systemic availability is 100% and that complete absorption and distribution of alcohol in the body water compartment has occurred at the time of sampling blood. Furthermore, individual variations in β and r introduce uncertainty in the calculated dose (A) or BAC (C_t) when average values are applied to a random subject from the population. The amount of individual variation was recently estimated as ±20% for 95% confidence limits in tests with over 100 subjects.[163] However, in the entire population of drinking drivers, these limits can be expected to be much wider.

5.2.6.2 Michaelis-Menten Model

Because the class I ADH enzymes have a low km (3 to 5 mg/dL), they become saturated with substrate after 1 to 2 drinks and the rate of disappearance of ethanol from blood therefore follows zero-order kinetics over a large segment of the post-absorptive elimination phase (Figure 5.2.6.1).[127,131] When the blood-alcohol concentration decreases below about 10 mg/dL, the ADH enzymes are no longer saturated and the curve changes to a curvilinear disappearance phase (first-order kinetics). However, these low blood-alcohol concentrations are not very relevant in forensic science work.

It was suggested and shown by Lundquist and Wolthers[164] that the entire post-absorptive elimination phase (zero-order and first-order stages) might be rationalized by an alternative pharmacokinetic model, namely that of saturation kinetics. They fitted the Michaelis–Menten equation to BAC-time data including the very low concentrations (<10 mg/dL) thanks to the availability of a highly sensitive ADH method for blood-alcohol analysis. By solving the integrated form of the M-M equation, the parameters V_{max} and k_m were determined. This approach based on the Michaelis–Menten equation or saturation kinetics was later strongly advocated by many specialists in pharmacokinetics, among others, Wagner, Wilkinson, and their colleagues[165-167] and values of 22 mg/dL/h and 5 mg/dL have been reported for V_{max}

and k_m, respectively.[167] Although the use of M-M kinetics has found some support among forensic scientists,[168] others have not considered its use worthwhile when dealing with actual casework[169] because so many other variable factors and uncertainties influence the absorption, distribution, and elimination of ethanol. Moreover, the mathematical concepts needed to understand and apply M-M kinetics are more challenging than those necessary to derive the Widmark equation. Explaining the scientific principles of pharmacokinetic modeling to a judge and jury, as is sometimes necessary in DUI litigation, is prohibitive. Moreover, the notion of multiple enzyme systems being involved in the metabolism of ethanol such as the various isozymes of ADH, including genetic variations and the contribution of P450IIE1 to the disposal of ethanol after chronic ingestion (metabolic tolerance), are not strictly compatible with the use of a single enzyme system required by the Michaelis-Menten equation.[170,171]

5.2.6.3 First-Pass Metabolism and Gastric Alcohol Dehydrogenase

Recent research efforts indicate that a small part of the alcohol a person consumes is metabolized before it reaches the systemic circulation and this process is known as first-pass metabolism (FPM).[172,173] Some of the alcohol ingested is seemingly cleared from the blood either in the stomach or during the first-pass of the portal blood through the liver. The magnitude of first-pass metabolism depends on many factors and seems to be greater when very small doses of ethanol (0.15 g/kg) are administered and particularly when alcohol is taken together with or after a meal presumably because under these conditions there is a longer time available for contact between alcohol and the enzyme-rich mucosal surfaces in the stomach.[172]

Distinguishing between first-pass metabolism occurring in the stomach as opposed to the liver has proven a difficult task and much debate has arisen about the significance of gastric ADH in accounting for FPM.[174,175] Some workers maintain that gastric metabolism of ethanol plays a significant role in the overall disposal of ethanol especially when emptying of the stomach is delayed and the absorption phase is more prolonged.[173] Others consider that negligible amounts of alcohol undergo presystemic oxidation in the stomach mainly because the amount of gastric ADH is only a small fraction of the amount of hepatic ADH available.[175] Moreover, the main advocates of gastric ADH and FPM of ethanol failed to consider the critical importance of stomach emptying and its influence on the rate of absorption of alcohol.[176] For drugs that obey saturation kinetics, the bioavailability should become more variable as a consequence of changes in absorption rate which modulates the fraction escaping first-pass metabolism.[177] Factors that influence the rate of absorption of alcohol from the gut (food, drugs, type of beverage, posture, time of day) also influence the concentration entering the liver and the corresponding degree of saturation of the metabolizing enzymes.[177]

Interest in FPM escalated even further after several common medications such as aspirin and H_2-receptor antagonists (cimetidine and ranitidine) were shown *in vitro* to inhibit ADH[178-181] extracted from gastric biopsies.[179] This observation lead to much speculation about adverse-drug reactions and the role of gastric-ADH as a protective barrier against the toxicity of ethanol in some individuals.[182] Proponents of FPM argued that if alcohol was taken together with these common medications, this would result in the peak BAC being higher than expected probably leading to a more pronounced impairment of body functions.[180,182] Accordingly, those individuals lacking gastric ADH or exhibiting reduced enzymatic activity such as women, oriental populations, and alcoholics, might be more susceptible to the toxic effects of heavy drinking.[173,183] These conclusions proved too hasty because many later studies into the effects of cimetidine and ranitidine on the pharmacokinetics of ethanol failed to confirm the initial reports showing higher peak BAC and AUC and enhanced bioavailability.[184-187]

What did emerge from this new wave of interest in clinical pharmacokinetics of ethanol was strong and convincing information documenting the large inter- and intra-individual variations

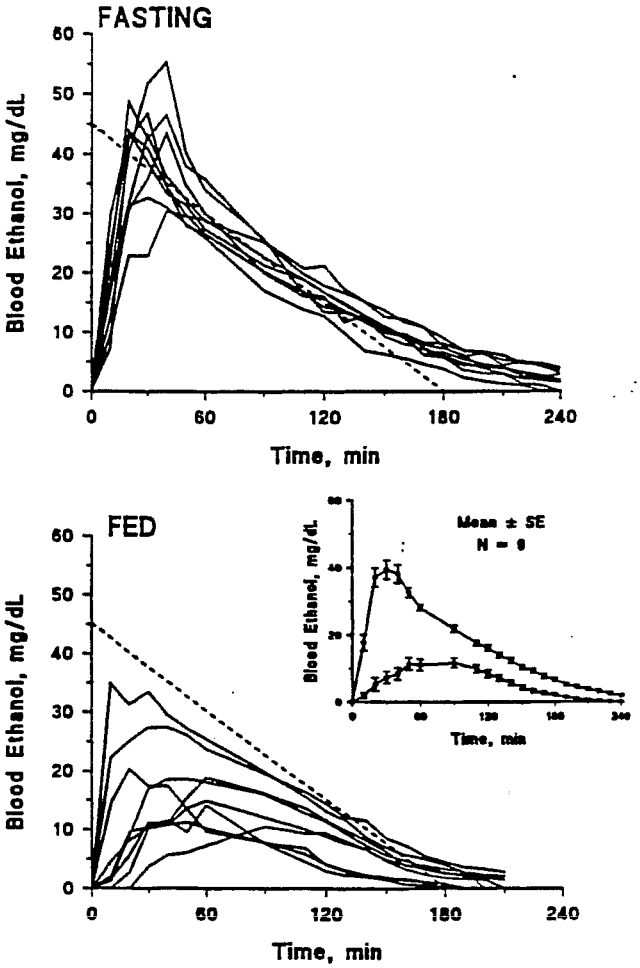

Figure 5.2.6.4 Individual concentration-time profile of ethanol in venous blood from experiments with nine subjects who drank 0.30 g ethanol/kg body weight in 10 min either after an overnight fast or after eating breakfast 1 h before drinking alcohol. Note the large inter-individual variations and much lower peak BAC with smaller area under the curve and the shorter time to eliminate the dose of alcohol in the fed-state. The insert figure shows mean ±SE for fed and fasting conditions.

in the pharmacokinetic profiles of ethanol especially when small doses (0.15 to 0.3 g/kg) were ingested after a meal.[188-191]

5.2.6.4 Food and Pharmacokinetics of Ethanol

Having food in the stomach before drinking retards the absorption of ethanol and the peak BAC and the initial impairment effects are considerably diminished compared with drinking the same dose on an empty stomach.[151,154,192,193] The bioavailability of ethanol is markedly reduced whenever consumption takes place after a meal and estimating values of r or V_d under these conditions give results that are too high, suggesting a loss of ethanol. The composition of the meal in terms of its fat, protein, or carbohydrate content was less important in this respect.[194-197] Figure 5.2.6.4 gives examples of pharmacokinetic profiles of ethanol in venous blood for nine subjects who drank 0.30 g ethanol per kg either on an empty stomach (overnight fast) or exactly 1 h after eating a protein-rich breakfast. The large inter-individual variations in peak BAC, area under the curve (AUC) are clearly evident. The broken diagonal lines show the slope and position of the zero-order elimination phase expected for a standard man with a volume of distribution of 0.70 liters/kg and an elimination rate constant of 15 mg/dL/h and when the bioavailability of alcohol was 100%. The observed BAC profiles were considerably lower than the values expected in every single case when the alcohol was consumed after the meal, which implies that some of the alcohol escapes being absorbed into the blood. Whether this reflects an active first-pass metabolism (stomach or liver or both) or whether an accelerated metabolism primarily takes place early after drinking during the absorption phase is not known.[134]

It seems that a prerequisite for finding an appreciable FPM was that the subjects had eaten food prior to drinking alcohol.[198] This raised a question about the role of stomach emptying and the speed of absorption of alcohol as a determinant of FPM.[176,181,199] In Figure 5.2.6.4 (insert), food taken 1 h before drinking not only lowers peak BAC and AUC but also seems

to boost the rate of elimination of ethanol.[193] This might be explained by a more efficient extraction of ethanol in the liver when the absorption from the gut is slow, and more prolonged, such as when alcohol is taken after eating a meal.[176] Food increases liver blood flow and this might facilitate a more effective exposure of ethanol to the metabolizing enzymes in the liver.[194,196] Whatever the mechanism, eating a meal before drinking is an effective way to produce a lower and later occurring peak BAC, a faster rate of clearance of ethanol from the body, and diminished feelings of inebriation.[192]

5.2.7 CONCLUSIONS

Ethanol tops the list of drugs of abuse in most countries, and too much drinking is a well-known cause of reckless behavior. Drunk drivers are over-represented in crashes and deaths on the highway and alcohol intoxication is the common denominator in many accidents within the home and in the workplace. Alcohol abuse and alcoholism are major public health hazards with enormous costs for the individual and society. Measuring alcohol in body fluids will continue to be the most frequently requested procedure in analytical toxicology for a long time to come. Few substances can be determined with such a high degree of accuracy, precision, and selectivity as the concentration of ethanol in a person's blood.[26] The analytical phase of the procedure is hard to fault especially when methods such as headspace gas chromatography are used at an accredited laboratory.[26] Making duplicate determinations is an effective safeguard against various mishaps occurring during the analysis and the aliquots of blood analyzed should be taken from two separate tubes.[26] Moreover, to enhance selectivity, at least two different chromatographic systems can be used thus providing different retention times for ethanol.[200] Alternatively, an independent assay method such as ADH or chemical oxidation can be run in parallel with GC.[73]

More attention needs to be given to the pre-analytical factors including the condition of the subject, the way the blood sample was taken, and the circumstances surrounding the sampling procedure. This becomes significant whenever blood samples are taken from victims of traffic accidents who require emergency hospital treatment, such as administration of drugs or intravenous fluids, to counteract shock. It is important to remember that the result of a chemical or biochemical test is only as good as the sample received. When the analytical results make the difference between punishment or acquittal, as in trials concerning driving under the influence of alcohol, pre-analytical factors are as important to control and document as analytical factors.

Widmark's method continues to dominate the way that forensic scientists and others deal with the pharmacokinetics of alcohol when requested to calculate the amount ingested from a single measurement of BAC.[131] Making back extrapolations of BAC is not recommended because of the wide variations in absorption, distribution, and elimination patterns of ethanol both within and between different individuals.[188-192,201] This becomes especially important when small doses of alcohol (0.3 g/kg) are taken after a meal because under these conditions the bioavailability of alcohol might be reduced by as much as 60% (Figure 5.2.6.4).[189] Warnings about adverse drug- alcohol interactions, e.g., after taking medication such as aspirin and H_2-receptor antagonists, before drinking alcohol and the risk of obtaining higher peak BAC and larger performance decrements seem to have been much exaggerated.[184-187]

Other ways of studying the pharmacokinetics of alcohol have been proposed including the use of Michaelis-Menten kinetics[202] and other saturation-type models.[203-205] Recently a three-compartment model[206] as well as a non-compartment model[207] were used to explore the disposition and fate of alcohol in the body in quantitative terms.

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5.3 MEASURING ALCOHOL POSTMORTEM

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The technical aspects of measuring ethanol in body fluid samples are little different if the sample is obtained from a corpse rather than a living person. However, the interpretation of the analytical results obtained from autopsy samples is confounded by problems such as the lack of homogeneity of blood samples, microbial alcohol production postmortem, alcohol diffusion from gastric residue and contaminated airways, and the lack of or unreliability of information on the clinical condition of the person immediately prior to death. On the other hand, autopsy offers opportunities for sampling body fluids and tissues not accessible or not readily available in the living. Sampling of blood from multiple vascular sites, the vitreous humour of the eye, gastric contents, sequested hematomas, as well as bile, brain, skeletal muscle, cerebrospinal fluid, and liver are all possible. Nevertheless, multiple sampling at autopsy can only partly compensate for the increased interpretative difficulties created by the various postmortem confounding factors. As a result, it is necessary to apply a greater degree of caution in the interpretation of postmortem ethanol analyses and to take into account the totality of the available information which should always include not only the results of the autopsy examination but also the scene of death examination and anamnestic data. A single autopsy blood ethanol level is commonly uninterpretable without concurrent vitreous humour and urine ethanol levels as well as information gleaned from the scene of death and case history.

5.3.1 POSTMORTEM BLOOD

5.3.1.1 Physical Properties and Site Dependence

Within a few hours of death, the blood within the vascular system clots and simultaneously there is clot lysis. The effectiveness of the clot lysis will determine whether a blood sample obtained at autopsy is clotted, completely fluid, or partly clotted and partly fluid. The fibrin clots invariably entrap large numbers of red blood cells so that the resulting clot is relatively red cell rich and serum poor. Occasionally the heart and great vessels may contain a large two-layered clot, the lower part typically red clot and the upper part pale yellow rubbery clot largely devoid of red cells (so-called "chicken fat"). Consequently "blood samples" obtained at autopsy are variable in their red cell and protein content and this will have some influence on the measured ethanol concentration because ethanol is distributed in the water portion of blood. Blood obtained from limb vessels is most likely to be fluid and largely devoid of clots and therefore provides as homogenous a-sample for analysis as can be hoped for. The presence of blood clots in themselves do not influence the accuracy of blood alcohol analysis using headspace gas chromatography.[1]

5.3.1.2 Water Content and Ethanol Content

Serum and plasma contain approximately 10 to 15% more water than whole blood. Because ethanol is distributed in the water portion of blood, it can be expected that the plasma ethanol content is approximately 10 to 15% higher than the corresponding whole blood concentration. This should be borne in mind whenever alcohol has been measured in serum or plasma in hospital prior to death or where a pre-mortem hospital sample of serum or plasma is subsequently analyzed for alcohol. In 134 blood samples from healthy men and women,[2] the mean blood alcohol concentration was 105.2mg/dL (range 21.8 to 154.8) and the mean serum alcohol concentration was 120.8mg/dL (range 25.0 to 183.1) with a mean serum:blood ratio of 1.15 (range 1.10 to 1.25, SD 0.02); 3 of the 134 serum:blood ratios were between 1.21 and 1.25.

A larger study on 235 subjects[3] produced a similar mean serum:blood ratio of 1.14 with a range of 1.04 to 1.26 and a normal distribution with SD of 0.041. Ethanol concentration in red blood cells was reported in 167 of these subjects and red cells:blood ethanol ratios ranged from 0.66 to 1.00 with a mean of 0.865 and a negatively skewed distribution with a SD of 0.065. Given this data it is evident that there may be significant differences in ethanol concentrations between different blood samples obtained at the same time from the same corpse because an autopsy "blood" sample may range in composition from being largely red cells at one extreme to largely plasma at the other. However, in practice, most autopsy blood samples will tend to be plasma-rich rather than red blood-cell rich because autopsy sampling procedures tend to avoid clots and favor clot-free fluid.

The whole blood water content decreases postmortem and, because ethanol is distributed only in the water phase of the body, this will cause the blood alcohol concentration to decrease. In a study of 71 cadavers,[4] a blood sample taken within 10 h of death (mean 2.1 h, range 0 to 9.6 h) the water content ranged between 72.4% and 89.3%, mean 80.4%, which is closely similar to the water content of blood from living persons (79.9 to 82.3% for women and 77.5 to 80.6% for men). Second samples taken from the same cadavers from 8 to 229 h postmortem had a lower water content ranging between 64.4 and 88.0%, mean 74.0%. However, observed differences in the blood alcohol concentration between the two sampling times were more strongly influenced by other postmortem factors, such as putrefaction, than by water content changes, so that correcting a postmortem blood alcohol for water content is not generally recommended.

5.3.2 BLOOD ETHANOL LEVELS

5.3.2.1 Reported Lethal Ranges

The lethal range of blood ethanol levels is based on published case reports of human fatalities and the experimentally derived LD50s of 500 to 550 mg/dL in rats, guinea-pigs, chickens, and dogs.[5] However, a review of actual cases suggests that the blood alcohol level which may be potentially lethal is 250 to 300 mg/dL rather than the higher figures commonly quoted.[6] (For fatal blood ethanol levels in various series, see Niyogi[7].) The often quoted lethal blood ethanol range of above 400 or 450 mg/dL may only apply to uncomplicated deaths as a result of acute alcohol poisoning in inexperienced drinkers. A review of fatalities with a blood ethanol level above 300mg/dL disclosed 502 attributable to acute ethanol poisoning alone, but 24 resulting from well-documented natural causes, 260 from obvious trauma or violence, and 28 with a combination of a high ethanol level and additional contributing or related abnormalities, emphasising the complexity of interpreting the significance of high blood ethanol levels at autopsy.[8]

5.3.2.2 Contributory Asphyxia in Fatalities

In a series of 115 deaths attributed to acute alcoholism, 59% showed some asphyxial element, either postural asphyxia or inhalation of vomit. For this reason it is particularly important to have accurate documentation of the position of the body as found and any evidence of inhalation of vomitus at the scene of death because passive regurgitation of gastric contents and contamination of the airways may occur postmortem during removal of the body to the mortuary. In many of these deaths in which asphyxia is a contributing factor, the urine alcohol is considerably higher than the blood alcohol suggesting that the mechanism of death was coma resulting from a high blood alcohol level with subsequent respiratory embarrassment and anoxia. In these fatalities, the blood alcohol level observed at autopsy is not the level causing death but rather the level with which the person dies.[6]

5.3.2.3 Effects of Tolerance

A high autopsy blood ethanol concentration, although indicating chemical intoxication at the time of death, does not necessarily imply that there were observable clinical manifestations of drunkenness and this is particularly so in chronic alcoholics.[9] Alcohol abusers develop a tolerance to ethanol to the extent that they can maintain ethanol levels in the potentially lethal range. In an Australian study,[10] blood alcohol levels were determined in chronic alcoholics presenting to a detoxification service. Of the 32 subjects, all appeared affected by alcohol with 23 showing altered mood or behavior, 6 appearing confused, and 3 appearing drowsy but none were stuporous or comatose. All displayed ataxia and dysarthria of varying degrees. The blood ethanol concentration ranged from 180 to 450 mg/dL with a mean of 313 mg/dL and 26 of the 32 were above 250 mg/dL. A similar Swedish study[11] identified 24 patients who attended a hospital casualty department and were found to have blood ethanol concentrations above 500mg/dL. Of 16 on whom full data were available, 8 were either awake or could be aroused by non-painful stimuli. All left hospital alive within 24 h. It is suggested that this tolerance to high blood ethanol levels seen in chronic alcoholics is primarily the result of neuronal adaptation. Physical dependence on ethanol, as demonstrated by the development of withdrawal signs and symptoms on stopping drinking, similarly indicates the existence of an adaptational process.

There are anecdotal descriptions of alcoholics surviving remarkably high levels of blood alcohol. In one instance,[12] a 24-year-old female chronic alcohol presented at the hospital with abdominal pain. She was agitated and slightly confused but alert, responsive to questioning,

and orientated to person and place, though unclear as to time. Her serum ethanol was 1510 mg/dL, which corresponds to a concentration in whole blood of about 1310 mg/dL. After 12 h treatment with intravenous fluids, electrolyte replacement, chlordiazepoxide, and intensive care monitoring, she felt well, and was symptomless at discharge two days later. Similarly a 52-year-old, 66-kg male was found unconscious in a bar with a blood ethanol concentration of 650 mg/dL and survived with minimum treatment comprising protection against aspiration and the occasional use of oxygen.[13] A 23-year-old, 57-kg female chronic alcoholic admitted comatose with a blood ethanol level of 780 mg/dL had apparently consumed 390 ml of absolute alcohol in the form of a bottle of bourbon. Eleven hours later she was discharged with a blood ethanol level of 190 mg/dL; the disappearance of ethanol from her blood seemingly followed a logarithmic function.[14] Two further case reports describe more stormy clinical courses but with survival, one with a serum ethanol level of 1127 mg/dL,[15] the other with a blood ethanol level of 1500 mg/dL.[16] This tolerance to high blood ethanol levels seen in chronic alcoholics makes it difficult to interpret the significance of a blood ethanol level obtained at autopsy from such a person.

On the other hand, non-lethal levels of ethanol may be of particular significance in some types of death. Ethanol adversely affects thermal regulation and, depending upon the ambient temperature, may cause either hypothermia or hyperthermia.[17] There is a large body of experimental and clinical data available regarding the hypothermic effect of alcohol on both animals and humans at different degrees of cold exposure. The importance of ethanol in hyperthermic deaths is less well appreciated but illustrated by Finnish sauna fatalities. In a series of 228 hyperthermic deaths (221 sauna related), alcohol had been consumed in 192 cases and the consumption was categorized as "heavy" in 61.[18] Similarly, complex cerebral dysfunctions induced by alcohol are thought to be significant in the syndrome of sudden, alcohol-associated, cranio-facial traumatic death.[19] In this syndrome, individuals who have collapsed and died at the scene of an assault are found at autopsy to have facial trauma insufficient to account for the death together with a high but non-lethal blood alcohol concentration.

5.3.2.4 Interaction of Alcohol with Other Drugs

The simultaneous presence of another drug with ethanol further complicates the interpretation of the concentrations measured at autopsy. Ethanol is a central nervous system depressant and a similar synergistic effect is found for other hypnotic drugs as well as antidepressants and narcotic analgesics so that allowance for ethanol-drug synergism is necessary when autopsy drug levels in blood are interpreted.[20] Although it has been proposed that carbon monoxide and ethanol may have an additive affect, there is no conclusive evidence of this.

The interplay between ethanol and both prescription drugs and drugs of abuse may be complex also. It has been suggested that ethanol enhances the acute toxicity of heroin and that ethanol use indirectly influences fatal heroin overdose through its association with infrequent (non-addictive) heroin use and thus a reduced tolerance to the acute toxic effects of heroin.[21] The use of cocaine with ethanol can produce a toxic metabolite, cocaethylene, which may be more important in determining lethality than the parent cocaine.[22]

The well-known interaction between ethanol and disulfiram (tetraethylthiuram disulphide) results from the inhibition of acetaldehyde dehydrogenese which converts acetaldehyde to acetate when ethanol is metabolized. Disulfiram is used in aversion therapy of chronic alcoholism, although its clinical effectiveness has been debated. In a person taking the drug, subsequent ingestion of alcohol produces numerous unpleasant symptoms which are probably the result of the toxic accumulation of acetaldehyde. Fatalities have been reported with relatively low blood ethanol levels and with acetaldehyde blood concentrations between 12 to 41 mg/L.[23]

Table 5.3.3.1 Prediction of Critical Values of BAC (as mean, or lower limit of 95% and 99% prediction intervals) from Vitreous Humor Alcohol Concentration (mg/dL)[37]

Observed VHAC mg/dL	Predicted BAC mg/dL		
	Mean	95% PI	99% PI
90	80	29 - 131	13 - 147
150	131	80 - 182	64 - 198
169	147	96 - 198	80 - 214
173	150	100 - 201	83 - 217
232	201	150 - 251	134 - 268
251	217	166 - 268	150 - 284

PI = prediction interval for the determination of a single BAC value. Thus, for an observed VHAC of 90 mg/dL the best estimate of BAC is 80 mg/dL; there is a 95% probability that the true value of BAC is between 29 and 131 mg/dL; and a 99% probability that the true value is between 13 and 147 mg/dL.

5.3.3 VITREOUS ALCOHOL

Analysis of vitreous humour is useful to corroborate a postmortem blood alcohol and assist in distinguishing antemortem intoxication from postmortem alcohol production. It can serve also as an alternative sample if a satisfactory postmortem blood sample is unavailable or contaminated. In most cases, the specimen is easily obtained and can be sampled without a full autopsy. Vitreous humour is a clear, serous fluid which is easy to work with analytically. Its anatomically isolated position protects it from bacterial putrefaction. In microbiological studies of vitreous obtained from 51 cadavers between one and five days postmortem, it was found that none of the samples contained large numbers of bacteria and only one contained fungi.[24]

5.3.3.1 Relationship to Blood Alcohol Concentrations

The predictive value of a known vitreous humour alcohol concentration (VHAC) in estimating an unknown blood alcohol concentration (BAC) in an individual case remains contentious, despite many studies.[25-36] Authors have provided various formulae, including a simple conversion factor, to predict BAC from VHAC but these do not take into account the uncertainty of the prediction for an individual subject. In the largest series,[37] of 345 cases, simple linear regression with BAC as outcome y-variable and VHAC as predictor x-variable (range 1 to 705 mg/dL) gave the regression equation BAC = 3.03 + 0.852 VHAC with 95% prediction interval ± 0.019 ([7157272 + (VHAC-189.7)²]. The practical application of the regression equation is shown in Table 5.3.3.1. Set out are the VHAC values which predict key BAC values of 80 and 150 mg/dL as the mean, or the minimum value at either the 95 or 99% prediction interval for the determination of a single BAC value. The prediction interval is too wide to be of much practical use. In addition, re-analysis of the raw data from previous publications gave significantly different regression equations in most instances. From an evidential viewpoint, it would be unreasonable to give an estimate of the mean BAC based upon the VHAC without also providing the 95% prediction interval which is a measure of the degree of confidence attached to the estimate in an individual case.[38-40]

Blood has a lower water content than vitreous so the expectation is that the blood:vitreous alcohol ratio will be less than unity. In cases where the ratio of blood to vitreous humour alcohol concentration exceeds 1.0, the most likely explanation is that death occurred before diffusion equilibrium had been attained and this observation may be of forensic significance.[27] Animal studies[41,42] indicate that, following intraperitoneal or intravenous injections of ethanol, BAC:VHAC ratios may be greater than 0.95 for 30 min or longer. A study of 43 fatalities[31] disclosed a bi-modal distribution of blood:vitreous alcohol ratios with the first mode from 0.72 to 0.90 and a positively skewed distribution from 0.94 to 1.37. It seems that the first mode of the distribution ratio represents the elimination phase of the blood alcohol curve and that the second mode represents the absorption phase prior to equilibrium being established. A second study of 86 cases confirmed this bi-modal distribution and suggested that a blood:vitreous ratio greater than 0.95 indicates that death occurred before equilibrium had been achieved, and therefore in the early absorptive phase.[34] The blood:vitreous ratio during this early phase had a mean of 1.09 (SD = 0.38) in contrast to the late absorptive and elimination phases where the mean was 0.80 (SD = 0.09). Others[33] failed to reproduce this bi-modal distribution. However, most deaths occur during the elimination phase and it is clear that the observation of a bi-modal distribution of blood:vitreous alcohol ratios in any study depends upon the inclusion of cases dying in the absorptive phase. It is likely that the proportion of absorptive phase cases included in published series has varied considerably and that this accounts, at least in part, for the differences in published BAC:VHAC ratios.

5.3.3.2 Postmortem Diffusion and Embalming

It seems reasonable to assume that ethanol may diffuse into or out of the vitreous postmortem. The chemical constituents of embalming fluid may diffuse into the vitreous humour after a body has been embalmed. Fortunately almost all commercial embalming fluids are free of ethyl alcohol, although they commonly contain methanol. Assessment of ethanol levels in 38 subjects pre- and post-embalming suggested that there was no significant effect on the vitreous humour ethanol concentration in the immediate aftermath of embalming.[43] However, in one case the embalmer cleaned the globus of the eye with ethanol on a cotton swab prior to placing an eye cap into position and this caused an elevation of vitreous ethanol from 0 to 340 mg/dL. Conversely, prolonged submersion of a body in water may result in diffusion of alcohol out of the vitreous. This was the proposed explanation for finding a zero ethanol concentration in vitreous but concentrations of 370mg/dL in urine and 223mg/dL in blood (blood acetone 46mg/dL) in a man submerged in cold fresh water for about 6 weeks. In a rabbit model which duplicated these circumstances, the vitreous ethanol level fell from a mean of 196mg/dL to 30mg/dL over the 6-week period.[44]

5.3.4 URINARY ALCOHOL

The ureteral urine, which is the urine as it is being formed, has an alcohol concentration approximately 1.3 times that of whole blood. In fatalities, the urine sample obtained is pooled bladder urine which has accumulated over an unknown time interval between last urination and death. Consequently, the bladder urine alcohol concentration does not necessarily reflect the blood alcohol concentration existing at the time of death. Instead it reflects the BAC prevailing during the period of urine accumulation since the bladder was last emptied.

Several studies have examined the range of ratios between BAC and pooled bladder UAC at autopsy. One study[45] quoted an average UAC:BAC ratio of 1.28:1 with a wide range of 0.21 to 2.66. Another study[30] quoted a UAC:BAC ratio of 1.21:1 with a range of 0.22 to 2.07 for a direct injection GC technique and a ratio of 1.16:1 with a range of 0.20 to 2.10 for a headspace GC technique. In a large series,[46] simple linear regression with BAC as outcome

variable and autopsy bladder UAC as predictor variable (n=435, range 3 to 587 mg/dL) gave the regression equation BAC=-5.6+0.811UAC with 95% prediction interval $\pm 0.026\sqrt{9465804+(UAC-213.3)^2}$]. In practice, a BAC of 80 mg/dL was predicted with 95% certainty by a UAC of 204 mg/dL and similarly a BAC of 150 mg/dL by a UAC of 291 mg/dL. The prediction interval is very wide so that autopsy UAC is of limited, if any, value in predicting an unknown BAC. Although an autopsy UAC should not be translated into a presumed BAC for legal purposes, it is possible to make a conservative estimate of the BAC existing during the time the urine was being produced and accumulated in the bladder, by dividing the observed autopsy UAC by 1.35 (or multiplying by .75). However, if the BAC profile was rising, which might be the case if death occured soon after drinking ended, the calculation UAC/1.35 underestimates the co-existing BAC.

When both urine and blood specimens are available at autopsy, then the UAC:BAC ratio may be of interpretive value. A ratio less than unity or not more than 1.2 suggests, but does not prove, the existence of a rising BAC. If the UAC:BAC ratio exceeds 1.3, this suggests that the subject was in the post-absorbtive stage at the time of death. However, establishing whether a deceased had consumed alcohol immediately prior to death is most easily achieved by obtaining an autopsy sample of stomach contents and analyzing for alcohol. A gastric contents concentration less then 500 mg/dL has been taken to indicate a post-absorptive state.[29] Unusually high UAC:BAC ratios reflect urine accumulation over a long period of time and extreme ratios are well recognized as occuring in delayed deaths from acute alcohol poisoning.[47] In delayed traumatic deaths, postmortem urine ethanol levels may help establish or exclude the role of alcohol. Urine ethanol levels up to and over 200 mg/dL may be found with no alcohol present in the blood.[48]

5.3.5 RESIDUAL GASTRIC ALCOHOL

5.3.5.1 Postmortem Concentrations

Opinions vary as to how much alcohol may be found in the stomach postmortem in case material. In one series,[49] the highest concentration found was 2.95 g/dL and the author quoted a similar high of 5 g/dL in a previous study. In another series,[50] only 1 of 60 cases had a concentration as high as 5.1 g/dL. In a small study,[51] the highest concentration observed was 8.7 g/dL and this was in a suicidal hanging. Given that alcohol is rapidly absorbed from the stomach, it seems likely that death must occur within about 1 h or less of ingesting substantial amounts of alcohol to detect a significant residue in the stomach postmortem.

5.3.5.2 Postmortem Diffusion

Researchers in the 1940s and 1950s debated the suitability of heart blood for quantitative analysis of ethanol on the grounds of possible artefactual elevation resulting from postmortem diffusion of alcohol present in the stomach at the time of death.[52-58] Later investigations by Plueckhahn[49,59-64] lead to the conclusion that postmortem ethanol diffusion from the stomach into the pericardial sac and left pleural cavity was significant and could contaminate blood samples allowed to pool there, but cardiac chamber blood, as such, was not susceptible to this diffusion artefact to any significant extent. However, more recent case studies and a cadaver model have shown that cardiac chamber, aortic, and other torso blood samples may be significantly affected by gastric diffusion artefact.[51]

In one study,[65] blood was obtained from the right atrium, ascending aorta, and the inferior vena cava in 307 subjects without significant decomposition and in whom the blood ethanol concentration was not less than 50 mg/dL in any sample. A total of 104 (33.9%) had one blood ethanol value 20% lower than the highest value. The most striking differences were found when gastric ethanol concentrations were greater than or equal to 800 mg/dL and with associated

evidence of aspiration. In a second study,[50] blood was obtained from the femoral vein, the aortic root, and the right atrium in 60 cases with blood ethanol concentrations of 50 mg/dL or greater and no gross trauma or significant decomposition. Although the mean alcohol concentrations for the different blood site samples were not significantly different, there were wide variations in alcohol concentration among the various blood sample sites in a number of individual cases. Of the cases, 20 (33.3%) had within-case blood alcohol differences greater than 25%; 4 had differences greater than 50%, with 1 of these cases exceeding 400%. Indeed three of the four latter cases had gastric alcohol concentrations between 1 and 5.1 g/dL and the fourth had a concentration between 0.5 and 1 g/dL, whereas, for the 60 cases as a whole, 22 were between 0.5 and 1 g/dL and 11 were above 1 g/dL. In a third study[51] of nine fatalities with known alcohol consumption shortly before death, two showed marked variations in blood ethanol concentrations in samples from 10 sites, with ranges (mg/dL) of 97 to 238 and 278 to 1395; pericardial fluid 1060 and 686; vitreous humour 34 and 225; stomach contents 300ml at 5.5 g/dL and 85 ml at 1.9 g/dL, respectively. These studies[50,51,65] suggest that postmortem diffusion of alcohol from the stomach into the blood may be a significant, although uncommon problem.

The above findings were corroborated by a human cadaver model[51] with multiple blood site sampling after introducing 400 ml of alcohol solution (5, 10, 20, or 40% weight/volume in water) into the stomach by esophageal tube. The pattern of ethanol diffusion showed marked between-case variability but typically concentrations were highest in pericardial fluid and, in decreasing order, in left pulmonary vein, aorta, left heart, pulmonary artery, superior vena cava, inferior vena cava, right heart, right pulmonary vein, and femoral vein. Diffusional flux was broadly proportional to the concentration of ethanol used, was time dependant (as assessed by 24- and 48-h sampling), and was markedly inhibited by refrigeration at 4°C. After gastric instillation of 400 ml of 5% solution for 48 h at room temperature in paired cadavers, ethanol concentrations (mg/dL) were: pericardial fluid 135, 222; aorta 50, 68; left heart 77, 26; right heart 41, 28; femoral vein 0, 0. With a 10% solution of ethanol in the stomach, concentrations (mg/dL) were: pericardial fluid 401, 255; aorta 129, 134; left heart 61, 93; right heart 31, 41; femoral vein 5, 7. The very high concentrations of alcohol found in the pericardial fluid emphasize the potential for serious contamination of any blood sample allowed to pool in the pericardial sac. Introducing 50 ml of 10% alcohol solution into the esophagus after esophago-gastric junction ligation produced similar aortic blood ethanol concentrations to those seen after gastric instillation. This suggests that postmortem gastro-esophageal reflux and diffusion from the esophagus is one mechanism of artefactual elevation of aortic blood ethanol.

Postmortem relaxation of the gastro-esophageal sphincter permits passive regurgitation of gastric contents into the esophagus, if body position and the volume of gastric contents permit. Thereafter, manipulation of the body during removal and transport might lead to contamination of the airways by gastric material, simulating agonal aspiration of vomitus. Alcohol in this gastric material could diffuse from the airways into the blood. An experimental study[66] demonstrated that a relatively small amount of ethanol introduced into the trachea of cadavers was readily absorbed into cardiac blood and also that there was direct diffusion from the trachea into both the aorta and superior vena cava.

5.3.6 ALCOHOL SYNTHESIS POSTMORTEM

Determining whether ethanol identified in postmortem blood represents alcohol ingested prior to death or was formed postmortem as a result of microbial activity is a frequent problem. Ethanol formation may occur in blood putrefying in a cadaver or in blood putrefying *in vitro*. It appears that ethanol is not formed postmortem except by microbial action. Germ-free mice do not putrefy because of the absence of micro-organisms[67] and postmortem autolysis of germ-

free mice produces low levels of acetone and acetaldehyde but no ethanol. By contrast, putrefying conventional mice also produced ethanol, propionic acid, isopropyl alcohol, and n-propyl alcohol.[67] Ethanol is both produced and utilized by micro-organisms, so that bodies with high initial levels may show a decrease, and bodies with low initial levels may show an increase.[68] However, in practice, it is ethanol production postmortem which represents the principal problem and this view is supported by an animal model.[69]

5.3.6.1 Chemical Pathways

Ethanol production in corpses[70] takes place by a pathway opposite to that of ethanol catabolism in the living body. The necessary alcohol dehydrogenase and acetaldehyde dehydrogenase enzymes are provided by the micro-organisms associated with putrefaction while the carbohydrate substrates are present in blood and tissues. The level of tissue glycogen available for postmortem glycolysis and subsequent microbial ethanol production varies considerably between tissues. Human liver contains about 1 to 8 g glycogen/100 g wet tissue; skeletal muscle 1 to 4 g/100 g, brain from a variety of animals 70 to 130 mg/100 g and retina (ox) 90 mg/100 g (all figures calculated from dry weight assuming 75% water).[71] Anaerobic glycolysis produces pyruvate, the main substrate for ethanol production. As well as glucose, lactate is a source of pyruvate through the action of lactate dehydrogenase. Because lactate is found in relatively high concentrations in all postmortem tissues (about 150 to 650 mg/100 g in all tissues),[68] it may well be an important source of ethanol. An *in vitro* study on putrefying postmortem blood under anaerobic conditions at room temperature demonstrated that ethanol formation occurred not only by way of glycolysis but also from lactate via conversion into pyruvate.[71]

5.3.6.2 Observed Ranges

Escape of large numbers of bacteria from the gut occurs in the first instance via the lymphatics and portal venous system, within a few hours of death. At room temperature, bacterial contamination of the systemic circulation occurs after about 6 h, and after 24 h there is direct bacterial penetration of the intestinal wall. Generally the tissues remain relatively free of viable bacteria during the first 24 h. Trauma immediately prior to death, intestinal lesions and neoplastic disease, generalized infection or gangrene are all conditions associated with early bacterial spread postmortem.[68] A wide variety of bacteria normally present in the gut and responsible for putrefaction can generate ethyl alcohol in blood, brain, liver, and other tissues.[72] Also, yeasts such as *Candida albicans,* as well as bacteria, may be responsible for postmortem alcohol production.[73]

There is considerable evidence that ethanol can be produced in corpses at levels up to 150 mg/dL after they have been stored for a few days at room temperature. In a study of 130 decomposing bodies,[74] there were 23 with presumed postmortem ethanol production. Of these 23, 19 had blood ethanol concentrations of 70 mg/dL or less and the other 4 had levels of 110, 120, 130 and 220 mg/dL. Because both bacterial growth and enzyme activity is temperature dependant, postmortem ethanol production is inhibited by refrigeration. For example, a series of 26 in-hospital deaths refrigerated within 1 h of death and stored at 6°C for 3 to 27 h before autopsy, showed no evidence of postmortem ethanol production despite positive blood cultures in 13 cases, 7 of whom had a blood glucose in excess of 20 mg/dL.[75] Similarly an inhibitor of bacterial growth, such as sodium fluoride, inhibits ethanol production. As with all blood specimens for ethanol analysis it is recommended that postmortem specimens should be preserved with 2% w/v sodium fluoride and that storage at temperatures above 4°C should be minimized.

5.3.6.3 Effects of Disease and Environment

Circumstances that can be expected to provide fertile ground for postmortem ethanol production include prolonged exposure to a high environmental temperature postmortem, terminal hyperglycemia, death from infectious disease with terminal septicemia, natural disease such as ischemia affecting the large bowel, abdominal trauma, and severe trauma with wound contamination. Body disruption of a severity which commonly occurs in aviation accidents is associated with extensive microbial contamination and a resultant higher probability of postmortem ethanol production. In a series of 975 victims of fatal aircraft accidents,[76] the blood alcohol concentration exceeded 40 mg/dL in 79 cases. Of these it was considered, based on ethanol distribution in urine, vitreous humour, and blood, that 27% represented postmortem production and 28% ingestion, but 45% were unresolved. In such cases, blood values as high as 300 mg/dL might be synthesised postmortem. In the USS Iowa explosion,[77] the highest ethanol level attributed to postmortem formation was 190 mg/dL.

5.3.6.4 Importance of Corroborative Analysis

In establishing whether there is postmortem production or *in vivo* ingestion of alcohol, circumstantial evidence and corroborative analyses of vitreous humour and urine is of considerable assistance. Vitreous humour is helpful because it is better protected from infiltration by the ethanol-producing bacteria of putrefaction. Urine is useful because it normally contains little or no substrate for bacterial conversion to ethanol. No significant ethanol production will occur as a result of bacterial contamination of urine unless the urine contains sufficient suitable substrate, such as glucose, as a consequence of some pathological abnormality, particularly diabetes mellitus.

After the early postmortem period, when decomposition begins, the problem of postmortem ethanol production increases because both decomposition and ethanol production are the result of micro-organism spread and proliferation.This putrefactive phase starts about two or three days after death in a temperate climate, but varies considerably depending on environmental conditions, primarily temperature. In early putrefaction, when a sample of vitreous humour is still obtainable, the presence of ethanol in this fluid is the best indicator of ethanol ingestion. The presence of ethanol in the urine, if it is available, is also a good indicator of ethanol ingestion. Once decomposition has progressed so that the vitreous is no longer available, due to collapse of the eyeballs, and blood cannot be obtained because the blood vessels are filled with putrefactive gases, then no reliance can be placed upon any sample and interpretation of analytical results is hazardous if not impossible. In these cases, anamnestic data may be more reliable than analytical data. In around 20% of decomposed bodies, the ethanol detected is probably derived from endogenous sources based upon its presence in blood but absence in vitreous or urine, but in many cases endogenous production cannot be distinguished from ingestion.[74] Postmortem ethanol production in decomposed bodies results in blood or putrefactive fluid concentrations less than 150 mg/dL in over 95% of cases,[74,78] but this general observation does not assist in evaluating a higher level found in an individual case. In decomposing bodies, endogenous ethanol production may occur in the bile as well as the blood and may be particularly marked in the sanguineous putrefactive fluid which accumulates in the pleural cavities.[78]

The importance of measuring ethanol concurrently in vitreous, urine, and blood samples was demonstrated in the assessment of low postmortem blood alcohol concentrations. A series[79] of 381 cases with autopsy blood alcohol concentrations less than 50 mg/dL were evaluated using the presence of ethanol in the vitreous and/or urine as indicators of ingestion rather than postmortem production. When the BAC was 10 to 19 mg/dL, then 54% of cases had positive vitreous or urine ethanol concentrations (greater than 10mg/dL); when the BAC

Table 5.3.7a Ethanol in Sequested Hematomas

Ref.	Survival Interval (hours)	Postmortem Ethanol Concentrations mg/dL Cardiac blood	Peripheral blood	Urine	SDH
81	12	0			120
	13	20		310	190
	6-12	160		310	300
	12-15	200			230(R) 360(L)
	>4	160		250	300
	UK	260		370	320
84	10	23	29	265	132
	1.5	121	121	232	206
	UK	58	47	226	104
	UK	151		322	192
82	13	0			120
	26	0			260
	9	40			100
	UK	50			150
	UK	40			1110

UK=unknown; SDH=subdural hematoma

was 20 to 29 mg/dL, this percentage increased to 63%; when the BAC was 30 to 39 mg/dL, the percentage was 73%; and when the BAC was 40 to 49 mg/dL, 92% of the cases had an alternative specimen positive for ethanol. Of the 165 cases where both vitreous and urine were available, over 90% demonstrated consistent results; however, in 14 there was an unexplained inconsistency with one specimen positive and the other negative.

5.3.6.5 Other Volatile Compounds

Bacterial production of ethyl alcohol is associated with the production of other volatile compounds such as methyl alcohol, formaldehyde, n-propyl alcohol, propionic acid, acetone, acetic acid, acetaldehyde, n-butyric acid, and iso-butyric acid. Of these, n-butyric acid and iso-butyric acid are said to be the most common associates of ethanol produced by putrefactive bacteria.[72] Others have advocated measuring n-propanol as a marker of microbial fermentation.[80] However, variability in metabolic pathways between micro-organisms leads to variability in the final products of glucose fermentation. This limits the potential value of measuring these other products to distinguish between alcohol ingestion and postmortem production.

5.3.7 SEQUESTERED HEMATOMAS

That ethanol might be measured in sequested hematomas was first suggested by Hirsch and Adelson,[81] although they claimed no originality, explaining that it is one of the "tricks of the trade". It has been most commonly applied to cases of head trauma with subdural or epidural hematomas.[81-84] but also to intracerebral clots.[85,86] Although principally used for ethanol, any toxicant might be measured in the hematoma. From the accumulated case data (Table 5.3.7a) it is clear that ethanol measurements in subdural hematomas may disclose levels markedly different from autopsy peripheral blood. In interpreting the significance of the results, several possibilities should be considered. The hematoma may have developed rapidly at the time of

Table 5.3.7b Ethanol in Antemortem Blood and Sequested Hematomas[83]

Time (hours) Injury to pre-mortem sample	Pre-mortem sample to death	Ethanol (mg/dL) pre-mortem	postmortem	SDH
1	21	535	0	170
3	7	486	30	190
5	8.5	183	0	90
3	41	161	0	40
1.5	4.5	164	70	110
2	18	93	0	120
1	25.5	240	0	110
1	58	101	0	40

SDH=subdural hematoma

injury, alternatively it may have been delayed and not developed for some hours, or it may have evolved over a period of time as the result of continuous or intermittent bleeding. If the hematoma accumulates over a period of hours, then its ethanol content will reflect changing blood ethanol levels during that time. Furthermore, the hematoma might not be perfectly sequested and ethanol may diffuse both out of it and into it. An animal model[84] has provided good experimental evidence that the current approach to determination of ethanol in sequested hematomas is well founded.

In cases of head trauma associated with subdural or extradural hematomas and with a prolonged survival time, the autopsy blood ethanol concentration may be very low or even zero, whereas the ethanol concentration in the hematoma may be substantial thus providing evidence that the deceased may have been intoxicated at the time of injury. In a study of 75 cases in which ethanol was measured in subdural hematomas and cardiac blood,[82] the analysis provided useful new information only in those cases with survival times greater than 9 h because it was these cases in which the blood ethanol had diminished markedly or been fully metabolized. In another case series consisting of 15 fatalities from penetrating and non-penetrating head injuries,[83] there was a pre-mortem blood ethanol level available. Findings in non-penetrating injuries (Table 5.3.7b) and penetrating injuries were similar in that intracranial hematoma levels of ethanol did not accurately reflect circulating blood ethanol levels at the time of injury. Therefore, quantitative interpretations must be guarded.

After trauma, the development of an intracranial haemorrhage, either subdural or intracerebral, may be delayed. If the victim was intoxicated at the time of injury, then this delay may be sufficient to allow clearance of ethanol from the blood. The intracranial hematoma will then contain no ethanol despite the history of injury when intoxicated. This apparent conflict between the history of the circumstances of injury and the absence of ethanol in the hematoma has been used to provide corroboration that development of the hematoma had been delayed.[87]

5.3.8 METHANOL

Methanol (wood alcohol) is used as antifreeze, photocopier developer, a paint remover, a solvent in varnishes, a denaturant of ethanol, and is readily available as methylated spirit. It may be used also as a substitute for ethanol by alcoholics.[88] The distribution of methanol in body fluids (including vitreous humour) and tissues was reported as similar to that of ethanol[89] but there may be preferential concentration in liver and kidney.[90] The lethal dose of methanol in

humans shows pronounced individual differences ranging from 15 to 500 ml. Clusters of poisonings are seen secondary to consumption of adulterated beverages.[91-93] Blindness and death may result also from dermal and respiratory absorption of methanol.

5.3.8.1 Methanol Poisoning

Acute methanol poisoning produces a distinct clinical picture with a latent period of several hours to days between consumption and the appearance of first symptoms. A combination of blurred vision with abdominal pain and vomiting are found in the majority of victims within the first 24 h after presentation. Visual disturbances, pancreatitis, metabolic acidosis, and diffuse encephalopathy may be seen in severe cases.[91] The characteristic delay between ingestion and onset of symptoms is thought to reflect the delayed appearance of metabolites which are more toxic than methanol itself. Methanol poisoning is characterized by a metabolic acidosis with an elevated anion gap. The serum anion gap is defined as (sodium + potassium) - (bicarbonate + chloride), and represents the difference in unmeasured cations and unmeasured anions, which includes organic acids. Both formic acid, produced by methanol catabolism, and lactic acid, resulting from disturbed cellular metabolism, are responsible for the metabolic acidosis.[94]

The severity of the poisoning correlates with the degree of metabolic acidosis more closely than the blood concentration of methanol.[95] Measuring formic acid concentrations may be of value in assessing methanol poisoning. Reported formic acid levels in two methanol fatalities were 32 and 23 mg/dL in blood and 227 and 47 mg/dL in urine.[96] The treatment of methanol poisoning includes the competitive inhibition of methanol oxidation using ethanol thus preventing the formation of toxic metabolites. Both methanol and ethanol are substrates for hepatic alcohol dehydrogenase (ADH), although the affinity of the enzyme is much higher for ethanol than methanol. Consequently, the biotransformation of methanol can be blocked by administration of ethanol.

5.3.8.2 Endogenous Methanol Production

Trace amounts of methanol (less than 1.0 mg/L) are produced in the body in the course of intermediary metabolism and the endogenous levels increase during a period of heavy drinking. Ingestion of methanol as a congener in various alcohol beverages add to this accumulation.[97] When alcoholics consume alcohol over a period of several days or weeks reaching blood ethanol concentrations of 150 to 450 mg/dL, then the methanol levels in blood and urine progressively increase to 20 to 40 mg/L. The elimination of methanol lags behind ethanol by 12 to 24 h and follows approximately the same time course as ethanol withdrawal symptoms leading to speculation on the role of methanol and/or its metabolites in alcohol withdrawal and hangover.[97] Below a blood concentration of about 10 mg/dL, liver ADH is no longer saturated with ethanol as substrate and the metabolism of methanol can therefore commence. At this low concentration, the elimination of ethanol follows first-order kinetics with a half-life of 15 min. The half-life of methanol, however, is about 10 times longer. As a result, elevated methanol levels will persist in blood for about 10 h after ethanol has reached endogenous levels and can serve as a marker of recent heavy drinking.[97]

Blood methanol levels in 24 teetotalers ranged from 0.1 to 0.8 mg/L with a mean of 0.44 mg/L so that these levels can be regarded as physiological. By contrast, blood methanol concentrations in samples taken on admission to hospital from 20 chronic alcoholics ranged from 0.22 to 20.09 mg/L.[98] The general extent to which methanol may accumulate in the blood of chronic alcoholics can be gauged from a study of ethanol and methanol in blood samples from 519 drunk driving suspects.[97] The concentration of ethanol ranged from 0.01 to 3.52 mg/g and the concentration of methanol in the same sample ranged from 1 to 23 mg/L with a mean of 7.3 (SD 3.6) and a positively skewed distribution. By contrast, in 15 fatalities

following hospital admission for methanol poisoning, postmortem heart blood methanol concentrations ranged from 23 to 268 mg/dL.[93]

5.3.9 ISOPROPYL ALCOHOL

Isopropyl alcohol (isopropanol) is used as a substitute for ethanol in many industrial processes and in home cleaning products, antifreeze, and skin lotions. A 70% solution is sold as "rubbing alcohol" and may be applied to the skin and then allowed to evaporate, as a means of reducing body temperature in a person with fever. Isopropanol has a characteristic odor and a slightly bitter taste. Deaths may occur following accidental ingestion or in alcoholics who use it as an ethanol substitute.[99] Death may occur rapidly as a result of central nervous system depression or may be delayed, when the presence or absence of shock with hypotension is the most important single prognostic factor.

Isopropyl alcohol has an apparent volume of distribution of 0.6 to 0.7 L/kg with maximum distribution occuring within 2 h. Elimination most closely approximates first-order kinetics although this is not well defined. It is metabolized to acetone, predominantly by liver alcohol dehydrogenase, and approximately 80% is excreted as acetone in the urine with 20% excreted unchanged.[100] The acetone causes a sweet ketotic odor. The elimination of both isopropanol and its major metabolite acetone obeyed apparent first-order kinetics with half-lives of 6.4 and 22.4 h, respectively, in a 46-year-old non-alcoholic female with initial serum isopropanol and acetone concentrations of 200 and 12 mg/dL, respectively.[101]

In a review of isopropanol deaths,[102] 31 were attributed to isopropanol poisoning alone, and the blood isopropanol ranged from 10 to 250 mg/dL, mean 140 mg/dL and acetone 40 to 300 mg/dL, mean 170 mg/dL. Four cases with low blood isopropanol levels (10 to 30 mg/dL) had very high acetone levels (110 to 200 mg/dL). For this reason, both acetone and isopropanol should be measured in suspected cases of isopropanol poisoning.

High blood levels of acetone may be found in diabetes mellitus and starvation ketosis with the possibility that alcohol dehydrogenase may reduce acetone to isopropyl alcohol. This is the suggested explanation for the detection of isopropyl alcohol in the blood of persons not thought to have ingested the compound. In 27 such fatalities, blood isopropyl alcohol ranged from less than 10 to 44 mg/dL with a mean of 14 mg/dL and in only 3 cases was the concentration greater than 20 mg/dL. Acetone levels ranged up to 56 mg/dL and in no individual case did the combined isopropanol and acetone levels come close to those seen in fatal isopropyl alcohol poisoning.[103]

5.3.10 OTHER BIOLOGICAL SAMPLES

There have been many attempts to correlate postmortem blood alcohol concentrations with the concentrations of alcohol measured in a creative variety of specimens. In addition to the usual urine and vitreous humour, these specimens have included saliva, cerebrospinal fluid, brain, liver, kidney, bone marrow, and skeletal muscle. All show a very wide range of variation in the ratio of the ethanol content in the target tissue or fluid compared with that in blood making them of little value in practice.

For blood alcohol levels greater than 40 mg/dL, the average liver:heart blood ratio in 103 cases was 0.56, SD 0.30, with a range of 0 to 1.40.[104] However, liver is not recommended as a suitable sample for postmortem ethanol analysis because it is rapidly invaded by gut micro-organisms and provides abundant glycogen as substrate for ethanol production by fermentation, as well as being subject to postmortem diffusion from gastric residue. For bile, in 89 cases with blood ethanol ranging from 46 to 697 mg/dL, the bile:blood ratio averaged 0.99, range 0.48 to 2.04. Bile has been suggested as superior to vitreous humour in the estimation or corroboration of a blood alcohol level[105] but, unfortunately, bile is vulnerable to postmortem

diffusion of unabsorbed alcohol in the stomach.[106] For cerebrospinal fluid (n=54, blood ethanol range 46 to 697 mg/dL), the average ratio was 1.14 and range 0.79 to 1.64.[29]

Because the effects of alcohol are on the brain, it would seem logical to analyze brain tissue for alcohol at autopsy. This is not the practice for two reasons. First, analysis of postmortem blood is more helpful because it allows for comparison with data available from living persons. Second, the brain is extremely heterogenous so that within the brain the ethanol concentration may vary two- to threefold between different regions; highest concentrations are found in the cerebellum and pituitary and lowest concentrations in the medulla and pons.[107] Brain tissue obtained from the frontal lobe (n=33 blood ethanol range 72 to 388 mg/dL) gave an average brain:blood ratio of 0.86 with a range of 0.64 to 1.20.[29]

5.3.11 CONCLUSIONS

Blood ethanol levels can be expected to be positive in around one half of all unnatural deaths so that routine screening of such deaths for ethanol is highly desirable. For natural deaths as a whole, the return of positives is not sufficiently high to justify screening, unless there is a history of chronic alcoholism or of recent alcohol ingestion. The autopsy blood sample should never be obtained from the heart, aorta, other large vessels of the chest or abdomen, or from blood permitted to pool at autopsy in the pericardial sac, pleural cavities, or abdominal cavity. If by mischance such a specimen is the only one available, then its provenance should be clearly declared and taken into account in the interpretation of the analytical results. Blind needle puncture of the chest to obtain a "cardiac" blood sample or a so-called "subclavian stab" is not recommended because at best it produces a chest cavity blood sample of unknown origin and at worst a contaminated sample.

The most appropriate routine autopsy blood sample for ethanol analyses, as well as other drug analyses, is one obtained from either the femoral vein or the external iliac vein using a needle and syringe after clamping or tying off the vessel proximally. The sample should be obtained early in the autopsy and prior to evisceration. Samples of vitreous humour and urine, if the latter is available, should also be taken. The interpretation of the significance of the analytical results of these specimens must, of necessity, take into account the autopsy findings, circumstances of death, and recent history of the decedent. To attempt to interpret the significance of an alcohol level in an isolated autopsy blood sample without additional information is to invite a medico-legal disaster.

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