ALCOHOL

Contents

Breath Alcohol Analysis

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Introduction

As early as 1904, alcohol was recognized as a significant contributor to the risk of having an automobile accident. Since then, abundant epidemiological evidence supports the causal association between alcohol concentration in drivers (usually measured in the breath) and the risk of automobile accidents. Alcohol remains a major threat to traffic safety and is the drug most commonly encountered by forensic toxicologists. In order to address these concerns efficiently, breath alcohol measurement has emerged as the predominant method employed in "driving under the influence" (DUI) enforcement throughout North America, Europe, Australia, and other western nations. Programs have been established that integrate analytical methods, protocols, and trained personnel, all within a sound legal framework. Despite significant advancements in technology and legal structure, many challenges remain. The term alcohol will refer to ethyl alcohol, the organic compound found in alcoholic beverages.

Relevance to Traffic Safety

The role of alcohol in traffic safety has been the focus of enormous research effort. The vast majority of this work has relied on breath alcohol data when assessing accident risk. Breath alcohol analysis has also become widely employed in other transportation, research, public safety, and workplace contexts.

Several reasons exist for the widespread forensic application of breath alcohol measurement. These include: (1) minimal training and ease of analysis; (2) instrument portability; (3) immediate results; (4) computerized instrumentation; (5) noninvasive sample collection; (6) analytical reliability and robustness; and (7) widespread legal acceptability. Many jurisdictions, therefore, have enacted laws prohibiting specific breath alcohol concentrations (BrAC) and requiring subjects to provide breath samples under implied consent. Implied consent describes legislation where, by the operation of a motor vehicle, an individual has implied to have given his/her consent to submit to a breath alcohol test if requested by a law enforcement officer. Failing to do so results in driver's license revocation. A long history of case law exists for these issues. The measurement of breath alcohol, therefore, has substantial scientific and legal foundation.

Biological Considerations

Alcohol is consumed orally in most cases of forensic interest. Ethyl alcohol is a small (atomic mass unit = 46), polar organic molecule that is rapidly absorbed into the blood by simple diffusion across the mucosa lining the stomach and small intestine. Absorption from the stomach can be highly variable, depending on food content, alcohol concentration of the beverage,

general gastric motility, and pyloric response. Approximately 25% of the ingested alcohol will be absorbed from the stomach while the remaining is rapidly absorbed in the upper portion of the small intestine. Upon entering the portal circulation, alcohol is transported to the liver where metabolism, predominantly via the alcohol dehydrogenase (ADH) pathway, occurs. The blood will distribute the alcohol to all parts of the body according to water concentration. Approximately 95% of the ingested alcohol is metabolized by enzymatic pathways in the liver with the remaining fraction being lost through sweat, urine, and exhaled breath. Alcohol pharmacokinetics generally follows a Michaelis–Menten model with linear (zero-order) elimination throughout most of the forensically relevant concentrations. Linear elimination has become an important assumption in cases requiring retrograde extrapolation.

The delivery of alcohol to the pulmonary circulation allows its measurement within the exhaled breath. The lungs of a healthy adult contain over 300 million alveoli and provide a surface area of 60 m^2. The alveolar–capillary interface allows for the exchange of volatile gases, including alcohol. In accordance with Henry's law, alcohol will partition itself between the capillary blood and the alveolar breath as a function of temperature. At 37 °C this partition coefficient ($K_{blood/air}$) is approximately 1780:1. Following exhalation and airway interaction, the alcohol concentration in the breath sample is significantly reduced. Measurement ratios determined from venous blood alcohol concentration (BAC) and end-expiratory BrAC are generally closer to 2300:1. Because early breath alcohol instruments were developed as surrogates for BAC and statutes prohibited specific BAC levels, BAC/BrAC ratios have been the focus of much research and litigation. Position statements by the National Safety Council's Committee on Alcohol and Other Drugs in the early 1950s agreed that "Available information indicates that this alveolar air–blood ratio is approximately 1:2100." In 1972 this ratio was reaffirmed by the Committee. From these and other position statements has emerged the practice in North America of reporting BrAC as grams per 210 liters. The assumed ratio of 2100:1 has resulted in many legal challenges owing to the many analytical and biological factors influencing it. This highly variable relationship between the within-subject BAC and BrAC is illustrated in Figure 1, which plots both the BAC and BrAC for the same subject over time along with the corresponding BAC/BrAC ratio. The ratio is clearly not constant, even within the same subject. Issues and challenges regarding the uncertainty of this ratio, therefore, have prompted many jurisdictions to adopt statutory language prohibiting specific BrAC (e.g., 0.08 g per 210 l) and BAC (e.g., 0.08 g per 100 ml) results separately. Moreover, one must use caution when reading and comparing the scientific literature regarding alcohol measurement since jurisdictions use a variety of different units. The measurement units used within each jurisdiction usually have a substantial procedural and legal history.

Differing within-subject pharmacokinetic models of BAC and BrAC have forensic significance. Since breath alcohol actually arises from arterial blood, it may differ in concentration from that of venous blood collected simultaneously. These arteriovenous differences are greatest during the absorption phase where BrAC (g per 210 l) frequently exceeds venous BAC (g per 100 ml). Following the peak (probably describing most subjects arrested for drunk driving), BrAC will generally be less than that of venous BAC, as illustrated in Figure 1.

Sampling is another very important consideration in measuring breath alcohol. While being voluntarily provided by an intoxicated individual, there can be significant variability in breath measurement results. Unlike blood, the subject is significantly involved in the breath-sampling process. Measurement results, therefore, are influenced by length of exhalation, preexhalation breathing pattern (e.g., hyper- or hypoventilation), breath temperature, preexhalation inhalation volume, and the alveolar composition of the sample. Modern instrumentation is designed to obtain representative and repeatable breath samples by employing several sampling criteria. Figure 2 illustrates two separate breath alcohol expirograms from the same individual collected within minutes of each other. The reported results (those obtained at the end of exhalation) show typical variation observed in forensic practice.

The variability observed in forensic breath alcohol measurement has two components – the analytical

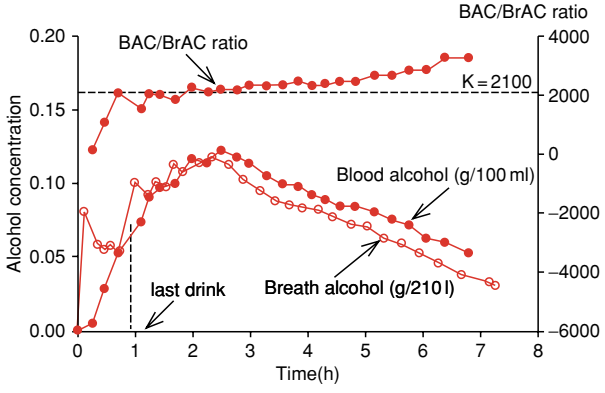

Figure 1 Corresponding blood (BAC) and breath alcohol concentration (BrAC)–time curves for the same individual along with the computed BAC/BrAC measurement ratio.

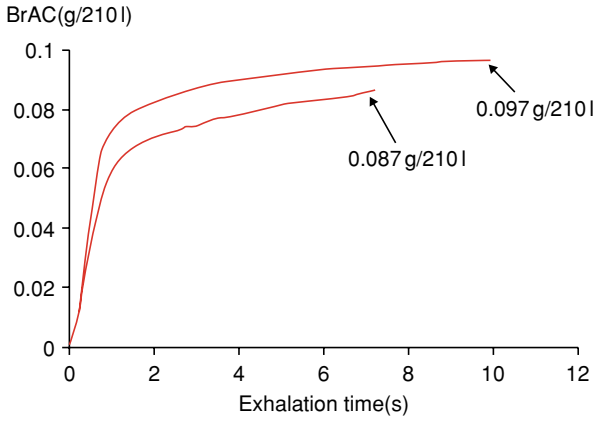

Figure 2 Two breath alcohol exhalation curves (expirograms) collected a few minutes apart from the same subject following two separate continuous exhalations into a computerized breath alcohol instrument. The large variability between samples is clearly illustrated.

and biological (sampling). If we consider these components as independent contributors to total variance, we can employ the equation:

$$S_T^2 = S_A^2 + S_B^2 \qquad [1]$$

where S_T^2 = total variance, S_A^2 = analytical variance, and S_B^2 = biological (sampling) variance. Consider as an estimate of the analytical component of variance an instrument that obtains a standard deviation of 0.0026 g per 210 l to 0.0009 g per 210 l on $n = 10$ sequential simulator standard results measured over 16 days near the concentration of 0.08 g per 210 l. The total variance, determined from an estimated standard deviation of 0.0033 g per 210 l, is obtained from evaluating duplicates on a large number of subjects also having concentrations near 0.08 g per 210 l. Employing these estimates in eqn [1] we determine that the biological (sampling) component contributes only 92% to the total measurement variance. This information can assist in identifying where efforts should be focused for minimizing the total variance. Sampling parameters based on exhalation time, volume, and flow rates can be developed within the analytical software that will require near-equivalent samples each time. These parameters must not be so strict, however, so that a large proportion of individuals are unable to comply. Similarly, features associated with the analytical, environmental, and simulator device can also be evaluated to minimize the analytical source of variance.

Analytical Methods

Work with animals in 1910 began to establish much of the physiological foundation for measuring alcohol in breath. Further work with humans in 1927 showed that breath was a suitable surrogate for blood alcohol. The work of Rolla Harger in 1931 led to the development of the Drunkometer – the first commercially available breath alcohol instrument. The analytical method was the oxidation of ethanol in a solution of potassium permanganate within which a color change was measured. Harger also recognized the importance of obtaining an end-expiratory sample and incorporated the measurement of carbon dioxide to estimate alveolar breath. The Drunkometer began to be used by law enforcement agencies during the late 1930s. Finally, the Breathalyzer, developed by Robert Borkenstein in 1954, became the most widely used evidential instrument for breath alcohol determination. The Breathalyzer also employed a colorimetric method using potassium dichromate in a sulfuric acid solution and was designed to collect only the last fraction (52.5 ml) of breath from a prolonged exhalation. The Breathalyzer continues to be used by several law enforcement agencies today.

Modern Instrumentation

Computerized instruments employing infrared absorption and electrochemical technologies have now become state of the art. Employing computerized technology, manufacturers have developed highly automated instruments that ensure end-expiratory samples, provide printout results, transfer data to a host computer, monitor system performance, reject defective test results, and provide several other "intelligent" features capable of monitoring and controlling the analytical process. These modern instruments are also capable of performing automatic internal and external (simulator or gas) standard measurements along with barometric pressure monitoring when employing gas standards.

A large number of instruments are available for use by law enforcement agencies today. The National Highway Traffic Safety Administration (NHTSA), an agency within the US Department of Transportation, has published a conforming products list containing over 75 different instruments produced by 20 different manufacturers. Only a brief description of a few of the more widely used instruments for forensic purposes will be presented. Since every jurisdiction purchasing modern instruments requests different features, the ones discussed here are only representative of the features as employed within specified jurisdictions. All of these features can be altered or employed by all of the manufacturers, owing to the flexibility offered by computerized technology. Moreover, where predetermined criteria are not met, all of the instruments can be programmed to abort tests,

require additional samples, and display and preserve message codes. Many of the analytical features of each instrument, therefore, are largely determined by the customized requests of end users.

The BAC Datamaster (National Patent Analytical Systems, Mansfield, OH) is widely used throughout North America and Europe. The instrument (Figure 3) employs infrared absorption to quantify the alcohol concentration in accordance with Beer's law.

The instrument employs two or more wavelengths (i.e., 3.37 and 3.44 μm) to distinguish between ethanol and one or more interfering substances (typically acetone). A basic condition of Beer's law requires that the same number of frequencies be employed as compounds to be distinguished. In addition, filters of these frequencies must be sufficiently separated to avoid overlap and of sufficient quality to approximate monochromatic light. These basic principles are appropriately applied in all modern infrared breath alcohol instruments discussed here. The BAC Datamaster is computerized with an attached keyboard. Customized software allows flexibility for a variety of analytical and data collection features. Breath-sampling parameters generally include: (1) minimum of 5 s of exhalation; (2) maximum slope for the breath alcohol expirogram; (3) minimum flow rate of approximately 4 l min^{-1}; and (4) a minimum breath volume of 1.5 l. The instrument also monitors several of its analytical systems including: (1) blank tests; (2) sample chamber purging; (3) internal standard; (4) external control standards; (5) sample chamber temperature; (6) duplicate breath test agreement; (7) pump operation; (8) software integrity; (9) presence of "mouth alcohol." Failing to comply with predetermined standards results in an aborted test.

Figure 3 The BAC Datamaster breath-testing instrument showing a wet-bath control simulator standard device attached.

The Intoximeter EC/IR (Intoximeters, St. Louis, MO) is another computerized instrument (Figure 4) employing electrochemical (fuel-cell) technology to quantify the breath alcohol. Infrared is also employed for the purpose of detecting "mouth alcohol" by monitoring the expirogram slope over time.

The instrument has a keyboard allowing for data collection. The breath-sampling parameters generally include: (1) minimum flow rate of 0.2 l s^{-1}; and (2) a minimum breath volume of 1.5 l. Several system parameters are also monitored, including: (1) blank tests; (2) fuel-cell solenoid operation; (3) diagnostic checks; (4) fuel-cell integrity; and (5) presence of "mouth alcohol." When failing to comply, status codes are preserved in memory for subsequent evaluation. The risk of acetone interference is further minimized by the fuel-cell characteristics and associated algorithms since the time for acetone reaction on the cell surface is largely different than that of ethanol.

The Intoxilyzer 5000 (CMI, Owensboro, KY) represents another widely used computerized instrument. This instrument (Figure 5) also quantifies the

Figure 4 The Intoximeter EC/IR breath-testing instrument. Reproduced with permission from Intoximeters, Inc., St. Louis, MO.

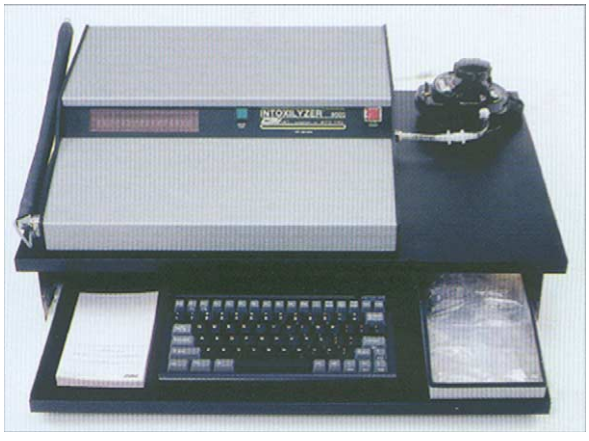

Figure 5 The Intoxilyzer 5000 breath-testing instrument. Reproduced with permission of CMI, Inc.

alcohol by means of infrared absorption. Multiple filters can be employed (depending on customer request) to distinguish between ethanol and several other potential interfering substances. The instrument also has a keyboard allowing data collection and printout capabilities. Self-diagnostics are also performed. Breath-sampling parameters generally include: (1) minimum flow rate of $0.15\,l\,s^{-1}$; (2) minimum exhalation time of 2 s; (3) minimum volume of 1.1 l; and (4) a maximum slope of 7% for the expirogram (Figure 2).

The Intoxilyzer 8000 represents one of the newest computerized evidential instruments offered by CMI. This instrument is smaller than the Intoxilyzer 5000 and, with 12 V power capability, can be employed in either a stationary or mobile environment. The instrument employs infrared absorption technology with frequencies at both 3.5 and 9 μm, thereby improving the specificity for ethanol. The sample chamber volume is 30 ml with an infrared path length of 25 cm (10 in.). A unique feature offered with this instrument is the attachment of either a magnetic strip or bar code reader for the acquisition of driver's license information. Software is encoded within flash read-only memory (ROM) devices with the capability of being remotely revised or updated. The instrument also contains an ethernet port for those requesting that capability. The capability of data collection, printouts, and the attachment of external standards is also included.

A newer evidential instrument is the Drager 7110 (National Drager, Durango, CO). This instrument (Figure 6) employs dual technology (infrared and fuel-cell) to quantify the alcohol and thereby improve specificity. The typical agreement required between the two analytical methods is 0.008 g per 210 l or within 10% of the infrared result, whichever is greater. Differences exceeding these limits are assumed to be the result of an interfering substance.

Figure 6 The Drager 7110 breath-testing instrument. Reproduced with permission from Intoximeters, Inc., St. Louis, MO.

Figure 7 The Alco-Sensor III handheld breath-testing instrument. Reproduced with permission from Intoximeters, Inc., St. Louis, MO.

Moreover, the infrared frequency is near 9 μm, thereby avoiding the many organic compounds with the carbon–hydrogen stretch near 3 μm. The instrument is also computerized, offering data collection, printout of results, and system diagnostics.

Several handheld instruments designed for portable screening tests at the roadside are also available. Many of these models also appear on the NHTSA conforming products list. One such instrument (Figure 7) is the Alco-Sensor III (Intoximeters, St. Louis, MO). Like most handheld models, this instrument employs electrochemical (fuel-cell) technology.

Some handheld units also offer an attachable printer, data collection, error detection features, and breath-sampling parameters. These handheld devices are largely employed for prearrest purposes to establish probable cause. Indeed, these handheld devices also find wide application in the workplace, schools, hospitals, alcohol treatment facilities, and jails because of their simple and inexpensive operation, immediate results, portability, and robustness.

Measurement Protocols and Quality Control

The serious consequences of a DUI conviction require both adequate instrumentation and sound

measurement protocols. Indeed, professional forensic practice should pursue the most reliable and confident analytical results possible. Results that are fit-for-purpose require a total program approach, including: (1) appropriate instrumentation; (2) sound testing protocol; (3) trained personnel; (4) initial and periodic evaluation of equipment; (5) sound administrative rules; (6) careful record-keeping; and (7) full disclosure.

Fitness-for-purpose is the assurance that appropriate confidence can be attached to measurement results sufficient for their intended application. All measurements possess error, resulting in uncertainty. A sound protocol will minimize both systematic and random errors to acceptable levels and identify cases where they are exceeded. Moreover, standards for acceptable results should be sufficiently strict so that not every subject tested will comply. An acceptable level of test rejection (i.e., 5%) will ensure that sufficiently rigorous forensic standards are being employed. There will not be admissible forensic breath alcohol evidence in those cases failing to comply with the predetermined criteria and other evidence (i.e., driving, field sobriety tests, etc.) must be relied upon.

The evidentiary weight assigned to breath test evidence is largely determined by the quality of the program/protocol producing them. At a minimum, the following should be considered in the program/protocol design: (1) specific language of the DUI statute; (2) critical concentrations prohibited by statute; (3) instruments employed and their capability; (4) training required; (5) printout results (information and format); (6) accuracy and precision requirements; (7) interpretability of results; (8) data collection capabilities; (9) unique legal challenges; and (10) program funding and control. Moreover, key individuals should be consulted regarding program/protocol development, including: (1) prosecutors; (2) law enforcement agencies; (3) judges; (4) traffic safety organizations; (5) forensic scientists; and (6) legislators. All of these have relevant interests and contributions regarding forensic breath alcohol measurement.

Quality control is largely determined by the careful integration of instrumentation and measurement protocol. Important considerations will include: (1) using preapproved instrumentation; (2) operation by qualified personnel; (3) preexhalation observation period (i.e., 15 min); (4) internal standard verification; (5) duplicate breath samples; (6) external control standard; (7) purging between all analyses; (8) error detection capability; and (9) printout of results. Preexhalation observation ensures that a recently consumed alcoholic beverage will not bias the result due to "mouth alcohol." The external standard is preferred over simply an internal standard check because it measures the analyte of interest. The internal standard is simply an optical or electronic signal generator that is useful but limited. The external standard may consist of a wet-bath simulator device heating an alcohol/water solution to 34 °C to provide a headspace vapor alcohol sample of known concentration. Although any constant simulator temperature could be employed, 34 °C has been selected by most manufacturers because of its proximity to end-expiratory human breath temperature. The external standard may also consist of a compressed gas standard of known alcohol concentration. Gas standards, however, need to account for atmospheric pressure and the absence of water vapor. Although the measurement correspondence required for external standards varies between jurisdictions, typical values are from 5% to 10%. Duplicate breath samples agreeing within predetermined standards (i.e., within 0.02 g per 210 l or $\pm$10% of their mean) are important to ensure precision and account for the largest source of total variance (the biological/sampling component). Purging between all sample analyses to predetermined levels (i.e., $\leq$0.003 g per 210 l) is also important to preclude a carryover effect. Although the third decimal place is informative for measuring precision, many jurisdictions prefer to report results truncated to the second decimal place as further forensic precaution. A carefully designed printout document showing all critical results and analytical units will further enhance interpretation. Finally, although not forensically necessary, data collection by a host computer can enhance program and instrument evaluation.

Legal Foundation and Challenges

There exists a long history of legal construction and case law regarding breath alcohol testing in both North America and Europe. The statutory framework generally consists of: (1) statutes prohibiting specified BrAC; (2) implied-consent statutes; and (3) statutes authorizing specified equipment, procedures, and personnel. Most jurisdictions possess *per se* legislation that prohibits motor vehicle operation while having specified BrACs. There will generally be coexisting legislation prohibiting the operation of a motor vehicle while "under the influence" of alcohol – accommodating the absence of a breath test. While simplifying the case by linking the offense to a measured result, *per se* statutes have also generated increasingly technical defense challenges regarding the analytical procedure. Challenges are particularly acute when results are near the prohibited limits. Nevertheless, jurisdictions continue to enact *per se* legislation while appellate courts continue to uphold

their constitutionality. Implied-consent legislation continues to provide the foundational leverage for obtaining breath alcohol evidence.

The statutory foundation for program implementation is also necessary. Program details are generally found within administrative rules authorizing all aspects of the program, including instrumentation, protocols, and personnel. Careful consideration must be given to the drafting of these rules. They should not be overly detailed, thereby interfering with legal interpretation and admissibility. Program details are best left to policy manuals that are easily amended and available to responsible personnel. There also exists a great deal of appellate case law proceeding from *US* v. *Frye* (1925) and *Daubert* v. *Merrill Dow Pharmaceuticals* (1993) supporting the analytical methodology.

Recent incentives from the US federal government have encouraged states to adopt legislation prohibiting alcohol concentrations of 0.08 g per 210 l or more while driving. Many political, economic, and public health and safety interests have motivated these efforts. Many jurisdictions have also increased the penalties associated with DUI conviction, including: jail time, license revocation, increased financial penalties, eliminating deferred prosecutions, and enhanced penalties at higher concentrations. Statutes also emphasize different groups, including: (1) 0.02 g per 210 l limit for minors (i.e., zero-tolerance); (2) 0.04 g per 210 l for commercial vehicle operators; (3) 0.08 g per 210 l for drivers in general; and (4) enhanced penalties for 0.15 g per 210 l or more. Moreover, some jurisdictions prohibit specified concentrations depending on the number of previous convictions. These efforts to link the offense or penalties to specific concentrations have important implications for breath alcohol analysis. Low concentration statutes, for example, must consider the limit-of-detection (LOD) capabilities. Similarly, estimating the uncertainty must consider the concentration, of which the variance is a function. Indeed, all of these analytically dependent issues fuel the continuing debate in DUI litigation.

Many legal challenges are raised by the defense regarding the admissibility of breath alcohol evidence in DUI litigation. Several of these are general and occur in most jurisdictions. Others are specific to a particular jurisdiction due to unique statutory language, nature of the offense, and its consequences, specific administrative rules, unique analytical/protocol features, previous case law, and unique rules of evidence. In many jurisdictions the defense effort is directed towards having the evidence suppressed in pretrial hearings, realizing the persuasive nature before a jury. Where suppression is not possible, the attempt is to minimize the weight of the evidence. Several common defense challenges and possible prosecution responses include:

1. Uncertainty in measurement results: this is often an issue for results near critical *per se* levels. The forensic scientist must be prepared to compute and discuss the quantitative uncertainty employing appropriate variance estimates.
2. Breath alcohol is not determined at time of driving: this often leads to performing some retrograde extrapolation, which is fraught with uncertainties. Many jurisdictions have enacted laws prohibiting specified BrACs within a specified time (i.e., 2 h) of driving.
3. Technical details regarding administrative rules: all technical aspects of the administrative rules become elements for challenge. If, for example, the temperature of simulator thermometers is specified, their accuracy, certifying records, and traceability all become an issue. These technical details are best left to policy manuals.
4. Instrument repair history: sound breath-test programs will maintain careful records documenting the certification and maintenance history on all instruments. The reliability of an instrument with a large set of maintenance records is often questioned by the defense. Prosecutors should focus on the protocol and safeguards under which the defendant's test was performed. A proper and complete record for the defendant's results in which all criteria were satisfied should be interpreted as independent of prior instrument problems. Moreover, modern instruments are designed to abort a test if any of the critical analytical criteria fail to be met.
5. The potential for interfering substances: historically, acetone has been considered the only organic compound remotely possible of interfering with forensic breath-testing. In recent years, other volatile organic compounds, primarily from occupational exposure, have also been suggested as potential biases. Instrument manufacturers have addressed these concerns through: (1) employing additional filters in the infrared region; (2) use of fuel-cell technology; (3) employing other less susceptible frequencies in the infrared region (i.e., 9 μm); or (4) use of dual technology. Significant literature exists documenting the minimal risk of interference in a properly performed forensic breath alcohol test.
6. Use of database records to discredit test results: instruments with a large number of "error" records may also be challenged. The prosecution should argue that the defendant's test is

independent of previous results. In addition, administrative rules can be framed so the loss of database records does not preclude the admissibility of test results.

7. Linearity cannot be inferred outside concentrations tested: for results greater than the limits tested by the instrument, the defense might argue that the same accuracy and precision cannot be inferred. Although technically correct, prosecutors should argue that the offense is exceeding a specific concentration, not that the measured result has a specified accuracy or precision.
8. Challenges based on biological considerations: the defense might argue that the longer the person exhales, the higher will be the result. Figure 2, however, reveals the small increase in BrAC over exhalation time. This issue is addressed by carefully defining in the administrative rules the sample and measurement objective. For example, the sampling objective might be defined as obtaining an end-expiratory breath sample following a full single exhalation.
9. Software reliability: the defense will often argue the instrument software is either inadequate or not properly evaluated. Fitness-for-purpose should be emphasized by the prosecution. Agencies purchasing instruments should ensure the manufacturer has appropriately tested and documented the software for the forensic context of breath alcohol testing.
10. Traceability of measurement results: traceability of breath alcohol results to some national authority (e.g., National Institute of Standards and Technology, International Organization for Standardization (ISO), OIML (International Organization for Legal Metrology)) may be challenged by the defense. Documentation showing traceability through control standards (simulator or gas) to the national authority should be maintained and provided.

Obviously, these reflect only a small number of the many challenges proffered by the defense regarding breath test evidence. Each jurisdiction will face many challenges unique to its programs. Forensic scientists must work closely with local prosecutors and law enforcement agencies to ensure a cooperative effort in order to address the many defense challenges they will face. Oftentimes, changes are necessary in analytical or procedural elements to achieve legal admissibility and confidence in court.

The Future

Breath alcohol analysis has become firmly established within the analytical, legal, and traffic safety communities as an important tool facilitating the apprehension and prosecution of the alcohol-impaired driver. Nevertheless, improvements can be made in many areas. Indeed, there remain many areas to be explored, amenable to creative research.

Improving quality control remains a priority as the seriousness of drunk driving convictions continues to escalate in most jurisdictions. Breath alcohol evidence, employed in over 90% of DUI cases in North America, must be obtained and presented in a manner that maximizes its informative and evidentiary value. Measurement protocols should include at a minimum those elements discussed earlier. In addition, measurement results should ideally be presented along with an assessment of their uncertainty. Many European jurisdictions accommodate this by including correction factors. Further work is also needed regarding the performance of wet-bath simulator versus dry-gas standards. Finally, the computerized features of instruments should be enhanced to allow monitoring of instrument performance over time.

Further research is needed regarding the potential risk of interfering substances. Unanswered questions remain regarding which volatile organic compounds and what exposure, biological or analytical conditions pose a measurable risk for undetected interference. Moreover, the optimal combination of analytical and procedural features to address the interference problem has yet to be defined.

"Intelligent measurement" and expert systems need further investigation to identify relevant applications for forensic breath alcohol measurement. Techniques may be available to make simulator devices more reflective of the dynamics and variability of human breath exhalation. Many "intelligent" features exist for enhancing system performance and measurement confidence.

Improving the communication and interpretation of analytical results for the court also presents an important challenge. Optimal analytical results are of no value if the court is left confused regarding the information. Improving this communication process may include some combination of: (1) clear and professional oral presentation; (2) use of visual aids; (3) printout document appearance and informative value; (4) use of analogies to explain technical detail; (5) providing estimates of uncertainty; and (6) full disclosure. Disclosure of material to the legal community, for example, can be enhanced through internet accessibility. Many areas capable of improving the communication and informative value of breath alcohol measurement have yet to be explored.

Many areas of program administration deserve further research. Such areas include: (1) records to be

retained; (2) optimal instrument certification schedules; (3) levels of personnel responsibility; (4) level of technical training for personnel; (5) instrument evaluation and approval process; (6) criteria used for field assignment of instruments; (7) advantages of employing a single instrument type; (8) internet provision of documentation; (9) computerized training of personnel; and (10) data collection and/or remote monitoring of instruments. Indeed, successful programs will involve thoughtful design, contributions from many different people, and the appropriate integration of instrumentation, protocols, and personnel.

Conclusions

Forensic breath alcohol measurement remains a prominent tool for confronting the problem of the alcohol-impaired driver. The biological understanding and analytical methodology are well established. The legal foundation is firm. Many challenges and opportunities, however, still remain. Quality control can still be improved while data collection and analysis need further application. The primary forensic objective remains, however, to provide the court with relevant and material evidence of the highest possible integrity.

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Blood and Body Fluid Analysis

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Introduction

Ethyl alcohol (EA), the psychoactive ingredient in alcoholic beverages, is universally available. Indiscriminate consumption is commonly associated with violence and disease. The most frequently detected drug by both clinical and forensic toxicology laboratories, EA is the leading cause of or contributor to drug-associated death, and constitutes the major catalyst in nonfatal trauma. For these reasons it has come to be regarded as unique, both historically and in current practice, and therefore is commonly discussed separately from other licit or illicit drugs of abuse.

In these contexts the task of the forensic pathologist or other forensic expert, as interpretive toxicologist, is to decide whether EA affected the subject's

antemortem psychological, behavioral, and physiological function (Table 1). In official medicolegal death investigation additional issues arise regarding the role of EA in the demise of the individual (Table 2).

Assessment of the effects of EA necessarily relies on properly collected, stored, transported, analyzed, and reported specimens from the subject. In order to reach scientifically sound conclusions on the range of effects and mode of action of EA, the medicolegal official must be familiar with the fate and disposition of consumed EA in the body. This intellectual, correlative process in turn rests upon a thorough understanding of the interplay between quantitated results from blood or other fluid matrices. Significantly coupled with the breadth of analytical results is a sound understanding of individual tolerance to EA. When these interconnected factors are mastered, the expert is judicially qualified to offer evidence-based opinions in medicolegal settings (Table 3).

Table 1 Primary role of medicolegal expert assessing effects of ethyl alcohol on the living

Determination of impact on physiological function
Evaluation of effect on behavior in multitask activities
Operation of motor vehicle (DUI or DWI)
Skill at control of machinery
Workplace-related activity
Assessment of influence on social behaviors

DUI, driving under the influence; DWI, driving while intoxicated.

Table 2 Questions by expert adjudging role of ethyl alcohol (EA) in medicolegal death investigation

Was EA solely causative in death?
Did EA act as a synergist to other toxins, causing death when no toxin alone is responsible for the fatality?
How did the BAC, quantitated postmortem, affect behavior shortly before death?
What was the BAC at the time of injury in cases of delayed death?

BAC, blood alcohol concentration.

Table 3 Responsibilities of the expert evaluating ethyl alcohol in blood and body fluids

Function as interpretive toxicologist
Master the state-of-the-art science on the fate and disposition of alcohol in the body
Understand the physiological, behavioral, and psychological effects of alcohol on humans
Oversee appropriate collection and analysis of fluid specimens
Maintain or confirm chain of custody for transport of specimens
Recognize the interrelationships between alcohol concentrations in blood and body fluids
Correlate laboratory findings with autopsy and the background of either death or event
Provide scientifically sound expert opinions on the effects of quantitated alcohol

Analytical toxicologists have reliably identified and quantified EA in virtually all body tissues, fluids, and secretions (Table 4). In the clinical setting the desired sample is venous blood appropriately collected via venepuncture, from which serum is typically segregated and analyzed enzymatically. Breath, saliva, and, with qualification, urine, serve as substitutes or complements when phlebotomy is legally or practically contraindicated.

The gold standard in testing postmortem fluid EA levels for medicolegal purposes is whole blood by headspace gas chromatography (HS-GC). In regard to the short postmortem interval prior to the onset of decomposition, countless studies have established the comparative ratio of postmortem whole-blood EA concentration (blood alcohol concentration or BAC) to other matrices (Table 5). Postmortem decomposition spuriously increases BAC due to endogenous production by overgrowth of normal, fermentative flora in the gut, with substantial (>0.20%) artifactual elevations reported in some cases. Vitreous humor is a reliable comparison medium to differentiate antemortem consumption from postmortem production. Intravascular fluids from embalmed bodies may be utilized selectively to estimate the antemortem BAC by comparison with constitutive volatiles in embalming fluid.

EA is the most frequently analyzed drug by the toxicologist in consultation with coroners, medical examiners, physicians in emergency departments, directors of poison control centers, and police. Optimal specimens are required for accurate analysis by the laboratory technician as practitioner of analytical toxicology, as well as for evaluation of the analytical results by the expert as interpretive toxicologist.

The BAC, which distinctively rests upon collection and analysis of the specimen obtained at a discrete time, is dependent above all on the individual's

Table 4 Body fluids suitable for analysis of ethyl alcohol in medicolegal investigation

Blood (whole blood, plasma, serum)[a]	Gastric and proximal small-bowel contents
Urine[a]	Bone marrow
Vitreous humor	''Decompositional'' fluid
Cerebrospinal fluid[a]	Fluid from embalmed bodies
Saliva[a]	Sequestered intracranial hematomas
Bile	Sweat[a]
Synovial fluid	Amniotic fluid[a]
Pericardial fluid	Maternal breast milk[a]
Dialysis fluid[a]	Lavage fluid[a]
''Cavity blood''	Aspirated vomitus[a]
Tears[a]	Pleural fluid

[a]Samples not limited to autopsy; they may be collected during life from hospitals or clinics.

Table 5 Summarized ratios: body fluid to whole blood alcohol concentration

Specimen	*Average ratio or range*
Serum or plasma	1.0–1.15
Vitreous humor	1.05–1.34
Urine	1.17–1.5
Bile	1.03–1.10
Cerebrospinal fluid	1.1
Saliva	1.08–1.12
Pericardial fluid	Variable
Synovial fluid	1.01–1.32
Tear fluid	1.08–1.20
Amniotic fluid	0.5
Bone marrow	0.34–0.53
Gastric contents	Variable
Pleural fluid	Variable
Solid organs not addressed in discussion	
Brain	0.65–0.96 (site-dependent)
Liver	0.6
Kidney	0.7
Skeletal muscle	0.89–0.91
Spleen	Variable
Testicle	''High correlation''

unique tolerance to and absorption, distribution, and metabolism of the drug. Correlation of the BAC to the level detected in a particular body fluid from one or more other body compartments is particularly important in death investigations for several reasons: (1) support of reliability of the blood level in evaluating the degree of intoxication; (2) resort to other body fluids when a satisfactory blood sample is unavailable or contaminated; and (3) quality assurance and proficiency of testing. Such correlative analysis requires the establishment of relative distribution ratios and standard deviations from the mean. Ideally, each investigatory agency should establish its own experience-based parameters. Experts may also refer to many studies establishing comparative ratios (with standard deviations and ranges) between whole-blood EA levels and other biologic fluids and tissues (Table 5).

Analytical Methodology

Specific analytical methods are necessary for the analysis of EA because of potential interference by a variety of volatile substances in postmortem specimens. Laboratory methods for the analysis of EA in biological specimens are classified as chemical, biochemical, and instrumental. Wet chemical analyses include distillation or microdiffusion utilizing the inherent volatility characteristics of alcohol, which allows for separation, oxidation, and subsequent detection. A well-known example of this chemical methodology is the Breathalyzer developed by Borkenstein in 1954. Biochemical methods utilize the enzyme alcohol dehydrogenase (ADH). Physiochemical methods include gas chromatography (GC), high-performance liquid chromatography (HPLC), and gas chromatography–mass spectrometry (GC-MS).

Gas Chromatography

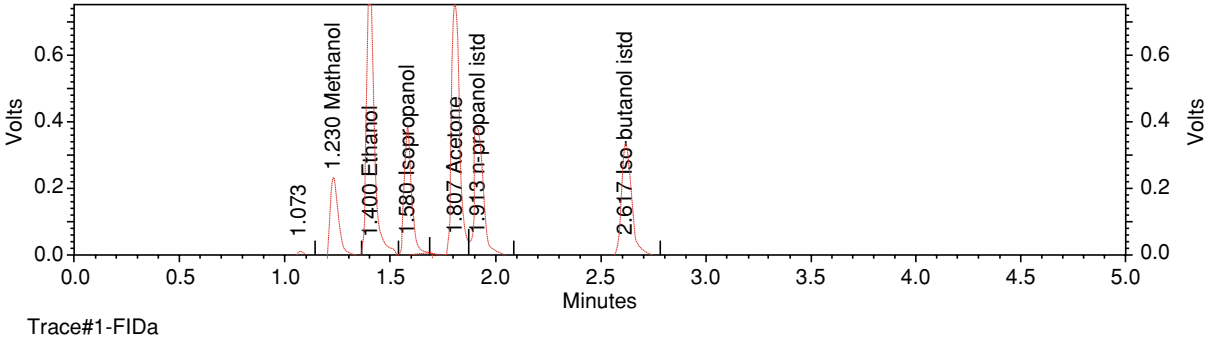
GC is the most common methodology for the measurement of EA in postmortem biological specimens, owing to its specificity, sensitivity, and reproducibility. EA has been measured by several GC methods, including solvent-based extraction, protein precipitation, and distillation techniques, direct injection, or headspace analyses. Headspace analysis and direct injection techniques are current applications of choice. Direct injection techniques generally require injection of a liquid sample into the gas chromatograph equipped with a flame ionization detector (FID). The specimen may be an undiluted sample, a protein precipitated sample, or a diluted sample of the specimen with an aqueous solution of an internal standard (commonly, 1-propanol and *t*-butanol).

Dual-column HS-GC is nearly completely specific for EA. It is a proven test method acceptable in most courts of law affording admissibility of analytical results on which to base expert testimony. HS-GC with FID precisely detects EA at concentrations as low as $0.01\,g\,dl^{-1}$. It also distinguishes EA from other alcohols, aldehydes, ketones, and other analytes in the mixture. Headspace analysis techniques physically rely on Henry's law wherein the ratio of a dissolved substance in solution is dependent on temperature, pressure, and concentration of the fluid medium. The measurable amount of volatile in the headspace above the liquid medium is proportional to the volatile liquid concentration in solution. The headspace procedure employs diluted blood samples of aqueous solution with internal standards, which are placed in small, capped bottles. After incubation produces the vaporized mixture, which includes an inert carrier gas, the headspace (gas phase) is injected into a closed system via the single injection port. The injection splits into two attached capillary columns (stationary phase(s)), variably coated to interact predictably with analytes of interest. EA is initially separated, based on the appropriately calibrated GC parameters and columns, and subsequently quantified using computer-aided techniques. Separation of the volatile compounds, as the vapor phase is carried through the column, depends on the relative differential affinity of each analyte for the stationary phase. A detector at the end of the column, which is designed for FID, creates over time electrical signals that are converted to quantitative results. In addition to temperature, optimal analysis also depends on the

condition at which the vapor is at equilibrium with the liquid specimen, flow rate of the carrier gas, material packing the column, column length, and the kind of detector. In sum, the discrete, absolute, or relative retention times (min) depicted on the gas chromatogram provide qualitative analysis, while the peak height or area for each analyte affords quantitative analysis (Figure 1). Although GC-MS techniques are the most definitive assay for EA analysis, these tests are not widely employed in forensic laboratories because they require considerable expertise and are more expensive.

Biochemical Methods – Immunoassays

Hospital and clinical laboratories commonly apply enzymatic methods, utilizing ADH, to determine EA in blood and urine because gas chromatographs are often unavailable. The instrument-based biochemical reaction is similar to *in vivo* enzymatic reactions controlling EA metabolism. The coenzyme, nicotinamide adenine dinucleotide (NAD), is reduced as a byproduct of the oxidation reaction of EA to acetaldehyde. Various trapping reagents, e.g., hydrazine or semicarbazide, trap acetaldehyde and drive the



Trace#1-FIDa
Results (Reprocessed)

Name	amt	Units	Area	Retention Time
	0.000		31232	1.073
Methanol	0.065	g/dl	612086	1.230
Ethanol	0.139	g/dl	2443108	1.400
Isopropanol	0.035	g/dl	951843	1.580
Acetone	0.050	g/dl	2080820	1.807
n-propanol istd	1.000	g/dl	1205693	1.913
Iso-butanol istd	0.941	g/dl	1133995	2.617

Trace#1-FIDb
Results (Reprocessed)

Name	amt	Units	Area	Retention Time
	0.000		28726	1.160
Methanol	0.058	g/dl	494231	1.513
Ethanol	0.143	g/dl	2110875	1.783
Acetone	0.048	g/dl	1875629	1.893
Isopropanol	0.036	g/dl	1025661	1.973
n-propanol istd	1.000	g/dl	1038570	2.737
Iso-butanol istd	1.003	g/dl	1041310	3.913

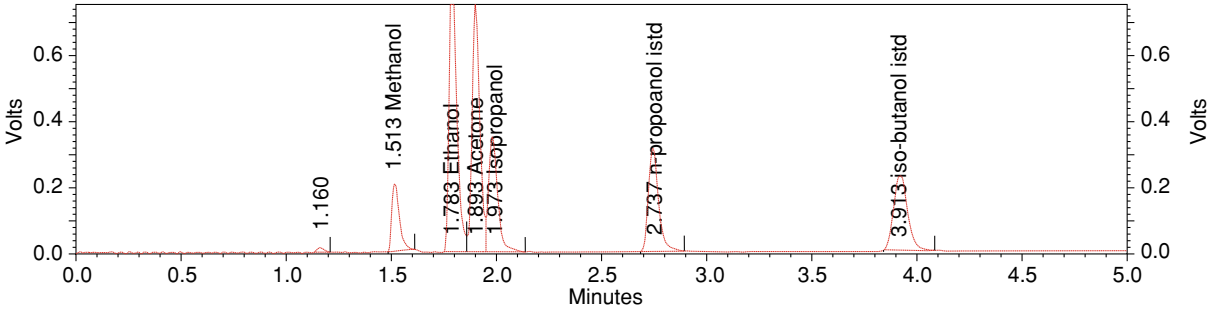


Figure 1 Gas chromatogram (dual-column headspace gas chromatography with flame ionization detector (HS-GC-FID)) with both columns depicting retention times and levels of methanol, ethanol, isopropanol, and acetone with internal standards, *n*-propanol and isobutanol.

oxidative metabolism of EA to the right. The reaction produces the reduced form of NAD, NADH, which can be measured spectrophotometrically at 340 nm. Radiative energy attenuation is a modified enzymatic method utilizing the NADH produced by an ADH-catalyzed ethanol/NAD reaction. The NADH product combines with a thiazole blue dye to form a chromogen. Measured fluorescence quantifies the EA in the liquid specimen.

The automated enzymatic method is a quick and easy way to detect EA. However, it lacks the specificity of HS-GC because the presence of other alcohols such as isopropanol may interfere chemically and yield an inconclusive, false-positive result. Unlike HS-GC, antigen–antibody reactions are subject to cross-reaction with other substances within the blood and for that reason are not regarded as a reliable method for testing BAC in a medicolegal or juridical context.

Specimen Selection and Collection in Living Subjects

Currently accepted venepuncture consists of cutaneous application of a nonalcoholic antiseptic (e.g., povidone iodine) and withdrawal of a sufficient aliquot of cubital venous or fingertip capillary whole blood by a sterile needle to a sealed sterile vial. Anticoagulants and microorganism-inhibiting chemicals are typically added. Importantly, venous blood does not precisely reflect the cerebral BAC, which ultimately defines the biochemical effects of EA, unless absorption and distribution of EA are complete at collection (Table 6).

Table 6 Accepted collection, transport, and storage of blood from living persons

Cutaneous application of nonvolatile antiseptic
Percutaneous venepuncture of cubital vein or fingertip capillary
Withdrawal of sample by sterile needle to sterile container
Vacuum glass collection tubes are acceptable legally
Filling container sufficiently to avoid evaporation
Use of clean container without anticoagulant allowing blood to clot (for serum)
Use of preservatives/anticoagulants (for whole blood and plasma):
1–2% sodium fluoride
EDTA or potassium oxalate
Proper labeling, laboratory request form, and chain of custody on or with container
Refrigeration (4 °C) or prompt delivery to analytical laboratory
Recording receipt and disposition of specimen by receiving analyst
Analysis or storage (refrigeration or frozen: −20 °C) of specimen

EDTA, ethylenediaminetetraacetic acid.

Randomly collected, first-voided urine is generally valuable only in confirming the presence of EA, because the urine alcohol concentration (UAC) is subject to multiple uncontrolled variables. Since the 1990s saliva, or oral fluid, has gained acceptance as a satisfactory matrix for on-the-spot testing for EA, both qualitative and semiquantitative, applicable to workplace or clinical settings such as emergency departments.

Specimen Selection and Collection – Postmortem

If possible, recovery of available antemortem or perimortem blood, blood components, or other body fluids (Table 4) from the victim, collected by investigators or medical personnel, is a recommended practice after declaration of death. The earliest collected sample frequently provides more accurate information about the question of antemortem intoxication by EA than do samples from autopsy, particularly when treatment over variable periods of time includes fluid resuscitation. This applies particularly in cases of traumatic exsanguination, urgent operative procedures, and prolonged hospitalization before death. For both living and deceased subjects, it is necessary to consider potential antemortem dilution from therapeutic administration of blood and other fluids.

In most postmortem cases, there is undoubtedly greater opportunity – in contrast to limitations within the clinical arena – to collect a variety of biologic specimens for laboratory analysis (Table 7). Under optimal circumstances, utilizing multiple specimens at autopsy from various compartments and subcompartments of the body helps to support the accuracy of a given quantitative result and thereby facilitates optimal interpretation.

The most critical, vigorously debated issue with regard to blood sample collection before putrefaction in contemporary postmortem medicolegal practice is the phenomenon of site-to-site differences in BAC, both within-case and between-case. Traditionally, "heart blood" or "central blood" (blood aspirated from either the intact cardiac chambers, the intrapericardial great vessels arising from and exiting the heart, or a mixture from these sites) has been routinely collected for EA analysis. This practice has been justified in view of various studies finding no statistical significance between heart blood and femoral blood ("peripheral blood") EA content. More recent controlled experiments addressing this issue were designed to collect fluid from multiple sites (pericardial fluid, left pulmonary vein, aorta, left heart, pulmonary artery, superior vena cava, inferior vena

Table 7 Accepted collection of routine samples for ethyl alcohol analysis after death

Intravascular blood (central and peripheral), as available:
≥50 ml, as available, from each site via clean wide-bore needle and syringe
Commercially available gray-top Vacutainer or
30 ml glass container with 250 mg NaF (= 1–2% NaF) or
Polypropylene test tubes with Teflon-lined screw caps
Aspirate from heart chambers, supravalvular aorta, pulmonary artery, vena cava
Percutaneous or direct phlebotomy from femoral (or subclavian) vein
Refrigerate/freeze (4–20 °C) promptly before delivery to analytical laboratory
Intravesical urine
≥250 ml, as available
Aspirate with needle to syringe through dome of distended urinary bladder
Aspirate directly after superior cystotomy of collapsed urinary bladder
Preserve in clean container, preferably with NaF
Refrigerate/freeze till timely analysis
Ocular vitreous humor
≥4–6 ml, as available, from both globes
Ophthalmocentesis with clean needle and 5 or 10-ml syringe
Avoid forceful aspiration to prevent retinal detachment
Preserve in clean container with NaF
Refrigerate/freeze till timely analysis
Cerebrospinal fluid
≥2–6 ml, as available
Preferred: aspiration via clean needle/syringe directly from cerebral cisterns or proximal cervical canal after opening skull
Avoid ''blind'' percutaneous suboccipital or lumbar puncture owing to potential contamination to analyte and paraspinal soft tissue
Preserve in clean container, NaF optional
Refrigerate/freeze till timely analysis
Synovial fluid
≥2–4 ml, as available
Percutaneous arthrocentesis (knee joint) via clean needle/syringe
Preserve in clean container with NaF
Refrigerate/freeze till timely analysis
Gastric/small-bowel contents
Following removal of block of esophagus, stomach, duodenum, pancreas:
Place all contents into clean volumetric container
via compression of stomach, forcing contents out of esophageal segment or
through gastrotomy in relatively avascular region
Quantitate and characterize contents (food, liquid, slurry, foreign bodies)
Preserve 50 ml uniform sample in clean container
Preserve 100 ml nonuniform sample (liquid/solid/semisolid) in clean container
Refrigerate/freeze till analysis
Bile
Collect and quantitate all liquid contents (<0.5–65 ml)
Choledochocystocentesis via clean needle/syringe before hepatic evisceration
Preserve contents in clean container
Refrigerate/freeze till analysis
Sequestered intracranial (epidural, subdural) hematomas
Quantitate and remove maximal amount of fluid and clot on opening skull
Preserve in clean container
Refrigerate/freeze till analysis

NaF, sodium fluoride.

cava, right heart, right pulmonary vein, femoral vein, and stomach), either simultaneously or at defined intervals, and at different environmental temperatures. Such experiments have demonstrated the chaotic, site-dependent unpredictability of BAC at autopsy in some cases, an artifact attributed to simple diffusion of EA from the stomach or esophagus to adjacent regions and to the circulation.

Blood and its Constituents

In postmortem sampling, available "whole blood" remains the most desirable specimen for analysis. In light of contemporary evidence, some recommend autopsy collection of at least two samples of blood, one peripheral and one complementary central sample, if case specifics permit. In order to avoid artifacts

and difficulties with postmortem redistribution, it is strongly recommended that the medicolegal investigator collect peripheral blood as the optimal sample, drawn by a wide-bore, clean needle with new syringe preferentially from the femoral vein and, when such a sample is not collectable, secondarily from external iliac vein or subclavian vein. It is necessary to avoid "milking" the vein to prevent admixing the blood with tissue fluid.

Specifically designed clean containers are utilized for specimen collection. Depending on the design and specimen type, these rubber-stoppered, glass collection tubes may contain sodium fluoride, heparin, potassium oxalate, EDTA, or no additives at all. The anticoagulant and bacteriostatic actions of sodium fluoride are optimal for preserving and storing whole blood drawn at autopsy. If blood is analyzed by GC, plastic containers are the most optimal receptacles. For analysis of volatiles, some sample should be retained in a Teflon-lined screw top to prevent diffusion.

In addition to potential postmortem artifactual site-to-site alterations, the BAC from various regions of the intact circulation and EA concentration in other body compartments vary during the absorption phase of EA metabolism. Therefore, it is necessary to specify unequivocally the source of the sample or the site of collection of whole blood. Arterial BAC may be at least 40% higher than venous BAC in the absorptive phase. It is equally important that the blood specimen should not be mixed from various sources, such as intermingled central and peripheral blood.

Pooled blood from the pericardial sac and bloody fluid recovered from extravascular body cavities (which are not blood!), especially in trauma, are less reliable toxicological specimens to quantitate EA, but may be used if these are the only blood-related source. The pooled or bloody fluid may have either a higher or lower level of EA than that in intravascular blood *per se* (central or peripheral), and accordingly may make meaningful interpretation of the reported "BAC" virtually impossible. If such samples are the only ones obtainable, antemortem BAC is merely an estimate. Bloody fluid from body surfaces or from the relevant scene are unreliable, inappropriate sources for toxicology evaluation.

In collecting blood samples at autopsy there are factors influencing the concentration of EA that are not pertinent to antemortem techniques. Diffusion of significant amounts of EA from the esophagus or stomach into the adjacent pericardial cavity and heart is likely to occur, and becomes increasingly significant as the postmortem interval increases. Yet, if there is a short period of time, measured in hours, between the last drink and death, diffusion of EA from the gut to the "heart blood" will not be substantial. Under circumstances where the autopsy is performed within 48 h of death, diffusion of alcohol from the gut to the heart is relatively insignificant. As noted, femoral or subclavian venous (peripheral) blood sites are preferable to central blood. These samples may be difficult to obtain secondary to insufficient volume and in cases of traumatic hypovolemia ("empty-heart sign"). As EA distributes to total body water, it is important prior to the onset of putrefaction to consider the water content of the blood sample in interpreting BAC. For example, the sample with a low hematocrit (volume of red cells to total blood volume) yields a higher level of EA due to the greater water volume. In cases of significant hypovolemia, sampling other compartments is necessary. When the remains are incinerated, the vascular compartment may contain only uniformly coagulated or anhydrous, "baked" clot. Such a sample should be collected even though the EA level has little meaning without correlating EA concentration in other available matrices.

Withdrawing a "blind" postmortem sample via precordial percutaneous pericardiocentesis to collect blood is indisputably flawed and to be avoided. Central blood specimens contain blood that is drawn by direct observation from the heart or the great vessels. In summary, external "blind" chest puncture is not considered an acceptable procedure for the collection of a blood sample for subsequent EA analysis. False elevations of EA in bloody fluid collected by external chest puncture can be confirmed by analysis of postmortem vitreous humor or urine. Without autopsy, it is recommended to collect peripheral blood.

In contrast to the practices of clinical laboratories, most forensic toxicology laboratories analyzing postmortem samples report BAC from whole blood preserved in sodium fluoride. Yet, as most forensic experts are frequently called upon either to interpret results from or analyze antemortem serum or plasma samples, it is incumbent on the expert to appreciate different results from various specimens. Researchers conclude that using serum, plasma, or whole blood for EA analysis produces essentially equivalent results for clinical and forensic purposes, as long as the final report clearly specifies the specimen (serum, plasma, whole blood). Under most physiological conditions, serum or plasma contains about 10–20% more water than an equal volume of whole blood. EA levels are correspondingly, but only slightly, higher in these samples. The average EA ratio of whole blood to serum or plasma is approximately 1:1.15.

Vitreous Humor

As a quality control measure, concomitant comparative quantitation of EA in the postmortem vitreous humor (VAC; vitreous alcohol concentration) is an excellent means of interpreting the reported BAC, whether central or peripheral. Because the intact, relatively avascular intraorbital globe is anatomically isolated from other tissues or fluid, it serves as an excellent compartment to obtain unadulterated, typically sterile vitreous humor for quantitation. Characteristically, VAC lags approximately 1–2 h behind BAC at metabolic equilibrium. Therefore, BAC in the absorptive phase is higher than VAC. At the plateau or equilibrium phase, the reported average ratio of BAC:VAC is 1:1.05–1.34 by virtue of the differential water content of these matrices. In the postabsorptive or elimination phase, VAC is higher than the BAC. Such comparative analysis is helpful in establishing whether the deceased was in the absorptive or elimination phase at death. Given the well-documented BAC:VAC ratios, reference to the VAC is also very useful in inferring the probable BAC at death when intravascular blood or other body fluids are not readily available.

As in all extrapolations based upon EA levels in extravascular matrices, a conservative approach is always prudent in estimating the BAC from the VAC at autopsy. The EA distribution ratio (VAC:BAC) (femoral blood) may exhibit wide variation in light of recent research. Investigators recommend a conservative approach by dividing the postmortem VAC by 2.0 to arrive at an estimate of the equivalent (femoral) BAC, which, although lower than the "true value," may then be offered with a higher degree of confidence in the medicolegal arena.

Other Body Fluids

When blood or vitreous humor is not available, such as in decomposition, trauma, or contamination, other aqueous body tissues may be used to quantitate EA, which is readily miscible in water. Ideally, because the level of alcohol in the central nervous system directly affects behavior and activity, the best sample for measurement of EA concentration is brain. Obviously, this is not feasible for living individuals. Although brain is usually readily available at autopsy, it is not the specimen of choice for several reasons:

1. Blood from the vascular compartment is usually easier to obtain and process.
2. The appropriately determined BAC adequately reflects the effect of EA on the brain.
3. Simultaneous sampling of various brain regions yields significant differences in the EA concentration.
4. It is more practical, technically efficient, and economically sound to analyze blood regularly when such high-caseload volumes are involved.

Other tissues and samples used for blood alternatives are urine, gastric contents, bone marrow, bile, intracerebral and paradural hematomas, synovial fluid, cerebrospinal fluid (CSF), and others catalogued in Table 4 (in addition to solid organs, e.g., liver, kidney, brain, spleen and lung, cardiac, smooth or skeletal muscle, a topic not relevant to this discussion). Many researchers have reported an established range and ratio of EA in these various body fluids to BAC (Table 5). Limited research suggests that synovial fluid from intact joints serves as a readily obtainable, adequate substitute for vitreous humor in estimating perimortem BAC. These tabulations are valuable and afford reasonable inferences with respect to BAC when intravascular blood is unavailable. BAC estimations must be expressed conservatively within a wide range when they are derived from extravascular biological fluids (or tissues). If biological fluids other than peripheral blood specimens are collected, the EA concentration derived from stomach or gastric contents (gastric alcohol content: GAC) may be referenced to improve the accuracy of the estimated BAC. One study of 60 autopsy cases suggested that a GAC $>0.5\,g\,dl^{-1}$ at death likely indicated "recent" ingestion and that the subject was in the preabsorptive phase; and, further, that a subject with a GAC $<0.5\,g\,dl^{-1}$ may be considered in the postabsorptive state. With the realization that the removal of EA from the stomach, especially in real-life drinking circumstances, is subject to multiple variables regarding end of drinking and time to peak BAC, this comparison may improve – in the absence of a reliable history – the estimation of the pharmacokinetic state of the individual at the time of death.

CSF can be used for EA analysis; however, use of CSF EA levels is of limited value in light of studies indicating that EA does not reach the CSF in maximum concentration until 3 h after the end of drinking and also exhibits delay in distribution equilibrium. If the posttraumatic survival of an individual is prolonged, the postmortem analysis of EA from sequestered intracerebral or paradural hematomas may be of value in estimating retrospectively the BAC at the time of injury.

Urine

With qualification, urine is potentially an acceptable medium to estimate BAC and to determine the pharmacokinetic phase of the subject at the time of collection. The preferred sample is ureteral urine. Excreted

EA from the renal circulation as a glomerular filtrate prior to mixing with water in the tubules is virtually identical to that in the water content of blood in that vascular compartment. Clinically or at autopsy, collection of ureteral urine is not practical. The urinary bladder is a storage container for eliminated urine until voiding. In the absence of pathological or drug-related conditions affecting urine production, urine continuously enters and collects in the bladder. It contains variable time-and-volume-dependent concentrations of EA.

Confounding factors in collection are inherent in the measurement of UAC. In living subjects, the stored urine must be voided and a subsequent urine specimen collected over time (30–60 min) with no EA consumption or postvoiding alteration. If, for example, after arrest for presumptive driving under the influence (DUI), a first void is followed promptly ($\leq$30 min) by withdrawal of a venous blood sample and then a second void (60 min (range 30–130)), reference limits for UAC/BAC have been established to estimate the subject's venous BAC. Once-voided urine should only be used as a qualitative test for EA. Toxicological urinalysis, though often based on one void in practice, is generally of little or no value *per se* to estimate an individual's BAC at a given time.

At autopsy, UAC represents the cumulative or integrated sum of different BACs *intra vitam* over time, encompassing various phases of EA metabolism. Pooled urine merely estimates an average urine concentration over the collection time. The quantitated UAC may be used for rough estimates of BAC in that timeframe. The reported average UAC:BAC ratio is 1:1.33, but the experimentally determined range is great, reportedly from 1:0.21 to 1:2.17–2.44. UAC:BAC comparisons may also be used to delineate the stage of metabolism the individual is in at the time of specimen collection: absorptive phase UAC:BAC $<$1.0; postabsorptive phase UAC:BAC $>$1.3.

Decomposed or Embalmed Specimens

Thorough intravascular embalming renders blood a medium unavailable for determining preembalming BAC. Vitreous humor may serve as a suitable substitute. In such cases the toxicologist must analyze a sample of the embalming fluid to compare with the VAC. In general, many embalming fluids, usually composed of formaldehyde, either do not contain EA or have relatively low levels compared to other volatiles. In commercially manufactured embalming fluid, other volatiles may include acetone, methanol, isopropanol, and occasionally EA. Typical formulas distinguishing the volatiles in embalming products are readily available. Another technical difficulty in analysis of EA in cases of exhumation–embalming arises when dehydration of tissue or postmortem synthesis of alcohol is present after prolonged burial. EA may be elevated in bodies that have not been embalmed in a timely manner. The BAC is therefore likely due to postmortem production of EA.

Postmortem decomposition, even at an early stage, falsely elevates BAC and complicates the task of the interpretative toxicologist. Fermentative flora, primarily bacteria, fungi, and yeast, enter the vascular compartment postmortem, metabolize glucose or protein, and produce endogenous EA chemically identical to that in alcoholic beverages. Because of relative isolation from the putrefactive processes, urine from the bladder and vitreous humor, which reside in relatively sterile compartments, are sometimes spared of this phenomenon.

Investigators report postmortem BAC as high as 0.22% attributable to endogenous production. In moderate-to-severe decomposition, simultaneous analysis of either vitreous humor or urine devoid of EA supports the conclusion that the postmortem BAC is due to endogenous fermentation by microorganisms. Bodies that have been stored in cold environments generally will have minimal endogenous alcohol production. Endogenous fermentation also applies to victims of drowning, who frequently undergo severe decompositional change even in temperate climates. Moreover, dilutional factors may occur, especially in fresh-water drowning. Therefore, the BAC quantified from postmortem samples may actually be lower than the true level. Specific variations are not known at this time due to the lack of research in this area.

The endogenous generation of EA by microorganisms is not unique to the postmortem period. Such considerations are also relevant to the living, particularly exemplified by subjects with metabolic complications of diabetes mellitus with urinary tract infections, or sepsis. In diabetics, discrepancies between BAC and UAC, where the latter demonstrates abnormally elevated amounts of EA, may be attributable to urinary retention and incontinence. As a result of this phenomenon, postmortem UAC in diabetics is unreliable.

Summary

In evaluation of the behavioral or lethal effects of EA, appropriately collected and handled specimens are required for both the analytical and the interpretative toxicologist. Currently there is near universal consensus that the preferred antemortem and

postmortem specimen is peripheral whole blood. A confounding factor affecting interpretation of the BAC in the early postmortem period is the occasional site-to-site difference in EA levels. For optimal evaluation and if resources permit, simultaneous collection of peripheral whole blood and a complementary, backup central sample is desirable. Other extravascular fluids or analytes with comparative ratios of EA to whole blood may be utilized as a gauge of its effects on the brain. Vitreous humor is a satisfactory complement to peripheral BAC and should be collected routinely for EA analysis. Several critical factors influence the distribution ratio and must be considered. Foremost among these is the stage of alcohol distribution at collection. The optimal specimen is collected at maximum BAC plateau or during the elimination phase. In spite of detailed historical investigation and thorough postmortem sampling, the medicolegal investigator may not be able at autopsy to pinpoint the pharmacokinetic phase of the individual at the time of death. If, for example, the specimen is collected during the absorption stage, then total body distribution has not been achieved. Evaluation of the reported BAC from that sample requires recognition of this limitation. Cautiously interpreted comparative ratios of whole-blood BAC to extravascular matrices are of value in making reasonable estimates of the BAC at death when a suitable blood sample is unavailable. With decomposition or embalming, interpretation of the BAC is fraught with difficulty even when other matrices are analyzed.

In living subjects plasma or serum is an acceptable body fluid for interpretation when designated as such. Properly collected urine samples may be used cautiously to estimate the BAC when blood is unavailable. Saliva has gained acceptance as a body fluid suitable for analysis of EA and monitoring intoxication in a variety of clinical settings.

See Also

Alcohol: Breath Alcohol Analysis; **Autopsy, Findings:** Postmortem Drug Measurements, Interpretation of; Postmortem Drug Sampling and Redistribution; **Crime-scene Investigation and Examination:** Collection and Chain of Evidence; **Toxicology:** Methods of Analysis, Antemortem; Methods of Analysis, Postmortem

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Acute and Chronic Use, Postmortem Findings

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Introduction

Mention the word alcohol to a chemist and this conjures up a family of organic compounds with broadly similar chemical properties and with each molecule containing one or more hydroxyl (-OH) groups. Alcohol is a generic name for a large group of organic chemical substances derived from hydrocarbons by replacing one or more of the hydrogen atoms with hydroxyl groups. Examples of alcohols commonly encountered in forensic medicine and toxicology are methanol, ethanol, isopropanol, *n*-propanol, and ethylene glycol. The basic properties of these primary alcohols (methanol, ethanol, and *n*-propanol), secondary alcohol (isopropanol), and the dihydroxy alcohol (ethylene glycol) are summarized in Table 1. The alcohol of prime concern in this review is ethanol or ethylalcohol, which is the ubiquitous psychoactive substance in alcoholic beverages (beers, wines, and distilled spirits).

Alcohol is a legal drug and, although most people drink in moderation, many progress to become heavy drinkers and sometimes become alcohol-dependent; in short they are addicted to alcohol and can be diagnosed clinically as alcoholics. People drink alcohol for different reasons. The first experience of this social drug often occurs during adolescence when teenagers and young adults perhaps out of curiosity or peer pressure make their drinking début. Some will then stop or curb their drinking owing to the nausea they experienced, whereas others continue to drink alcohol throughout adult life. Excessive drinking and abuse of alcohol lead to problems within the family, with the police, and at the workplace.

Alcohol has been referred to as the Jekyll and Hyde of the drug world because moderate drinking has a number of beneficial effects on a person's health and gives feelings of well-being. Drinking small amounts of alcohol tends to relax people by lowering their inhibitions and encouraging social interaction. Recent research has shown that 1–2 drinks per day, especially in the form of red wine, is an effective prophylactic treatment for cardiovascular diseases such as stroke. However, for about 10–15% of the population who choose to drink alcohol, particularly men, initial moderate consumption escalates into abuse, which wrecks lives and causes considerable morbidity and mortality. Alternatively, the production, advertisement, and sale of alcoholic beverages represent a major source of government income via taxation, making this legal drug a double-edged sword.

In most countries alcohol-related injuries constitute a major public health problem with enormous costs for society both directly owing to the medical intervention necessary for alcohol-related diseases and also indirectly through alcohol-related accidents. Impairment of body function and diminished performance after heavy drinking are responsible for 30–40% of traffic fatalities in most countries, these being caused by drunk drivers. In addition, many problems in the workplace and in the home, including domestic violence, are another consequence of heavy drinking. Besides driving under the influence, overconsumption of alcohol and drunkenness are underlying factors in many criminal offenses including murder, sexual assaults, and rape as well as the aggressive behavior of drunken hooligans. Autopsy reports show that people committing suicide as well as those who die by drowning have high blood alcohol concentration (BAC). The analysis of alcohol in biological specimens therefore represents the most commonly requested service from forensic science and toxicology laboratories.

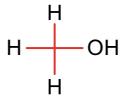
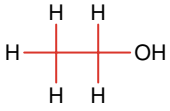
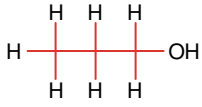
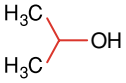
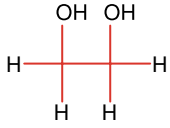
An overview of the forensic science aspects of alcohol is presented, particularly acute and chronic effects on the individual, and issues of importance and concern in relation to postmortem toxicology of alcohol, including a correct interpretation of the results.

Reporting Blood Alcohol Concentrations

Because of the well-established relationship between the concentration of alcohol in a person's blood or breath and the risk of causing a traffic accident, most countries have established threshold limits of alcohol concentration above which it is an offense to drive a motor vehicle. However, these punishable limits differ between countries owing to tradition, lifestyle, and not least various political forces and public opinion. In most European countries a BAC limit of 50 mg per 100 ml is enforced, whereas Norway and Sweden have adopted a threshold of 20 mg per 100 g blood (21 mg per 100 ml). The UK, Ireland, and most US states as well as the provinces of Canada are more tolerant to driving after drinking: the legal blood alcohol limit is 80 mg per 100 ml. In a few remaining US states the threshold alcohol limit for driving is set at 100 mg per 100 ml (Table 2).

The concentration units used to report the results of forensic alcohol analysis depend in part on the kind of biological fluid analyzed, whether blood, breath, or urine. Moreover, some countries use mass/mass units (Germany and the Nordic countries) although

Table 1 Characteristic features of various alcohols commonly encountered in forensic toxicology and legal medicine

Property	*Methanol*	*Ethanol*	*n-propanol*	*Isopropanol*	*Ethylene glycol*
CAS number	65-46-1	64-17-5	71-23-8	67-63-0	107-21-1
Molecular weight	32.04	46.07	60.09	60.09	62.07
Molecular formula	C_2H_4O	C_2H_6O	C_3H_8O	C_3H_8O	$C_2H_6O_2$
Chemical formula	CH_3OH	CH_3CH_2OH	$CH_3CH_2CH_2OH$	$(CH_3)_2CHOH$	$(CH_2OH)_2$
Structure	Primary aliphatic alcohol	Primary aliphatic alcohol	Primary aliphatic alcohol	Secondary aliphatic alcohol	Dihydroxy aliphatic alcohol (diol)
Structural formula					
Common name	Wood alcohol	Beverage or grain alcohol	Propyl alcohol	Rubbing alcohol	Antifreeze
Boiling point	64.7 °C	78.5 °C	82.6 °C	82.5 °C	197 °C
Melting point	−95.8 °C	−114.1 °C	−126.5 °C	−88.5 °C	−13 °C
Density	0.791 at 20 °C	0.789 at 20 °C	0.805 at 20 °C	0.785 at 20 °C	1.11 at 20 °C
Water solubility	Mixes completely	Mixes completely	Mixes completely	Mixes completely	Mixes completely
Main metabolites	Formaldehyde and formic acid	Acetaldehyde and acetic acid	Propionaldehyde and propionic acid	Acetone	Glycolic, glyoxylic, and oxalic acid

CAS, Chemical Abstract Service Registry Number.

Table 2 Threshold concentration limits of alcohol in whole blood and breath for operating a motor vehicle in various countries and the blood:breath ratios of alcohol used to establish the breath alcohol limits

Country	*Blood alcohol concentration*	*Breath alcohol concentration*	*Blood:breath ratio of alcohol*
Most European countries	0.50 mg ml^{-1}	0.25 mg l^{-1}	2000:1
The Netherlands	0.50 mg ml^{-1}	220 μg l^{-1}	2300:1
Norway, Sweden[a]	0.20 mg g^{-1}	0.10 mg l^{-1}	2100:1
Finland	0.50 mg g^{-1}	0.21 mg l^{-1}	2400:1
USA	0.08 or 0.10 g per 100 ml	0.08 or 0.10 g per 210 l	2100:1
UK and Ireland[b]	80 mg per 100 ml	35 μg per 100 ml	2300:1
Canada	0.08 g per 100 ml	0.08 g per 210 l	2100:1

[a]Because a blood alcohol concentration of 0.20 mg g^{-1} is equivalent to 0.21 mg ml^{-1}, the actual blood:breath ratio operating is 2100:1.
[b]If urine is the specimen collected and submitted for analysis, the threshold concentration of alcohol is 107 mg per 100 ml.

most use mass/volume units. Because the specific gravity of whole blood is 1.055 on average, 100 mg per 100 ml blood is close to 95 mg per 100 g blood (Table 2). When the analysis of alcohol is done at hospital or clinical laboratories the specimens used are plasma or serum and these contain more water and therefore more alcohol than an equal volume of whole blood. In addition, the unit of concentration used to report results is mmol l^{-1}, where 21.7 mmol l^{-1} = 100 mg per 100 ml. The average plasma-to-whole-blood ratio of alcohol determined empirically is 1.15:1, which leads to a 15% higher concentration of alcohol in the plasma after the red cells are removed by centrifugation.

Most countries now use breath alcohol instruments in traffic-law enforcement to establish whether a person has consumed too much alcohol for driving. This has necessitated creating threshold breath alcohol concentration (BrAC) limits and thus avoiding the need to translate results into the equivalent BAC in every case. The critical BrAC limits were derived from the preexisting BAC limits by assuming a population average blood:breath ratio of alcohol (BAC:BrAC). Unfortunately, different countries opted for different BAC:BrAC ratios when their threshold BrAC limits were being set. Moreover, the units of concentration used to report blood and breath alcohol measurements differ between countries;

Table 3 Interrelationships between the concentration units used to measure and report blood alcohol concentrations for clinical and legal purposes

UK and Ireland (mg per 100 ml (mg%))	*USA and Canada (g per 100 ml (g%))*	*Most European countries ($g\,l^{-1}$)*	*Nordic countries and Germany ($mg\,g^{-1}$ or $g\,kg^{-1}$)*[a]
50	0.05	0.50	0.47
80	0.08	0.80	0.76
100	0.10	1.00	0.95
150	0.15	1.50	1.42
200	0.20	2.00	1.89

[a]The specific gravity of whole blood is taken as 1.055, whence density is 1.055 g ml^{-1}.

examples of the current BAC and BrAC limits and the blood:breath ratios used in different countries are shown in (Table 3).

In connection with alcohol use in the workplace, especially by those engaged in safety-sensitive work, a BAC of 40 mg per 100 ml is enforced throughout the USA. Furthermore, for people below 21 years a zero-tolerance policy has been instituted for driving, which in practice means a legal blood alcohol limit of 20 mg per 100 ml. This is motivated by the fact that young people are overrepresented in alcohol-related road traffic crashes, which emphasizes the need for stricter control of their drinking habits. The legal drinking age in the USA is 21 years, although teenage drinking is a fact of life and is virtually impossible to control. Nevertheless, establishing a minimum legal drinking age (21 years) and a low BAC (<20 mg per 100 ml) for driving has resulted in a decline in alcohol-related highway deaths among young people.

Alcoholic Beverages

The concentration of alcohol in alcoholic beverages is expressed as percent by volume (% v/v), namely ml alcohol per 100 ml beverage. When required to calculate the amount of alcohol ingested from a given number of drinks these v/v percentages need to be converted to weight percent (% w/v) or g per 100 ml, which is done by multiplying v/v with the specific gravity of alcohol (0.79), leading to the equivalent w/v concentrations shown below.

- Beers 2.5–6.0% v/v – 2.0–4.7% w/v (g per 100 ml)
- Table wines 8–12% v/v – 6.3–9.5% w/v (g per 100 ml)
- Sherry/port 16–20% v/v – 12.6–15.8% w/v (g per 100 ml)
- Spirits 35–50% v/v – 27.6–39.4% w/v (g per 100 ml)

Besides the problem posed by different alcohol concentrations in similar drinks, the situation is further complicated because different volumes are dispensed as a standard measure depending on country and establishment where the alcoholic beverages are served. In the USA, the alcohol equivalent of a standard drink corresponds to a 12 oz (approximately 360 ml) bottle or can of beer, a 5 oz (approximately 150 ml) glass of wine, or a $1\frac{1}{2}$ oz (approximately 45 ml) serving of distilled spirits. Assuming that beer is 5 vol%, wine 12 vol%, and spirits 40 vol%, a standard drink thus corresponds to 14 g pure ethanol or an amount that requires 2 h to become eliminated from the body by metabolism in the liver. Obviously, the exact quantity of ethanol depends on the alcoholic strength of the beverage, which can vary widely for beers (2–10% v/v).

In the UK, a standard drink is referred to as a unit of alcohol and this corresponds to 8 g ethanol, and is considered broadly equivalent to half a pint of beer, a small glass of table wine, or a single measure of distilled spirits. The current recommendation for sensible drinking without risk of damaging health is 1–2 units per day. For men, risky drinking implies consumption of 8 units of alcohol daily (64 g) over a long period of time, which will eventually lead to alcohol-related health problems. For women, the amount of alcohol considered harmful is 6 units of alcohol or 48 g per day. The smaller size and lower body weight in women mean less body water to dilute the alcohol and therefore a higher BAC for the same dose ingested compared with men. In addition, hormonal differences might make the female gender more vulnerable to the untoward effects of alcohol and its metabolites.

Methods of Measuring Alcohol in Body Fluids

The methods used to measure alcohol in body fluids are the same regardless of whether the specimens are taken from the living or dead. However, differences exist depending on whether alcohol is measured in breath as opposed to liquid specimens like blood, urine, or saliva. Over the years, the methods for measuring alcohol in body fluids have undergone radical changes. Between 1900 and 1950

nonspecific wet-chemical oxidation methods dominated. In the early 1950s more selective enzymatic procedures appeared using the enzyme alcohol dehydrogenase (ADH) extracted from horse liver and/or yeast. Today highly selective physicochemical methods are used for analysis of alcohol in body fluids such as gas–liquid chromatography and mass spectrometry.

The first methods of breath alcohol analysis used the principles of chemical oxidation with dichromate and photometric detection of the endpoint as with the famous Borkenstein Breathalyzer® instrument, which was widely used by police forces in the USA, Canada, and Australia. More modern instruments for breath alcohol analysis rely on infrared (IR) spectrometry for quantitative analysis of ethanol or electrochemical (EC) oxidation, which is the basis of the so-called fuel-cell instruments. Some breath alcohol instruments make use of both analytical principles (IR and EC), thus furnishing an enhanced selectivity for the analysis and identification of ethanol. Breath alcohol testing is noninvasive and therefore ideal for conducting on-the-spot tests in drivers. Several kinds of handheld devices are available for testing motorists at the roadside. Such breath alcohol screening tests are also being used in accident and emergency departments to test for alcohol intoxication in casualty patients. Point-of-care testing is currently in vogue and minimally invasive procedures such as the analysis of exhaled air have many advantages over blood sampling. Another noninvasive approach uses saliva (oral fluid) as a biological specimen for analysis of alcohol and a number of enzymatic test kits are available for this purpose.

The principles and basic features of the various methods used to analyze ethanol in blood and breath are summarized in Table 4. The current method of choice in forensic science and toxicology laboratories is headspace gas–liquid chromatography (HS-GC), which first appeared in the early 1970s. Besides the determination of ethanol, the same HS-GC technique can be applied to analyze a wide range of low-molecular-weight volatile substances that might be present in the biological specimens, such as methanol, acetone, isopropanol, and toluene. In brief, the HS-GC method entails sampling the air or vapor phase, called the headspace, above the liquid specimen (e.g., blood or urine) contained in an airtight glass vial kept at a constant temperature of 50 or 60 °C. After air–liquid equilibrium is established, an aliquot of the vapor phase is removed either using a gas-tight syringe or with some automated system and transferred into the HS-GC column for gas chromatographic analysis.

Table 4 Summary of the analytical methods used to determine ethanol in body fluids

Method of analysis	*Basic principle of the analytical method*
Chemical oxidation	The ethanol is first separated from the biological matrix by distillation, diffusion, aeration, or protein precipitation. The resulting aqueous ethanol is then oxidized, usually with a mixture of potassium dichromate and sulfuric acid, and the reaction endpoint is determined by volumetric titration or by spectrophotometry
Enzymatic oxidation	Ethanol is first separated from the biological matrix as above; the pH of the aqueous distillate is adjusted to between 8 and 9 with semicarbizide buffer, and the coenzyme (NAD^+) is added. Oxidation of ethanol is achieved by adding the enzyme alcohol dehydrogenase derived from yeast and the reaction is monitored by formation of the reduced coenzyme (NADH) at 340 nm by ultraviolet spectrometry
Gas chromatography using liquid injection	An aliquot of blood or other body fluid is diluted 1:5 or 1:10 with an aqueous solution of internal standard (either *n*-propanol or *t*-butanol). About 1–5 μl of the diluted specimen is injected into the gas chromatograph fitted with a polar stationary phase (e.g., polyethylene glycol) and a flame ionization detector is used for quantitation
Gas chromatography using headspace analysis	An aliquot of blood or other body fluid is diluted 1:5 or 1:10 with an aqueous internal standard as above. The diluted specimen is allowed to equilibrate in an airtight glass vial for 20 min before an aliquot of the vapor phase (called the headspace) is removed with a gastight syringe or other means (instruments fitted with automated injectors are common) and transferred into a gas chromatograph for analysis
Infrared spectrometry	Ethanol in the vapor phase (e.g., breath) is quantitatively determined by infrared spectrometry according to the Lambert–Beer law. Ethanol absorbs infrared radiation at wavelengths of 3.4 μm corresponding to the C-H stretch and at 9.5 μm corresponding to the C-O stretch
Electrochemical oxidation	Ethanol in the vapor phase (e.g., breath) is quantitatively determined by electrochemical oxidation with a platinum black catalyst and an acid electrolyte mounted with electrical connections to form a fuel cell. The ethanol molecules enter one side of the cell and are oxidized via acetaldehyde to acetic acid; the current produced is proportional to the concentration of ethanol in the sample

A recommended practice in forensic science is to make all determinations of BAC in duplicate. To enhance the selectivity of the assay the chromatographic conditions should differ for each aliquot of the duplicate and this requires the use of two different stationary phases that give unique retention times (RTs) for ethanol. The RT is defined as the time in minutes measured from the point of injection to the appearance of the apex of the peak on the chromatogram. RT is characteristic of the substance analyzed and is used for qualitative analysis or identification by comparison with pure known compounds. In practice it is rare that different substances have the same RT under the same chromatographic conditions and even rarer if two different GC systems are used for the analysis. Quantitative analysis is achieved by measuring the height or area under the GC peak response and for many volatile compounds a flame ionization detector (FID) is the universally accepted method. The detector response from the FID is remarkably linear over a wide range of concentrations encountered in forensic toxicology from 0 to 600 mg per 100 ml and higher.

Alcohol in the Body

Alcohol is a small polar molecule (molecular weight 46.07) and mixes with water in all proportions. Alcohol is easily absorbed from the stomach and small intestine by passive diffusion according to the concentration gradient existing. Drinking alcohol in the form of whisky (40% v/v), wine (10% v/v), or beer (5% v/v) will be expected to show different rates of absorption. The alcohol from the stronger drink is likely to become absorbed faster and give higher peak BACs for the same dose. Some basic characteristics of ethanol in the body and body fluids are summarized in Table 5.

Trace amounts of alcohol are produced naturally in the body mainly through the action of microorganisms and yeasts in the jejunum and colon that utilize dietary carbohydrates as substrates for biosynthesis of alcohol. However, the concentration of endogenous ethanol reaching the peripheral venous blood remains very low (<0.1 mg per 100 ml) as determined by highly sensitive and specific methods. Indeed, if any alcohol is produced in the gut it first has to enter the portal venous blood and pass through the liver before reaching the peripheral circulation. The alcohol-metabolizing enzymes located in the liver can effectively metabolize low concentrations of endogenously produced alcohol and only trace amounts are detectable in the peripheral circulation. Endogenous ethanol production therefore lacks any clinical or forensic significance.

Absorption of Alcohol

After drinking beer, wine, or spirits, the alcohol (ethanol) present in these beverages mixes with the total body water without binding to plasma proteins and the solubility of ethanol in fat and bone is negligible. How fast alcohol enters the blood stream depends on many variable factors, particularly the amount ingested, the rate of drinking, and especially the speed of gastric emptying. Alcohol can be absorbed

Table 5 Characteristic features of ethanol and its distribution in body fluids

Property	*Value*
Molecular weight	46.07
Density	0.79 g ml^{-1}
Critical diameter	4.4 Å
Dielectric constant	26
Energy value	~ 7.1 kcal g^{-1}
Spirits 40 vol%	31.6 g per 100 ml
Wine 12 vol%	9.5 g per 100 ml
Beer 5 vol%	4.0 g per 100 ml
SI units 21.7 mmol l^{-1}	100 mg per 100 ml
Standard drink contains	8–10 g ethanol
Plasma:whole blood distribution ratio	1.15:1 (wide range)[a]
Urine:whole blood distribution ratio	1.30:1 (wide range)[b]
Distribution volume	0.6 l kg^{-1} (women) 0.7 l kg^{-1} (men)
Proportion metabolized and excreted	95% and 5%
Elimination rate from blood (range)	10–25 mg per 100 ml per h[c]
Elimination rate from body (range)	6–18 g ethanol per h[c]

[a]The values in any individual case depend on the water content of the specimen, which in turn depends on blood hematocrit.
[b]The urine:blood alcohol concentration ratio is lower on the ascending limb compared with the descending limb of the blood alcohol curve and increases as blood alcohol concentration decreases.
[c]Values apply to the vast majority of people.

through the stomach and also from the small intestine (duodenum and jejunum) where the rate of absorption is faster owing to the larger internal surface area provided by the villi. The alcohol contained in beer and wine tends to be absorbed more slowly than alcohol from whisky and vodka, not only because of the lower concentrations present but also because malt beverages and wines contain sugars as well as other constituents that tend to delay gastric emptying owing to an altered gastric pH caused by the buffer capacity of the drink.

Eating a meal during or before drinking alcohol diminishes the rate of alcohol absorption into the blood because the food tends to delay stomach emptying. The resulting peak BAC is lowered after food and the time that alcohol remains in the body is shorter under these conditions compared with taking the same amount on an empty stomach. This is illustrated in Figure 1, which shows mean concentration–time profiles of ethanol derived from analysis of whole blood from 10 subjects who drank 0.3 g ethanol per kg either after an overnight fast (empty stomach) or after eating a standardized breakfast (after food).

Another factor influencing the absorption rate of alcohol is a person's blood sugar level. It seems that hyperglycemia slows and hypoglycemia accelerates gastric emptying, which means that the time of day when drinks are consumed and other determinants of blood sugar, e.g., eating low-carbohydrate diets, pregnancy, and diabetes, are important to consider. Hormonal changes depending on age, menstrual cycle, and menopause in women might account for gender differences in gastric motility and rate of alcohol absorption. Some commonly used medications (aspirin, cimetidine, ranitidine) can alter gastrointestinal motility and this is reflected in drug-induced changes in ethanol absorption rate. Smoking cigarettes is known to delay the opening of the pyloric sphincter, thereby slowing the absorption of alcohol into the portal venous blood. It seems that a host of environmental and possibly gender-related differences exist that modulate gastric emptying and alter the peak BAC and the acute impairment effects seen after a given dose of alcohol.

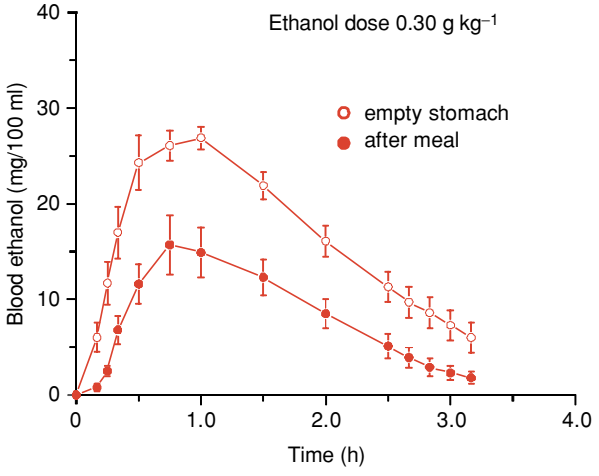


Figure 1 Comparison of the concentration–time profiles of ethanol in blood after drinking a moderate dose of alcohol (0.3 g kg^{-1}) on an empty stomach (10-h fast) or after eating a meal.

Distribution of Alcohol

A person's BAC depends not only on the dose and the rate of absorption of alcohol from the gut but also on the body weight and particularly the amount of muscle and fatty tissues in the body. Having a high proportion of fat instead of lean tissue means a higher BAC for a given dose of alcohol because leaner individuals have more body water into which the alcohol ingested becomes diluted. Since women tend to be smaller than men and also have more fatty tissue per kg body weight and therefore less body water, a given amount of alcohol in a female drinker is expected to produce a higher BAC and therefore a greater intoxicating effect. This makes women more susceptible to the health hazards of prolonged heavy drinking.

All body fluids and tissues take up alcohol in proportion to their water content and the ratio of blood flow to tissue mass determines the speed of equilibration into the various body compartments. Most of the body water resides in the skeletal muscles so only a part of the ingested alcohol is circulating in the blood stream. The ratio of blood flow to tissue mass is high for organs such as the lung, the brain, and the kidney, which rapidly equilibrate with the absorbed alcohol. However, for the resting skeletal muscles in the arms and legs the ratio of blood flow to tissue mass is considerably less and a longer time is necessary to attain equilibrium with the concentration of alcohol in the arterial blood. This leads to arterial–venous difference in ethanol concentration, which are particularly marked during the absorption and distribution stages of ethanol metabolism. By 60–120 min postingestion, the arterial–venous differences are abolished and for the remainder of the time alcohol is present in the body, providing no further drinks are taken, the concentration in the venous blood is slightly higher than in the arterial blood.

The relationship between a person's BAC and the amount of alcohol absorbed and distributed in all body fluids and tissues is given by the following simple equation:

$$A = \text{BAC} \times V_d \times \text{body weight} \qquad [1]$$

Table 6 Relationship between blood alcohol concentration (mg per 100 ml) and the amount of alcohol (g) absorbed and distributed in all body fluids at time of sampling. Values are shown for the average nonobese healthy adult person with body weights (kg) ranging from 50 to 90 kg

Blood alcohol (mg per 100 ml)	*Subject*[a]	*50 kg*[b]	*60 kg*	*70 kg*	*80 kg*	*90 kg*
20	Male	7.0	8.4	9.8	11.2	12.6
	Female	6.0	7.2	8.4	9.6	10.8
50	Male	17.5	21.0	24.5	28.0	31.5
	Female	15.0	18.0	21.0	24.0	27.0
80	Male	28.0	33.6	39.2	44.8	50.4
	Female	24.0	28.8	33.6	39.4	43.2
100	Male	35.0	42.0	49.0	56.0	63.0
	Female	30.0	36.0	42.0	48.0	54.0
150	Male	52.5	63.0	73.5	84.0	94.5
	Female	45.0	54.0	63.0	72.0	81.0
200	Male	70.0	84.0	98.0	112.0	126.0
	Female	60.0	72.0	84.0	96.0	107.9

[a]The volumes of distribution of ethanol were assumed to be 0.7 l kg^{-1} for men and 0.6 l kg^{-1} for women.
[b]Conversion factor: 1 kg = 2.2 lb and therefore 50 kg is 110 lb.

where A is the amount of alcohol in grams absorbed and distributed in all body fluids at the time of sampling blood, BAC is the person's BAC in units of g l^{-1} (not mg per 100 ml), body weight in kilograms, and V_d is the volume of distribution of alcohol expressed as liters per kilogram (l kg^{-1}) body weight. The average V_d parameter for healthy nonobese males is 0.7 l kg^{-1} and for healthy nonobese females 0.6 l kg^{-1}. By rearranging the above equation it becomes obvious that V_d corresponds to the ratio of the concentration of alcohol in the whole body (dose $= A/kg$) to the concentration of alcohol in the blood (BAC). Since alcohol only distributes into the total body water, the ratio of alcohol in the body to alcohol in the blood is the same as the ratio of percent water in the body to percent water in the blood.

Assuming a total body water of 60% for men and 53% for women and a blood water of 82% w/v for men and 86% w/v for women (because of their lower hematocrit) predicts a V_d of 0.73 l kg^{-1} for men and 0.62 kg^{-1} for women. Obviously there are appreciable inter- and intraindividual variations in the actual values depending on the person's age, gender, and the amount of adipose tissue in the body, as indicated by studies of body composition and body mass index (BMI). The magnitude of variation in V_d is about ± 20% within the same gender. Table 6 was constructed using the above equation to give the amounts of alcohol in grams absorbed and distributed in all body fluids for healthy men and women with body weights ranging from 50 to 90 kg. The calculations were based on measured blood ethanol concentrations from 20 to 200 mg per 100 ml.

Metabolism and Elimination of Alcohol

Once absorbed from the gut, the alcohol molecules are transported to the liver by the portal venous blood where hepatic enzymes begin to clear the drug from the blood stream. The principal alcohol-metabolizing enzyme is ADH, which converts ethanol into its primary toxic metabolite acetaldehyde (Figure 2) which fortunately is swiftly transformed into acetate by another hepatic enzyme called aldehyde dehydrogenase (ALDH).

The same enzymes are also involved in the metabolism of methanol, as illustrated in Figure 2. Indeed, the classic treatment for patients poisoned with methanol is to administer ethanol intravenously to achieve an initial BAC of 100–150 mg per 100 ml and keep this constant by administering more ethanol at a constant rate of 7–10 g h^{-1}. This treatment prevents the oxidation of methanol into its toxic metabolites formaldehyde and formic acid, and any unmetabolized methanol can be removed from the blood stream by dialysis. Bicarbonate is also given to the patient to counteract acidosis caused by excess formic acid in the blood. Another more modern antidote for methanol poisoning is the drug fomepizole (4-methylpyrazole), which is a competitive inhibitor of ADH.

The catalytic activities of both ADH and ALDH display racial and genetic variations including polymorphism and isoenzymes exist with different characteristics including specificity for substrates and k_m and V_{max} values (Figure 2). Many people of Asian descent (40–50%) have an inherent low tolerance to alcohol and experience nausea even after a couple of drinks because they inherit a defective form of the ALDH enzyme. The enzyme is less capable of

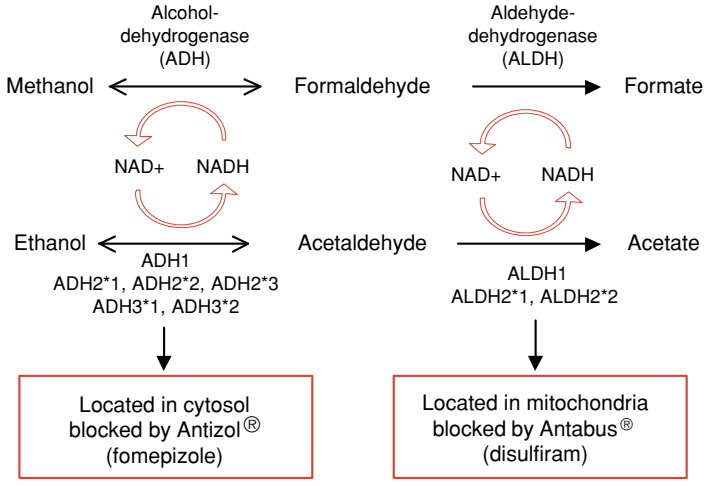


Figure 2 Scheme showing the enzymatic oxidation of ethanol and methanol via alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) and the various isozymes involved and examples of drugs that inhibit ADH (fomepizole or 4-methylpyrazole) and ALDH (disulfiram).

effectively metabolizing the acetaldehyde produced during the oxidation of ethanol and abnormally high concentration of acetaldehyde appear in peripheral blood. The high blood acetaldehyde triggers a range of unpleasant effects including facial flushing, nausea, tachycardia, and breathing difficulties, and this deters people from continuing to drink. These individuals are afforded a protection from becoming heavy drinkers and alcoholics owing to their inability to metabolize acetaldehyde effectively. The same effect can be achieved by giving Antabuse® (disulfiram), a drug treatment known as aversion therapy for alcoholics (Figure 2). This medication works by blocking the action of ALDH so if a treated person drinks alcohol he/she suffers the consequences caused by the high concentration of blood acetaldehyde.

Oxidative Metabolism

The bulk of the dose of alcohol ingested (93–95%) undergoes oxidative metabolism. This process occurs primarily in the liver, whereby ethanol is converted enzymatically first to acetaldehyde and then to acetate by the action of ADH and ALDH, respectively. These enzymes are located in the cytosol fraction of the liver (ADH) and mitochondria (ALDH) (Figure 2). Only small amounts of ethanol (5–7%) are excreted unchanged in breath, sweat, and urine, which means that drinking water to increase production of urine or hyperventilating the lungs or exercising to increase the formation of sweat are not effective ways of lowering the BAC to sober up quicker. There is an abundance of ADH in the liver so even people with serious liver dysfunction, such as hepatocellular carcinoma or cirrhosis, are capable of metabolizing ethanol, albeit at a slightly slower rate.

Another enzyme system engaged in the metabolism of ethanol is known as cytochrome P4502E1 (CYP2E1), which is located in a subcellular component of the hepatocyte know as smooth endoplasmic reticulum, particularly the microsomal fraction. The CYP2E1 as well as many other microsomal enzymes (e.g., CYP2D6, CYP2C9, CYP2C19) are important for the metabolism of endogenous substances as well as drugs and xenobiotics taken into the body. The CYP2E1 enzyme has a higher k_m for oxidation of ethanol (40–60 mg per 100 ml) and therefore comes into play when BAC reaches higher concentrations, as in heavy drinkers and alcoholics. Moreover, the CYP2E1 enzyme is inducible after a period of binge drinking so that alcoholics and others with very high BAC can clear ethanol more effectively from the blood stream. This accounts for the faster rates of disappearance of ethanol from blood reported during detoxification of alcoholics (30–35 mg per 100 ml per h). Many adverse drug–alcohol interactions are caused by the CYP2E1 enzyme, which is also involved in metabolism of the over-the-counter medication acetaminophen (paracetamol). Hyperactive CYP2E1, caused by heavy drinking, can result in liver damage and cell death owing to a toxic metabolite of acetaminophen. Furthermore, many dangerous environmental chemicals (e.g., chlorcarbons and hydrocarbons) are substrates for CYP2E1 and these can also be converted into toxic metabolites. The fate of ethanol in the body and the proportions metabolized and excreted are illustrated in Figure 3.

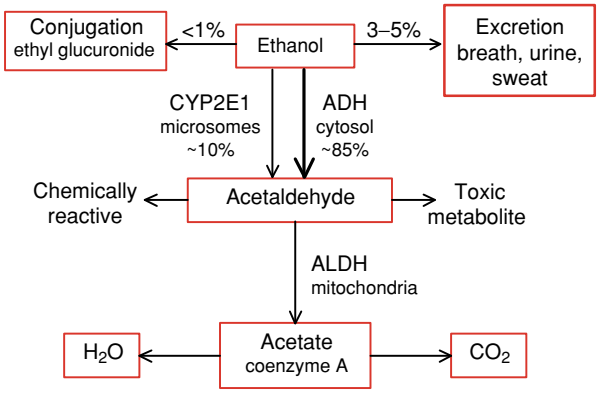


Figure 3 Scheme showing the fate of ethanol in the body, the relative amounts oxidized via alcohol dehydrogenase (ADH) and cytochrome P450 (CYP2E1), and conjugation and excretion in breath, sweat, and urine.

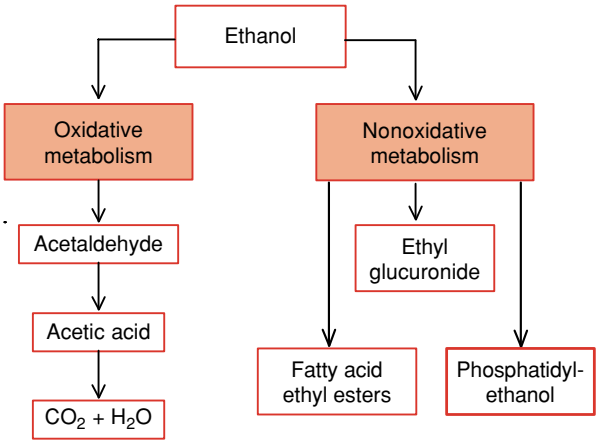


Figure 4 Comparison of the metabolites of ethanol produced by oxidative and nonoxidative metabolic pathways.

Gastric Metabolism of Ethanol

Although ethanol is primarily metabolized in the liver where most of the enzymes are located, studies have shown that ADH also occurs in the lung, kidney, and gastric mucosa. Great interest was aroused some years ago when investigators claimed that a considerable fraction of the dose of ethanol consumed was already eliminated in the stomach by class IV isozymes of ADH in the gastric mucosa. Interestingly, the activity of gastric ADH was found to be lower in women than in men and also less in alcoholics compared with moderate drinkers. It was argued that women and problem drinkers were more vulnerable to the ill-effects of ethanol and reached higher BAC for the same pattern of drinking because they lacked the capacity for ethanol metabolism in the stomach. This would mean an enhanced systemic availability of ethanol and a greater risk of organ and tissue damage as well as more pronounced acute effects on the person's performance and behavior. The magnitude of gastric first-pass metabolism was found to be greater during repetitive drinking, which is more in keeping with daily life, as compared with ingesting a bolus dose.

In another series of publications, it was shown that gastric ADH was rendered less effective when certain medication, such as aspirin, Tagamet®, and Zantac®, had been taken before drinking. This was explained by drug-induced inhibition of the gastric ADH enzyme so people who combined alcohol with this medication were more liable to reach a higher BAC because less was metabolized presystemically in the stomach. However, the significance of gastric ADH as a protective barrier against alcohol's effects is still a matter of conjecture and debate. It was found that the difference in BAC curves with and without the drug was highly dependent on the dose of alcohol ingested and the fed/fasted state of the individual. Whether the liver or the gut is the primary site for first-pass oxidation of ethanol is therefore still unresolved.

Nonoxidative Metabolism

It has been known for many years that a very small fraction (<1%) of the dose of ethanol consumed undergoes a phase II conjugation reaction with glucuronic acid to produce ethyl glucuronide (EtG), which is then excreted in the urine. Recent studies have shown that EtG has a much longer elimination half-life than ethanol itself and can therefore be detected in blood and urine long after ethanol is no longer measurable. This means that analysis of EtG in blood or urine could serve as a marker to detect recent consumption of alcohol. This would be useful when monitoring outpatient alcoholics and others in rehabilitation programs who are required to refrain from drinking.

Other examples of nonoxidative pathways of ethanol metabolism are the synthesis of fatty acid ethylesters (FAEE) and phosphatidylethanol (PEth) products formed during enzymatic reactions between ethanol and various free fatty acids (esterification) and phospholipid adducts, respectively. Both FAEE and PEth are being actively researched as markers of excessive drinking and also as possible explanations for ethanol toxicity to body organs and tissues. The oxidative and nonoxidative metabolites of ethanol are compared in Figure 4.

Rate of Alcohol Disappearance from Blood

The speed at which alcohol disappears from the blood stream is often discussed in forensic and legal medicine because this information is needed when retrograde estimations of a person's BAC are required,

such as in drink driving cases. It sometimes happens that the BAC at the time of driving or involvement in a traffic accident needs to be estimated from the BAC measured several hours later at a time when the blood was drawn. In properly conducted experiments the rate of ethanol disappearance from the blood, which is commonly referred to as the β-slope or burn-off rate, usually ranges from 10 to 25 mg per 100 ml h^{-1} in the vast majority of people. These rates were obtained from controlled drinking experiments and tracing the concentration–time profiles of ethanol. Only those blood samples on the postabsorptive elimination phase of the BAC profile can be used to calculate the disappearance rate of ethanol from blood (Figure 5). This rate is given by the slope of the line (β-slope) or the ratio C_o/min_o in units of mg per 100 ml h^{-1}. The volume of distribution of alcohol is derived from the ratio dose (g kg^{-1})/C_o and takes the units l kg^{-1}.

In healthy people with moderate drinking habits a disappearance rate of 15 mg per 100 ml h^{-1} is considered a good average value, although in heavy drinkers, including many apprehended drunk drivers, higher mean rates are found, such as 19 mg per 100 ml h^{-1}. Alcohol starts to become metabolized from the moment it enters the body and this process continues at a constant rate per unit time (zero-order kinetics). When a measured BAC needs to be converted into the amount of alcohol a person has consumed, the amount lost through metabolism since beginning to drink must be considered. This can be done by using eqn 2, where ($\beta \times t$) is the amount of alcohol eliminated since the start of drinking, that is 0.15 mg per 100 ml h^{-1} multiplied by the number of hours elapsed.

$$A = [\text{BAC} + \beta \times t] \times V_d \times \text{body weight} \qquad [2]$$

To obtain an estimate of the rate of elimination of alcohol from the whole body one needs to consider the volume of distribution of alcohol, which depends on, among other things, the size of the individual and the total body water. The amount of alcohol eliminated from the body is given by the product of β and V_d and has units of g kg^{-1} h^{-1}. As a rule of thumb, a human being can eliminate 0.1 g 100% ethanol per kilogram body weight per hour regardless of gender, so a man or woman of 80 kg body weight eliminates 8 g ethanol per hour or approximately one unit of alcohol.

Table 7 shows likely rates of alcohol elimination from the blood stream (β-slope) and the body as a whole and also the conditions under which these values might be observed in practice.

Distribution of Alcohol into Body Fluids

Many different body fluids and tissues have been used for the determination of ethanol in clinical and forensic medicine and the choice depends on whether samples are taken from living or dead bodies. The specimens most commonly obtained are listed in Table 8, although it should be noted that the concentration of alcohol is not the same in the various fluids or tissues listed. The main reason for this is the different amounts of water and the time after end of drinking when the samples are obtained or how long after drinking death occurred in the cause of forensic autopsy work.

Urine

Much has been written about the relationship between alcohol in blood and urine in both living

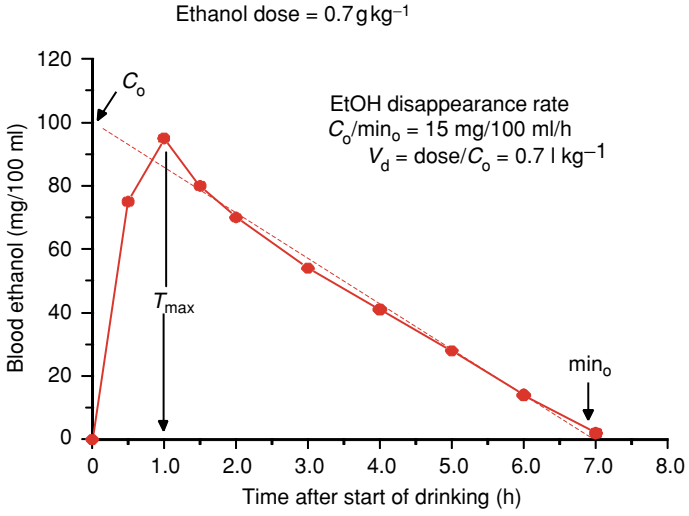


Figure 5 Concentration–time profile of ethanol after drinking 0.7 g kg^{-1} (160 ml whisky) on an empty stomach and the method of calculating disappearance rate from blood (β-slope) and the volume of distribution (V_d).

Table 7 Expected elimination rate of alcohol from blood and the whole body under different treatments or conditions

Elimination rate from blood (mg per 100 ml per h)	*Elimination rate from whole body (g h^{-1})*[a]	*Conditions/treatment necessary*
8–10	4–5	People with liver dysfunction (e.g., owing to cirrhosis or carcinoma) or those who are malnourished or eat low-protein diets. Treatment with the drug fomepizole (4-methylpyrazole) will also slow elimination of ethanol
10–12	5–6	Healthy individuals who drink moderate amounts of alcohol after an overnight (10-h) fast
12–16	6–8	After ingestion of a moderate dose of ethanol under nonfasting conditions
16–25	8–12	Healthy individuals who reach appreciably high blood alcohol concentration (>120 mg per 100 ml) such as drunk drivers
25–35	12–17	Alcoholics or very heavy drinkers immediately after a drinking spree (e.g., during detoxification). Even heavy drinking for several days might show enhanced rates. Treatment with protein-rich diets or conditions that cause hypermetabolic conditions (e.g., burn trauma, hyperthyroidism)

[a]The above values apply to a healthy nonobese individual with a body weight of 70 kg and an ethanol volume of distribution of 0.7 l kg^{-1}.

Table 8 Examples of biological specimens used for determination of alcohol in forensic casework when dealing with living and dead subjects

Living subjects	*Deceased subjects (postmortem)*
Whole blood	Whole blood
Cubital vein[a]	Cubital or jugular vein
Radial artery	Femoral vein[a]
Capillary or fingertip sample	Cardiac (heart) blood
Plasma or serum[a]	Stomach contents
Freshly voided urine[a]	Bladder urine[a]
Tears	Vitreous humor[a]
Cerebrospinal fluid	Cerebrospinal fluid
Saliva	Bile
Sweat	Bone marrow or synovial fluid
Breath	Various tissues
Free-expired	Brain
End-expired[a]	Muscle
Rebreathed	Liver

[a]Recommended specimens if available.

subjects and also in cadavers. Indeed, in postmortem toxicology urine is an important biological specimen for the analysis of alcohol and comparing the concentration in urine and blood can help to resolve whether postmortem synthesis might have occurred. Except in conditions like diabetes or other disturbances in carbohydrate metabolism, bladder urine does not normally contain sugar, which is the usual substrate for microbial synthesis of ethanol. This means that the risk of postmortem synthesis of ethanol is seemingly less in bladder urine compared with blood specimens.

In healthy people, urine is produced in the kidneys and enters the bladder at a rate of about 1 ml min^{-1} or 60 ml h^{-1}, although this production might increase 10-fold during a period of alcohol-induced diuresis close to the peak BAC. Drinking water will not dilute the concentration of ethanol in the urine because this depends on the concentration of ethanol in renal artery plasma, which cannot be lowered by drinking liquids. Urine is therefore an excellent body fluid to verify that a person has used a particular drug, including alcohol, and urine drug testing is a large commercial enterprise in most countries. However, interpreting the concentrations of ethanol determined in urine and blood taken at autopsy is not always easy because urine tends to pool in the bladder, during which time the blood ethanol concentrations might have changed considerably, especially during the absorption phase. Urine is secreted in batches so the ethanol concentration in a voided sample will not reflect the concentration of ethanol in renal artery blood at the time of voiding. Much depends on the particular stage of alcohol pharmacokinetics and how long the person might have survived after drinking alcohol before death occurred. But also in living subjects the relationship between urine and blood

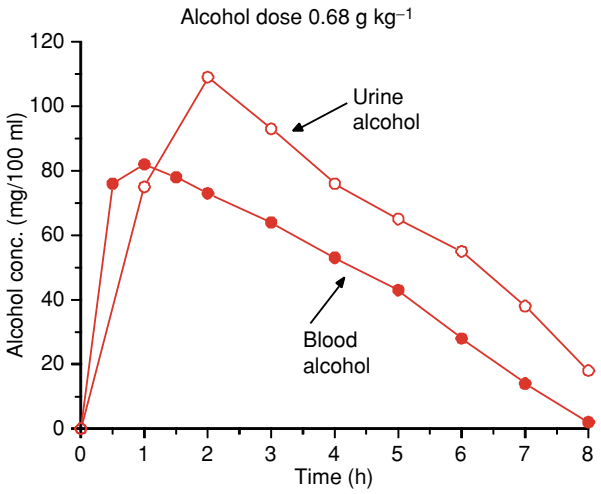


Figure 6 Concentration–time profiles of ethanol in blood and urine after drinking a moderate amount of alcohol (0.68 g kg^{-1}) in 20 min on an empty stomach. Alcohol was in the form of neat whisky and the bladder was emptied before the start of drinking.

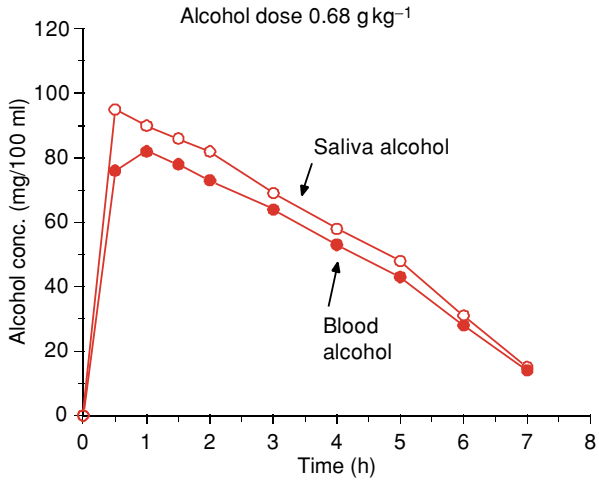


Figure 7 Concentration–time profiles of ethanol in blood and saliva after drinking a moderate amount of alcohol (0.68 g kg^{-1}) in 20 min on an empty stomach. Alcohol was in the form of neat whisky.

alcohol can differ widely depending on the position of the blood alcohol curve and the storage time in the bladder before voiding (Figure 6).

The magnitude of variation in urine vs blood alcohol concentration ratios is an important consideration whenever attempts are made to estimate BAC indirectly from the concentration determination in urine. Although the average urine:blood ratio of ethanol in the postabsorptive phase is about 1.3:1, the values vary widely in any individual case depending on many factors. A conservative estimate of BAC from UAC can be obtained by using a considerably higher UAC:BAC ratio such as 1.5:1 or higher, depending on legal requirements, such as beyond a reasonable doubt or more likely than not. Figure 6 shows the relationship between alcohol in blood and bladder urine in one subject who consumed 0.68 g ethanol per kg body weight on an empty stomach in 20 min. Note that the bladder was emptied before drinking started and then every hour for up to 7 h.

Saliva

Saliva is a watery fluid produced by the parotid, the submaxillary, and the sublingual glands, although the mixed oral fluid collected for determination of drugs also contains mucous secretions from the mouth. The use of saliva as a body fluid for analysis of alcohol and other drugs has expanded greatly over the past decade. A number of methods have been developed for sampling saliva such as by chewing on cotton wool or parafilm to stimulate production of an appropriate specimen. For drugs like ethanol, which enter the saliva by simple diffusion from the arterial blood supply to the salivary glands, the time lag between alcohol entering the blood stream and appearing in the saliva is very short. Only the nonprotein-bound fraction of a drug enters the saliva, which makes alcohol an ideal candidate for oral fluid analysis because of its negligible binding to albumin and other plasma proteins. Accordingly, the concentration of alcohol in saliva should be the same as that in the water fraction of the blood. Studies have found that the mean saliva:blood alcohol ratio is about 1.08:1 and this was remarkably constant during absorption, distribution, and elimination of alcohol in the body. The main disadvantage of saliva as a biofluid for drug analysis is the small volume available and the fact that some people, owing to a dry mouth, will not be able to produce the required sample on demand.

Figure 7 shows an example of the pharmacokinetic profiles of ethanol in saliva and blood in one subject who drank 0.68 g ethanol per kg body weight on an empty stomach in 20 min. Note that the saliva profile is closer to the blood alcohol profile than the urine alcohol profile (Figure 6) owing to a shorter time lag for alcohol to enter the oral fluids compared with the urine in the bladder, which is stored until voided.

Breath

A small fraction (1–2%) of all the alcohol ingested is exhaled in the breath. Alcohol diffuses from the pulmonary capillary blood across the alveolar–capillary membrane into the alveolar spaces and into the respiratory passages. The amount of ethanol leaving the body via the lungs depends primarily on the blood/air

partition coefficient for alcohol, which is about 2000:1 at body temperature (37 °C). With a BAC of 100 mg per 100 ml the concentration of ethanol in alveolar air is only 0.05 mg per 100 ml (100/0.05 = 2000). If ventilation of the lungs is 4 l min^{-1} or 240 l h^{-1}, only 120 mg of ethanol (0.12 g) leaves the body with the exhaled air every hour. This amount is negligible compared with the amount lost by hepatic metabolism, which corresponds to 6–8 g of ethanol per hour.

The analysis of alcohol in breath has found many applications in both clinical and forensic medicine as a rapid noninvasive test for alcohol consumption and if necessary as a way to estimate the amount of alcohol in the body. A wide variety of breath alcohol analyzers have been developed and used for both roadside screening of drivers and also for evidential purposes, as discussed elsewhere in this encyclopedia.

Figure 8 compares venous blood ethanol and breath ethanol concentrations in one subject tested with an infrared breath alcohol analyzer (Intoxilyzer 5000). Note the slightly higher breath readings at the first sampling point just 15 min after drinking ended and the lower results at all later times, which can be explained by arterial–venous differences in blood ethanol concentration. The alveolar and end-exhaled concentration of alcohol is closer to arterial BAC and not venous BAC.

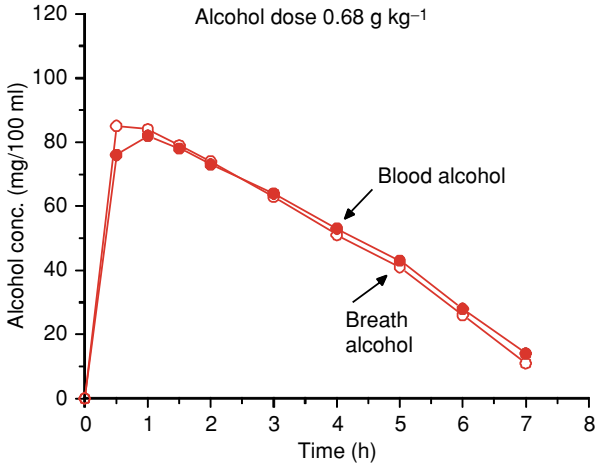


Figure 8 Concentration–time profiles of ethanol in blood and breath after drinking a moderate amount of alcohol (0.68 g kg^{-1}) in 20 min on an empty stomach. Alcohol was in the form of neat whisky and the breath alcohol analyzer was Intoxilyzer 5000 based on infrared analysis.

Vitreous Humor

The vitreous humor (VH) of the eye is a clear fluid widely used in postmortem toxicology not only for determination of ethanol but also for measuring glucose, potassium, chloride, and lactate as well as certain drugs of abuse. VH is particularly useful when bodies are badly damaged or when putrefaction has occurred. VH specimens are easy to obtain using syringe and needle and this procedure can be done even before a complete autopsy is performed. The remoteness of the eye from the gut where bacteria start to spread makes VH less prone to artifacts caused by contamination with microbes and postmortem diffusion of alcohol from the stomach and chest cavity. Indeed, VH can sometimes be used as a specimen for alcohol analysis in embalmed bodies. The concentration of alcohol in VH helps to verify the BAC at autopsy and if necessary BAC can be estimated from VH concentration, albeit with large uncertainty.

Table 9 shows ethanol distribution ratios of VH/blood and urine/blood from a large autopsy material.

Effects of Alcohol on the Body

The effects of alcohol on human performance have been investigated extensively and the cardinal signs and symptoms of drunkenness are common knowledge – lack of judgment and restraint, slurred speech, unsteady gait. The effects of alcohol depend not only on the amounts consumed (the dose) but also on the speed of drinking: larger doses and faster drinking times lead to a more pronounced effect on the person's performance and behavior. An unusually rapid absorption of alcohol such that BAC passes 120–150 mg per 100 ml within 30 min after the end of drinking often results in nausea and vomiting caused by an action of alcohol triggering a vomit reflex in the brain.

Table 9 Mean distribution ratios of ethanol for urine/blood and vitreous humor/blood in specimens taken at autopsy. The vitreous data represent all causes of death but the urine data were alcohol-related deaths only (alcoholism or acute alcohol poisoning)

Body fluids	n	*Mean blood alcohol concentration (median)*	*Mean vitreous humor concentration or urine alcohol concentration (median)*	*Mean ratio*[a] *(median)*	*95% range*
Vitreous humor/blood	505	170 (150)	199 (180)	1.17 (1.18)	0.63–1.45
Urine/blood	1118	309 (320)	372 (380)	1.25 (1.21)	0.85–2.0

[a]Calculated for cases with blood alcohol concentration exceeding 50 mg per 100 ml because ratios increase sharply as blood alcohol concentration decreases.

Table 10 Typical signs and symptoms of acute alcohol influence as a function of a person's blood alcohol concentration when observations were made close to the maximum value after a single oral dose

Blood-alcohol (mg per 100 ml)	*Signs and symptoms of alcohol influence*[a]
<20	No untoward effects or outward signs
30–50	Mild euphoria and impairment of certain skilled tasks that require divided attention
50–100	Reduced inhibitions, increased talkativeness, sensory and motor disturbances, slower reaction time, especially in choice situations
100–150	Lack of coordination, unsteady gait, slurred speech, prolonged reaction to sights and sounds
150–200	Obvious drunkenness, significantly slower reaction time even for simple tasks, nausea and vomiting in some people, ataxia, aggressiveness
200–300	Inability to stand upright and walk without support, incoherent speech, motor areas of the brain severely depressed with distorted perception and judgment
300–400	Confusion, stupor, or coma with shallow breathing and risk of death
>400	Heightened risk of death through respiratory paralysis and cardiopulmonary arrest

[a]Large intersubject variations exist within each blood-alcohol concentration range owing to different drinking patterns and the development of tolerance to alcohol, and individuals may exhibit very different effects.

Table 10 lists some of the typical signs and symptoms of alcohol influence at various BAC intervals, although it is important to note that wide variations exist both between and within individuals from occasion to occasion. Much depends on the person's age and experience with drinking alcohol and particularly the speed of intake, beverage type and whether food was eaten, and not least the development of acute and chronic tolerance. Impairment is more pronounced on the rising part of the blood–alcohol curve compared with the declining phase several hours after end of drinking, and this is known as the Mellanby effect.

Acute Intoxication

Drinking alcohol interferes with many bodily functions including reaction time and the ability to perform skilled tasks, especially those that require divided attention. Cognitive functions are initially influenced (e.g., impairment of thinking, learning, memory) followed by motor skills and vision, all of which increases the likelihood of an accident, especially when skilled tasks like driving are performed. Alcohol reaches the brain almost immediately after drinking starts and the initial effects are felt after just one drink. There is a strong dose–effect relationship between the BAC and degree of inebriation, especially when the BAC curve is in the ascending phase, that is, during absorption of alcohol into the blood stream as it crosses the blood–brain barrier to influence brain functions. After the peak BAC or BrAC is reached in the descending phase of the curve, a marked recovery in both objective and subjective feelings of intoxication is evident. Seemingly the brain adapts to the alcohol environment and several hours after the maximum BAC is reached, highly sensitive tests are needed to detect any residual alcohol impairment.

Alcohol exerts its effects on the brain by interfering with the normal functioning of nerve cells and chemical messengers (neurotransmitters). The wide spectrum of ethanol's effects, progressing from euphoria and excitement to muscle relaxation and ataxia, sedation, and stupor, and ending in coma and respiratory failure (Table 10), suggest the involvement of several different receptor systems including dopamine, gamma-aminobutyric acid ($GABA_A$), glutamate (*N*-methyl-D-aspartate receptor), and serotonin as well as others.

Tolerance

People react to drugs in different ways and accordingly they display wide intersubject variation in how much alcohol is required to elicit a certain effect or cause a change in behavior. Some people tolerate alcohol better than others, especially after a long period of continual drinking. Intake of the same dose of alcohol to reach the same BAC causes less effect in a tolerant person as evidenced by both performance tasks and objective ratings. Alternatively, an increasing amount of drug is necessary to produce the same effect and this as illustrated in Figure 9, is demonstrated by a shift in the concentration–effect curve to the right.

There are several different kinds of tolerance to alcohol:

- Acute tolerance can be defined as an adaptation to the effects of alcohol within a single drinking session in a person hitherto alcohol-free. Measures of alcohol-induced motor impairment at a given BAC

on the rising limb of the curve are more pronounced than at the same BAC on the descending limb after the absorption and distribution terminates. Acute tolerance is particularly marked for subjective feelings of intoxication recorded at various times after drinking. The development and recording of acute tolerance in humans and dogs were first noted about 100 years ago by the British pharmacologist Sir Edward Mellanby.

- Metabolic or dispositional tolerance develops after a period of continuous heavy drinking and this is reflected in a more rapid rate of elimination of alcohol from the blood compared with after drinking a single dose. The mechanism of metabolic tolerance has been traced to a specific group of enzymes located in the microsomal fraction of the liver cell denoted CYP2E1. These enzymes are activated during chronic drinking and "learn" to dispose of alcohol more effectively. This form of enzyme induction is associated with an increased rate of alcohol degradation with less time being needed to clear alcohol from the body, thereby reducing the duration of alcohol's effects on performance and behavior.
- Chronic tolerance is represented by a progressive change brought about by continuous heavy drinking and this tends to develop over months or years of alcohol exposure. A given BAC produces less of an effect on the individual for measurements made at the same time after end of drinking, thus eliminating the confounding influence of acute tolerance. The effect–concentration relationship is shifted to the right in an alcohol-tolerant subject (Figure 9). One consequence of chronic tolerance after prolonged heavy drinking is the emergence of physical dependence. This means that when drinking stops abruptly the tolerant person experiences abstinence, which is associated with a range of unpleasant and often life-threatening effects including anxiety, restlessness, convulsions, delirium tremens, and hallucinations, and many have died after abrupt withdrawal of alcohol.

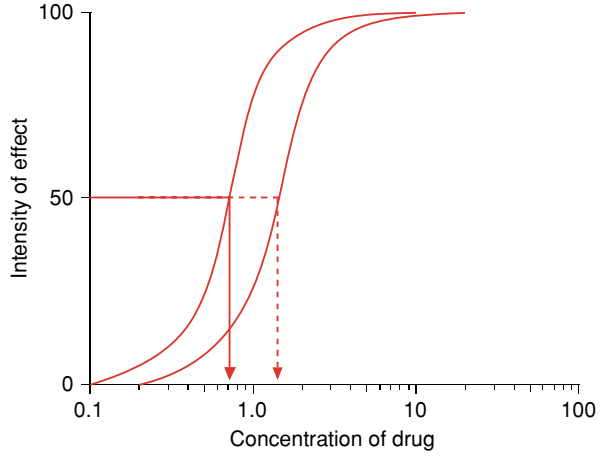


Figure 9 Illustration of the development of chronic tolerance with a parallel shift in the effect–concentration curve to the right.

Depressant drugs used to treat abstinence symptoms include barbiturates and, more recently, benzodiazepines such as diazepam and lorazepam, all of which are agonists for the $GABA_A$-receptor complex. Despite long interest in the phenomenon of acute and chronic alcohol tolerance and dependence, the exact cellular mechanisms involved are obscure, although several neurochemical synapses and receptors are probably involved (mainly $GABA_A$ and glutamate).

The phenomenon of acute alcohol tolerance is well illustrated in Figure 10, where it can be seen that after drinking neat spirits on an empty stomach, the

Figure 10 Relationship between blood-alcohol concentration and the well-known signs and symptoms of alcohol intoxication at various times after drinking alcohol as a bolus dose (1.36 g kg^{-1}) as neat spirits in 15 min and on an empty stomach.

symptoms of alcohol influence were no longer measurable by 6 h postdrinking, although the mean BAC was still relatively high, being close to 110 mg per 100 ml.

The temporal variations in signs and symptoms of alcohol influence, depending on the rising or falling phase of the BAC curve, are shown by the data in Table 11, which come from a Finnish study by Alha. The data show that symptoms of intoxication are more prevalent at lower BACs on the absorption phase compared with the postpeak period several hours postdosing. In most subjects the signs and symptoms of being under the influence of alcohol were no longer evident by 2–2½ h postpeak. This provides experimental verification of what has become known as the Mellanby effect or acute tolerance to alcohol.

Metabolic Effects of Alcohol

Ethanol is unique among drugs of abuse in that it exerts two completely different actions: one of these is nutritional, providing energy (7.1 kcal g^{-1}) and another is impairment of the central nervous system and disruption of intermediary metabolism. During the hepatic oxidation of ethanol (Figures 2 and 3) there is a marked change in the redox state of the liver and the coenzyme NAD^+ is reduced to NADH, which offsets other NAD-dependent metabolic reactions. Among other things, pyruvate is reduced to lactate, causing varying degrees of lactic acidosis, which in turn inhibits the renal excretion of uric acid, which accumulates in joints and precipitates attacks of gout. The ethanol-induced increase in NADH/NAD^+ ratio also explains the characteristic fatty liver seen in heavy drinkers and alcoholics.

In some individuals who drink excessively over many years, fatty liver progresses to hepatitis and eventually liver cirrhosis and death. The change in redox state in the hepatocyte leads to inhibition of fatty acid oxidation, which in turn increases the synthesis of triglycerides. The excess NADH also hampers hepatic gluconeogenesis so if glycogen stores are deleted, as often happens in heavy drinkers who neglect to eat properly, this leads to an alcohol-induced hypoglycemia.

Acetaldehyde, the toxic metabolite of ethanol by all known oxidative pathways (Figure 3), has been incriminated in many of the untoward effects of heavy drinking, including hepatotoxicity, cancer, and cell death and also addiction and dependence.

Identifying Problem Drinkers

Judging whether a person drinks too much alcohol is not always easy because some alcoholics furiously deny their actual pattern of consumption. Obtaining an accurate drinking history by self-reports, clinical interviews, and questionnaires is notoriously difficult. Accordingly, more objective ways to identify heavy drinkers are needed for use in preventive medicine and clinical practice to validate self-reported alcohol consumption. Biochemical markers such as altered urinary metabolites or the activity of certain serum enzymes or an abnormal blood chemistry after a period of continuous heavy drinking have attracted much attention. Laboratory testing for hazardous drinking has become an important area of addiction medicine and in rehabilitation programs, such as in drunk drivers who reapply for a driving permit.

Well-controlled population surveys have shown that only about half of the known total consumption of alcohol in a country can be accounted for by results from questionnaire surveys. Accordingly, various laboratory tests have been developed to aid in the diagnosis of hazardous or harmful drinking. Among others, carbohydrate-deficient transferrin (CDT) and γ-glutamyltransferase (GGT) are well-known

Table 11 Percentage of individuals (healthy men) diagnosed as being under the influence at various times after they drank neat spirits on an empty stomach. Comparisons were made on the rising (absorption) phase and declining phase of the blood-alcohol curves 1–1½ and 2–2½h postpeak

Blood alcohol concentration (mg per 100 g)	*Percentage under the influence on rising (absorption) phase*	*Percentage under influence 1–1½ h postpeak*	*Percentage under influence 2–2½ h postpeak*
12–50	50% (40/80)[a]	#	0% (0/4)
51–80	57% (47/83)	5% (1/18)	0% (0/28)
81–100	66% (33/49)	4% (1/23)	4% (1/24)
101–120	77% (40/52)	36% (8/22)	21% (4/19)
121–140	69% (29/42)	38% (8/21)	15% (3/20)
141–160	91% (30/33)	#	#

[a]Proportion of individuals under the influence of alcohol.
#, None with these blood-alcohol ranges.

Table 12 Biochemical markers or indicators of acute and chronic intake of alcohol

Biochemical marker	*Specimen for analysis*	*Comments*
Ethanol (EtOH)	Blood, breath, saliva, urine	Highly specific and useful to prove acute alcohol intake; sensitivity depends on amount of alcohol consumed
Ethyl glucuronide (EtG)	Blood or urine	More sensitive than analysis of ethanol, this metabolite is a useful marker for recent drinking up to 24 h after a drinking spree
5-hydroxytryptophol (5-HTOL)	Urine	The predominant urinary metabolite of serotonin is 5-hydroxyindoleacetic acid (5-HIAA), although this shifts towards 5-HTOL during catabolism of alcohol. This leads to an increased ratio of 5-HTOL:5-HIAA, which remains elevated for 10–20 h after end of drinking
γ-glutamyl transferase (GGT)	Serum	This serum marker is elevated after chronic drinking and although fairly sensitive it lacks specificity because other factors can elevate the readings and cause positive results (e.g., various drugs, other liver diseases)
Carbohydrate-deficient transferrin (CDT)	Serum	A widely used marker with good specificity for detecting long-standing heavy drinking
Mean corpuscular volume (MCV)	Red blood cells	Routine clinical laboratory test
Transaminases (AST, ALT)	Serum	Routine clinical laboratory tests, although not very sensitive or specific for alcohol abuse

examples of biochemical tests that can signify prolonged heavy drinking and early damage to organs and tissue. Some markers (EtOH, EtG, and 5HTOL/5HIAA: see Table 12 for abbreviations) are useful to detect relapse to drinking in connection with rehabilitation of alcoholics or drug abusers. This places high demands on the sensitivity of the test, which is reflected in a high percentage of true-positive results. If tests are used for medico-legal purposes the results should have high specificity, that is, a small likelihood of obtaining a false-positive result.

The ideal marker should exhibit 100% sensitivity and 100% specificity, but this is never achieved in practice because reference ranges for normal and abnormal values tend to overlap. Nevertheless, the use of biochemical tests for monitoring a person's drinking habits is increasing and is now used in connection with granting life insurance policies and sometimes in connection with job applications.

The main features of some widely used alcohol markers are summarized in Table 12.

Toxicity of Alcohol

The toxicity of alcohol is low compared with many other drugs and toxins when one considers that tens of grams (25 g and more) are necessary to bring about a pharmacological effect compared with milligram amounts of other drugs (e.g., 5–10 mg diazepam, 10 mg morphine, 100 mg codeine) in first-time users. However, the ratio of effective dose to lethal dose for ethanol is fairly narrow, being only about 8:1, considering that a BAC of 50 mg per 100 ml causes euphoria whereas 400 mg per 100 ml causes death. Alcohol can kill in various ways besides sudden deaths associated with acute alcohol poisoning and chronic alcoholism. In deaths on the roads and in the workplace as well as in suicides and other kinds of trauma, alcohol intoxication and drunkenness are overrepresented.

Table 13 Postmortem blood-alcohol concentration in men and women when death was attributed to acute alcohol poisoning and when alcohol was the only drug present

Gender	n	*Age ± SD (years)*	*Blood alcohol concentration (mean ± SD) (mg per 100 ml)*
Men	529	54 ± 11	355 ± 87
Women	164	53 ± 12	373 ± 83
Both	693	54 ± 11	360 ± 86

Deaths ascribed to acute alcohol intoxication are often the result of asphyxia caused by a depression of the respiratory center in the lower brainstem (medulla oblongata). This usually occurs when BAC is between 300 and 500 mg per 100 ml depending on tolerance. Another mechanism of death is suffocation by inhalation of vomit because a deeply comatose person might lack a gag reflex or die through positional asphyxia when lying face-down or in some other compromising position. Deaths resulting from inhalation of vomit need to be verified by histological examination of the lungs.

The BACs measured in femoral venous blood at autopsy when death was ascribed to acute alcohol intoxication are given in Table 13 for a large case series of postmortem examinations. The age of the men and women was about the same; being in their

mid-50s, but the women had a somewhat higher mean BAC of 373 versus 355 mg per 100 ml compared with the men. However, the BAC at autopsy is probably an underestimation of the highest BAC reached, owing to the metabolism (breakdown) of alcohol that takes place up to the time of death.

If nothing remarkable is found at autopsy apart from a fatty liver, then death may have resulted from severe metabolic disturbances after binge drinking combined with food deprivation or malnutrition. A metabolic acidosis may be caused by an accumulation of ketone bodies in the blood (ketoacidosis) as well as excess lactic acid, both of which are common in alcoholics since normal metabolic processes are disrupted during ethanol metabolism.

Postmortem Aspects

The quantitative and qualitative analysis of ethanol in postmortem specimens is a relatively simple task and the methods available are no different from those applied to specimens from living subjects. However, interpreting the results of alcohol analysis in postmortem specimens requires care owing to numerous analytical and physiological artifacts. The recommended blood-sampling site for toxicological specimens is a femoral vein after cross-clamping the femoral artery and transection of the vein before draining the femoral venous blood. Taking blood from the heart or pleural cavity is not recommended because of the risk of contamination with alcohol possibly remaining in the stomach if there was drinking just before death. How the body was handled and transported from the place of death to the postmortem examination and whether some agonal event might have caused stomach contents to enter the lungs heightens the risk of a postmortem artifact occurring.

In bodies without signs of putrefaction and when the specimens are preserved in a refrigerator at +4 °C there is little risk of alcohol being produced or destroyed through the action of bacteria and yeasts. Moreover, if the sampling and analysis of ethanol are done the same day as the autopsy then chemical preservatives are not necessary. For longer delays such as when specimens are transported or sent by mail to another laboratory it is imperative to include sodium or potassium fluoride as a preservative to give a final concentration of 1–2%. The fluoride ion functions as an enzyme inhibitor and prevents the production of ethanol by microbial and fermentation processes.

Results of postmortem blood alcohol analysis are strengthened if additional body fluids are submitted for toxicological analysis, particularly bladder urine, VH, and cerebrospinal fluid (CSF). These liquids, which are almost 100% water, are obtained from the urinary bladder, the eye, and the base of the neck (cisternal fluid), and are expected to contain more ethanol than an equal volume of blood, which is only 80% w/w water. Besides different water content, however, there are also temporal variations in the concentrations reaching body fluids and cavities. The urine:blood, VH:blood, and CSF:blood ratios of alcohol change as a function of time after drinking.

During drinking and on the ascending limb of the BAC profile when the alcohol is being absorbed into the blood stream, the concentrations of ethanol in urine, VH, and CSF are lower than or about the same as in venous blood. On the descending limb of the BAC profile, corresponding to the postabsorptive phase, the concentration of ethanol in urine, VH, and CSF are always higher than in the blood. Indeed, alcohol might still be measurable in these alternative specimens even though BAC is reported as negative. In autopsy work a blood ethanol concentration below 10 mg per 100 ml is usually reported as being negative.

Many alcohol-related fatalities involve traumatic events, resulting in open wounds and massive blood loss, which increase the risk of bacteria entering the body and postmortem synthesis of ethanol occurring. These risks are heightened at elevated environmental temperatures (summer months) and when a long time is needed to recover the bodies, e.g., after air disasters or drowning. Although a fluoride preservative is routinely added to blood specimens taken at autopsy it should not be overlooked that some alcohol might have been synthesized in body cavities between the time of death and autopsy.

Obtaining blood for alcohol analysis from a subdural hematoma or clot in the brain can sometimes furnish useful information because of the reduced or nonexistent blood circulation to the clot. The person's BAC at the time of sustaining the injury and formation of the clot decreases owing to hepatic metabolism but the poor circulation in the clot means that alcohol concentrations remain elevated. The concentration of ethanol in the sequestered hematoma gives an indication of the person's BAC several hours earlier, e.g., at a time when the trauma occurred. For example, if a drunken person suffers a blow to the head but survives, albeit being unconscious for several hours prior to death, the subdural or epidural hematoma might contain an appreciable concentration of alcohol. With a survival period of say 10 h and a rate of alcohol elimination corresponding to 15 mg per 100 ml h^{-1}, a person's BAC decreases by 150 mg per 100 ml from the time of the trauma until the time of death. At autopsy, the concentration of ethanol in the blood clot is expected to be considerably higher than in a femoral venous blood sample. In practice,

the rate of formation of the clot and other factors need to be considered.

Conclusion

Knowledge about the disposition and fate of alcohol in the body, including the relative amounts metabolized and excreted and the rates of distribution into various body fluids and tissues, has not changed much since the 1950s. However, much has been learnt about the biochemistry of alcohol and the effects on various metabolic pathways, particularly those related to liver pathology after chronic drinking. The mechanism of action of alcohol in the brain and effects on other body organs (e.g., pancreas) has also advanced considerably since the 1950s.

Ethanol can now be determined in body fluids and tissues with a high degree of precision, accuracy, and specificity and on-the-spot methods using the analysis of saliva and breath are currently available. The limited selectivity of older wet-chemical methods of analysis had always posed a problem in postmortem toxicology because of the risk of cross-reaction with other organic volatiles possibly present in body fluids, such as the products of putrefaction. Although carbon monoxide poisoning was once the major cause of death, especially in suicides, it seems that acute alcohol intoxication, alcohol-related disease, road traffic fatalities, and drowning now dominate among out-of-hospital deaths. Because alcohol is a legal drug its negative impact fails to receive the same publicity and media attention as illicit drugs like heroin, cocaine, and cannabis.

Gender and genetic differences in the metabolism of alcohol continue to be a popular research field and subtle differences have been noted, especially between different racial groups. A smaller volume of distribution for ethanol in women, a faster and more variable absorption from the gut, a lower activity of gastric ADH enzyme, a swifter hepatic clearance, and a higher concentration of acetaldehyde are physiological factors that make females more sensitive to alcohol than males. Although a person's drinking habits depend on a complex interaction between social, cultural, and genetic factors, there is a host of nutritional, biochemical, and hormonal influences that seem to make some people more vulnerable than others to the untoward effects of alcohol consumption.

Alcohol intoxication not only figures in a large proportion of unnatural and suspicious deaths but also in natural deaths, and some feel that blood alcohol analysis is needed in all out-of-hospital deaths. The concentration of ethanol in a specimen taken from a single sampling site is virtually impossible to interpret without additional information such as reliable case history and circumstances surrounding the death as well as measuring alcohol concentration in other biofluids (urine, VH, CSF). Great care is needed when interpreting the results of analyzed postmortem blood specimens and when a statement is made for legal purposes about the person's state of inebriation at the time of death.

Alcohol has always been and probably will remain the number-one drug of abuse in modern society and requests to measure alcohol in body fluids and to interpret the results for legal purposes will remain the most commonly requested service from forensic science and toxicology laboratories.

See Also

Alcohol: Breath Alcohol Analysis; Blood and Body Fluid Analysis; **Forensic Psychiatry and Forensic Psychology:** Drug and Alcohol Addiction

Further Reading

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Alcohol Back-tracking Calculations *See* **Back-tracking Calculations**