



Review

# Interpreting results of ethanol analysis in postmortem specimens: A review of the literature

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## Abstract

We searched the scientific literature for articles dealing with postmortem aspects of ethanol and problems associated with making a correct interpretation of the results. A person's blood-alcohol concentration (BAC) and state of inebriation at the time of death is not always easy to establish owing to various postmortem artifacts. The possibility of alcohol being produced in the body after death, e.g. via microbial contamination and fermentation is a recurring issue in routine casework. If ethanol remains unabsorbed in the stomach at the time of death, this raises the possibility of continued local diffusion into surrounding tissues and central blood after death. Skull trauma often renders a person unconscious for several hours before death, during which time the BAC continues to decrease owing to metabolism in the liver. Under these circumstances blood from an intracerebral or subdural clot is a useful specimen for determination of ethanol. Bodies recovered from water are particularly problematic to deal with owing to possible dilution of body fluids, decomposition, and enhanced risk of microbial synthesis of ethanol. The relationship between blood and urine-ethanol concentrations has been extensively investigated in autopsy specimens and the urine/blood concentration ratio might give a clue about the stage of alcohol absorption and distribution at the time of death. Owing to extensive abdominal trauma in aviation disasters (e.g. rupture of the viscera), interpretation of BAC in autopsy specimens from the pilot and crew is highly contentious and great care is needed to reach valid conclusions. Vitreous humor is strongly recommended as a body fluid for determination of ethanol in postmortem toxicology to help establish whether the deceased had consumed ethanol before death. Less common autopsy specimens submitted for analysis include bile, bone marrow, brain, testicle, muscle tissue, liver, synovial and cerebrospinal fluids. Some investigators recommend measuring the water content of autopsy blood and if necessary correcting the concentration of ethanol to a mean value of 80% w/w, which corresponds to fresh whole blood. Alcoholics often die at home with zero or low BAC and nothing more remarkable at autopsy than a fatty liver. Increasing evidence suggests that such deaths might be caused by a pronounced ketoacidosis. Recent research has focused on developing various biochemical tests or markers of postmortem synthesis of ethanol. These include the urinary metabolites of serotonin and non-oxidative metabolites of ethanol, such as ethyl glucuronide, phosphatidylethanol and fatty acid ethyl esters. This literature review will hopefully be a good starting point for those who are contemplating a fresh investigation into some aspect of postmortem alcohol analysis and toxicology.

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**Keywords:** Alcohol; Analysis; Autopsy; Interpretation; Legal medicine; Postmortem

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## 1. Introduction

Over-consumption of alcoholic beverages and drunkenness have always played a major role in fatal accidents, trauma deaths, drowning, suicide, and many crimes of violence as evidenced by police reports and accident and emergency department records [1–7]. Moreover, heavy drinking and alcohol-induced impairment are common underlying factors in road-traffic crashes as well as accidents in the workplace and the home [8–10]. Alcohol tops the list of psychoactive substances encountered in postmortem toxicology (Table 1) and the analysis and interpretation of blood-alcohol concentration (BAC) in autopsy specimens represents a large part of the

Table 1  
The 20 substances most frequently encountered in femoral venous blood samples in forensic autopsies performed in Sweden in 2002

Rank	Substance	Number of instances	Comments on drug class
1	Ethanol <sup>a</sup>	2094	Alcoholic beverages
2	Paracetamol	568	Over the counter antipyretic
3	Diazepam	286	Benzodiazepine anxiolytic
4	Citalopram	238	SSRI <sup>b</sup> antidepressant
5	Morphine <sup>c</sup>	207	Narcotic analgesic
6	Propoxyphene	204	Centrally active analgesic
7	Propiomazine	199	Sedative/hypnotic
8	Zopiclone	197	Sedative/hypnotic
9	Codeine	187	Analgesic
10	Alimemazine	139	Neuroleptic/sedative
11	Carbon monoxide	139	Toxic combustible gas
12	Carbamazepine	137	Antiepileptic
13	Amphetamine	135	Stimulant drug of abuse
14	Tramadol	121	Strong analgesic
15	Mirtazapine	120	Newer antidepressant
16	Sertraline	117	SSRI antidepressant
17	Flunitrazepam	109	Benzodiazepine hypnotic
18	Tetrahydrocannabinol	103	Active substance in cannabis
19	Venlafaxine	96	Newer antidepressant
20	Nitrazepam	93	Benzodiazepine hypnotic

<sup>a</sup> Blood concentrations exceeding 10 mg/100 mL.

<sup>b</sup> SSRI stands for selective serotonin reuptake inhibitor.

<sup>c</sup> Metabolite of heroin.

workload at forensic medicine and toxicology laboratories [5,9,10]. The kinds of drugs encountered in autopsy blood specimens and the frequency of occurrence of positive ethanol findings depends on many social-medical factors that might be different in other countries.

In general, the concentration of ethanol measured in postmortem blood needs to be interpreted in relation to whether the person had consumed alcohol and might have been drunk at the time of death or if the concentration exceeded some threshold limit [11,12]. Such conclusions are often contentious and caution is needed owing to various postmortem artifacts. The diagnosis of alcohol influence has deep-rooted social-medical ramifications owing to the existence of punishable BAC limits for driving in most countries, such as 0.20 mg/g in Sweden, 0.50 mg/g or 0.50 mg/mL in most European nations and 0.80 mg/mL (0.08 g% or 80 mg/100 mL) in UK, USA and Canada [13]. Accident and insurance claims might be null and void if the person involved in a fatal crash was declared above the legal limit for driving.

The qualitative and quantitative determination of ethanol in postmortem specimens has become a relatively simple analytical procedure and accurate, precise, and specific results are possible [14,15]. However, interpreting postmortem BAC results and drawing correct conclusions about antemortem levels and the person's state of inebriation and degree of behavioral impairment at the time of death is fraught with difficulties [11,12,16–18]. The condition of the body, the time between death and autopsy, the environmental conditions (temperature and humidity), and the nature of the specimen collected for analysis are important factors to consider. Under some circumstances alcohol might be produced after death by microbial activity and fermentation of glucose, which is a real problem if the corpse has undergone decomposition [19,20]. Postmortem diffusion of alcohol from the stomach to central blood sampling sites is another complicating factor if a person died shortly after a period of heavy drinking [21,22]. Care is needed to ensure that biological specimens are not contaminated with ethanol or other extraneous solvents during any life-saving treatment or in connection with external examination of

the body or if a blood sample for alcohol analysis was taken before performing a complete autopsy [23].

Many articles have been written dealing with postmortem aspects of alcohol as well as scores of presentations made at conferences devoted to forensic medicine and toxicology. The published proceedings from these conferences have not been included in this survey because such compilations are hard to locate through usual channels and the quality and rigor of the peer-review of manuscripts is unknown. A vast amount of blood-alcohol research in both living and dead has been published in German language journals, such as *Blutalkohol* and *Zeitschrift für Rechtsmedizin* and the more important articles are included in this presentation. A recent comprehensive review of issues relating to analysis of ethanol in postmortem toxicology with a major focus on research published in German language journals was presented by Huckenbeck [24].

Historically, two classic investigations of postmortem analysis of ethanol and the problems associated with interpretation of results were published by scientists from Scandinavian countries. The first was a 1930 monograph written by Sjövall and Widmark from Sweden [25], professors in forensic pathology and physiological chemistry, respectively, which was a seminal work. The doctoral thesis (from 1958) by Wolthers [26] from Denmark was also a classic investigation in the field of analytical toxicology. He described in detail one of the first applications of a gas chromatograph, known then as a vapor fractometer to separate ethanol from other volatile agents produced during decomposition and putrefaction. Owing to the mode of publication and the language barrier, neither of these pioneer efforts received the international recognition they deserved.

After drinking alcoholic beverages, the alcohol they contain is absorbed from the gut into the portal vein where it is transported through the liver then on to the heart before distribution throughout all body fluids and tissues. The concentrations reached in the various body organs and tissues at equilibrium depend on their relative water contents and the speed of equilibration depends on the ratio of blood flow to tissue mass [13,14,27,28]. In general, any body fluid or tissue that contains water could serve as a specimen for determination of ethanol and, indeed, the literature contains reports of many different materials being used for toxicological analysis.

Besides the water content of the biological specimens analyzed, another factor to consider when the concentration of ethanol is compared and contrasted is the stage of alcohol absorption and distribution at the time of death. A recent study documented the magnitude and time-course of arterial-venous differences in blood-ethanol concentration in healthy volunteers [29]. Accordingly, the concentration of ethanol in arterial blood was higher than in venous blood during the time that alcohol was being absorbed from the gut, whereas in the post-absorptive phase, the venous blood contained a slightly higher concentration of ethanol than the arterial blood.

The question of ethanol being produced in the body after death often arises when mass transportation fatalities are investigated [16–19]. A paper by Corry [20] is considered a

standard reference work for questions related to the biochemistry and microbiology of formation and degradation of ethanol in postmortem blood specimens. Speedy recovery and refrigeration of the bodies helps to prevent synthesis of ethanol by the action of molds, yeasts and bacteria. The review by Corry was initiated following the Moorgate underground train crash in London (28 February 1975) in which 43 people died, including the driver [30]. The body of the train-driver was trapped in the wreckage for a few days before being recovered and extensive trauma and exposure to elevated temperatures raised the question of possible postmortem synthesis of ethanol. A four-fold difference (20–80 mg/100 mL) in the concentration of ethanol was reported in the driver's blood taken from different sampling sites during the postmortem examination [31]. The media were quick to report that the driver had been drinking before the crash, although an equally plausible explanation for variations in the analytical results might have been the synthesis of ethanol owing to microbial activity [32]. Moreover, the toxicology report of positive blood-ethanol stood in sharp contrast with other evidence, which suggested that the driver of the train had moderate drinking habits (personal communication, R.L. Williams, Metropolitan Police, London).

The aim of this literature survey was to collect together in one place the bulk of published work relevant to forensic analysis of ethanol in autopsy specimens and to consider factors that might influence the correct interpretation of results. We have not made a critical appraisal of every cited article and, indeed, most of them have already undergone a peer-review before publication. Instead, we have grouped the papers together according to the type of question or theme being investigated and reported upon.

## 2. Sampling of body fluids for determination of ethanol

Among analytical chemists, the widely quoted adage that “*the result of an analysis is only as good as the sample received*” is particularly valid in the field of postmortem forensic toxicology. Several sets of guidelines have been published for collecting the most appropriate specimens for toxicological analysis [33–35]. In the case of ethanol, the blood samples should be taken from a femoral vein and whenever possible additional specimens, such as urine and vitreous humor (VH), should also be obtained and sent for analysis [33–35].

The tubes used to collect and transport blood specimens to the laboratory are best prepared before the autopsy and should contain sodium or potassium fluoride as preservative to ensure a final concentration of 1–2% w/v [36,37]. The fluoride ions function as enzyme inhibitor, which is important to prevent any further production of ethanol between the time of the autopsy and dispatch and transport to the laboratory for analysis. If blood-ethanol is determined on the same day as the autopsy is performed, then addition of fluoride as preservative is probably unnecessary. The common grey-stopper 4- or 5-mL Vacutainer tubes used for blood glucose measurements only contain about 16 mg NaF and this is an insufficient amount of preservative for postmortem blood specimens. If the amount of fluoride added

to blood or urine specimens is challenged, this might need to be verified by measuring the concentration of fluoride ions, such as by using a fluoride-sensitive electrode [38,39].

All containers used to collect autopsy blood specimens should be carefully labeled with the kind of material, the anatomical site of origin, the date and time and important details of the case including identification of the deceased. Blood and urine specimens intended for determination of volatiles like ethanol should have a small air-space to minimize evaporation. Finally, the containers should be made airtight with tamperproof seals and if possible transported to the laboratory refrigerated (4 °C). In forensic casework the chain-of-custody of specimens is important to document and this helps to guarantee the integrity of the results in case these are called into question in later court proceedings.

Some forensic practitioners consider that blood from the intact heart chambers is suitable for toxicological analysis of ethanol [40], whereas others recommend using a peripheral venous sampling site preferably a femoral vein after visualization and cross-clamping proximally [41–46]. Clark [47] discussed methods of specimen collection and proper routines for sampling and handling autopsy materials intended for clinical laboratory analysis. International comparisons of postmortem drug concentrations are simplified if the blood sampling site, the method of sampling as well as the assay methods are as far as possible standardized. In this connection femoral venous blood is considered the best possible specimen for toxicological analysis [48].

The concentrations of ethanol measured in blood drawn from different sampling sites tend to vary much more than expected from inherent variations in the analytical methods used [49]. Studies have shown that concentrations of ethanol and other drugs determined in heart blood are generally higher than in blood from a peripheral vein although in any individual case there are likely to be considerable variations [50–53]. The worst possible specimen is a blind-stick into the chest or blood scooped from the chest cavity on opening the body [54,55].

If the production of ethanol between the time of death and autopsy can be ruled-out, the concentration of ethanol in a peripheral venous blood sample with fluoride (1% w/v) added provides the best possible measure of the deceased's BAC. Table 2 compares the body fluids and tissues available for analysis of ethanol from living and dead persons. Threshold concentrations of ethanol in blood, breath and urine have been established in most countries above which it is not permitted to drive a motor vehicle.

### 3. Determination of ethanol in body fluids

Analytical methods used for determination of ethanol in body fluids are the same regardless of whether clinical specimens from hospital patients or autopsy specimens are submitted to the toxicology laboratory. The method of choice worldwide for qualitative and quantitative determination of ethanol in body fluids is gas chromatography with a flame ionization detector using either a direct injection technique or by headspace sampling [56–59].

Table 2

Examples of body fluids and tissues suitable for determination of ethanol in living and dead subjects

Living subjects	Dead subjects
Venous blood <sup>a</sup>	Femoral blood <sup>b</sup>
Capillary blood	Heart blood
Plasma/serum	Blood clot
Urine (fresh void) <sup>a</sup>	Bladder urine <sup>b</sup>
Tear fluid	Vitreous humor <sup>b</sup>
Cerebrospinal fluid (lumbar fluid)	Cerebrospinal fluid (cisternal)
Saliva	Bile
Perspiration/sweat	Synovial fluid
Breath <sup>a</sup>	Brain, skeletal muscle, liver

<sup>a</sup> Punishable blood-, breath- and urine-alcohol concentration limits exist for driving in most countries.

<sup>b</sup> These specimens are recommended for postmortem alcohol analysis.

Headspace sampling seems to be the preferred method for determination of volatile substances and offers the advantage that the chromatographic column is protected from being overloaded with non-volatile blood constituents. Headspace gas chromatography (HS-GC) entails removing a portion of the vapour phase in equilibrium with the biological specimen, which is kept in an air-tight glass vial at a constant temperature of 50 or 60 °C. When HS-GC is used for quantitative analysis, care is needed to minimize or eliminate matrix effects when aqueous solutions of ethanol are used for calibration of the instrument and quality control of accuracy [60,61]. The best way to eliminate matrix effects is to dilute the blood specimen 1:5 or 1:10 with an aqueous solution of an internal standard, such as *n*-propanol or *t*-butanol. Another approach, although this is not recommended by us, is to saturate both the biological specimens and the aqueous standards with an inorganic salt such as sodium chloride or sodium sulphate [62]. This salting-out technique raises the vapor pressure of non-electrolytes (e.g. ethanol) in the flask and boosts the sensitivity of the HS-GC analysis. This might be a worthwhile strategy if trace amounts of volatile substances are of interest. The calibration method of known addition is also suitable when ethanol or other drugs are determined in a complex or unusual matrix.

The biological specimens sent for analysis of ethanol should be analyzed in duplicate on two different chromatographic systems thus providing different retention times for ethanol and internal standard. Some laboratories encourage using two different internal standards (e.g. *n*-propanol and *t*-butanol) to dilute the blood specimens. Indeed, the tertiary alcohol is recommended in connection with autopsy materials because under some circumstances small amounts of *n*-propanol might be produced during decomposition and putrefaction processes [57].

The precision of routine blood-alcohol analysis is high and inter-laboratory coefficients of variation (CV), according to several studies, are only 3–5% compared with within laboratory CVs of less than 1% [14,63]. However, when the concentrations of ethanol in blood from different sampling sites are compared, the CVs are much greater, sometimes several fold. This can be explained, at least in part, by the varying fluidity of the specimens and the amounts of plasma, red cells and clots present [49,64]. This site-to-site biological uncertainty needs to



be considered when results of postmortem alcohol analysis are interpreted and compared with a threshold concentration, such as the statutory BAC for driving [65]. The notion of making a deduction from the mean result of analyzing postmortem blood as a way to compensate for analytical and sampling variations has, to our knowledge, not been applied in practice.

By use of highly specific gas chromatographic methods of analysis, ethanol can be determined reliably even in the presence of potential interfering substances (e.g. acetaldehyde, ethyl acetate, *n*-propanol, 2-propanol, *n*-butanol), that might be produced during decomposition of the body. The significance attached to fairly low BAC in autopsy specimens (<30 mg/100 mL or 0.3 mg/mL) is debatable without supporting evidence from analysis of ethanol in urine and vitreous humor.

In postmortem toxicology, a BAC less than 10 mg/100 mL (0.1 mg/mL) should be reported as negative and, indeed, this is close to the limit of quantitation by most routine HS-GC methods. This policy helps to avoid unnecessary speculation and debate about the significance and meaning of low BACs. Most laboratories report the concentrations of ethanol in biofluids in units of mass/volume, such as mg/100 mL in UK. Some countries however prefer to use mass/mass units and comparison between the two requires a correction for the density of whole blood (1.05 g/mL on average) [66]. However, owing to the variable quality of autopsy blood specimens, including the proportion of red cells to plasma, degree of lysis and the presence of clots, making such a correction depending on whether mass/volume or mass/mass concentration units were used is probably unnecessary.

#### 4. Autopsy blood samples

##### 4.1. Blood-ethanol in acute alcohol poisoning

Of the multitude of specimens available at autopsy for toxicological analysis, blood is crucial to allow making valid conclusions of whether the deceased had consumed alcohol and was under the influence or impaired at the time of death. According to Plueckhahn [67], who has published extensively on interpreting BAC in postmortem specimens, the following basic information is required when results are evaluated:

- Site and method of collection of the blood sample.
- Time after death and state of body when sample was collected.
- Conditions of storage of sample, preservative used, and time elapsed before initial analysis.
- The analyst (laboratory) and method used for analysis of the sample.

In addition to the above, one might also consider the appearance and condition of the blood specimen such as its color, smell, fluidity, presence of clots and if necessary a determination of the water content could be made.

Gross intoxication caused by heavy drinking might prove life threatening in several ways besides the direct toxicity of ethanol on depression of respiratory centers in the brain, which

often occurs at a BAC of 400 mg/100 mL or more. Accordingly, many other factors must be considered when a diagnosis of acute alcohol poisoning is recorded as the cause of death [67–72].

Administration of intravenous solutions such as the osmotic diuretic mannitol (a sugar alcohol) to help relieve swelling in the brain provided a good substrate for postmortem synthesis of ethanol [73]. Care is needed to ensure that the biological specimens sent for analysis are not inadvertently contaminated by extraneous solvents or aerosols used at the morgue [23,74]. One study showed that cleaning abraded and lacerated skin with 70% ethanol before performing an emergency operation led to contamination of femoral venous blood obtained later at autopsy [23]. Obviously, the skin should not be disinfected with ethanol before drawing blood if the intention is to determine BAC, because this would cast doubt on the reliability of the result. However, studies have shown that absorption of ethanol through intact skin and the risk of carry-over from use of an ethanol swab was negligible [75–77].

Although blood from a femoral vein is the recommended specimen for toxicological analysis some pathologists still persist in submitting cardiac blood or worse still fluid scooped from the chest or pleural cavity as substitute for a proper blood specimen [54]. These questionable procedures increase the potential for contamination of specimens with ethanol that might have diffused from the stomach into surrounding tissues or entered the lungs by inhalation of stomach contents owing to some agonal event. Femoral blood is least susceptible to postmortem changes and accordingly this is the specimen recommended for toxicological analysis. Blood from the intact chambers of the heart is also considered suitable as a supplementary specimen to compare with femoral BAC or when the volume of the latter specimen might be limited [40].

The BAC necessary to cause death is often an open question and much depends on the person's age, drinking experience and degree of tolerance development [78]. The speed of drinking plays a role in alcohol toxicity as does the kind of beverage consumed, whether beer (5% v/v) or neat liquor (40% v/v) and particularly any masking of the taste of alcohol by adding sugar or fruit flavorings [79]. Many drunk drivers have been apprehended with a blood-ethanol concentration over 400 mg/100 mL and a few have exceeded 500 mg/100 mL [80].

Drunkenness and hypothermia represent a dangerous combination and deaths tend to occur at a lower BAC when people are exposed to cold, such as, when an alcoholic sleeps outdoors in the winter months [78]. Drinking large amounts of alcohol to produce stupor and unconsciousness combined with positional asphyxia or inhalation of vomit are common causes of death in intoxicated individuals who die of suffocation [81–83]. The toxicity of ethanol is often considerably enhanced by the concomitant use of other drugs with their site of action in the brain, especially opiates, propoxyphene, antidepressants and some sedative hypnotics [84].

Two recent studies looked at the frequency distributions of BACs in deaths attributed to acute alcohol poisoning and similar mean and median concentrations were found, namely 360 mg/100 mL (0.36 g%) [78,84]. In both studies ethanol was

determined in femoral venous blood and no other drugs were present that might confound or exaggerate the toxicity of ethanol and complicate making a diagnosis of acute alcohol poisoning.

It seems reasonable to assume that the BAC at autopsy will almost always be lower than the maximum BAC reached during a drinking binge, owing to metabolism of ethanol taking place up until the moment of death [85–87]. During the time after discontinuation of drinking until death, the BAC might decrease appreciably depending on the speed of alcohol elimination from blood, which in heavy drinkers could exceed 20 or 30 mg/100 mL per h (0.02 or 0.03 g% per h) [88].

Fig. 1 shows the frequency distribution of ethanol concentrations in femoral blood at autopsy when death was attributed to acute alcohol poisoning [78]. The fit of the data to a normal distribution (Gaussian curve) was remarkable good and in 95% of the population the BAC at autopsy ranged from 220 to 500 mg/100 mL. Besides the concentration of ethanol in autopsy blood other factors to consider are the person's general state of health, age, asphyxia, and tolerance to alcohol when the cause of death is assigned by the pathologist as acute alcohol poisoning [72].

#### 4.2. Analysis of subdural or epidural hematomas

Obtaining a blood specimen from a subdural or epidural hematoma is a useful strategy in deaths caused by a blow on the head [89–98]. A victim often survives for several hours after a fall or blunt trauma to the head with circulation remaining intact until the time of death. Owing to the reduced circulation in the damaged region of the brain, alcohol in the blood clot is not metabolized to the same extent as blood circulating through the liver. Accordingly, the blood clot will contain a higher concentration of alcohol compared with a specimen of peripheral venous blood obtained at autopsy. The sampling

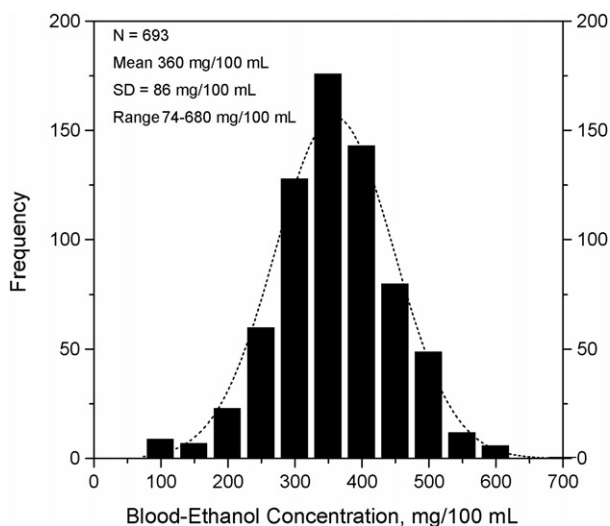


Fig. 1. Frequency distribution of the concentrations of ethanol in femoral venous blood in deaths attributed to acute alcohol poisoning. The dotted line shows a normal Gaussian curve fitted to the data and 95% of the distribution was between 220 and 500 mg/100 mL [78].

and analysis of intracranial blood clots might therefore furnish useful complementary information about the person's BAC several hours before death, such as, when the trauma occurred [99].

The usefulness of alcohol analysis in intracranial blood clots and comparisons with concentrations in peripheral or heart blood at autopsy has been reported by several workers [89–98]. However, it should be kept in mind that some people might survive the initial trauma and formation of the hematoma and still continue to drink more alcohol before falling into a coma and dying. An injury that results in destruction of the skin surface and surrounding tissue means that bacteria and infection can enter the wound, which increases the potential for microbial synthesis of ethanol in the blood clot after death. Alcohol can be determined directly in the blood clot after mixing with water using conventional gas chromatographic methods although the variable water content of such blood specimens need to be considered when the results are interpreted [100].

#### 4.3. Should the water content of blood samples be considered?

After drinking alcoholic beverages, the ethanol they contain distributes fairly rapidly into the total body water compartment and binding to plasma proteins and tissues and the solubility in bone and lipid are negligible. The concentration of ethanol in blood, body organs and tissues at equilibrium and the speed of distribution depend on the water content of the materials analyzed and the tissue perfusion rate. The water content of fresh whole blood is normally about 80% w/w on average, being slightly less for men than for women, owing to the lower hematocrit of female blood [66]. The figure 80% w/w is however a good average value regardless of gender and this corresponds to 84% w/v assuming a density of 1.05 g/mL for whole blood [101].

Both the water content and also the concentration of alcohol tends to decrease in blood after death and with a long postmortem interval, the blood-water might be abnormally low (mean 71.9% w/w, range 65–85% w/w,  $N = 76$ ) [102]. In connection with interpreting postmortem BAC, some workers recommend that the concentration of ethanol should be corrected to a blood-water content of 80% w/w [103–108]. However, if the corpse shows definite signs of putrefaction and the autopsy was delayed, then factors other than blood-water content were considered more important as determinants of the blood-ethanol concentration [105].

The water content of blood and tissues is easy to determine either by freeze drying or more simply by desiccation after the blood-ethanol concentration has been determined [106,107]. The need to correct the concentration of ethanol to a blood-water of 80% w/w water has been advocated mainly by forensic pathologists in Germany [109] although to our knowledge this practice has not been followed in other countries. The water content of the specimen analyzed becomes more important when blood clots or tissue samples are analyzed and calculations are made about the amount of alcohol absorbed

and distributed in the body at the time of death. The distribution of ethanol between erythrocytes and whole blood in specimens from living individuals was 0.865–1 (range 0.66–1.0,  $N = 167$ ) and similar values should apply to autopsy blood specimens [110].

## 5. Alcohol in blood and urine obtained at autopsy

The two fluids most commonly submitted for analysis of ethanol after completing an autopsy are blood and urine and a wealth of information is available about the urine/blood alcohol relationship in living and dead. Urine should be sampled directly from the intact bladder by penetrating the organ with a sterile syringe and needle and transfer to a container with a fluoride preservative present (1–2%) before shipment for analysis of ethanol [111,112].

The quantitative relationship between urine–alcohol concentration (UAC) and BAC has been extensively studied [113]. Besides the higher content of water in urine (~99–100%) compared with whole blood (~80%), the concentration–time curves are shifted in time [114]. Thus by calculating the ratio of UAC/BAC furnishes useful information about the status of alcohol absorption at the time of death [115–118]. Finding a ratio less than or close to unity suggests incomplete absorption of alcohol in all body fluids at time of death, which indicates fairly recent drinking and some of the ingested alcohol probably remains unabsorbed in the stomach, whereas finding a ratio of 1.25 or more suggests that absorption and distribution of ethanol was complete by the time of death [117]. If the question of recent drinking has legal significance, then a sample of the stomach contents should be obtained and submitted for determination of ethanol [87]. Additional human studies are needed to document the rate of decrease in the ethanol concentration in the stomach after intake of various kinds of alcoholic beverages (e.g. beer versus spirits) in both the fed and fasted state [119].

Urine is a useful specimen for analysis of ethanol because it is mainly water and the risk of microbes or yeasts invading the urinary bladder after death appears to be less compared with the risk of blood specimens being contaminated. Moreover, the urine produced by healthy individuals does not contain any significant amounts of glucose although this is a major limitation if the deceased suffered from diabetes and glycosuria [120–123]. Glucose is the ubiquitous substrate for postmortem synthesis of ethanol in both blood and urine. Finding an elevated UAC in a specimen from a diabetic and a negative concentration in blood usually means that ethanol was produced in the urine after death, e.g. by yeast fermentation of glucose [124,125]. The determination of glucose in VH, if available, is helpful to support the notion of concomitant glycosuria. Whether or not a person suffered from diabetes (Types I or II) is therefore important to know about when the postmortem alcohol concentrations are interpreted [126]. There is also a possibility that alcohol was produced in blood and urine before the autopsy was performed so adding fluoride to the specimen sent for analysis does not exclude that ethanol was produced prior to the postmortem examination.

A long time period between end of drinking and time of death might be associated with a zero BAC owing to on-going hepatic metabolism but a high UAC is found [127]. The total volume of urine in the bladder also provides useful information and larger volumes tend to be associated with higher UAC/BAC ratios [128]. Metabolism of ethanol does not occur in the urinary bladder and diffusion back into the blood is seemingly a slow and insignificant process [129].

The concentration of ethanol in blood and urine at autopsy are highly correlated (Pearson's  $r > 0.9$ ) although BAC should not be estimated indirectly from UAC in any individual case. The degree of scatter of individual values around the regression is large (pronounced residual standard deviation) and this leads to a wide prediction interval [130–132]. However, much depends on what the estimated BAC is intended for and whether there is other evidence to support heavy drinking and drunkenness at time of death. The level of proof differs between criminal and civil litigation.

## 6. Analysis of vitreous humor

Vitreous means glassy and humor means fluid so the watery fluid from within the eye is a useful specimen for postmortem ethanol determination. The mechanism of transfer of small molecules from the blood into the fluids of the eye was studied in the mid-1940s [133] and the first publication describing use of VH for analysis of ethanol in medical examiner cases dates from 1966 [134]. Since then scores of publications have compared and contrasted the concentrations of ethanol in blood and VH at autopsy [135–153]. VH is useful not only for analysis of alcohol, but also for other drugs as well as endogenous biochemical constituents of the body. For example, the concentrations of lactate and glucose in VH have been utilized as an indicator of antemortem hyperglycemia [154,155]. Studies have shown that between-eye differences in the concentrations of ethanol and other biochemical constituents are fairly small [156–160].

The main advantage of VH over blood, besides its watery nature, is that anatomically it is remote from the gut and therefore less prone to contamination by spread of bacteria. This is important if the corpse has undergone decomposition or has been subjected to severe trauma [161]. Under these circumstances the spread of bacteria is exaggerated as is the risk of ethanol being produced after death in blood taken from a central sampling site. Owing to the remoteness of the eyes from the large blood vessels and the gut, VH provides a very useful specimen whenever the corpse has already undergone decomposition so that postmortem synthesis is a real possibility. However, VH might contain glucose, which otherwise is a viable substrate for postmortem synthesis of ethanol [162].

Studies have shown that the mean VH/blood ratio of ethanol is very close to values expected from the distribution of water in these two biological specimens, namely about 1.15–1.20:1. However, there are wide individual variations so using an average ratio to estimate BAC from VH or vice-versa is not recommended. A study from Germany based on 592 autopsies found that the correlation coefficient between VH and blood

was  $r = 0.936$  and the corresponding regression equation was  $VH = -0.18 + 1.24 \text{ BAC}$ . From this work, the authors recommend using a factor of 0.81 to compute BAC indirectly from VH, namely  $VH \times 0.81 = \text{BAC}$  [163].

In a study that involved 672 forensic autopsies [164] the concentration of ethanol in VH was compared with femoral venous blood and the Pearson's correlation coefficient was  $r = 0.979$ . The mean VH/blood ratio was 1.19:1 (S.D. 0.285) and this translates into a 95% range ( $\pm 1.96 \times \text{S.D.}$ ) of 0.63–1.75. This means that if the vitreous alcohol is divided by 1.75 this gives a very conservative estimate of the coexisting concentration in femoral blood. Another large study comprising 349 autopsies found a high correlation between VH alcohol and BAC although the residual standard deviation (S.D.) was 26 mg/100 mL (0.26 g/L) [165]. This suggests that BAC might be predicted from analysis of VH within  $\pm 51$  mg/100 mL ( $\pm 1.96 \times \text{S.D.}$ ) in 95 of 100 cases from the same population.

A 2005 publication from USA [166], which surprisingly neglected to cite these earlier investigations, also found a high correlation ( $r = 0.958$ ) between VH and BAC. In those cases when the concentrations in VH exceeded that in blood, the mean VH/blood ratio was 1.24:1 (median 1.19:1), suggesting a somewhat skewed distribution of individual values [166]. The utility of using the concentration of alcohol in VH to draw conclusions about the person's BAC at the time of death was the subject of litigation after a road-traffic crash because there was no blood specimen available for analysis [167]. The plaintiff in the case requested a so-called *Frye Hearing* to determine the reliability of translating the result of analyzing ethanol in VH into the coexisting BAC. A court in Florida (USA) upheld the conversion within certain limits and the fact there was supporting evidence from ethanol determined in liver tissue.

Measuring the concentration of ethanol in VH has also been advocated if a postmortem and toxicology needs to be performed on embalmed bodies [168,169]. Embalming fluids might contain diverse preservatives, such as germicides, anticoagulants (EDTA), perfuming materials and are usually rich in aldehydes, such as formaldehyde, paraformaldehyde, and glutaraldehyde [170]. Owing to occupational health hazards when handling formaldehyde, the composition of embalming fluids today is dominated by aliphatic alcohols (e.g. methanol) and could thus include traces of ethanol.

The time required for ethanol to enter the bloodstream and penetrate the fluids of the eye seems to be fairly short [171,172]. This means that the concentration of ethanol in VH and in blood follow a similar time-course with only a short lag-time evident. Furthermore, ethanol and many other abused drugs and medication seem to be fairly stable in VH during prolonged periods of storage at 4 °C provided a fluoride preservative is present [173,174]. After 12 months storage of VH at 4 °C with fluoride added, the mean ethanol concentration was 200 mg/100 mL compared with 121 mg/100 mL in specimens without fluoride. In the same study, the concentration of ethanol in femoral blood samples ( $N = 16$ ) with fluoride added decreased by 8% after 12 months storage at 4 °C [173]. The mean starting BAC was 175 mg/100 mL (range 39–360), which dropped to 161 mg/100 mL (range 30–340) after 12

months storage and the change was statistically highly significant ( $p < 0.001$ ).

Obtaining proper specimens of VH for toxicological analysis might not be feasible if the corpse is appreciably dehydrated, incinerated or badly decomposed. It was also pointed out that VH might be unsuitable for biochemical and toxicological analysis because of its abnormal viscosity or cellular composition or if the deceased had suffered from some disease of the eyes and had undergone ophthalmic operations [175,176].

## 7. Unconventional specimens

Interpreting postmortem ethanol concentration is simplified thanks to a much larger selection of body fluids and tissues available for sampling and analysis of alcohol. The traditional and recommended body fluids for analysis of alcohol and other drugs are femoral blood, bladder urine and VH (Table 2). However, when these are unavailable, other biological specimens or tissues are desirable and should be taken by the pathologist and sent for toxicological analysis. Specimens such as liver, brain, skeletal muscle, spleen, bone marrow, cerebrospinal fluid (CSF), and synovial fluid as well as bile, have occasionally served as material for toxicological analysis [177–190].

A large study comparing alcohol concentrations in CSF and blood ( $N = 509$  bodies) reported a correlation coefficient of  $r = 0.943$  and a regression equation defined as  $\text{CSF} = -0.11 + 1.35 \text{ BAC}$  and a factor of 0.74 was recommended to compute BAC from the concentration of ethanol measured in CSF [189].

Some publications describe use of more imaginative specimens such as testicle and putrefactive blister fluid as well as fluid from the paranasal sinus in cases of drowning [191,192]. Another possibility might be to obtain fluid from the inner ear (perilymph fluid), which is protected by the skull, and a few hundred microliters might be available for determination of ethanol [186,193].

The water and lipid content of these more unusual body fluids and tissues and the stability of ethanol after sampling are important to know about for better interpretation of the results [194–199]. Organs such as liver and kidney retain some enzymatic activity after death as the body cools and depending on ambient temperature and availability of cofactor  $\text{NAD}^+$  ethanol might be metabolized to some extent after death. This probably explains, at least in part, the finding that the liver/heart blood ratios of alcohol ( $N = 103$ , mean 0.56 and standard deviation  $\pm 0.3$ ) were considerably lower than values expected based on the liver/blood ratios of water being roughly 1:1 [200]. To minimize the risk of postmortem diffusion of ethanol from gastric residue, the liver specimen should be taken from deep within the right lobe rather than the left lobe, which is less protected and located closer to the stomach. Some body organs and tissue are probably more susceptible than others to putrefaction processes depending on their glucose and glycogen content and proximity to the bowel thus facilitating spread of bacteria and fungal growth [194–199].

In decomposed or exhumed bodies, skeletal muscle is probably the most appropriate specimen for forensic analysis of



ethanol and other drugs and is convenient to take from the large thigh muscle [180,201]. The muscle tissue (about 1 g) should be cut into small pieces, macerated or homogenized and mixed with water prior to ethanol being determined by HS-GC analysis. Care is needed to compensate for matrix effects and dilution during calibration and standardization of the gas chromatograph when quantitative determinations are made.

## 8. Microbial contamination and decomposition

The possibility of ethanol being produced or degraded in the body after death has always been and still is a dilemma when concentrations of ethanol in postmortem specimens are interpreted [25,26,202–211]. Depending on circumstances, varying periods of time (days, weeks or months) might elapse after death until a body is discovered and the autopsy performed.

When the supply of oxygen to the body ends, the integrity of cell membranes and tissue compartments gradually disintegrate through the action of various digestive enzymes. This reflects the process of autolysis (self digestion) resulting in a softening and liquefaction of the tissue (freezing the body prevents autolysis). During this process, bacteria from the bowel invade the surrounding tissue and vascular system and the rate of infiltration depends on many factors including the ambient temperature, position of the body and whether death was caused by bacterial infection. Glucose concentrations increase in blood after death and this sugar is probably the simplest substrate for microbial synthesis of ethanol [20,68]. Within a few days after death, the first sign of bacterial activity is usually greenish discoloration of the skin covering the lower abdomen, which eventually spreads to other parts of the body and a putrid smell becomes apparent. Odorous gases (e.g. hydrogen and alkyl sulfides and methane) are produced in a putrefied corpse along with ethanol and many other low-molecular weight volatiles. These putrefaction products include diverse reducing volatiles and this represented a major analytical problem more than 50 years ago when non-specific chemical oxidation methods of analysis were used in forensic toxicology laboratories [25,26].

The speed of decomposition of the body depends on many factors including the time elapsed after death, in-door or out-door conditions, invasion by insects, temperature and humidity of ambient air, circulation of the air, immersion in water and extent of trauma and damage to the corpse [212]. Skin slippage, bloating, purging, and skin discoloration, presence of maggots, and bad-smelling corpses are strong indications of well established putrefaction [213,214]. A chemical preservative, such as the enzyme inhibitor sodium fluoride (1–2% w/v), should be added to specimens intended for determination of ethanol, which should prevent any further production of ethanol [215]. The fluoride ion is seemingly effective in inhibiting the activity of several kinds of enzymes, such as enolase a component in the glycolytic pathway, and is important for the action of yeasts, fungi and many micro-organisms responsible for fermentation [216].

Low concentrations of ethanol (<30 mg/100 mL) are more likely to be formed postmortem than high concentrations.

Supporting evidence of antemortem alcohol ingestion, such as that furnished by finding the expected concentrations in urine and VH proves useful in such cases [150]. Extensive trauma to a body likewise increases the potential for spread of bacteria and heightens the risk of ethanol production after death [217]. Blood-ethanol concentrations as high as 190 mg/100 mL have been reported in postmortem blood after particularly traumatic events such as explosions and when no evidence existed to support ingestion of ethanol before the disaster [218].

The comparison of ethanol concentrations in blood from different sampling sites as well as in alternative body fluids such as urine, VH and CSF can help to evaluate the origin of ethanol in dead bodies [219]. Finding an atypical distribution pattern of ethanol in the specimens sent for analysis or positive findings in some but not other materials points towards postmortem synthesis having occurred. When body organs and tissues are sent for toxicological analysis they are not pre-treated with fluoride salts, which should be borne in mind when comparisons are made with blood and urine specimens with the added preservatives. Ethanol might be formed preferentially in some organs and tissues more so than others [220,221], depending on glucose content, anatomical location, proximity to the abdomen, etc. [222]. Use of standardized sampling technique and multiple specimens can simplify the interpretation of postmortem ethanol concentrations [223–225].

Research from Japan using rats made use of deuterium labeled ethanol as a novel way to distinguish a pre-existing blood-ethanol concentration from non-deuterated ethanol produced by microbial processes [226–228]. Other animal models (mice and rats) have been described to investigate the production of ethanol in dead bodies under various conditions of temperature and storage [229]. Germ-free mice were killed and stored under sterile conditions to document the importance of micro-organisms or fungal fermentation in the biosynthesis of ethanol. Conventional mice used as control produced increasing amounts of ethanol over time but this was not the case in the animals bred and maintained germ-free after sacrifice [230].

Studies designed to investigate the rate of production of endogenous ethanol in dead bodies are hampered by ethical constraints on this kind of research. The time course of ethanol synthesis under different conditions of storage temperature and humidity as influenced by nutrition, cause of death and degree of abdominal trauma would be welcomed [225]. Reports of positive blood-ethanol in infant fatalities are particularly sensitive and care is needed to ensure that appropriate specimens are obtained for toxicological analysis. Death caused by bacterial infections and the likelihood that infant food formula might contain good substrates for postmortem synthesis of ethanol warrant careful consideration [205,231].

Besides the biosynthesis of ethanol, a wide variety of micro-organisms are capable of utilizing ethanol as a source of carbon and energy [20,161]. This would tend to lower or eliminate completely a true pre-existing BAC before death and lead to incorrect conclusions. Some investigators recommend that postmortem blood specimens are subjected to microbiological

examination to identify the kind and number of microbes present including various strains of *Candida*, *Clostridium*, and *Klebsiella* species, *Escherichia coli*, etc. [36]. Today, molecular biological methods are available to identify the particular species of micro-organisms in body fluids and tissue obtained at autopsy [232,233].

## 9. Biochemical markers of postmortem synthesis

In forensic and legal medical practice the need to distinguish between antemortem ingestion and postmortem synthesis of ethanol still persists [234]. To help resolve this problem, investigators have tried to develop a practical and useful biochemical indicator and in this connection, various non-oxidative metabolites of ethanol have been used. Much attention has been given to ethyl glucuronide (EtG), a minor metabolite of ethanol, because if measurable amounts are present in body fluids this means that ethanol must have undergone metabolism during life [235–242]. However, finding EtG does not necessarily prove the person died with an elevated BAC because the half-life for elimination of EtG is a lot longer than for ethanol itself [241].

### 9.1. Ethyl glucuronide

The pioneers in drug metabolism demonstrated that low-molecular weight alcohols undergo conjugation reactions with glucuronic acid to produce water soluble glucuronides, which are then excreted in the urine [243]. Schmitt et al. [235] should be credited with revitalizing interest in this non-oxidative pathway of ethanol metabolism thanks to the availability of improved analytical methods such as gas chromatography/mass spectrometry (GC–MS) and more recently liquid chromatography/mass spectrometry (LC–MS). Analysis of EtG furnishes a more sensitive way to monitor recent drinking because of its longer elimination half-life compared with ethanol itself [241]. EtG is already being used as a biochemical marker to detect covert drinking in individuals who are undergoing rehabilitation or medical treatment for alcoholism [239,240]. Analysis of EtG and also ethyl sulfate have attracted interest in occupational medicine when people must refrain from drinking as a condition of employment or when they need to perform highly skilled tasks and safety-sensitive work, e.g. surgeons or airline pilots [239].

Trace quantities of EtG are produced during enzymatic metabolism of ethanol (<0.1% of dose) but more importantly, EtG is seemingly not produced by the action of microbes and yeasts on glucose. Accordingly if ethanol was only produced in the body after death one would not expect to find any measurable quantities of EtG in the samples analyzed. The risk of ethanol being produced in the urinary bladder is exaggerated if there is a fermentable carbohydrate such as glucose, which is common in poorly controlled diabetes. Diabetics also tend to suffer more from urinary tract and fungal infections enhancing the risk of postmortem synthesis [244]. Urinary EtG can disclose recent drinking for

about 6–10 h after ethanol is no longer measurable, depending on the dose ingested [244]. This presence of EtG and its longer elimination time could be misinterpreted if ethanol had been produced in blood by microbial activity, whereas the antemortem BAC had reached zero owing to metabolism. This scenario would probably be incorrectly interpreted to mean that the individual had alcohol in the blood at the time of death.

The concentration of EtG were found to decrease in urine specimens infected with *E. coli* although this reaction was preventable by having fluoride ions in the sample containers [216]. EtG concentrations remained fairly constant in urine samples held in air-tight containers when these were stored at room temperature for 5-week [242]. In the same study, EtG concentrations in liver and skeletal muscle decreased by 28% during 4-week of storage at room temperature. Importantly, when EtG-free blood and liver samples were spiked with ethanol (100 mg/100 mL) there was no new in vitro formation of EtG during storage.

A few studies have investigated the presence of EtG in hair strands obtained from dead bodies [245–247]. The analysis of drugs of abuse in hair provides a way to monitor prior use and abuse of the substance, which could serve as a way to diagnose long-term heavy drinking. However, dose-response studies of EtG incorporation into hair of living subjects are necessary before any valid conclusions about the utility of EtG analysis in hair can be made in postmortem material.

### 9.2. Other non-oxidative ethanol metabolites

Other non-oxidative metabolites of ethanol currently receiving attention as markers of antemortem consumption of alcohol as opposed to postmortem synthesis are phosphatidylethanol [248–250] and various esters formed between ethanol and short-chain fatty acids [251,252]. These non-oxidative metabolites are measurable in blood and tissues by highly sensitive methods and are excreted in urine with half-lives longer than ethanol itself [253]. The sensitivity, specificity and practical usefulness of these newer markers compared with analysis of EtG remains to be established.

### 9.3. Metabolites of serotonin

The urinary metabolites of serotonin, namely 5-hydroxytryptophol (5HTOL) and 5-hydroxyindoleacetic acid (5HIAA) have also been used to resolve whether a positive BAC stems from antemortem ingestion of ethanol or postmortem synthesis. Finding an elevated urinary ratio of 5HTOL/5HIAA (>15) indicates that ethanol has undergone metabolism, which points to antemortem ingestion [254–256]. Accurate assignment of ethanol origin in postmortem urine samples was described using an improved GC–MS method for simultaneous determination of 5HTOL and 5HIAA [257]. The same group of investigators went on to use this approach for investigating the origin of ethanol in victims of civil aviation disasters [254].

#### 9.4. Low molecular weight volatiles

During the microbial synthesis of ethanol from various endogenous substrates, other low-molecular volatiles are generated in blood and tissues and these include higher aliphatic alcohols (isoamyl alcohol, *n*-propanol, isopropanol, *n*-butanol), acetaldehyde, propionic acid as well as other organic acids. Of these *n*-butanol and isobutyric acid are considered reliable indicators of putrefaction and if present in blood mean that the concentration of ethanol is also suspect [258–260]. However, the spectrum of metabolic products varies depending on the available substrate, the kind of microorganism present and the conditions of growth. Methanol is seemingly not a product of microbial synthesis so elevated concentrations of this toxic alcohol in postmortem blood specimens suggests accidentally or intentionally ingested or contamination from embalming fluids [24].

Some investigators have focused attention on *n*-propanol as an indicator of putrefaction, although recent work questions this strategy [37,261]. The quantitative relationship between the amount of *n*-propanol detected and the amount of ethanol produced in postmortem blood was not strong and the concentrations of *n*-propanol tended to be 15–20 times less than those of ethanol [261]. If the gas chromatographic trace shows a signal at the same retention time as *n*-propanol or *n*-butanol, this raises a warning flag that the concentration of ethanol should be questioned. In bodies recovered from water, the presence of *n*-butanol is considered a good indicator that postmortem synthesis of ethanol might also have occurred during immersion [262].

Other more conventional biochemical markers for over-consumption of alcohol, such as carbohydrate deficient transferrin (CDT), have been investigated in blood and VH taken at postmortem with limited success [263,264]. Routine alcohol testing is recommended in all out-of-hospital deaths and elevated BACs can be expected in a large proportion of these cases [265].

### 10. Postmortem diffusion of alcohol

A long known and much debated source of error in postmortem toxicology is passive diffusion of ethanol and drugs through the stomach wall after death to falsely raise the concentration of these substances in surrounding tissues and also in central or peripheral blood [70,266–271]. The problem posed by postmortem redistribution of both licit and illicit drugs has aptly been referred to as a toxicological nightmare [41]. The passive diffusion of alcohol across a mucous membrane depends on the concentration gradient across the membrane and its permeability. With a high concentration of alcohol in the stomach at the time of death this means the concentration gradient and risk of diffusion is exaggerated and contamination of pericardial and pleural fluids and left lobe of the liver with alcohol becomes a real possibility. If the deceased had suffered a traumatic death and the stomach was ruptured, this naturally enhance the risk of gastric alcohol spreading into surrounding tissue to cause contamination problems.

The significance of postmortem diffusion of alcohol depends on time of last drink before death, the quantity and strength of the alcohol it contained and any dilution with food in the stomach. There is little quantitative data about the rate of decrease in stomach-alcohol concentration after the end of drinking. However, judging from the time needed to reach  $C_{max}$  in blood from many controlled drinking studies, it appears that most of the ingested alcohol is fully absorbed by 2-h and in most cases after 1-h [272]. The time for complete absorption is delayed if ethanol is consumed together with or after a meal [273].

The subject of postmortem diffusion and redistribution has been studied for many substances and seems to be more of a problem for drugs other than ethanol, especially those with large volumes of distribution, such as tricyclic antidepressants. Drugs that are sequestered in a particular tissue or when concentrations are sensitive to changes in pH after death are especially problematic [41–46]. Postmortem redistribution is less of a concern for unionized drugs like ethanol that are absorbed fairly rapidly from the gut and do not bind to tissue components and instead distribute evenly into the total body water [50].

If there is circumstantial evidence suggesting that the deceased had consumed a lot of alcohol or took drugs before a fatal accident then diffusion into surrounding organs and tissues cannot be ignored [73,102,274–279]. Sampling and analysis of gastric residue is not a routine procedure but this is advisable if there is evidence of drug overdose or recent antemortem drinking. Finding a gastric ethanol concentrations above 500 mg/100 mL (5 mg/mL or 0.5 g%) is thought to corroborate fairly recent drinking [67,112]. Another postmortem artifact arises if the deceased because of some agonal event inhales vomit enriched with alcohol, which then contaminates the pulmonary tract and blood from the pericardial sac or pleural cavities [276–278]. Aspiration deaths need to be verified by a careful histological examination of lung tissue.

The use of any life-saving emergency treatment including administering of drugs or intravenous fluids to counteract shock or vigorous heart massage should be considered along with the toxicology report. The positioning of the body at the death scene, the initial inspection, transport and storage of the corpse to the morgue are not trivial and careless handling might promote redistribution of alcohol by reflux of gastric residue.

Some investigators have used human cadaver models to investigate the potential for various drugs, including ethanol, to spread from the stomach into organs and vascular spaces after death [274–276]. In verified alcohol-free corpses, ethanol at different initial concentrations was instilled into the stomachs and blood for determination of ethanol was taken from various vascular sites over the next few days. In one such study, the concentrations of ethanol determined in blood from the intact chambers of the heart agreed well with blood from peripheral vessels despite a high concentration of alcohol in the stomach [270]. Nevertheless, the general conclusion from this kind of work seems to be that the best practice is to always obtain femoral venous blood for toxicological analysis of ethanol and other drugs.

## 11. Immersion deaths and drowning

Bodies recovered from water and drowning deaths present a special problem for the forensic pathologist when manner of death is ascertained – whether accident, suicide or murder [280–283]. The significance and interpretation of any elevated BAC also presents difficulties in interpretation. Both losses and increases in the concentration of ethanol in body fluids can occur when a body has been submerged in water for an extended period. Decreases in the concentration of ethanol are likely owing to dilution of body fluids with water as time passes before recovery [284]. Environmental factors particularly the temperature of the water during summer months, the degree of trauma to the body and whether putrefaction processes were advanced should be considered when postmortem concentrations of ethanol are interpreted [285].

In a recent study, based on 562 drowning deaths, the decrease in weight of the lungs that occurs over time in water was used as an indirect way to monitor the time course of decomposition. The lung weight was then related to the prevalence of finding measurable BAC in the bodies recovered from water [285]. The authors concluded that production of ethanol might start by 12–24 h after submersion in water during the warmer non-winter months. Longer submersion times were associated with a greater proportion of elevated BAC at autopsy.

The complexity of interpreting BAC in drowning deaths was highlighted in a recent paper by Cullen and Mayes [123]. The material consisted of 44 bodies recovered from the sea with no evidence of consumption of alcohol before death. Ethanol was positive in both blood and urine in 14 cases, which hitherto was taken to mean that alcohol must have been ingested before death. However, in these cases the mean UAC/BAC ratio was abnormally low, being only 0.56:1 compared with a ratio of 1.25:1 or more expected if absorption and distribution of alcohol was complete. The elevated UAC in these cases were thought to arise from ethanol being produced in the abdomen by bacteria and then diffusion through surrounding tissue into the urinary bladder [123]. The authors also noted a statistically significant and positive correlation between blood ( $r = 0.65$ ) and urine ( $r = 0.83$ ) ethanol concentrations and the number of days that bodies were in the sea before recovery.

## 12. Alcohol and aviation disasters

The problem of alcohol use by pilots is a highly contentious matter when aviation disasters are investigated and this issue continues to attract a lot of attention [286]. During 1989–1990 the US Civil Aeromedical Institute received specimens from 975 victims of fatal aircraft crashes and 79 of these were positive for ethanol (>40 mg/100 mL) [287]. Based on the distribution of ethanol in urine, vitreous, blood and tissue it was determined that 21 of the positive cases could be attributed to postmortem synthesis of ethanol whereas 22 reflected drinking alcohol and 36 could not be interpreted in a satisfactory way. In two cases the production of ethanol postmortem reached as

much as 150 mg/100 mL [287]. Similar findings were reported in a more recent compilation involving 1587 civil aviation pilot fatalities 1999–2003 [288].

Concentrations of ethanol above 10 mg/100 mL were found in 14.8% of 377 Federal Aviation Administration fatalities [289]. Of these positive cases, the ethanol concentrations were between 10 and 50 mg/100 mL in 36 cases, between 51 and 100 mg/100 mL in 10 cases and 10 others were in excess of 100 mg/100 mL. The occurrence of other volatiles in blood besides ethanol was used as an indicator of possible postmortem synthesis. Using this chemical marker, only 4.5% of cases were thought to reflect antemortem ingestion of ethanol.

Aviation fatalities are extremely difficult to deal with when it comes to recovery of bodies and obtaining the best possible samples for toxicological analysis [290–292]. The postmortem examination and the analytical toxicology are complicated owing to extensive trauma in victims of plane crashes including rupturing of the stomach and bursting of the bladder [293]. Obtaining biological specimens for toxicological analysis after an aircraft disaster presents a great challenge and findings of positive BAC needs to be interpreted with caution because of the heightened risk of postmortem synthesis. A scheme for toxicological processing of postmortem specimens from pilots was recently described based on long experience of many airplane crashes in USA [294].

The special problems associated with aviation medicine and toxicology have been discussed in a number of publications [287,290–293,295–300]. Multi-site sampling of blood and tissue including VH, CSF, brain, liver and skeletal muscle if and when available is strongly recommended [288,295]. The use of biochemical markers to distinguish ingestion of ethanol and hepatic metabolism from microbial synthesis is urgently needed when deaths caused by blunt trauma or burns are investigated.

The problem of interpreting low postmortem BAC in victims of trauma was exemplified by a report of the USS Iowa naval disaster in which 47 men died [218]. Autopsies were started 48 h after the explosion and were completed 48 h later, although the time delay to commencing analytical toxicology was not reported. An explosion in a gun turret caused extensive blunt force and thermal injury to the bodies, many of which were significantly decomposed after being submerged in water. Of the 47 deaths, 23 had positive blood-ethanol (>10 mg/100 mL) although the source of blood and whether a fluoride preservative had been added was not mentioned [218]. Some of the positive blood-ethanol findings might have arisen during transport and storage of specimens prior to analysis. The blood of most victims contained less than 30 mg ethanol per 100 mL and the range was from 10 to 190 mg/100 mL. Most of the cases with positive BAC were associated with negative results in urine, vitreous, bile, kidney or brain tissue, which strongly suggests postmortem production in the blood samples. For example, a person with 190 mg/100 mL in blood had no alcohol present in urine, which obviously raises suspicion about the integrity of the BAC report.



### 13. Ketoacidosis as cause of death in alcoholics

Many people die alone at home and the postmortem examination and toxicological report for presence of drugs and poisons show no obvious cause of death. The autopsy findings are usually unremarkable apart from fatty liver and the blood-ethanol concentration is low or zero. Evidence sometimes surfaces that many of these individuals were known to be heavy drinkers and clinically might have been diagnosed as dependent on alcohol. A growing number of studies have implicated ketoacidosis as a likely cause of death, which is supported by analysis of high levels of ketone bodies in body fluids, namely acetone, acetoacetate and particularly  $\beta$ -hydroxybutyrate [301–312]. This designation of a ketoacidosis death in binge drinkers and alcoholics has received considerable attention when no other obvious explanation exists [303]. In this connection, the concentration of  $\beta$ -hydroxybutyrate is seemingly more important than acetone and acetoacetate [305–311]. However, the threshold concentration of this intermediary metabolite in blood or other tissue to allow making such a diagnosis has not yet been properly established.

During the metabolism of ethanol the redox state in the hepatocyte changes to a more reduced potential and  $\text{NAD}^+$  is converted to NADH, which has important consequences for normal metabolic functions of the liver. Among other things, the ratio of lactate to pyruvate increases appreciably during ethanol oxidation and heavy drinkers are likely to suffer from lactic acidosis and gout [307]. Furthermore, and perhaps more importantly, alcoholics on a drinking binge neglect to eat properly, which leads to depletion of glycogen stores in liver and muscle tissue. The altered redox state in the hepatocyte also means that gluconeogenesis is diminished or stopped completely. This triggers lipolysis and conversion of triglycerides into free fatty acids, which in turn are metabolized in the liver into ketone bodies. Taken together these conditions can precipitate a dangerous state of ketoacidosis and also alcohol-induced hypoglycemia, which might be augmented by vomiting during a period of alcohol withdrawal [304,308–312].

### 14. Concluding remarks

Toxicological analysis constitutes an essential element in all investigations of unnatural and sudden deaths and in this connection alcohol intoxication and drunkenness play an important role [313,314]. Elevated BAC is a prominent finding in all out-of-hospital deaths (Table 1) and much depends on alcohol control policy, availability and the price of alcohol in different countries [9,315]. The literature base underpinning postmortem alcohol toxicology is large and has a long history with relevant information contained in many textbooks of forensic and legal medicine, the mainstream forensic science and legal medicine journals as well as journals devoted to substance abuse, general medicine and pathology.

Methods for quantitative and qualitative analysis of ethanol in body fluids are the same regardless of whether specimens are taken from the living or dead. The presence or absence of

ethanol can be ascertained with a high degree of accuracy, precision and selectivity using HS-GC and if necessary by GC-MS to furnish an unequivocal identification [316,317]. This stands in contrast to wet-chemical and enzymatic methods of analysis, which are not specific tests for ethanol and fail to distinguish it from certain other low-molecular volatiles. Despite use of gas chromatographic methods of analysis, the interpretation of toxicological results of ethanol analysis has sometimes proven difficult. In two cyanide deaths caused by drinking acetonitrile, this compound showed the same retention time as ethanol on two different chromatographic systems [318]. A more selective method such as GC-MS or a completely independent method, such as chemical oxidation, was necessary to resolve this problem [319].

The BAC at autopsy can be converted into the amount of alcohol absorbed and distributed in all body fluids at the time of death using the well-known Widmark equation [14,320]. However, if there was alcohol unabsorbed in the stomach at time of death, this calculation results in an underestimation of the total amount consumed. Such concentration-dose calculations are valid and feasible for drugs like alcohol, methanol, acetone, and 2-propanol because these substances distribute evenly throughout the body according to the water content of organs, fluids and tissues. The relationships between dose and blood-concentration of other drugs, such as those given in many clinical pharmacology textbooks, are not practical to use in postmortem toxicology. Many drugs bind tightly to plasma proteins, are sequestered to tissue depots or dissolve in lipids and their distribution volumes and half-lives are often not well defined, especially after toxic doses. Furthermore, the important serum/whole blood distribution ratios of many drugs are unavailable making it difficult to compare the concentration in autopsy blood with the therapeutic range established in plasma or serum.

The phenomenon of postmortem diffusion and redistribution means that the concentration of a drug determined in autopsy blood is not necessarily the same as the concentration present at the time of death. Artifacts of this kind are more of a problem for drugs other than ethanol, such as opiates and antidepressants [41]. Movement of a relatively small amount of substance from a tissue depot, e.g. the liver into the vascular system after death could produce a substantial change in the drug concentration determined in central blood obtained later at autopsy.

Comparing the concentrations of ethanol in different body fluids such as cardiac and femoral blood, urine and VH is virtually essential to ensure reaching a correct diagnosis of whether a person was under the influence of alcohol at the time of death. Besides the toxicological report, the entire case scenario and particularly the deceased's medical history, information from the scene and the circumstances leading up to death need to be carefully considered and weighed in relation to the toxicological report. A person's BAC should not be interpreted in a vacuum and the totality of information gleaned from toxicology and interviews with witnesses, police reports, etc., are important considerations. Interpreting the BAC at autopsy in terms of impairment or culpability in a traffic crash often becomes a contentious issue in legal

proceedings that follow and when insurance claims are made [321].

Resolving whether a positive blood-ethanol arose from postmortem synthesis or antemortem ingestion is a recurring question in legal medicine and toxicology and has spawned the development and evaluation of various biochemical markers [211]. The origin of ethanol in postmortem blood might become a particularly thorny issue in mass transportation disasters and when highly traumatic deaths are investigated, such as a plane crash or an explosion, or when there is evidence that the corpse had undergone decomposition and putrefaction [213,214,217–219,322,323].

This review of the forensic alcohol literature has focused on issues related to the analysis and interpretation of ethanol concentrations measured in a wide variety of postmortem specimens. The long list of references, which were double checked with PubMed for accuracy, will hopefully prove useful to colleagues who are contemplating a fresh investigation into this area of forensic toxicology.

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