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Interpreting Postmortem BAC Levels: Influencing Factors

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Abstract

Introduction: The forensic toxicologist is challenged to provide scientific evidence to distinguish the source of ethanol (antemortem ingestion or microbial production) determined in the postmortem blood. For this reason, the aim of this literature survey is to determinate of factors that may influence BAC postmortem and the correct process to interpret postmortem blood alcohol levels.

Methodology: We searched the scientific literature for articles dealing with postmortem aspects of ethanol and problems associated with making a correct interpretation of the results.

Results and Discussion: Possible sources of postmortem EtOH have been the ante-mortem ingestion, the ante-mortem endogenous production and the postmortem microbial neo-formation, which has been considered the most critical factor that could complicate the results.

The condition of the body, the time between death and autopsy, the environmental conditions, the nature and state of the specimen collected for analysis, the clinical history of the deceased, and the circumstance of death 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. Postmortem diffusion of alcohol from the stomach to central blood sampling sites is another complicating factor.

This brief review investigates the complementary approaches necessary for the interpretation process.

Conclusions: The evaluation of the postmortem BAC remains a complicated, multifunctional procedure. More studies are needed to clarify this problem.

Keywords: Blood alcohol concentration; forensic toxicology; alcohol origins; factors influencing

Introduction

Ethanol, the most commonly encountered psychoactive substance in forensic investigations, presents a unique challenge when interpreting blood alcohol concentration (BAC) in postmortem cases. While BAC remains the primary method for assessing alcohol intake, its interpretation faces significant difficulties due to the multitude of factors influencing BAC levels after death. This complexity necessitates that forensic toxicologists critically evaluate various sources of evidence to distinguish between antemortem (before death) alcohol consumption and postmortem production by microbes. Information regarding the deceased's medical history, autopsy findings, sample collection and storage methods all play a crucial role in accurately interpreting BAC results. Additionally, complementary approaches, such as comparing ethanol levels in different tissues and analyzing biomarkers of chronic alcohol consumption, are essential for confirming the origin of the measured BAC in postmortem cases.

This literature review aims to comprehensively analyze published research on the forensic analysis of ethanol in autopsy specimens. We will explore factors influencing the interpretation of postmortem BAC results and discuss the role of complementary approaches in confirming the origin of measured ethanol levels.

Background on Ethanol

Ethanol, commonly known as "alcohol", is the most widespread human toxicant whose toxic risk is often underestimated by the general population. Alcohol is a solvent widely used in chemical synthesis and in the paint, varnish, ink, plastic, adhesive, cosmetics, and pharmaceutical industries. It is also used in laboratories. Therapeutic use is limited to skin disinfection, adjunctive treatment of ethanol withdrawal syndrome, methanol or ethylene glycol intoxication, and venous sclerosis. Ethanol is the basic component of alcoholic beverages (INRS, 2019).

Toxicokinetics

Ethanol is rapidly absorbed by the oral and respiratory routes, with little absorption through skin contact (INRS, 2019).

Digestive absorption is by simple diffusion, mainly from the duodenum and proximal jejunum (70-80%) and, to a lesser extent, from the stomach. There are a number of factors that can slow down or accelerate the process. Among the parameters that slow it down are food intake and the speed of gastric emptying, which is a determining factor. Factors that speed it up include the emptiness of the stomach, the acceleration of gastrointestinal motility, and the elevation of the alcoholic strength of the beverage, as well as the presence of CO_2. After a single ingestion, blood alcohol levels peak after 1 hour if the alcohol was ingested without food, after 2 hours otherwise; the rate of absorption is highest for concentrations between 10 and 30%. As ethanol is highly volatile, its penetration by the respiratory route is significant. On average, it is estimated at 60%. Penetration through the skin is negligible in adults, at around 1%. However, it must be taken into consideration on injured or highly permeable skin such as that of newborns and infants [1].

One of the essential characteristics of ethanol, linked to its low molar mass and high water solubility, gives it the property of diffusing rapidly and uniformly throughout the body, following the movements of water, mainly in highly vascularized organs such as the brain, lungs, and liver; concentration is highest in cerebrospinal fluid and urine, where it reaches 1.3 times the plasma concentration, itself slightly higher (1.1 times) than the average organ concentration, and is negligible in bone and fat, given its low liposolubility. The average volume of distribution is 0.5 L/kg in women and 0.6 L/kg in men. Thus, in obese subjects, an identical quantity of alcohol ingested per unit of weight gives a higher BAC than in slim subjects. Moreover, during the absorption phase, arterial blood contains more alcohol than venous blood. Ethanol freely crosses the fetoplacental barrier, and similar concentrations are found in maternal and fetal blood.

Ethanol metabolism essentially involves complete oxidation to CO2 and water, which takes place in 3 stages. The first stage, which leads to acetic aldehyde, takes place for the most part (80 - 90%) in the liver under the action of alcohol dehydrogenase. The cytochrome P450 (inducible) and catalase-peroxidase systems are also involved at this stage. There is also a gastric metabolism, linked to gastric ADH (alcohol dehydrogenase) activity, of the order of 10%, which decreases in the event of damage to the gastric mucosa. The second step, leading to acetic acid, is dependent on aldehyde dehydrogenase present in the liver (90%) and kidney (10%). Thus, due to genetic polymorphism of aldehyde dehydrogenase, certain ethnic groups may degrade acetic aldehyde more slowly. Accumulation can also occur in the presence of a specific aldehyde dehydrogenase inhibitor (e.g. disulfiram). The acetic acid formed is released into the blood and the third stage takes place mainly in peripheral tissues and certain organs: muscles, heart, brain, where it is oxidized to CO_2 and water with the oxidation of acetate to CO_2. Other minor non-oxidative metabolic pathways, mainly hepatic, account for less than 1% and are involved in the formation of fatty acid ethylesters, phosphatidilethanol, ethylglucuronide and ethylsulfate. These metabolites are of vital importance for the specific biological diagnosis of alcohol consumption, as well as for its monitoring in many clinical contexts [2]. It should be noted that there is endogenous alcohol production in the gut microbiota via fermentation.

Unmetabolized ethanol is eliminated through exhaled air, sweat and urine. A very small amount is eliminated as ethylglucuronide and ethylsulfate. Pulmonary elimination is the basis for estimating BAC from concentrations in exhaled air. In fact, the ratio of alcohol concentrations in blood to exhaled air is of the order of 2100, making it possible to estimate BAC (in g/L).

Toxicodynamics

The main actions of ethanol are attributable to its action as a central nervous system (CNS) depressant [3]. Ethanol is a narcotic, causing excitation then general depression of the CNS, acting as a GABA A receptor agonist and NMDA glutamatergic receptor antagonist. Ethanol stimulates the brain's reward pathways, facilitating influx into the dopaminergic, noradrenergic, and serotonergic pathways [3].

The mechanism of its effects on lipid metabolism is more complex. It can lead to; an increase in triglyceride synthesis in the liver; an increase in glycerol incorporation into phosphatidylcholine with relative choline deficiency; a release of catecholamines that accelerate the mobilization of fatty deposits; and a decrease in the rate of fatty acid oxidation.

Acute Intoxication

Alcoholics react differently to equivalent BAC. The clinical effects of ethanol depend mainly on the quantity consumed. The toxic dose is estimated at around 0.8 g/kg (1 mL/kg) of pure ethanol (about 3 to 4 glasses), which will produce a BAC of 1 g/L. The clinical effects of acute intoxication are summarized as a function of blood concentration by K. M. Dubowski Table 1 [3].

Table 1: The clinical effects of acute intoxication

Phase	Concentration	Symptoms
Subclinical	0 – 0,4 g/L	Functional changes occur in the upper cerebral cortex and affect the perception and processing of information received by the senses. Learned motor responses are spared.
Euphoria	0,5 – 1,4 g/L	Decreased attention span, judgment and control with progressive loss of motor response. There is a slight euphoria, an increase in self-confidence and sociability.
Excitement	1,5 – 2,4 g/L	Changes in personality and behavior are unpredictable, with decreased inhibition and poor judgment. There are memory and comprehension disorders, a decrease in sensory response with an increase in motor reaction time and muscle incoordination and muscle incoordination.

Confusion	2,5 – 3,4 g/L	Speech disorders, a staggering gait with a decrease in the perception of pain, balance disorders and a disturbance in the perception of colours, shapes, sizes and movement. Increased mental confusion with exaggerated emotional states (fear, anger and grief) is very characteristic of this stage.
Stupor	3,5– 4 g/L	The individual is apathetic with a markedly diminished response to stimuli, altered consciousness coexisting with awake sleep.
Coma	> 4 g/L	Complete unconsciousness without awakening; death occurs in 95% of cases when the coma lasts more than 12 hours.

Chronic Intoxication

Chronic ethanol consumption leads to varying degrees of physical dependence and tolerance, with characteristic withdrawal symptoms. There are three types of tolerance: metabolic, pharmacodynamic and behavioral, or adaptive.

When increased amounts of ethanol are consumed chronically, delayed toxic biological effects occur, ensuing and including cytotoxicity with clinically evident functional disturbances in various organ systems. Significant pathologies with pathophysiological sequelae may involve the liver (hepatic steatosis, acute hepatitis, cirrhosis with liver failure, hepatocellular carcinoma), heart (cardiomyopathy, hypertension), brain (Wernicke-Korsakoff encephalopathy due to vitamin B1 deficiency, superior cerebellar atrophy, Marchiafava-Bignami disease, stroke, and seizure disorders), esophagus (submucosal varices, Mallory-Weiss syndrome, rupture, carcinoma), stomach and duodenum (gastritis, gastric atrophy, peptic ulcer, carcinoma), pancreas (acute and chronic pancreatitis with pseudocyesis), and genitourinary system (reduced testosterone production, testicular atrophy, disturbance of menstrual cycles, infertility in both sexes). Ethanol toxicity also manifests during pregnancy (fetal alcohol syndrome, spontaneous abortion, fetal withdrawal, teratogenesis, growth retardation, disruption of the CNS, and external malformations). Concomitant poor nutritional intake leads to general malnutrition. The IARC has classified ethanol in alcoholic beverages as a Group 1 human carcinogen. It acts as a tobacco cocarcinogen.

Toxicological Diagnosis Ethanol Consumption in Postmortem

At postmortem autopsies, several samples are likely to be of interest in interpreting the alcohol content at the time of death. Whole blood is the gold standard for determining BAC, for a number of reasons; it is generally easier to obtain and process, and BAC adequately reflects the effect of ethanol and the state of impregnation at the time of analysis; it is more practical, technically efficient and economically rational to analyze blood regularly when such high volumes are required [2, 3, 5]. However, some laboratories use serum or plasma to perform this assay. Under most physiological conditions, serum or plasma contains around 10-12% more water than the same volume of whole blood, so ethanol levels are proportional, but slightly higher in these samples. The following ethanol ratios are obtained for the conversion of these blood components to whole blood: serum = 1.12-1.17; plasma = 1.10-1.35 [3]. Blood taken from the body cavity is generally a poor sample for toxicological analysis, as it is likely to be contaminated by the contents of the intestines, urine or other body fluids, for example after severe trauma. In the laboratory, black or greenish blood with a strong putrefactive odor may indicate postmortem ethanol production. These criteria are generally useful; however, there is no correlation between the degree of decomposition and the amount of ethanol produced, as all bodies and conditions are different [6].

The use of multiple samples from different body compartments is beneficial, as the analysis of more than one sample tends to guarantee the accuracy of a given quantitative result and thus facilitates interpretation. Urine is a useful sample for ethanol analysis, as the risk of microbial or yeast invasion of the bladder after death appears to be lower than the risk of contamination of blood samples [2]. Vitreous humor is one of the biological specimens widely used in forensic toxicology, mainly when the body is severely damaged or affected by putrefaction [4]. It is anatomically distant from the intestine and therefore less likely to be

contaminated by the spread of bacteria [2]. Vitreous humor is easy to collect and shows sample stability over time after death. Its close correlation with blood ethanol concentration makes it an excellent matrix that could be used as an alternative. However, certain limitations are also present, such as a limited sample volume and that it dries out quickly [4]. Other tissues and samples can be used, such as bile, gastric contents, bone marrow, solid organs, e.g. liver, kidney, brain, spleen and lung, cardiac, smooth or skeletal muscle, intracerebral and paradural hematomas, synovial fluid and cerebrospinal fluid.

Blood samples should be taken in sterile tubes, preferably containing sodium fluoride, heparin or another anticoagulant, or without additives. The anticoagulant and bacteriostatic actions of sodium fluoride are optimal for the preservation and storage of whole blood. Body fluid samples should be transported rapidly. If necessary, samples should be refrigerated at the best storage temperatures, between -20°C and -4°C. At -20°C, samples can be stored for 3 to 4 months.

In terms of analysis, specific methods are required due to interference between volatile substances present in postmortem samples. Gas chromatography is the gold standard for BAC analysis, enabling specific identification and quantification of ethanol. Only results obtained by gas chromatography are legally valid for road safety purposes. The Cordebard chemical method is one of the official methods for alcohol determination and is also the oldest. It is virtually no longer used today. A technique widely used by laboratories is the enzymatic method. These methods are fast, easy, and automated, but unlike gas chromatography, enzymatic reactions are prone to cross-reactivity, leading to false-positive or overestimated results. As a result, they are not considered reliable methods for determining BAC in a forensic or legal context [3].

Around the world, legal blood alcohol limits vary widely, from 0.2 g/L in Norway and Sweden to 0.4 g/L in China and Japan, 0.5 g/L in most European countries, and Australia, and up to 0.8 g/L in the USA, the United Kingdom, and Malta. In Indonesia, however, there is no legal limit. In Algeria, the limit for blood alcohol content measured in whole blood has been raised from 0.1 to 0.2 g/L.

For a proper forensic interpretation of a subject's state of alcohol impairment, it is essential to estimate the BAC at the time of the incident, or the maximum BAC. In addition, the magistrate may ask the toxicologist to determine the quantity of alcohol ingested; this question can be answered with sufficient approximation using Widmark's formula.

Factors Influencing the Interpretation of Postmortem BAC

The problem with postmortem analytical investigations is not the analysis itself, which must of course be carried out with the utmost precision, but the interpretation of the results, which may depend on a number of factors. Indeed, several individual and extra-individual factors are likely to influence blood alcohol levels.

Factors related to the deceased

Age

A number of age-related anatomical and physiological factors have a negative effect on ethanol elimination, including increased liver size. The activity of enzymes such as alcohol and acetaldehyde dehydrogenase and cytochrome P450 2E1 are also affected by age. Gastric ADH has also been reported to vary with age. The activity of this enzyme is also influenced by gastric atrophy and chronic gastritis frequently observed in the elderly, and gastric emptying and volume of distribution decrease with age.

Gender

The bioavailability of ethanol is much higher in women than in men, as they have less gastric first-pass metabolism of ethanol.

This is associated with lower gastric ADH activity in women. Moreover, women have less total body water per fraction of body weight, so the same amount of ethanol consumed by a woman would reasonably result in a higher BAC than in a man of equal weight.

Circumstances of Death

Drowning

Loss and increased concentration of ethanol in body fluids can occur when a body has been immersed in water for an extended period. Environmental factors, in particular, water temperature, degree of body trauma, and advanced putrefaction processes must be considered when interpreting postmortem ethanol concentration [2]. Ethanol production can begin 12 to 24 h after immersion in water during the warmer months. Longer immersion times have been associated with a higher proportion of high BAC at autopsy. An abnormally high urinary ethanol concentration signifies microbial production of ethanol in the abdomen, followed by diffusion through the surrounding tissues into the bladder. There is a significant, positive correlation between blood and urine concentrations and the number of days the body remains submerged before recovery.

Polytrauma

The influence of trauma, blood loss and possible transfusion on BAC is often controversial. If alcohol remains unabsorbed in the stomach when trauma occurs, reduced splanchnic circulation will tend to slow the absorption of alcohol into portal vein blood) When gastrointestinal traumatic injury occurs and unabsorbed ethanol is present in the stomach, contamination of blood samples occurs and artificially high BAC is obtained. Severe trauma also increases the potential for the spread of bacteria and increases the risk of ethanol production after death [2].

It is recommended that in the event of traumatic injury, cardiac blood samples from the intact heart cavity, together with additional biological fluid samples, should be taken to exclude the possibility of contamination and to ensure that the BAC is accurate.

Medical history

Diabetes

In diabetics, urine contains a significant amount of glucose, which is the main substrate for ethanol formation postmortem [2]. Diabetics tend to suffer more from urinary disorders and urinary tract infections, thus increasing the risk of postmortem ethanol synthesis.

Auto-Brewing Syndrome

The term "endogenous alcohol" applies to ethanol production in the body via carbohydrate fermentation by intestinal microflora. Generally, these values are extremely low. However, individuals suffering from certain pathologies (diabetes, Crohn's disease, short small bowel syndrome, pseudo-obstruction, hepatic cirrhosis, etc.) can produce significant quantities of alcohol which is then absorbed into the bloodstream, constituting a syndrome known as "auto-brewing syndrome" which was initially described in Japanese. Lifestyle, abdominal surgery, disturbance of the intestinal tract or repeated antibiotic therapy seem to favor stagnation of the alimentary bolus and proliferation of agents responsible for alcoholic fermentation. Various yeasts of the Candida and Saccharomyces families, as well as bacteria such as Klebsiella pneumonia, Enterococcus Faescium and Citrocacter freundi, have been implicated in this syndrome. As a result, the possibility that endogenous alcohol may modify BAC.

Medication

Many medications interact with alcohol and can change the metabolism of alcohol and vice versa. This includes antibiotics, antidepressants, antihistamines, and medications for pain, anxiety, or sleep. Some of these may increase BAC. The mechanism of some of them are presented in Table 2.

Table 2: Interactions between alcohol and various drug classes (Alan, 1998; Weathermon, 1999)

Drugs	Type of interactions
-Acetylsalicylic acid-H_2 antihistamines:Ranitidine, Cimetidine	Increased gastric emptyingFaster absorption of alcohol in the small intestineInhibition of gastric ADH
Erythromycin	Increased gastric emptying
Cisapride	Faster absorption of alcohol in the small intestine

Moreover, any medication increasing peripheral blood flow such as antihypertensive medications will likely decrease BAC. The use of any emergency treatment, including the administration of intravenous drugs or fluids to treat shock, or vigorous cardiac massage, must be considered. The positioning of the body at death, initial inspection, transport and storage of the corpse at the morgue are not trivial, and careless handling could promote the redistribution of alcohol through reflux of gastric residues [2].

Factors Involved in the Production and Degradation of Ethanol in Vivo

Postmortem Ethanol Production

In routine practice, endogenous ethanol production after death is directly related to the numbers and nature of microorganisms present, the type and quantity of substrates available, temperature and time elapsed between death and autopsy, humidity, oxygen availability, water immersion and the extent of body trauma [6, 7]. These factors pose a problem when it comes to correctly interpreting the results obtained in a postmortem analysis [7, 12].

Decomposition of the human body is a complicated process that differs between cadavers, and even between different parts of the same body, and is affected by environmental conditions [12]. When the supply of oxygen to the body ends, the integrity of cell membranes and tissue compartments gradually disintegrates through the action of various digestive enzymes. This reflects the autolysis process [2]. During this process, intestinal bacteria can enter after death and be distributed in the bloodstream, thus invading surrounding tissues [2, 5, 6]. Blood glucose concentrations increase after death, providing a simpler substrate for microbial ethanol synthesis [9]. There are at least 58 species of bacteria, 17 species of yeast and 24 species of mold capable of producing ethanol under various conditions. The yeast Candida albicans was the main cause of ethanol production, but the greatest increase observed was the result of contamination by Escherichia coli [10].

Postmortem Ethanol Degradation

In addition to ethanol biosynthesis, a wide variety of microorganisms are capable of using ethanol as an energy source [2, 5], This could reduce BAC and lead to erroneous conclusions [7].

Postmortem Redistribution Factors

Passive diffusion of ethanol through the stomach wall after death could falsely increase its concentration in surrounding tissues and also in the blood [2, 5]. This atypical distribution could be facilitated by traumatic lesions, alcohol-rich aspirated vomit, blood coagulation, as well as mechanical factors, such as postural changes during postmortem inspection and body transport.

Biological sampling factors

Nature and Condition of Biological Sample

The concentration of ethanol in different parts of the body may vary in cases where death has occurred during the absorption phase. Differences have been reported between arterial and venous blood [5]. An excellent correlation was observed between ethanol concentrations in right heart blood and femoral venous blood. However, alcohol levels in cardiac blood may be overestimated due to possible diffusion from the stomach postmortem. Concentrations may also be increased due to autolysis of cardiac tissue or trauma. Blood accumulated in the pericardial sac is not currently recommended, due to the risk of contamination. The more the condition of specimens deteriorates as body decomposition progresses, the greater the likelihood that the ethanol present is due to postmortem synthesis [8, 9]. In addition, other problems related to the state of the sample, including coagulation, hemolysis and hemoconcentration, can influence blood alcohol levels Tab.3. In vitro ethanol production in urine has also been recorded [5].

Table 3: Influence of the state of the sample on BAC (Sammis Law Firm, 2018)

Blood sample status	Impact
Coagulated	Artificially raising the alcohol content in a blood sample by altering the liquid/solid ratio.
Hemolyzed	Change in blood color following haemolysis leading to inaccurate results by enzymatic methods which is color dependent.A dramatic increase in alcohol concentration by the method of gas chromatography.
Hemoconcentrate	Hemoconcentration increases the alcohol content in a blood sample.

A finding of high ethanol concentration in the vitreous humor is generally accepted as evidence in support of lifetime alcohol consumption. In traumatic deaths, when decomposition has begun, the discovery of alcohol accompanied by a negative or very low concentration in the vitreous humor may indicate that ethanol has been neoformed [4].

Sample Preservation

Numerous studies have reported on the generation of microbial ethanol after sampling in postmortem samples [8]. It is worth mentioning that temperature increase and storage time are recognized as the main factors influencing post-sampling alcohol production, in addition to the microbial species and microbial load present in the specimen, the presence or absence of glucose in the sample is suggested to be the absolute determinant of post-sampling ethanol production [8]. Synthesis by microbial contamination can be avoided by proper preservation with refrigeration preservation with sodium fluoride at 1 to 2% w/v [4-6]. Fluoride ions function as an enzyme inhibitor, which is important to prevent further ethanol production [4].

Suitability of the sample for Analysis

The appearance and condition of the blood sample, such as its color, odor, fluidity, presence of clots and, if necessary, determination of water content, are very important in interpreting the results [2]. The suitability of collected biological samples for analysis can be established by subjecting them to microbiological tests to identify the type and number of microbes present, including various strains of Candida, Clostridium and Klebsiella species, Escherichia coli, etc. [2]. If microbiological studies show that the sample contains a large number of micro-organisms or supports considerable microbial activity, the sample is probably not optimal for analysis, and endogenous alcohol production is likely and certainly cannot be ruled out. If, however, no microbial activity is demonstrated, the probability of ethanol in the specimen being due to ingestion is much higher, although endogenous production may not be completely ruled out. There are other methods that use PCR and microbial DNA primers de-

signed to identify common ethanol-producing microorganisms (Vu, 2000).

Complementary Approaches to Confirm the Origin of BAC

Comparison of Alcohol Levels between Several Matrices

Testing several matrices to determine alcohol concentration is common practice in forensic analysis to verify the ethanol absorption/elimination phase. The most commonly tested biological matrices include: blood, urine and vitreous humor. In general, BAC is higher than the alcohol concentration in urine and the alcohol concentration in vitreous humor during the absorption phase, and the reverse during elimination. In addition, arterial blood can have alcohol concentrations up to 40% higher than venous blood during the absorption phase. The ratio of urine ethanol concentration to BAC provides useful information on the state of alcohol absorption at the time of death and indicates whether postmortem production has taken place. Finding a ratio of less than or close to 1 suggests incomplete alcohol absorption, indicating that alcohol consumption is fairly recent and some ingested alcohol probably remains unabsorbed in the stomach, whereas finding a ratio of 1.25 or more suggests that ethanol absorption and distribution was complete at the time of death [2].

Research and Assay of Biological Markers of Alcoholism

Researchers have attempted to develop a practical and useful method for the determination of biological markers of alcoholism. Much attention has been focused on ethylglucuronid, an ethanol metabolite formed from a minor metabolic pathway. This metabolite is not produced by the action of microorganisms [2]. Urinary ethylglucuronid can reveal recent consumption for around 6 to 10 h [2].

Another biomarker developed more recently, phosphatidylethanol, has a number of advantages over ethylglucuronid. It can be assayed in a more conventional matrix (whole blood), with shorter analysis times (a few days to a week), lower assay costs and easier, more informative interpretation [2]. Blood testing detects ethanol consumption over the past 3 weeks. The specificity of its blood measurement is 100%, and its sensitivity, higher than that of transferrin, varies between 86% and 95% depending on the study.

Urinary serotonin metabolites 5-hydroxy-tryptophol (5-HTOL) and 5-hydroxyindoleacetic acid (5-HIAA) have also been used to determine whether BAC are due to antemortem ethanol ingestion or postmortem synthesis [2]. Ethanol produces a switch to the serotonin-reducing pathway, increasing the ratio of 5-hydroxy-tryptophol to 5-hydroxyindoleacetic acid. The ratio remains elevated for several hours after ethanol ingestion (Robertson, 2010). Both 5-HTOL and 5-HIAA are relatively stable in urine. The main value of this test may lie in its ability to confirm alcohol production postmortem. A normal value of 5-HTOL/ 5-HIAA in the urine confirms that ethanol in the blood has not been ingested [11]. Finding a high urinary ratio of 5HTOL/5HIAA (>15) indicates that ethanol has undergone metabolism, pointing to antemortem ingestion [2].

Transferrin is a protein involved in the transport of iron throughout the body. The presence of ethanol and/or its metabolite acetaldehyde affects protein synthesis, including transferrin [11]. Its levels will return to normal after a few days to a few weeks of abstinence. Problems of sample stability limit the usefulness of this test, which has no forensic value.

Determination of Volatile Solvents

When alcohol is produced postmortem by microorganisms, the resulting compound is generally not pure ethanol. This is in contrast to ingested alcohol, which generally consists solely of ethanol. It therefore follows that if volatile substances other than ethanol are present in autopsy samples, it is likely that endogenous alcohol production has taken place. Volatile solvents of interest include n-butanol, n-propanol, 2-propanol, isobutanol, methanol, acetone, acetaldehyde, ethyl acetate, diethyl ether, and

formaldehyde, some of which can be used as markers of postmortem ethanol formation, as they do not naturally exist in the body [7, 9, 12]. More specifically, the presence of 1-butanol, alone or with 1-propanol, suggests postmortem microbial production of ethanol [9].

The simultaneous presence of two or more higher alcohols in a postmortem blood sample is suggested as a warning signal that the ethanol origin of this sample should be questioned [2]. More recently, a semi-quantitative approach, the 1-propanol concentration of 1.04 mg/L has been suggested as a threshold concentration to flag a sample as positive or negative for postmortem ethanol production [9]. An approach to modeling microbial ethanol production has been developed, generally involving simple linear computational models designed from the concentrations of higher alcohols found in the sample while taking into consideration the microbial strain involved [8, 9].

Conclusion

Resolving whether a positive blood-ethanol arose from postmortem synthesis or antemortem ingestion is a recurring question in legal medicine and toxicology. Much debate has arisen on whether and how the origin of postmortem ethanol could be determined. In general, achievable accuracy in interpreting the results of ethanol postmortem analysis could be achieved to some extent, provided proper sample handling (collection, preparation, and storage), determination of other volatiles, and analysis multispecimen are performed [2, 5].

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