INTERPRETATION
OF
MEASURED ALCOHOL LEVELS
IN
FATAL AVIATION ACCIDENT VICTIMS

Overview

By

Dr Shelley Robertson
MBBS, LLB, FRCPA, DMJ, FACLM, DAvMed, MHealSc (AvMed).

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SUMMARY

The determination of blood ethanol concentration in a deceased pilot is an important part of the accident investigation. The finding of an elevated blood alcohol level in such a case may have significant implications, both medico-legal and social. It is therefore important to ensure that the finding of an elevated blood alcohol concentration is valid.

It is known that micro-organisms involved in the process of putrefaction after death can produce alcohol, usually a mixture of ethanol and other volatile substances. This process occurs when a body is not refrigerated soon after death and is hastened by environmental conditions such as high temperatures and when the body has been traumatised.

Older methods of analysis could not distinguish between ethanol and mixtures of other volatile compounds. Current methodology (gas chromatography) can isolate ethanol and identify other substances.

There is a range of specimens in which ethanol can be measured. Their suitability for analysis can be determined by microbiological studies although this would not be routinely performed in most laboratories.

Medico-legal and forensic implications are associated with a 'blood alcohol concentration'.[49] It therefore seems most useful to measure the ethanol level in a specimen of blood, but this may not always be available depending on the state of the body. Vitreous is the next specimen of choice, and valid conclusions regarding the ingestion of alcohol can usually be made based on the results of its analysis.

Urine analysis may also be helpful, particularly in conjunction with blood and vitreous. Comparison of levels of these three specimens is probably the ideal means of interpreting blood alcohol concentrations. If none of these specimens is available, resort can be made to other organ and tissue samples but there are difficulties in both methodology and interpretation of results relating any alcohol present to ingested ethanol.

Ethanol in gastric contents generally indicates recent ingestion, but the rapid absorption of ethanol and post-mortem diffusion from the stomach may limit the usefulness of analysis of gastric contents.

The presence of volatile compounds in addition to ethanol (seen by gas chromatography methods) may suggest post-mortem production by micro-organisms but also needs to be interpreted cautiously.

It is possible to measure parameters which are associated with or indicate ethanol consumption. These are qualitative only and do not enable the blood ethanol concentration to be calculated or estimated. They have applications in a clinical setting where they address the issue of alcohol consumption in previous days. This is not usually the main issue in a fatal aviation accident investigation, where the "bottle to throttle" rule applies, and the issue is what factors were influencing the pilot's capacity to fly the aircraft. Two of these measurements, ethyl glucuronide and the 5-HTOL: 5-HIAA may have some application in the future of fatal aviation accident investigation but they are not currently performed routinely.

INTRODUCTION

Assessment of a pilot's fitness to fly an aircraft is an integral part of a fatal aviation accident investigation.

Ethanol (ethyl alcohol, 'alcohol') is a commonly ingested substance which has been implicated in the causation of many types of accidents. These accidents take place in domestic, workplace or recreational settings, and often involve use of machinery, performance of complex tasks and control of vehicles including motor cars, marine vessels and aircraft. This is due to the depressant effect of ethanol on the central nervous system, the body's "control centre".

These effects have been well described elsewhere [3,38] but can be summarised as loss of control of movements, decreased ability to process information and make decisions, decreased awareness of surroundings and situations, prolonged reaction times.

In determining the cause of a fatal aviation accident, the possibility of a pilot being affected by ethanol must be considered, given that ethanol use is so common and it can significantly impair fitness to fly.[1,8,21,29.] Numerous studies have implicated ethanol as a causal or contributing factor in fatal aviation accidents.[3,15,17,25,26,34,49.]

There are legal and social issues associated with a pilot's use of ethanol. Civil Aviation Safety Authority (CASA) regulations state that pilots may only fly eight hours or more after ingesting alcohol.[11] The "eight hour bottle-to-throttle" rule is designed to prevent a pilot taking to the air when affected by previous ethanol ingestion or still having ethanol in the blood following a heavy bout of drinking. Insurance claims may be rendered invalid if the pilot is confirmed to have been under the influence of alcohol. Social stigma may surround the family and associates of a pilot involved in a fatal aviation accident, where a coroner or other investigators make a finding of pilot incapacitation due to ethanol. There may also be allegations of culpability and criminal negligence.

It is therefore important to make a correct assessment of the pilot's blood alcohol (ethanol) level at the time of the accident. In practice, this usually means at the time of death, but there may be situations when the time of death and the time of the accident are not the same, as when there has been a post-crash survival period.

This paper will discuss the means of determining the pilot's blood ethanol concentration based on specimens collected after death. Background information including the basic chemistry and metabolism of ethanol, the process of putrefaction and the production of alcohol by micro-organisms after death will be provided. Laboratory methods of ethanol analysis and the range of specimens used will be described.

Difficulties associated with assessment of post-mortem blood alcohol levels will be discussed in detail, particularly the issue of post-mortem alcohol production. Factors important in the interpretation of the results of laboratory analysis, which may lead to a pilot being falsely accused of having ingested ethanol and being under its influence when the fatal crash occurred, will also be discussed.[26,51.] Finally, laboratory measurements of parameters which relate to alcohol consumption will be described[60,65].

These discussions will be summarised, then recommendations made for the optimum collection and handling of post-mortem specimens and interpretation of results.

BACKGROUND

Chemistry of Ethanol

Ethanol or ethyl alcohol belongs to the group of chemical compounds known as alcohols. These compounds are comprised of carbon, hydrogen and oxygen molecules arranged in specific configurations that give them certain properties such as solubility in water and lipid (fat) and volatility (ease of vapourisation).

In the biological world, ethanol is formed by the fermentation of sugar (glucose), first described by Gay-Lussac in 1810.[20]

$$C_6H_{12}O_6 \rightarrow 2(CH_3CH_2OH) + 2(CO_2)$$

This occurs by a series of chemical reactions facilitated by enzymes, known as the "Embden-Meyerhof pathway".

Ethanol is the specific type of alcohol present in 'alcoholic' drinks and is usually the only alcohol present, an exception being fruit brandies. These are distilled from fermented mashed fruit rather than the more common process of fermenting fruit juices, and the end product may also contain butanol.[6] In the manufacture of alcoholic drinks, yeasts (micro-organisms) are added to a substrate (sugar-containing medium) to produce alcohol by fermentation.

'Blood alcohol concentration' (BAC) is measured for medico-legal and forensic purposes but it is the ethanol level in blood that is correlated with the decrement in human performance. The two levels are often assumed to be the same and the terms 'blood alcohol' and blood ethanol' are used interchangeably. Some laboratory methods used for the determination of BAC measure other alcohols in addition to ethanol.

Metabolism of Ethanol

When ethanol is ingested (usually in the form of an 'alcoholic' beverage) it is rapidly absorbed into the circulating bloodstream. It passes readily through the wall of the stomach by the simple process of diffusion.

According to Fick's law, the rate of diffusion across a membrane is proportional to the concentration gradient on either side of the membrane.[20] It follows that the more ethanol there is in the stomach, the quicker it will be absorbed. There are other factors such as the presence of food in the stomach, temperature and gastric motility (the mechanism whereby stomach contents pass into the small intestine) which affect this rate.

Ethanol that has not been absorbed whilst it was in the stomach is rapidly absorbed in the upper part of the small intestine. The ethanol passes from the gastro-intestinal tract into blood in the portal venous system (blood vessels draining the gastro-intestinal tract and carrying nutrients in blood to the liver where metabolism takes place). A small proportion of ethanol is removed from the blood by the liver the first time that the portal blood flows through the liver ('first pass metabolism').

The remainder mixes with circulating blood and equilibrates rapidly in other body organs and tissues. Since ethanol is highly soluble in water, once equilibration has occurred, the actual

levels of ethanol in different tissues depend on the water content of that tissue (most organs and tissues have a high water content).

Ethanol is then metabolised (broken down) in the liver by several different enzyme systems, the main one being the 'alcohol dehydrogenase' system. Carbon dioxide and water are the end products after formation of intermediaries acetaldehyde and acetic acid.

Some ethanol is filtered by the kidneys and passes unchanged into the urine.

After a period of time, the ethanol level of blood and tissues decreases as it is continually removed from circulation and metabolised in the liver. The rate of metabolism is variable, but formulae have been created which try to predict the amount of ethanol an individual had ingested based on the measured BAC.

Putrefaction

The definition of death used to be "cessation of respiration and circulation". With the advent of artificial respiration, cardiac bypass machines and organ transplant programmes however, the definition has had to change. The legal definition of death now includes 'brain death' where a series of neurological tests demonstrate the absence of any brain function although respiration is provided by mechanical means and circulation continues.[27]

When a person 'dies', not all of that person's bodily functions and tissues 'die' at the same time. With respiratory and circulatory failure, oxygen is no longer delivered to organs and tissues and the dependence of a particular organ or tissue on oxygen will determine how quickly the organ or tissue 'dies'. The corollary of this is that some metabolic processes may continue for some time after brain death has occurred.

Included in these processes are the body's 'defence mechanisms'. The human body has a number of features which protect it from infection and invasion by micro-organisms and toxic substances. The most obvious of these is the skin, which, when intact, provides an efficient barrier to bacteria and some toxins. Another example is the gastro-intestinal tract or 'gut' (where bacteria normally reside and may assist in the breakdown of food substances so they can be absorbed or eliminated). Whilst the person is alive and well, the defence mechanisms prevent gut organisms from reaching other parts of the body where they may cause harm. After death, the mechanisms become inactive or break down, and bacteria can disseminate throughout the body.

In a deceased body, one of the early signs of this is the greenish discolouration of the abdomen, most pronounced on the right, where the skin overlies the caecum (that part of the intestine containing large numbers of bacteria). Other signs include bloating or gaseous distension of the abdomen and genitalia (where gas is formed by bacterial activity) and 'marbling', which is a red-green abourising pattern seen on the skin due to the formation of sulfhaemoglobin by bacteria breaking down blood in blood vessels.[16,30].

The above changes occur at a variable time after death.

The post-mortem spread of bacteria and other micro-organisms can be facilitated by a number of conditions, including disease states, adverse environmental conditions and trauma. Unfortunately, it is these last two which are often present in aviation accident fatalities.

These putrefactive changes complicate the autopsy by potentially masking or obliterating pathological changes in organs and tissues, mimicking injuries and contaminating specimens or rendering them unsuitable for analysis.

Micro-organism Production of Alcohol

As mentioned previously, micro-organisms such as yeasts and bacteria are capable of producing alcohol from glucose (sugar) in the process of fermentation. This does not only occur when yeasts are deliberately introduced into the alcohol manufacturing process. They can produce alcohol whenever environmental conditions sustain their activity, and suitable substrates (eg glucose) are present. A deceased body may provide ideal conditions for micro-organisms to flourish.[16,23.]

Alcohol production by micro-organisms has been demonstrated in animals and humans.[4,16,22,40.] In one study, various groups of bacteria were isolated from deceased tissue, then inoculated into blood and the amount of alcohol produced was measured.

In general, it has been shown that micro-organisms can produce alcohol in deceased bodies and that this process occurs within a few days of death, when an unpreserved body is stored at room temperature (~20-25^{0}C) and more rapidly at higher ambient temperatures.

The alcohol produced by micro-organisms is usually not pure ethanol. A number of volatile products including alcohols may be produced. These include butanol, 2-propanol, acetone, methanol, 1-propanol.[9,35]

Micro-organisms can use a number of different substrates to produce alcohol, the main one being glucose but others include glycogen, glycols, pyruvate, lactate, amino acids, ribose. The specific pathway, by-products and end-products of the process vary according to the substrates available and the enzymes present in the micro-organisms. Organisms capable of producing alcohol in deceased bodies include Candida albicans (yeast), Clostridium sp., Escherichia coli, Streptococcus faecalis, Lactobacillus sp. and Proteus vulgaris.[13,16,35.] Many of these organisms are present within the bowel during life. Other species that are present on the skin or in soil may enter the body after death, particularly when the skin has been breached, as in the case of traumatised bodies.

Methods of Analysis

There are four main laboratory methods for the analysis of ethanol in biological specimens.

(i) Widmark method

First described in 1922, this is a method for quantifying alcohol based on the oxidation of potassium dichromate in the presence of sulphuric acid, followed by a titrimetric analysis.[1,28.] It is non-specific, as alcohols other than ethanol (eg methanol) and related compounds such as acetone and ether can all be involved in the oxidation reaction.

(ii) Alcohol dehydrogenase

This method uses one of the enzymes which metabolises alcohol in animals and humans. The enzyme was first obtained from horse liver and yeast in the 1940's. This method largely

replaced the earlier Widmark method, but there were still problems with specificity, as primary alcohols (including ethanol) such as isopropanol and n-propanol also reacted positively.[28] This method is still used in some laboratories.

(iii) Gas chromatography

In this method, a sample of the fluid being tested (eg whole blood, vitreous, urine) is injected directly into a column and the result detected by a flame ionization detector. The method gives rapid results, and is specific for ethanol. Other alcohols can be readily detected by the pattern of peaks on the chromatograph.[18,50.]

(iv) Head-space Gas Chromatography

This method is the "gold standard" for volatile analysis. The principle is the distribution between a carrier gas and a liquid phase of a volatile substance, sampled from the vapour above a heated solution of the specimen. The differential distribution of the volatile substances depends on their physicochemical properties (eg solubility, boiling point), allowing compounds to be identified and quantitated specifically.[36]

The majority of laboratories today use either Gas Chromatography or Head-space analysis to determine alcohol concentrations.

Specimens

(i) Blood

Blood is the usual specimen provided for ethanol analysis. Transport legislation states acceptable ethanol (alcohol) concentrations in blood (either whole blood or plasma) and the results are usually expressed as mg% (eg 0.05) or mg/dL.[20] The legislation also states how a specimen must be obtained in a living person and the method of storage.[48] No such legislation governs the taking of samples from a deceased person.

The site from which post-mortem blood is obtained is important.[39,46.] The preferred site is the femoral vein. Blood from the pericardial sac or chest cavity can have falsely elevated alcohol levels due to post-mortem diffusion of alcohol from the stomach.[42,44.] Blood should be collected into containers with appropriate preservative (eg 200mg sodium fluoride) and anticoagulant (eg 30mg potassium oxalate), depending on how the analysis is to be performed (eg on 'whole blood' or plasma).[41,43.]

Because of ethanol's uniform distribution through the body, other fluids and tissues can be used for analysis.

(ii) Vitreous

This is the fluid within the eye.[24] It is colourless, transparent and gel-like, consisting of 99% water with small amounts of salts and mucoprotein, and it is enclosed by the vitreous membrane. No blood vessels connect directly to the vitreous, which receives nutrients from vessels supplying the retina and adjacent structures. It is not normally subject to contamination by micro-organisms and its high water content means that measured ethanol levels (ethanol crossing readily through the vitreous membrane) are comparable to blood levels.[50] Due to its anatomical site, it is usually well-preserved after death, only disappearing

if there have been extensive eye injuries or advanced decomposition with collapse of the eyeball.[10,14,19,32,45.]

` (iii) Urine

Urine is the fluid filtered by the kidneys which collects in the bladder. Like vitreous, it is resistant to post-mortem contamination by micro-organisms. Post-mortem alcohol production in urine only occurs when the urine contains large amounts of glucose (as is sometimes found in persons with diabetes) or there has been a bacterial or yeast infection (urinary tract infection) during life.[18]

If urine is present in the bladder after death, it is easily sampled at autopsy. The bladder must be intact, however, or the urine may be subject to contamination by other fluids. If the deceased passed urine shortly before or at the moment of death, the bladder will be empty and no urine available for sampling.

A urine alcohol measurement does not reflect the alcohol content of urine at any point in time (eg time of death). It represents an average of the levels in urine collected in the bladder which may have taken several hours.[31] As long as this is recognized, urinary alcohol estimation is a useful measurement.

(iv) Bile

Bile is a fluid excreted by the liver containing enzymes which aid digestion. It is stored in the gall-bladder. Like any other body fluid, it may contain alcohol after ingestion of ethanol, but the correlation with blood levels is generally poor and dependant on many variables. Bile is also subject to contamination by post-mortem diffusion and contamination by bacteria, with possible endogenous alcohol production.[53]

(v) Other fluids

It may be possible to test cerebrospinal fluid for alcohol but this fluid is often difficult to sample, and it may be lost if there has been significant trauma to a body, particularly head and spinal injuries.

Saliva is not routinely used for post-mortem analysis, the main difficulties being specimen collection and contamination but current studies may demonstrate its usefulness.[47]

(vi) Solid Organs

One of the main problems associated with the measurement of alcohol content of solid organs such as liver, brain, muscle is meaningful correlation with blood alcohol concentrations. Although these organs have a high water content, they also contain variable amounts of lipid (fat) which must be measured and taken into account when comparing alcohol levels with those in blood. There are also technical difficulties with the preparation of these specimens which limit their usefulness.[19]

(vii) Gastric Contents

Studies have shown that alcohol does not remain in the stomach for very long following ingestion. It is absorbed rapidly into the blood during life, as previously described, and diffuses into surrounding tissues after death. Gastric contents is therefore not a reliable specimen for assisting the estimation of blood ethanol levels.[20,42,44.] It does have some value,

in that a high gastric ethanol content is almost certainly indicative of recently ingested alcohol.

(viii) Bone Marrow

Bone marrow has been suggested as a specimen for alcohol determination.[53] Red marrow (containing blood components and precursors) can be obtained in adults from the 'flat' bones such as pelvis, sternum and ribs. It is well protected from contamination and may still be present when other suitable specimens have been lost due to trauma or decomposition.

This study demonstrated good correlation between BAC and bone marrow alcohol concentration when correction was made for the lipid content of marrow. This method is not used generally but it has potential for future development.

DIFFICULTIES ASSOCIATED WITH THE ASSESSMENT OF POST-MORTEM ALCOHOL LEVELS

Post-accident Survival

The time of death in a fatal accident may not necessarily be the same as the time of the accident. Even if lethal injuries are sustained, there may be a period of time before death actually occurs. If the post-accident survival period is 1-2 hours or more, even if the pilot had an elevated blood ethanol concentration at the time of the crash, metabolism of ethanol would continue and at the time of death, the blood alcohol level may not be detectable. This scenario should be considered whenever there is the possibility of a significant post-crash survival period.

In vitro Consumption of Alcohol

A blood ethanol level may appear artificially low if there has been inappropriate or inadequate storage of the specimen. This is usually the result of contamination of the specimen by micro-organisms which metabolize the ethanol in the specimen to other compounds. In order to minimise this problem, specimens should be collected with appropriate preservatives (eg sodium fluoride) and refrigerated at 4^0C immediately after collection.[33,39,40,41,43.]

Post-mortem Diffusion/Contamination

Contamination occurs when there has been disruption of the stomach or other hollow organ with spillage of contents, possibly containing ethanol, into other body cavities. If blood is collected from these cavities, (eg pooled blood in the chest), it may show falsely elevated ethanol levels.

Post-mortem diffusion of ethanol across the gastric or bowel wall can occur in intact and disrupted bodies.[40,41,42,44,54.] A blood sample taken from a peripheral vein, eg the femoral vein (large vein in the groin) is not generally subject to these processes and is therefore the site of choice.[39,46.] Similarly, urine, vitreous (and probably bone marrow) are less likely to be affected compared with more centrally located organs and sites such as liver and gall bladder.

Post-mortem Production of Alcohol

The mechanism whereby this occurs has previously been discussed.[4,16,22,35,40,43,51.] It is usually not a problem in at least the first 24-48 hours following death. As the post-mortem interval increases, so does the likelihood of endogenous alcohol production by micro-organisms, particularly when environmental temperatures are high and the body has been traumatised.[13,23.]

It is difficult to state with any certainty, the time period after death at which post-mortem alcohol production commences. It is also difficult to state with certainty, the time interval after death when post-mortem alcohol production has occurred to a significant degree and is contributing to measured alcohol levels. The reason for this uncertainty is the large numbers of variable factors which affect the process. These include environmental conditions,

particularly temperature, airflow and humidity, the state of the body (eg intact or traumatised, burnt or immersed), body size and presence of clothing. The state of health of the deceased during life is also a factor.

Given this uncertainty, only a very approximate time period can be offered as a guide as to when post-mortem alcohol production occurs. In general, at temperatures less than 5°C, post-mortem alcohol production would not be expected to occur for at least a week and possibly not at all. When ambient temperatures are between 5–20°C, post-mortem alcohol production may occur at a significant level after 48 hours. At temperatures in excess of 20°C, post-mortem alcohol production may occur after 24 hours to significant levels as discussed elsewhere in this paper.

It must be emphasized that the above time frames are approximate, and ambient temperature is not the only factor influencing the rate of post-mortem alcohol production. An assessment of the extent of the body's putrefactive changes at autopsy is a better guide to the likelihood of significant post-mortem alcohol production.

INTERPRETATION OF THE RESULTS OF ANALYSIS FOR ETHANOL

Suitability of Sample for Analysis

In order to draw valid conclusions from the results of post-mortem ethanol analysis, it is important to assess the reliability of the specimen. Details of collection including time and site of collection are necessary. The state of the body (eg 'normal', burnt or showing mild/moderate/advanced decomposition changes) should also be known. If the body is showing external signs of decomposition, the results of ethanol analysis must be interpreted with care. Suitability for analysis of the actual biological specimens collected can be made by subjecting them to microbiological testing. This can be done by the following methods:

(i) Direct Microscopy

Micro-organisms can be seen in a sample of the specimen under the microscope, with special staining techniques.[25]

(ii) Culture

\Colonies of micro-organisms can be grown following inoculation of suitable culture media by a small sample of the specimen.[25]

(iii) DNA testing

This method uses PCR (polymerase chain reaction) and microbial DNA primers designed for identifying three common ethanol-producing micro-organisms, Candida albicans, Proteus vulgaris and Escherichia coli. It is probably not available in most laboratories.[52]

If microbiological studies show the specimen to contain large numbers of micro-organisms or sustain considerable microbial activity, the specimen is likely not to be optimal for analysis, and the production of endogenous alcohol is probable and certainly cannot be excluded. If however, no microbial activity is demonstrated, the likelihood of any ethanol present in the specimen being due to ingestion is much higher, although endogenous production may not be able to be completely ruled out.

Comparison of Multiple Specimens

Given that vitreous is a useful fluid to sample (if the eyeball is intact after death), many studies have been performed attempting to correlate vitreous alcohol concentration (VAC) with blood alcohol concentration (BAC), and in doing so, determine if the measured BAC is due to ingested ethanol or endogenously-produced alcohol.[29]

One study found that a BAC of <0.03g/dL was often associated with a negative VAC, and could therefore be attributed to post-mortem (endogenous) alcohol formation. A BAC of 0.05 had an 85% chance of being associated with a positive VAC, and > 0.05 had a 99% chance.[10] These figures are based on statistical analysis and therefore cannot be definitively applied to an individual case, limiting their usefulness. The principle remains sound, however, that if

the VAC is negative in the face of a positive BAC, endogenous alcohol formation is a strong possibility.

Other studies have attempted to calculate a conversion factor whereby BAC can be determined from a measured VAC according to the formula

$$BAC = \text{conversion factor} \times VAC$$

This 'conversion factor' has been variously calculated as being between 0.75 and 1.01, but is probably lies between 0.85 and 0.95.[14,19,32,45,50,53.]

The same general principles regarding the relationship between VAC and BAC can be applied to urinary alcohol concentration (UAC) and BAC. If BAC is positive and UAC is negative, the likelihood is that alcohol measured in the blood has been produced post-mortem. The exception is when ethanol has only recently been ingested and has not yet had time to be filtered into the urine and collect in the bladder. Conversion factors for UAC into BAC have been devised but are not generally useful as the UAC is an averaged measurement over a period of time.[31]

Absorptive/Elimination Phase of Ethanol

As outlined earlier, ingested ethanol is rapidly absorbed from the gastro-intestinal tract and reaches equilibrium in blood with organs and tissues. It is then gradually eliminated by metabolism in the liver. In the absorptive phase, BAC may be higher than VAC and UAC. One study used the alcohol level in gastric contents to indicate whether the alcohol is in an absorptive or equilibrium phase.[2] If gastric alcohol concentration was less than 0.5g/100ml, it could be assumed that the alcohol was no longer in the absorptive phase and that BAC could be calculated from VAC. The usefulness of trying to assess the phase is limited however, and alcohol concentration of gastric contents is not a test that is usually performed.

Formation of Other Compounds

When alcohol is produced post-mortem by micro-organisms, the resultant compound is usually not pure ethanol. This is in contrast to ingested alcohol which usually consists solely of ethanol. It follows then, that if volatile substances other than ethanol are present in autopsy samples, the likelihood is that endogenous production of alcohol has taken place.[9] One study has even suggested that the ratio of ethanol to another volatile compound, n-propanol, can be used to calculate the amount of ethanol produced post-mortem, thereby allowing the amount of ingested ethanol in a specimen to be determined. Other studies have failed to support this proposition. Whilst the presence of other volatile compounds (eg propanol, isopropanol, acetone, acetaldehyde, butanol) may be a useful indicator that there is alcohol-producing activity by micro-organisms, it is not definite evidence of such.[20]

OTHER LABORATORY MEASUREMENTS ASSOCIATED WITH ETHANOL INGESTION

There are a number of parameters other than direct measurement of ethanol concentrations which can indicate ethanol consumption. These are generally used in a clinical setting where they can serve as markers of ethanol consumption and monitor patient compliance in alcohol rehabilitation and workplace abstinence. They have not been routinely applied in a forensic context and their usefulness in the area of fatal aviation accident investigation is probably limited. They will briefly be discussed below.

Ethyl Glucuronide (EG)

This is a compound formed from a minor metabolic pathway of ethanol[55]. Less than 0.5% of ingested ethanol is conjugated in the liver with glucuronic acid to form this non-volatile, water-soluble compound which circulates in blood and is filtered by the kidneys to be excreted in urine where it was first detected in 1967[22,7]. It has also been detected in hair, and this is currently being used in some alcohol and drug monitoring plans clinically[56,57].

Ethyl glucuronide is a stable compound and is also a specific indicator for ethanol ingestion[58,62,63,66,67,69,70,72]. This would suggest that it has forensic applications, however, measurement of EG levels in both blood and urine do not bear a constant relationship to BAC or UAC[59,60,64,68]. The demonstration of EG in a specimen is of qualitative value, that is, it only indicates recent (within the last 40 hours or even longer) ingestion of ethanol, not how much was ingested or at what time[17]. This limits its usefulness in fatal accident investigation, but if the only answer required of an investigator is "had the pilot been drinking in the 40 hours prior to death?", it may provide an answer[73,74]. Although it is a stable compound and is not produced by post-mortem activity of micro-organisms, there may still be problems with specimen availability (blood or urine) and contamination of specimens by decomposition products generally which may technically affect the assay.

Ratio 5-hydroxytryptanol to 5-hydroxyindoleacetic acid (5–HTOL: 5–HIAA)

5–hydroxytryptamine (serotonin) is a neurotransmitter substance, normally broken down into 5–hydroxyindoleacetaldehyde (5–HIAL) which is either oxidized to 5–HIAA or reduced to 5-HTOL. Ethanol produces a shift to the reductive pathway, thereby increasing the ratio of 5-HTOL to 5–HIAA. The ratio remains elevated for several hours after ethanol ingestion. 5-HTOL and 5–HIAA are relatively stable in urine.

The main value of this test may be in its ability to confirm post-mortem alcohol production[65,60]. For example, post-mortem alcohol production may be suspected in a blood specimen which is positive for ethanol in the presence of a negative urine sample. A normal 5-HTOL: 5–HIAA in the urine confirms that the ethanol present in blood has not been ingested. It has similar limitations to the other tests, however, in that it is a qualitative indicator only of ethanol ingestion and a urine sample must be available.

Fatty Acid Ethyl Ester (FAEE)

This compound is formed from free fatty acids and ethanol[55,60]. It is a marker of ethanol consumption, accumulates in fatty tissue and is detectable in hair. It is not necessarily stable in blood and its measurement is complex and affected by a number of pre-analytical variables. Its presence in hair may be useful as a qualitative marker in living patients but it does not appear to have forensic application.

Carbohydrate-Deficient Transferrin (CDT)

Transferrin is a protein involved in iron transport throughout the body. The presence of ethanol and/or its metabolite acetaldehyde affect synthesis of proteins. Daily ethanol intake of 50-80g for a week will produce elevated levels of this abnormal transferrin molecule, measurable in blood[65,60]. These levels will return to normal following some days to weeks of abstinence. Elevated CDT levels are not specific for ethanol consumption, and can be found in association with liver disease including chronic viral hepatitis and other conditions. Problems with stability in samples also limit the usefulness of this test which has no forensic application.

Gamma Glutaryl Transferase (GGT)

This is an enzyme present in the liver which is induced (or has its activity increased) by ethanol. It is one of the 'routine' liver function tests performed on a blood specimens and is useful in predicting a patient's recent ingestion of ethanol. Increases in GGT however, are not always due to ethanol, so like the measurement of MCV, it is not specific and has limited application in postmortem investigation

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Mean Cell Volume (MCV)

This is a measurement of the mean volume of a circulating erythrocyte (red blood cell). The MCV is usually increased in alcoholics (ie their red cells are bigger than normal). Factors other than ethanol can produce red cell enlargement however, so the test is not specific for ethanol. Haematological parameters such as blood cell measurements are poorly preserved in post-mortem blood samples so this test has no practical application in fatal accident investigation.

RECOMMENDATIONS FOR THE INVESTIGATION OF FATAL AVIATION ACCIDENTS

The following guidelines may be useful in obtaining optimum results from the analysis of post-mortem specimens obtained from victims of fatal aviation accidents.

Preservation of the Body

It is not often that the bodies of fatally injured aviation accident victims are recovered immediately after the fatal accident. There may be a considerable time period before the wreckage is located. Access to the site of the accident may be difficult and delayed, and then the investigation itself may further delay the retrieval of bodies. It is crucial to the investigation that bodies are photographed in situ within the wreckage and viewed by accident investigators. During this time, the process of post-mortem putrefaction of the bodies may be progressing at a rate accelerated by environmental conditions.

The main factor here in obtaining suitable specimens for toxicological analysis (and obtaining other important information from the autopsy such as injury patterns, survivability, presence of natural disease) is removing the bodies as soon as possible from the wreckage and transporting them to a refrigerated facility, preferably a mortuary.

The aim is to minimise putrefaction of the bodies to enable optimum specimen collection at autopsy. If a long delay (greater than 24 hours) is anticipated, especially in the setting of high ambient temperatures (greater than 20^{o}C), a temporary solution may be to erect some sort of shelter for the bodies, at least protecting them from direct sunlight. A makeshift tarpaulin or similar will afford some shade and allow airflow around the bodies, thus avoiding the 'heated in a sealed container' effect.

Gross insect infestation should also be prevented if possible, but if insecticides are used in the vicinity of the body, the type and way in which it was used should be noted in case there is contamination of specimens for toxicological analysis.

Specimen Collection at the Scene

Ideally, biological specimens for toxicological analysis should be collected as soon after death as possible. In a fatal aviation accident, the time taken to locate and access the wreckage is usually the most significant period from time of death to body handling. Further delays in reaching an appropriate mortuary, such as awaiting attendance of key personnel and photography of the accident scene, may only add around 24 hours and are probably not going to cause significant changes in the state of the bodies.

Whilst it may seem theoretically advantageous to obtain biological specimens when the bodies are first recovered, the practicalities of this limit its usefulness. For example, collection of specimens must be carried out by trained personnel, who not only have the required expertise, but are also aware of risks associated with the procedure such as infectious and toxic hazards.

A forensic pathologist attending the scene may be in a position to collect specimens (provided appropriate equipment is available) but there may be difficulties such as access to and position of the body, presence of clothing and equipment etc which hamper access to the preferred collection site eg femoral vein. In the absence of a forensic pathologist, there are other considerations. Obtaining a sample of vitreous from a deceased body is technically a fairly straightforward procedure, but unless the person performing it is experienced, the sample may be lost or contaminated. A request for emergency medical or paramedical personnel to remove vitreous is not likely to be met favourably, as it is outside their range of experience and expertise. Mortuary workers are really the only group of persons trained in this technique.

In summary, biological specimen collection at the accident scene is best avoided. Preferable is the removal of bodies as rapidly as possible to a suitable mortuary facility where specimens can be collected by specially-trained staff in controlled conditions. If delays are inevitable, some protection to the bodies should be afforded.

Mortuary Specimens

The more specimens collected, the greater is the potential for a wide range of analyses to be performed and information made available. The following samples should be collected as follows:

(i) Blood

This is best collected from the femoral vein, and the specimen preserved (eg with sodium fluoride) and refrigerated at 4^0C. If femoral vein blood is not obtained, cardiac blood is the next best choice. If resort must be made to pericardial or chest/abdominal cavity blood, the collection site must be noted and the results of analysis interpreted cautiously.

(ii) Vitreous

This should always be obtained where possible and refrigerated after collection.

(iii) Urine

This is obtained from direct bladder puncture at autopsy, ideally before evisceration of organs (where the bladder may rupture and the urine become contaminated). The specimen should be refrigerated after collection.

(iv) Other Specimens

These include liver, gastric contents, skeletal muscle. It may be worth taking samples and refrigerating them, not necessarily performing analysis in the first instance, only if there are difficulties with the interpretation of results of analysis of other specimens.

Interpretation of Results

The state of the body should always be noted, along with the estimated interval from time of death to when specimens obtained.

The site of specimen collection should be documented.

If the question of post-mortem alcohol formation is an issue, comparison between blood, vitreous and urinary ethanol levels should be made. Gas chromatograph results should be reviewed to determine if other alcohols or volatile compounds are present.

If putrefaction is suspected of complicating the results of analysis, suitability of samples should be addressed by microbiological studies.

It should be borne in mind that 'interpretation' means just that. There may not be an absolute solution to the problem of ingested ethanol versus post-mortem production, however with the maximum amount of information available, the likelihood of the presence of alcohol in a specimen being due to one or other (or both) can be expressed.

Finally, there will be cases where valid interpretation of toxicological analysis is not possible, either due to absolute unavailability of specimens (eg body not located, extent of trauma) or total unsuitability of specimens (charred remains or advanced decomposition). In these cases, it is important not to make speculative assessments of ethanol ingestion.

APPENDIX:

Checklist for Obtaining Optimum Biological Specimens in Aviation Accident Investigation

1. Recovery of bodies and despatch to mortuary/refrigerated facility as soon as practicable.

2. If long delay anticipated, protect bodies eg tarpaulin-type cover +/- insecticide or netting. Ensure airflow around bodies is maintained.

3. No advantage in taking biological specimens at accident scene unless very long delay anticipated. If, in consultation with forensic pathologist, decision is made to take specimens, this should be done by trained personnel (eg forensic pathologist or mortuary technician).

4. In situation 3. above, most useful specimens are femoral vein blood and vitreous.

5. Specimens should be collected under controlled conditions (as set out in laboratory procedure manuals) with appropriate preservatives and refrigeration of samples.

6. At autopsy, the more specimens taken, the greater the potential for useful information. Femoral blood, vitreous and urine should always be taken where possible. Additional specimens such as gastric contents, liver, skeletal muscle should also be taken for storage and future analysis if required.

7. If putrefaction is pronounced, microbiological studies on specimens may be performed to assess their suitability for analysis.

8. The presence of volatile compounds in specimens should be reported or drawn to the forensic pathologist's attention.

9. The forensic pathologist needs to be aware of relevant information including estimated time of accident, duration of exposure to, and type of, environmental conditions.

10. If the above is followed, the likelihood of valid interpretation of analytical results is high, however, there will be cases where no suitable samples are obtained. Interpretation in these cases is at best, speculative.

REFERENCES

1. Alha AR, Tamminen V. Detection of alcohol in aviation and other fatalities in Finland. Aerospace Med 1971; 42(5): 564-568.

2. Backer RC, Pisano RV, Sopher IM. The comparison of alcohol concentrations in postmortem fluids and tissues. J. Forensic Sci 1980; 25: 327-331.

3. Billings CE, Wick RL Jnr, Gerke RJ, Chase RC. Effects of ethyl alcohol on pilot performance. Aerospace Med 1973; 44(4): 379-382.

4. Blackmore DJ. The bacterial production of ethyl alcohol. J Forens Sci Soc 1968; 8: 73-78.

5. Bogusz M, Guminska M, Markiewicz J. Studies on the formation of endogenous ethanol in blood putrefying in vitro. J. Forensic Med 1970; 17(4): 156-168.

6. Bonte W. Formation of fusel alcohols during storage of blood samples. Proc 24th International Meeting TIAFT, Banff, Canada 1987: 101-104.

7. Briglia EJ, Bidanset JH, Dal Cortivo, LA. The distribution of ethanol in post-mortem blood specimens. J Forensic Sci 1992; 37(4): 991-998.

8. Canfield DV, Hardinsky J, Millett DP, Endecott B, Smith D. Prevalence of drugs and alcohol in fatal civil aviation accidents between 1994 and 1998. Aviat Space Environ Med 2001; 72: 120-124.

9. Canfield DV, Kupiec T, Huffine E. Postmortem alcohol production in fatal aircraft accidents. J Forens Sci 1993; 38(4): 914-917.

10. Caplan YH, Levine B. Vitreous humor in the evaluation of postmortem blood ethanol concentrations. J Anal Toxicol 1990; 14: 305-307

11. CASA regulations: No. CAR 256(3).

12. Chaturvedi AK, Smith DR, Soper JW, Canfield DV, Winnery JE. Characteristics and toxicological processing of post-mortem pilot specimens from fatal civil aviation accidents. CAMI report: August, 2002. US Department of Transportation, FAA.

13. Clark MA, Jones JW. Studies of putrefactive ethanol production 1: lack of spontaneous ethanol production in intact human bodies. J Forens Sci 1982; 27: 366-371.

14. Coe JI, Sherman RE. Comparative study of postmortem vitreous humor and blood alcohol. . Forensic Sci. 1970; 15: 185-190

15. Cook CCH. Alcohol and aviation. Addiction 1997; 92(5): 539-555

16. Corry JEL. Possible sources of ethanol ante- and post-mortem: its relationship to the biochemistry and microbiology of decomposition. J Appl Bacteriol 1978; 44: 1-56.

17. Davis GL. Postmortem alcohol analyses of general aviation pilot fatalities, Armed Forces Institute of Pathology 1962-1967. Aerospace Med 1973; 44(1): 80-83

18. Drummer OH. The Forensic Pharmacology of Drug Abuse. Arnold, London 1999: 300.

19. Felby S, Olsen J. Comparative studies of postmortem ethyl alcohol in vitreous humor, blood and muscle. J. Forensic Sc. 1969; 14: 93-101

20. Garriott JC (ed). Medicolegal aspects of alcohol; 3rd ed. Lawyers and Judges Publishing Company Inc. Littleton, USA 1996.

21. Gibbons HL. Alcohol, aviation and safety revisited: a historical review and a suggestion. Aviat Space Environ Med. 1988; 59: 657-660.

22. Gilliland MGF, Bost RO. Alcohol in decomposed bodies: post-mortem synthesis and distribution. J Forens Sci 1993; 38(6): 1266-1274.

23. Gormsen H. Alcohol production in the dead body. J Forens Med 1954; 1(5): 314-315.

24. Gray's Anatomy 35th ed. Longman 1973:1120

25. Harper CR, Albers WR. Alcohol and general aviation accidents. Aerospace Med. 1964; 35: 462-464.

26. Hill IR. Toxicological findings in fatal aircraft accidents in the United Kingdom. Amer J Forens Med and Pathol 1986; 7(4): 322-326.

27. Human Tissue Act 1982 (Vic) s41.

28. Karch SB. Drug Abuse Handbook. Boca Raton, Florida: CRC Press, 1997.

29. Klette K, Levine B, Springate C, Smith ML. Toxicological findings in military aircraft fatalities from 1968-1990. Forensic Sci Int 1992; 53: 143-148.

30. Knight B. Forensic Pathology. Arnold; Great Britain, 1991 : 58.

31. Kuroda N, Williams K, Pounder DJ. Estimating blood alcohol from urinary alcohol at autopsy. Amer J Forens Med Pathol 1995; 16(3): 219-222.

32. Leahy MS, Farber ER, Meadows TR. Quanitiation of ethyl alcohol in the postmortem vitreous humor. J Forensic Sci 1968; 13: 498-502.

33. Levine B, SmithML, Smialek JE, Caplan YH. Interpretation of low postmortem concentrations of ethanol. J Forensic Sci 1993; 38: 663-667.

34. Li G, Hooten EG, Baker SP, Butts JD. Alcohol in aviation-related fatalities: North Carolina 1985-1994. Aviat Space Environ. Med. 1998; 69: 755-60.

35. Mayes RW. The post-mortem production of ethanol and other volatiles. Proc 24th International Meeting, TIAFT Banff, Canada 1987: 94-100.

36. McLean GA, Wilcox BC, Canfield DV. Selection criteria for alcohol detection methods. CAMI report: August 1991. US Department of Transportation, FAA.

37. Nanikawa R, Ameno K, Hashimoto Y, Hamada K. Medicolegal studies on alcohol detected in dead bodies-alcohol levels in skeletal muscle. Forens Sci Int 1982; 20: 133-140.

38. Newman DG. Alcohol and human performance from an aviation perspective: a review. ATSB Research Report, March, 2004.

39. Plueckhahn VD. Alcohol levels in autopsy heart blood. J Forensic Med 1968; 15: 12-21.

40. Plueckhahn VD, Ballard B. Factors influencing the significance of alcohol concentrations in autopsy blood samples. Med J Aust. 1968; 939-943.

41. Plueckhahn VD. The evaluation of autopsy blood alcohol levels. Med Sci Law 1968; 8: 168-176.

42. Plueckhahan VD, Ballard B. Diffusion of stomach alcohol and heart blood alcohol concentration at autopsy. J. Forensic Sci 1967; 12: 463-70.

43. Plueckhahn VD. The significance of blood alcohol levels at autopsy. Med J Aust 1967; 2(3): 118-124.

44. Pounder DJ, Smith DRW. Postmortem diffusion of alcohol from the stomach. Amer J Forens Med Pathol 1995; 16(2): 89-96.

45. Pounder DJ, Kuroda N. Vitreous alcohol is of limited value in predicting blood alcohol. Forensic Sci Int 1994; 65: 73-80.

46. Prouty R, Anderson W. A comparison of post-mortem heart blood and femoral blood ethyl alcohol concentration. J Anal Toxicol 1987; 11(5): 191-197.

47. Ranson DL; Reasearch proposal, VIFM 2004.

48. Road Safety Act 1986 (Vic) s49 (1).

49. Ruehle CJ. Toxicologic studies on USAF aircraft accident casualities 1973-1984. Aviat Space Environ Med. 1989; 60(10, suppl) 86-88.

50. Sturner WQ, Coumbis RJ. The quanitiation of ethyl alcohol in vitreous humor and blood by gas chromatography. Amer J Clin Pathol 1966; 46: 349-351.

51. Turkel HW, Gifford H. Erroneous blood alcohol findings at autopsy. JAMA 1957; 164(10): 1077-1079.

52. Vu NT, Chaturvedi AK, Canfield DV, Soper JW, Kupfer DM, Roe BA. DNA-based detection of ethanol-producing microorganisms in post-mortem blood and tissues by polymerase chain reaction. CAMI report: May, 2000. US Department of Transportation, FAA.

53. Winek CL, Esposito FM. Comparative study of ethanol levels in blood versus bone marrow, vitreous humor, bile and urine. Forensic Sci Int 1981; 17:27-36.

54. Zumwalt RE, Bost RO, Sunshine I. Evaluation of ethanol concentrations in decomposed bodies. J Forensic Sci 1982; 27(3): 549-554.

55. Yegles M, Labarthe A, Autwarter V, Hartwig S, Vater H, Wennig R. Comparison of ethyl glucuronide and fatty acid ethyl ester concentration in the hair of alcoholics, social drinkers and teetotallers. Forens Sci Int 2004; 145 (2-3): 167-73.

56. Jurado C, Soriano T, Gimenez MP, Menendez M. Diagnosis of chronic alcohol consumption. Hair analysis for ethyl-glucuronide. Forens Sci Int 2004; 145 (2-3): 161-6

57. Skipper GE, Weinmann W, Thierauf A, Schaefer P, Wiesbeck G, Allen JP, Miller M, Wurst FM. Ethyl glucuronide: a biomarker to identify alcohol use by health professionals recovering from substance use disorders. Alcohol Alcohol. 2004; (5) 445-9.

58. Weinmann W, Schaefer P, Thierauf A, Schreiber A, Wurst FM. Confirmatory analysis of ethylglucuronide in urine by liquid-chromatography/electrospray ionization/tandem mass spectrometry according to forensic guidelines. J Am Soc Mass Spectrom. 2004; 15(2): 188-93.

59. Bergstrom J, Helander A, Jones AW. Ethyl glucuronide concentrations in two successive urinary voids from drinking drivers: relationship to creatinine content and blood and urine ethanol concentrations. Forens Sci Int 2003; 133(1-2): 86-94.

60. Musshoff F. Chromatographic methods for the determination of markers of chronic and acute alcohol consumption. J Chromatogr B Analyt Technol Biomed Life Sci. 2002; 781 (1-2): 457-80.

61. Droenner P, Schmitt G, Aderjan R, Zimmer H. A kinetic model describing the pharmacokinetics of ethyl glucuronide in humans. Forens Sci Int 2002; 126 (1): 24-9.

62. Schmitt G, Droenner P, Skopp G, Aderjan R. Ethyl glucuronide concentration in serum of human volunteers, teetotalers and suspected drinking drivers. J Forensic Sci. 1997; 42(6): 1099-102.

63. Wurst FM, Wiesbeck GA, Metzger JW, Weinmann W. On sensitivity, specificity, and the influence of various parameters on ethyl glucuronide levels in urine – results from the WHO/ISBRA study. Alcohol Clin Exp Res. 2004; (8): 1220-8.

64. Wurst FM, Skipper GE, Weinmann W. Ethyl glucuronide – the direct ethanol metabolite on the threshold from science to routine use. Addiction 2003 Suppl 2: 51-61.

65. Helander A. Biological markers in alcoholism. J Neural Transm Suppl. 2003; (66): 15-32.

66. Stephanson N, Dahl H, Helander A, Beck O. Direct quantification of ethyl glucuronide in clinical urine samples by liquid chromatography-mass spectrometry. Ther Drug Monit. 2002; 24 (5): 645-51.

67. Wurst FM, Metzger J, WHO/ISBRA Study on State and Trait Markers of Alcohol Use and Dependence Investigators. The ethanol conjugate ethyl glucuronide is a useful marker of recent alcohol consumption. Alcohol Clin Exp Res 2002; 26 (7): 1114-9.

68. Dahl H, Stephanson N, Beck O, Helander A. Comparison of urinary excretion characteristics of ethanol and ethyl glucuronide. J Anal Toxicol. 2002; 26(4): 201-4.

69. Seidl S, Wurst FM, Alt A. Ethyl glucuronide – a biological marker for recent alcohol consumption. Addict. Biol 2001; 6(3): 205-212.

70. Zimmer H, Schmitt G, Aderjan R. Preliminary immunochemical test for the determination of ethyl glucuronide in serum and urine: comparison of screening method results with gas chromatography- mass spectrometry. J Anal Toxicol. 2002; 26(1): 11-6.

71. Wurst FM, Kempter C, Metzger J, Seidl S, Alt A. Ethyl glucuronide: a marker of recent alcohol consumption with clinical and forensic implications. Alcohol 2000; 20(2): 111-6

72. Nishikawa M, Tsuchihashi H, Miki A, Katagi M, Schmitt G, Zimmer H, Keller T, Aderjan R. Determination of ethyl glucuronide, a minor metabolite of ethanol, in human serum by liquid chromatography-electrospray ionization mass spectrometry. J Chromatogr B Biomed Sci Appl 1999; 726 (1-2): 105-10.

73. Wurst FM, Schuttler R, Kempter C, Seidl S, Gilg T, Jachau K, Alt A. Can ethyl glucuronide be determined in post-mortem body fluids and tissues? Alcohol Alcohol. 1999; 34(2): 262-3.

74. Wurst FM, Kempter C, Seidl S, Alt A. Ethyl glucuronide – a marker of alcohol consumption and a relapse marker with clinical and forensic implications. Alcohol Alcohol. 1999; 34(1): 71-7.

75. Helander A, Beck O, Jones AW. Urinary 5HTOL/5HIAA as biochemical marker of postmortem ethanol synthesis. Lancet 1992; 340: 1159.

76. Jaakonmaki P, Knox KL, Horning EC, Horning MG. The characterization by gas-liquid chromatography of ethyl β-D-glucuronic acid as a metabolite of ethanol n rat and man. Eur J Pharmacol. 1. 1967; 63-70.