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Postmortem Ethanol Testing Procedures Available to Accident Investigators

Dennis V. Canfield¹
Captain James D. Brink²
Robert D. Johnson¹
Russell J. Lewis¹
Kurt M. Dubowski¹

¹Civil Aerospace Medical Institute
Federal Aviation Administration
Oklahoma City, Oklahoma 73125

²Investigative Services Crime Laboratory
State Highway Patrol
Columbus, Ohio 43209

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16. Abstract An ethanol positive fatal case reported as being from ingestion was ultimately determined to be from postmortem ethanol production using the ratio of two serotonin metabolites found in urine. This case involved a transportation accident that could have resulted in additional hardships for the victim's family through loss of compensation and reputation.					
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POSTMORTEM ETHANOL TESTING PROCEDURES AVAILABLE TO ACCIDENT INVESTIGATORS

CASE HISTORY

On a stormy night in late September 2006, an Ohio State Highway Patrol Trooper and a Patrol Sergeant in a patrol cruiser were assumed to be en route to assist another Trooper whose ill child was being taken to the hospital in an emergency situation. While en route, the cruiser, traveling at an estimated speed of 61 to 72 mph, hydroplaned on the wet highway, spun around, crossed the center line, and crashed into a vehicle headed in the opposite direction. Both vehicles were engulfed in flames, resulting in fatal thermal and trauma injuries to all three occupants. Laboratory tests on non-autopsy specimens collected at the scene from all victims shortly after death by the Ohio State Highway Patrol Crime Laboratory (OSPCL) revealed no ethanol in the blood of the victims. Autopsies were performed approximately 34 hours after the crash at a contract autopsy facility on 2 of the victims. To reduce the decomposition of these two victims, formalin powder was utilized. Toxicology results from the autopsy found no ethanol present in one victim, and the other victim had a BAC of 0.01%. However, the autopsy for the driver of the patrol car was delayed by 60 hours. No form of preservation was used, so the body was in an advanced stage of decomposition at the time of autopsy. The specimens collected at autopsy from the Trooper driving the cruiser revealed 0.07% ethanol in urine and 0.08% ethanol in both heart and cavity blood. Glucose test strips were negative for glucose in the urine, and no vitreous fluid was available for testing. Because of the coroner's report, the Trooper driving the patrol car was erroneously reported by the media as being drunk at the time of the crash. An investigation found no evidence that this officer had consumed ethanol or had acted in an intoxicated manner before or during his tour of duty. The OSPCL was informed of a laboratory test that could, in some cases, differentiate between ingested ethanol and ethanol produced postmortem by microbial action. It was decided that the specimens would be subjected to this test to ascertain whether the officer had indeed recently consumed ethanol. This novel technique was developed and reported in the peer-reviewed literature by the Federal Aviation Administration's (FAA's) Bioaeronautical Sciences Research Laboratory in Oklahoma City, OK (1). This procedure involves the simultaneous analysis of two serotonin metabolites whose ratio is significantly altered when ethanol is ingested. Urine from both officers

was analyzed using this procedure, and in each case, was found to have a ratio of 5-Hydroxyindole-3-Acetic Acid (5-HIAA) to 5-Hydroxytryptophol (5-HTOL), indicating that the ethanol found at autopsy was from postmortem ethanol formation and not from ingestion.

Frequency of Postmortem Ethanol Formation

Postmortem ethanol production in human bodies has been well documented by many forensic scientists over the past 70 years (2-4). A recent postmortem ethanol article referenced 323 postmortem ethanol papers (5). A study reported in 1993 by the FAA laboratory found that postmortem ethanol occurred in 27% of all ethanol-positive aviation accidents, and that the differentiation of postmortem ethanol production from ingestion could not be determined in 43% of all ethanol positive aviation accidents (6). Ethanol ingestion could only be confirmed in 30% of all positive aviation ethanol cases.

Distribution of Ethanol in Biological Specimens

Knowing vitreous fluid ethanol concentrations are approximately 18% higher than blood ethanol, and urine ethanol concentrations are approximately 30% or more than the blood ethanol concentrations (assuming the existence of the post-absorptive or elimination phase), it has been suggested that the best approach to differentiate ethanol ingestion from postmortem ethanol is the distribution of ethanol in blood, urine, and vitreous fluid (7-9). Vitreous fluid is considered an excellent specimen for determining ingested ethanol, even though it may contain nutrients (glucose) needed for postmortem ethanol formation, because it is protected by the skull from the microorganisms responsible for postmortem ethanol production (10). Under normal conditions, urine lacks the nutrients required for postmortem ethanol production; however, certain medical conditions can cause elevated glucose concentrations in urine and increase the chances of postmortem ethanol production in urine.

Trauma as a Factor in Postmortem Ethanol

Postmortem specimen contamination with ethanol-producing microorganisms increases with an increasing extent of trauma to the body. Therefore, postmortem ethanol formation is far more likely to occur in cases involving severe trauma from high speed crashes such as aviation accidents. Furthermore, if there is a fire that damages the protective dermal layer of the body, there is

an increased opportunity for ethanol producing microorganisms to invade the body and produce postmortem ethanol.

Time as a Factor in Postmortem Ethanol Formation

Time is another factor in the amount of postmortem ethanol produced (11); however, due to the many other variables that affect the production of ethanol, it is impossible to predict postmortem ethanol production solely from the amount of time before specimen collection. In one aviation fatality investigated by several of the authors, an accident occurred at an airport, and postmortem specimens were collected two hours after the accident. An ethanol concentration of 0.055% was found in the pilot's blood, but no ethanol was found in vitreous fluid, brain, or muscle (Fig. 1). The variability of postmortem ethanol formation can also be seen in accidents involving multiple victims where one body forms postmortem ethanol and the other does not, even though they were in the same accident.

Nutrients as an Indicator of Postmortem Ethanol Production

Urine does not normally contain the nutrients necessary for microbial ethanol production. Tests are routinely performed on urine specimens to check for the presence of glucose. The presence of glucose may suggest postmortem ethanol, but the absence of glucose does not exclude postmortem ethanol because ethanol-producing microorganisms can produce ethanol from substrates other than glucose (sucrose, mannose, lactose, etc). Further, the possibility exists that glucose originally present in the urine was consumed by microorganisms prior to the specimen being collected or analyzed. In individuals who have recently eaten a meal, the nutrients available in the body for postmortem ethanol production are increased.

Serotonin Metabolite Ratios as an Indicator of Postmortem Ethanol Production

Due to the high rate of postmortem ethanol formation in fatal aviation accidents and the frequency at which no determination could be made regarding ethanol origin, it was found to be advantageous to develop a new analytical procedure to determine the origin of ethanol found in pilots who had died in an aviation accident. In 1967, it was reported that the ratio of two serotonin metabolites, 5-HIAA and 5-HTOL, were altered by the ingestion of ethanol and remain altered for up to 16 hours after the individual stopped consuming ethanol (12). Researchers discovered that this ratio of serotonin metabolites in urine could be used to determine whether a person had recently

consumed ethanol (13). This ratio was initially used in ethanol cessation programs to monitor patients diagnosed with alcoholism. Under normal circumstances, 5-HIAA is found at concentrations up to 100 times as great as 5-HTOL. When ethanol is consumed, the production of 5-HTOL is favored, and the ratio shifts from 100:1 (5-HIAA/5-HTOL) up to 60:40 (5-HIAA/5-HTOL). The determination of this ratio has been used successfully in aviation accident investigations to differentiate between ingested and postmortem ethanol (14). A ratio of 5-HTOL/5-HIAA below 15 has proven to be evidence that any ethanol found in the urine specimen is from postmortem ethanol production and not from ingestion. Ratios of 5-HTOL/5-HIAA above 15 usually indicate that the person consumed ethanol within 8-12 hours prior to death. An elevated ratio does not exclude the possibility that the ethanol found in the specimen is from a combination of ethanol ingestion and postmortem ethanol production, but a ratio below 15 does exclude ethanol ingestion as a source of ethanol in a specimen.

Identification of Ethanol Producing Microorganisms

Procedures have been developed at the FAA Civil Aerospace Medical Institute (CAMI) to identify the ethanol-producing microorganisms present in a specimen containing ethanol. This procedure uses a set of DNA probes developed to identify DNA sequences associated with microorganisms capable of ethanol production. The procedure has been used in aviation fatalities to show that ethanol-producing microorganisms were absent or present in the specimen (15,16).

Other Volatiles as Indicators of Postmortem Ethanol Formation

It has been proposed that the presence of volatiles other than ethanol indicates postmortem ethanol formation (17). Research has shown the presence of other volatiles to be an indication of specimen putrefaction but not necessarily a conclusive indicator of postmortem ethanol production (6,18).

Ethanol Concentration as an Indicator of Postmortem Ethanol

Some investigators believe that concentrations of ethanol above a certain concentration (0.04%) indicate the ingestion of ethanol (19). However postmortem ethanol concentrations above 0.350% have been found in postmortem specimens taken from aviation accidents. Therefore, no conclusions can be made regarding the absence or presence of postmortem ethanol based solely on the concentration of blood ethanol found.

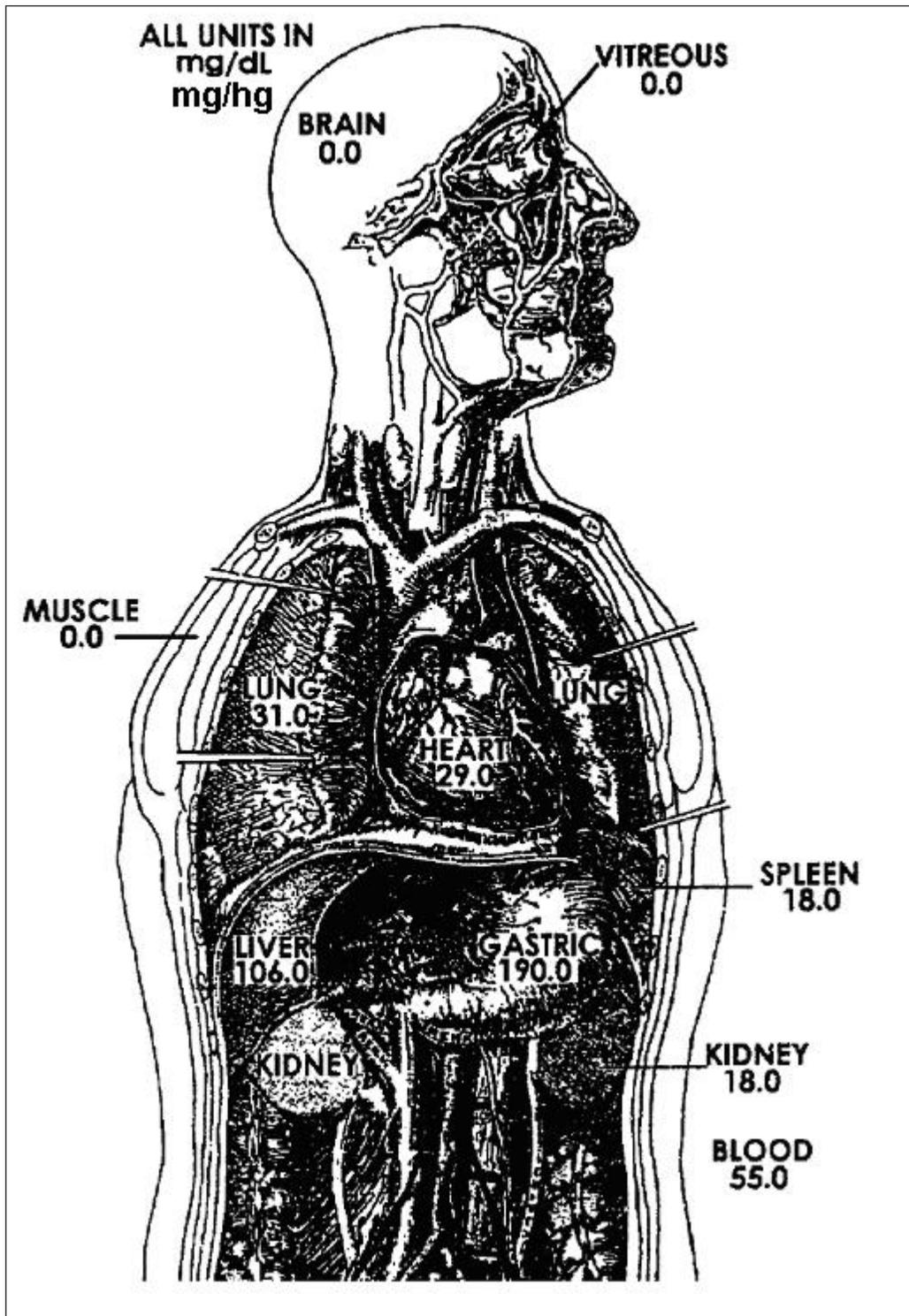


Figure 1: Postmortem alcohol distribution in a fatal aviation case.

CONCLUSION

Postmortem ethanol production can and does occur in fatal accidents. Therefore, care is necessary in investigating fatal accidents involving severe trauma to the body or in cases where long delays occurred prior to the collection of specimens for toxicological analysis. When abnormal ethanol distribution is found in blood, urine, and vitreous fluid, further testing may be warranted using serotonin metabolite ratios. Finding elevated blood ethanol concentrations but no ethanol in vitreous fluid and urine should be reported as negative for ingested ethanol and should not require further testing. It is important to realize that an incorrect finding of "intoxication," when the person did not consume ethanol, can result in extreme hardship for the family of the deceased through loss of pension, workers compensation, life insurance, and the reputation of the individual.

REFERENCES

1. R.D. Johnson, R.J. Lewis, D.V. Canfield, et al. Accurate assignment of ethanol origin in postmortem urine: Liquid chromatographic-mass spectrometric determination of serotonin metabolites. *J Chromatogr B Analyt Technol Biomed Life Sci* 805:223-34 (2004).
2. D.J. Blackmore. The bacterial production of ethyl alcohol. *J Forensic Sci Soc* 8:73-8 (1968).
3. M. Nicloux. Neoformation d'alcool ethylique dans le cadavre humain en voie de putrefaction. *Compte Rendu des Seances de la Societe de Biologie* 121:975-8 (1936).
4. J.E. Corry. A review. Possible sources of ethanol ante- and post-mortem: its relationship to the biochemistry and microbiology of decomposition. *J Appl Bacteriol* 44:1-56 (1978).
5. F.C. Kugelberg and A.W. Jones. Interpreting results of ethanol analysis in postmortem specimens: A review of the literature. *Forensic Sci Int* 165:10-29 (2007).
6. D.V. Canfield, T. Kupiec, and E. Huffine. Postmortem alcohol production in fatal aircraft accidents. *J Forensic Sci* 38:914-7 (1993).
7. A.W. Jones and P. Holmgren. Uncertainty in estimating blood ethanol concentrations by analysis of vitreous humour. *J Clin Pathol* 54:699-702 (2001).
8. B.E. Stone and P.A. Rooney. A study using body fluids to determine blood alcohol. *J Anal Toxicol* 8:95-6 (1984).
9. A.W. Jones. Urine as a biological specimen for forensic analysis of alcohol and variability in the urine-to-blood relationship. *Toxicol Rev* 25:15-35 (2006).
10. Y.H. Caplan and B. Levine. Vitreous humor in the evaluation of postmortem blood ethanol concentrations. *J Anal Toxicol* 14:305-7 (1990).
11. R. J. Lewis, R.D. Johnson, M.K. Angier, et al. Ethanol formation in unadulterated postmortem tissues. *Forensic Sci Int* 146:17-24 (2004).
12. V.E. Davis, H. Brown, J.A. Huff, et al. The alteration of serotonin metabolism to 5-hydroxytryptophol by ethanol ingestion in man. *J Lab Clin Med* 69:132-40 (1967).
13. A. Helander, O. Beck, and A.W. Jones. Urinary 5HTOL/5HIAA as biochemical marker of postmortem ethanol synthesis. *Lancet* 340:1159 (1992).
14. R.D. Johnson, R.J. Lewis, D.V. Canfield, et al. Utilizing the urinary 5-HTOL/5-HIAA ratio to determine ethanol origin in civil aviation accident victims. *J Forensic Sci* 50:670-5 (2005).
15. D.M. Kupfer, A.K. Chaturvedi, D.V. Canfield, et al. PCR-based identification of postmortem microbial contaminants--a preliminary study. *J Forensic Sci* 44:592-6 (1999).
16. N.T. Vu, A.K. Chaturvedi, D.V. Canfield, et al. DNA-based detection of ethanol producing microorganisms in postmortem blood tissues by polymerase chain reaction. Federal Aviation Administration. Report No. DOT/FAA/AM-00/16, 2000.
17. K. Ziavrou, V.A. Boumba, and T.G. Vougiouklakis. Insights into the origin of postmortem ethanol. *Int J Toxicol* 24:69-77 (2005).
18. D. Yajima, H. Motani, K. Kamei, et al. Ethanol production by *Candida albicans* in postmortem human blood samples: Effects of blood glucose level and dilution. *Forensic Sci Int* 164:116-21 (2006).
19. B. Levine, M.L. Smith, J.E. Smialek, et al. Interpretation of low postmortem concentrations of ethanol. *J Forensic Sci* 38:663-7 (1993).