Dead Drunk? - Ethyl Glucuronide and Ethyl Sulphate as Indicators of Post-Mortem Fermentation

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INTRODUCTION

Interpretation of ethanol (alcohol) concentrations post-mortem can be challenging, owing to post-mortem artefacts including production and diffusion (1). It is well established that ethanol may be produced *in-vitro*, due to fermentation of sugars by bacteria. This process, also common in urine samples obtained from diabetics or those with urinary tract infections, is accentuated by humidity and warm temperatures. The passage of time, location of the body and trauma can also compound production.

Concentrations of ethanol resulting from post-mortem fermentation are typically low (<50mg/dL) but may exceed 150mg/dL if the conditions for production are optimal (1-2). Whilst fermentation can be inhibited by correct storage and preservation of samples with fluoride, significant concentrations of ethanol may already have been formed prior to sampling. Distinguishing between ethanol present due to consumption and that produced *in-vitro,* via fermentation, is of particular importance in road traffic collisions (RTC).

EtG & EtS: Ethyl glucuronide (EtG) and ethyl sulphate (EtS) are water soluble, stable, non-volatile and non-oxidative, direct metabolites of ethanol (3). As EtG and EtS are formed as a result of ethanol metabolism, their detection can assist the differentiation of ante-mortem consumption and post-mortem production of ethanol.

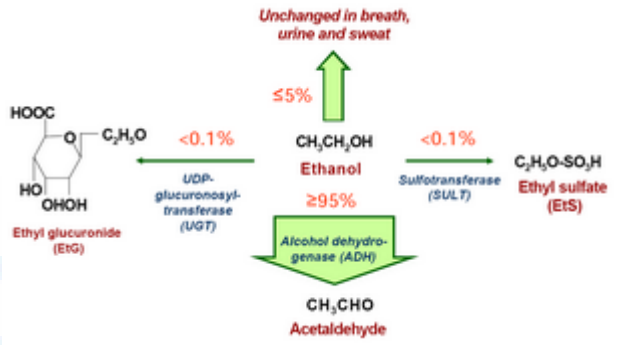

Figure 1. Ethanol Elimination Pathways (A. Helander, 2007)

CASE REPORTS

Case Report 1: As a result of a pending forced eviction and relocation to unsatisfactory accommodation, a 91 year old female was found suspended from the hanging rail of her wardrobe by her dressing gown cord, in what appeared to be self suspension. She suffered from depression and had previously attempted suicide and had self harmed. She suffered from Parkinson's disease and had limited mobility, requiring the assistance of daily carers and use of a Zimmer frame.

Case Report 2: A normally fit and well 45 year old male was found dead lying face down in bed, gripping his pillow. A small amount of blood was coming from his mouth. The cause of death was found to be aspiration but the reason for this occurrence was unknown. The Coroner recorded an open verdict.

Case Report 3: A 61 year old male was found lying dead on his back, next to his bed. Vomit was found in the toilet and the deceased had a wound to the back of his head. Neighbours had not seen the deceased for approximately 10 days and notified the police after noticing a build up of post outside his door. The television was still on and the TV listing magazine was open at a date 9 days prior to his discovery.

EXPERIMENTAL

Samples: Unpreserved post-mortem femoral vein blood and unpreserved urine were submitted for cases 1 and 2. Sodium fluoride preserved post-mortem femoral blood and urine samples were submitted for case 3.

Ethanol: Ethanol concentrations were measured by headspace GC-FID on a Shimadzu GC 2014 coupled to a HTA, HT200H headspace auto sampler (LLOQ: 10mg/dL).

EtG: EtG was measured in urine only using the Microgenics DRI® EtG Enzyme Immunoassay on the Olympus AU400 (LLOQ: 100ng/mL).

EtG & EtS: EtG and EtS analysis and confirmation were performed using a Waters® ACQUITY UPLC® System coupled to a Waters® TQ Detector (LLOQ: EtG: 250ng/mL, EtS: 50ng/mL) (4).

RESULTS

Case Report	Ethanol (mg/dL)		EtG (ng/mL)		EtS (ng/mL)
	Blood	Urine	DRI-EA	UPLC/MS/MS*	UPLC/MS/MS*
1	99	ND	ND	ND	ND
2	157	ND	ND	ND	ND
3	103	13	ND	ND	ND

ND = None detected * = Blood and urine

DISCUSSION

In the 3 case reports shown, the presence of significant ethanol concentrations (>80mg/dL – legal UK driving limit) in blood with low or absent urine ethanol, raises the question of post-mortem fermentation. This possibility is further supported by the absence of ethanol metabolites; EtG and EtS. Had these blood samples originated from RTCs, and no supporting specimens had been available, as is frequently the case, the conclusions drawn could have been incorrect and had legal consequences. It should, however, be noted that;

- Bacterial ß-glucuronidases can result in the breakdown of EtG (5-6).
- ß-glucuronidase, but not sulfatase, activity is particularly prominent in most strains of *E. coli* (5).
- Refrigerating or freezing samples and fluoride preservation greatly reduces hydrolysis of EtG (5).
- EtG can be produced by *E. coli* if ethanol is present or produced *in-vitro* (7).
- Production of EtG may not be prevented by optimising storage conditions (7).
- Depending on the type and concentration of bacteria present, EtS can be degraded (8).
- There is no evidence to suggest that EtS is subject to *in-vitro* synthesis (5,7).
- EtS appears to be a more reliable marker than EtG.

CONCLUSIONS

Post-mortem production of ethanol is a well known and documented phenomenon. Collecting samples into fluoride preservative can halt this process. However, correctly preserved samples may offer a false sense of security, particularly if there has been a prolonged delay between death and sample collection, during which fermentation may have occurred. Comparison of blood, urine and vitreous humour ethanol concentrations, where possible, pathological findings and case history all assist in determining whether ethanol present is the result of ante-mortem consumption or *in-vitro*, post-mortem production. EtG and EtS can be used as additional parameters to identify post-mortem fermentation.

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