INTRODUCTION

Besides the acute effects of ethanol on a person's performance and behavior, the after-effects of heavy drinking, commonly referred to as the hangover state, causes impairment of mental and body functioning, such as fatigue, attention deficit, and diminished psychomotor skills (Kelly et al., 1970; Seppälä et al., 1976; Lemon et al. 1993). It seems likely that many accidents on the roads and in the workplace might be attributed to decreased attention and slower reaction time associated with the residual effects of an evening's heavy drinking (Karvinen et al., 1962; Törnros and Laurell 1991; Roehrs et al., 1994). Although neither dose-response relationships nor the prevalence of hangover have been clearly established, experience has shown that a person's blood alcohol concentration (BAC) should exceed approximately 1.0 g/L during the acute phase of intoxication (Smith et al., 1983; Collins and Chiles 1980). The hangover starts to develop when the BAC decreases again and is approaching zero. However, the intensity and duration of hangover shows large inter- and intra-individual variations and the reduced performance in some people might persist for many hours after all ethanol has been cleared from the body (Goldberg, 1961).

BIOCHEMICAL MARKERS OF HANGOVER

Low concentrations of ethanol and methanol (0.5-1.0 mg/L) can be determined in body fluids even without drinking alcoholic beverages (Majchrowicz and Mendelson, 1971). These endogenous alcohols are oxidized in the liver to their respective aldehydes by alcohol dehydrogenase (ADH) and the affinity of this enzyme is roughly 10 times higher for ethanol
as its substrate compared with methanol. This means that the concentrations of methanol in blood and other body fluids increase during the time people drink alcoholic beverages because the enzyme ADH is fully engaged in the metabolism of ethanol (Majchrowitz and Mendelson, 1971). Methanol occurs as a congener in alcoholic beverages, which also contributes to the raised concentrations observed in body fluids after a drinking spree (Majchrowitz and Mendelson, 1971). The elimination half-life of methanol in humans is between 2-4 hours (Jones 1987; Haffner et al. 1992) so that methanol can be detected in blood, breath, and urine for long after ethanol has returned to its endogenous concentration (Jones, 1986).

Acetaldehyde is the primary metabolite of ethanol oxidation and this noxious substance is rapidly converted to acetate through the action of aldehyde dehydrogenase (ALDH). However, ALDH is also involved in the oxidation of biogenic aldehydes such as those formed during catabolism of dopamine, noradrenaline and serotonin (5-hydroxytryptamine). Without drinking any alcohol, the urinary excretion products of serotonin metabolism are 5-hydroxyindoleacetic acid (5HIAA) and 5-hydroxytryptophol (5HTOL), and the ratio of 5HTOL/5HIAA is normally very low (Helander et al., 1993). When acetaldehyde derived from ethanol competes with the biogenic aldehyde formed from serotonin for available ALDH, there is a switch in the metabolic pathway and the ratio 5HTOL/5HIAA in the urine increases appreciably and does not recover to baseline levels until after ethanol has been cleared from the blood (Helander et al., 1993).

Analyzing methanol and the metabolites of serotonin (5HTOL and 5HIAA) in urine therefore furnishes a way to disclose recent drinking even after ethanol has been cleared from the body (Helander et al., 1996; Jones and Helander 1996).

CLINICAL AND FORENSIC APPLICATIONS

During rehabilitation of alcoholics and others who are forbidden to drink alcohol as part of their treatment, a sensitive and specific test of drinking is often necessary. Breath-ethanol testing alone is not sufficiently sensitive to control whether a patient has remained abstinent because many people can regulate their intake of alcohol. Studies have shown that the analysis of methanol and 5HTOL/5HIAA can serve as markers of relapse and as a way to
monitor whether a person has recently consumed alcohol. This is useful in connection with the treatment of recidivist drunk drivers who are not allowed to drink as a condition of relicensing. During medicolegal investigations into the likely cause of serious accidents in the workplace it is obviously important to establish whether or not recent consumption of alcohol was a factor involved. This might be relevant in civil and criminal litigation when responsibility for the accident and insurance claims are made (Ames et al., 1997).

We have conducted a controlled drinking experiment to investigate the usefulness of methanol and 5HTOL/5HIAA as biochemical markers of alcohol ingestion and to test how long after the end of drinking these markers remain valid compared with measuring ethanol in blood, breath, and urine.

**EXPERIMENTAL**

Twenty healthy subjects (9 women and 11 men) with a mean age of 39 ± 9.4 y (± SD) and mean body weight 72 ± 11.6 kg (± SD) participated in this study as paid volunteers. They drank either 80 g or 50 g of ethanol according to choice as white table wine (11% v/v) and export beer (5.2 % v/v). The alcohol was consumed over a period of 2 hours together with a meal in an attempt to mimic social drinking conditions. Fifteen minutes after the end of drinking, breath-alcohol tests were made with an Alcolmeter S-D2 and the results were reported as equivalent blood-alcohol concentration. Thereafter, a specimen of urine (10 ml) was collected into a plastic tube containing 100 mg sodium fluoride as preservative. Another breath-alcohol test was made and a specimen of urine collected just before bedtime. Breath-alcohol concentration was measured in the morning after arising from bed and a sample of urine was also collected. Breath and urine samples were taken on two further occasions during the morning or early afternoon. The times for collecting the consecutive samples of urine were not exactly the same for each subject. Furthermore, a control specimen of urine was either collected before ingestion of alcohol began or on a separate occasion when the subjects had abstained from drinking alcohol.

The concentrations of ethanol and methanol in urine were determined by headspace gas chromatography as reported in more detail elsewhere (Jones and Schuberth, 1989; Jones and Löwinger 1988). For the analysis of ethanol, the urine was diluted 1 + 10 with n-propanol
(0.8 g/L) as an internal standard and the peak height ratios ethanol/n-propanol were used for quantitative analysis by comparison with the response from analysis of known strength alcohol standards (0.50, 1.00 and 1.50 g/L). The limit of quantitation of the method is 0.01 g/L and the within-run precision expressed as coefficient of variation is 1% at a mean urine-ethanol concentration of 1.0 g/L. For the analysis of methanol a salting-out procedure was used to enhance the analytical sensitivity. This entailed adding the aliquot of urine (0.5 ml) to a headspace vial containing 1.2 g sodium chloride. The vials (22 ml volume) were immediately made air-tight with crimped-on rubber septums before being analyzed. The precision (CV) of analyzing methanol in urine by this method was 3.4 % at a mean concentration of 2.3 mg/L.

The concentrations of 5-hydroxytryptophol (5HTOL) and 5-hydroxyindoleacetic acid (5HIAA) in urine were determined by gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC), respectively. These methods have been validated and described in detail elsewhere (Beck et al., 1982; Helander et al., 1991). To compensate for variations in urine flow rate and also dietary intake of serotonin, which are factors that might influence results, the ratio of 5HTOL/5HIAA was calculated.

The concentrations of methanol and the ratio 5HTOL/5HIAA in the pre-drinking specimens of urine were compared with results for samples collected after drinking by a Student’s t-test for paired observations (dependent t-test).

RESULTS

Figures 1-3 show the concentrations of ethanol, methanol, and the ratio 5HTOL/5HIAA in urine samples collected before drinking alcohol and on five occasions after intake of either 50 g or 80 g ethanol. As expected, the UAC, UMC, and the ratio of 5HTOL/5HIAA were higher after the subjects had consumed 80 g ethanol compared with 50 g.

In the morning after drinking 50 or 80 g ethanol, the breath-test results were zero for all except three individuals who registered 0.01, 0.05, and 0.09 g/L on the Alcolmeter S-D2 device. The highest BrAC was obtained for the person with highest morning UAC of 0.76 g/L. The first morning voids as a rule contained measurable amounts of ethanol, being 0.38 ±
0.10 g/L after drinking 80 g ethanol and 0.09 ±0.03 g/L after intake of 50 g ethanol. However, ethanol was not measurable in urine (< 0.01 g/L) at the time of the second and third voids obtained later in the morning or early afternoon, indicating alcohol-free status in all subjects by this time.

Figures 2 and 3 show that urinary methanol and the ratio of 5HTOL/5HIAA follow a different time course compared with UAC, being shifted in time. This time lag is particularly evident for urinary methanol, which peaks in the first morning void when the BrAC had already reached zero in most subjects. Table 1 compares the statistical significance of differences between the pre-drinking levels of methanol and the 5HTOL/5HIAA ratio with values determined in urine specimens collected during the morning and afternoon (1st, 2nd and 3rd voids). The results for the second and third morning voids have practical importance as a test for recent drinking because by the time of sampling ethanol was no longer detectable in blood, breath, or urine and the hangover was most troublesome.

Figure 1. Concentrations of ethanol in urine after subjects drank 50 g (N = 11) or 80 g (N = 9) ethanol as beer and white wine.
Figure 2. Concentrations of methanol in urine collected before and after intake of 50 g (N = 11) or 80 g (N = 9) ethanol as beer and white wine.

Figure 3: Concentration ratios of 5HTOL/5HIAA (pmol/nmol) in urine samples collected before and after intake of alcohol 80 g (N = 9) or 50 g (N = 11) in the form of white wine and beer.
Table 1: Differences between urinary concentrations of methanol (mg/L) and the ratio of 5HTOL/5HIAA in pre-drinking specimens of urine and voids made in the morning after an evening's heavy drinking. Statistical testing was by Student's t-test for paired observations, where; * p < 0.05, ** p<0.01, *** p<0.001.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>EtOH Dose</th>
<th>Urine-4 1st morning void</th>
<th>Urine-5 2nd morning void</th>
<th>Urine-6 3rd morning void</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>50 g</td>
<td>3.06 ± 0.40**</td>
<td>0.73 ± 0.16**</td>
<td>0.26 ± 0.09*</td>
</tr>
<tr>
<td>80 g</td>
<td>5.06 ± 0.55***</td>
<td>2.28 ± 0.67**</td>
<td>0.49 ± 0.32</td>
<td></td>
</tr>
<tr>
<td>5HTOL/5HIAA</td>
<td>50 g</td>
<td>134 ± 28.7***</td>
<td>4.6 ± 1.23**</td>
<td>1.54 ± 0.41*</td>
</tr>
<tr>
<td>80 g</td>
<td>296 ± 47.3***</td>
<td>35.0 ± 8.41**</td>
<td>6.22 ± 2.26*</td>
<td></td>
</tr>
</tbody>
</table>

The first morning void was collected between 7-10 hours after bedtime, the second morning void 3-6 hours later and the third and last void between 5-8 hours later. However, although concentrations of methanol and the 5HTOL/5HTOL ratio could be distinguished from the pre-drinking values, the last specimen of urine (3rd morning void) would not be of much practical use without knowledge of a person's pre-drinking levels. This information is mostly not available.

**DISCUSSION**

It is common knowledge that people do not function normally when they are suffering from a hangover and this can be demonstrated when skilled tasks must be performed (Kelly et al., 1970; Seppälä et al., 1976; Lemon et al. 1993). For this reason ingestion of alcohol is strictly regulated for those engaged in safety-sensitive work (Dubowski and Kaplan, 1996). Nevertheless, too much drinking leads to many accidents and premature deaths and minimum periods of alcohol deprivation have been introduced for those engaged in highly demanding tasks such as aviation pilots. Until now the hangover effects of too much drinking have not been seriously considered as a cause of accidents because reliable markers of alcohol consumption have not been available after ethanol has been metabolized. When investigating the causes of serious accidents alcohol and drug use should always be considered as likely contributing factors. However, a low blood or urine alcohol concentration at autopsy, e.g. less than 0.10 g/L, is not considered relevant and previous alcohol ingestion and intoxication are
usually written off as likely causes of the accident. However, an autopsy BAC of 0.01 g/L might reflect a BAC of 2.0 g/L the previous evening and the person concerned might therefore have been suffering from a hangover when the accident happened. Sensitive and specific biochemical tests of recent drinking even after ethanol has been cleared from the body would be useful in medicolegal investigations of fatal accidents. In this study, most of the subjects had eliminated all alcohol from the blood sometime during the night so that the UAC in the first morning void reflects the BAC prevailing since the bladder was last emptied, that is, before bedtime. The concentrations of methanol and the 5HTOL/5HIAA ratio were elevated above pre-drinking baseline values in the first, the second, and sometimes even the third urine voids collected during the morning and early afternoon and therefore up to 5-10 hours after BrAC was no longer measurable.

Some investigators advocate measuring methanol in body fluids as a marker for chronic consumption of alcohol and alcoholism (Iffland, 1996). If the concentration is above 10 mg/L in serum this is considered a marker of heavy and continuous drinking for days or weeks. By contrast, we are advocating the analysis of methanol in urine as a marker of acute intake of alcohol and this test is sufficiently sensitive to disclose ingestion of alcohol even after alcohol has been cleared from the body. However, the analysis of methanol alone is not completely specific as a test for alcohol consumption because eating fresh fruits and drinking large volumes of fruit juice containing traces of free methanol or methyl esters can account for the urinary excretion of this alcohol. Even the artificial sweetner aspartame can be converted in the body to methanol (Davoll et al., 1986). Accordingly an elevated concentration of methanol in urine needs to be supported by other evidence or a more specific marker of alcohol consumption such as an elevated ratio of 5HTOL/5HIAA.

These new biochemical markers (methanol and 5HTOL) furnish a way to disclose recent drinking for up to 5-10 hours after alcohol is no longer measurable by conventional breath-alcohol tests. There will certainly be applications as "relapse markers" during rehabilitation of alcoholics and drug addicts as well as convicted drunk drivers who must refrain from drinking as a part of their treatment. Finding an elevated urinary concentration of methanol and an elevated ratio of 5HTOL/5HIAA provides convincing evidence of recent alcohol consumption even if ethanol cannot be determined in body fluids. Because a person's pre-drinking levels of urinary methanol and the ratio 5HTOL/5HIAA are not usually known, we
consider that threshold values of 2.0 mg/L for methanol and 15 pmol/nmol for 5HTOL/5HIAA are reasonably values for clinical and forensic purposes when evaluating random samples of urine.

REFERENCES


