The aim of this study was to investigate the concentrations of ethyl glucuronide (EtG) in oral fluid, blood, and urine after healthy volunteers drank two doses of ethanol, 0.5 \((n = 11)\) and 1.0 g/kg \((n = 10)\), after an overnight fast. Samples of oral fluid, blood, and urine were collected before drinking started and at \(1.5, 3.5, 5.5, 8.5, 11.5,\) and \(24\) h post-dosing. Following ingestion of low dose of ethanol, the Cmax for EtG was 0.36 mg/L (range 0.28–0.41 mg/L) in blood and 69.8 mg/L (range 47.1–96.5 mg/L) in urine. In oral fluid, the concentrations were < 1% of those in blood, and only three subjects exceeded the limit of quantification for EtG in oral fluid. After ingestion of the high dose of ethanol, the Cmax for EtG was 1.06 mg/L (range 0.8–1.22 mg/L) in blood, 159.9 mg/L (range 97.2–225.5 mg/L) in urine, and 0.032 mg/L (range 0.013–0.059 mg/L) in oral fluid. The median oral fluid/blood ratio was 0.029 (range 0.012–0.054) for EtG. The detection time for EtG was median 11.5 h (range 3.5–11.5 h) in oral fluid. According to this, the detection time for EtG in oral fluid is therefore only a few hours longer than for ethanol itself and represents limited additional value.

Introduction

In a number of situations, such as in workplace drug testing and during alcoholism treatment, monitoring of a person’s alcohol use might be desirable. Traditionally, ethanol itself has been measured in blood, breath, or urine to detect recent alcohol ingestion, but because of the rapid clearance of ethanol from the body (1), its nonoxidative metabolite ethyl glucuronide (EtG) (2) has gained popularity. A number of studies have investigated the kinetics of EtG in urine (3–6), where this metabolite has been detectable for about 24 h after ingestion of a low dose (< 0.5 g ethanol per kg body weight) and up to five days or more after ingestion of large and repeated doses of ethanol. In blood, pharmacokinetic studies have shown terminal half-lives for EtG of 2–4 h and detection times of about 10 h after ingestion of low dose of ethanol and up to 24 h after large and repeated doses (7–10). In addition, it has been shown that EtG in hair is a sensitive and specific marker of chronic heavy drinking (11,12).

Oral fluid has become an important specimen in drug testing, especially in roadside drug screening and workplace drug-testing programs (13). There are mainly two advantages with this. First, the sampling procedure is superior because it is performed in a noninvasive manner and could be supervised more easily. Second, the detection of alcohol or drugs in oral fluid may reflect the presence of those compounds in blood and thus confirm a more recent intake (14). Oral fluid kinetics and analytical methods have been published during recent years for many drugs of abuse, such as ethanol, cannabis, amphetamines, opiates, and cocaine (15–22). As it is preferable to use the same matrix for control of all drug intakes including alcohol, we previously suggested that oral fluid could be used to measure EtG. A method for the analysis of EtG in oral fluid was recently published (23), and this publication also indicated a considerably longer detection time of EtG in oral fluid compared to ethanol itself. These data were obtained in an uncontrolled study of only three subjects. The aim of the present study was therefore to investigate the kinetics of EtG in oral fluid compared to blood and urine in a controlled, kinetic drinking experiment with ingestion of both low and high doses of ethanol.

Materials and Methods

Study protocol

Eleven healthy volunteers (three men and eight women)
with a median age of 22 years (range 19–30 years) and a median body mass index of 21.8 kg/m² (range 18.4–26.0 kg/m²) participated in a controlled drinking experiment. They were all social drinkers with a median use of 144 g pure ethanol/month (range 48–240 g) and had abstained from alcohol during the week preceding the study, according to self-reports. Exclusion criteria were somatic or psychiatric illness, use of regular medication, and pregnancy. Pregnancy tests were performed for all women before start of alcohol ingestion.

The subjects gave informed consent, and the study protocol was approved by the National Committee for Research Ethics in Norway and the Directorate for Health and Social Affairs.

Each subject participated in two sessions separated by two weeks. The first day, 0.5 g ethanol per kg body weight (low dose) was ingested for 15 min, and on the second day, the dose was 1.0 g ethanol per kg body weight (high dose) consumed for 1 h. Otherwise, the protocol was similar for the two days. One subject (no. 5) participated on the first day only.

After an overnight fast, the participants signed in for the study at 8:00 a.m. Samples of oral fluid, blood, and urine were collected before start of drinking. Ethanol (vodka 60%, v/v) was consumed over a 15-min period (0.5 g/kg dose) or 1-h period (1.0 g/kg dose). The vodka was confirmed by ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS–MS) (24) not to contain EtG (limit of detection (LOD) 0.17 mg/L). Samples of oral fluid, blood, and urine were then collected at 1.5, 3.5, 5.5, 8.5, 11.5, and 24 h after start of drinking. Oral fluid was collected using Statsure Saliva Sampler (Saliva Diagnostic Systems, Brooklyn, NY) containing 1 mL of buffer. The total weight of the sample (including both buffer and oral fluid) was measured before analysis and the result corrected for the individual dilution factor. Urine was collected in Sterilin tubes (Bibby Sterilin, Staffordshire, U.K.) without any additives, whereas for the blood samples, 5-mL Vacutainer® tubes (BD, Franklin Lakes, NJ) containing 20 mg fluoride and 143 I.U. Heparin were used. All samples were stored at 4°C immediately after sampling and then stored at –20°C until analysis.

No food or drink apart from water was ingested until 1.5 h after start of drinking, at which point a meal consisting of bread, cheese, ham, and nonalcoholic drinks was consumed. This was repeated 5.5 h after start of drinking, and dinner was consumed 9.5 h after start of drinking. The participants were not allowed to consume any other food during the study period. One subject suffered from very minor vomiting 1 h and 45 min after start of drinking (1.0 g/kg dose).

### Measurement of ethanol and creatinine

Ethanol was determined by an alcohol dehydrogenase method in oral fluid, blood, and urine (25). The analytical cutoffs for ethanol in oral fluid-buffer mixture, blood, and urine were 0.06, 0.02, and 0.1 g/L, respectively. Only values above these levels were reported as positive results. The quantitative results reported for ethanol in oral fluid were corrected for their individual dilution factor. The creatinine concentration in urine was determined according to a previously published photometric method (26).

### Measurement of EtG in oral fluid

EtG was determined in oral fluid using a previously published UPLC–MS–MS method (23). LOD for this method was 0.0022 mg/L, and limit of quantification (LOQ) was 0.0044 mg/L (for oral fluid-buffer mixture). The concentrations above the LOQ and LOD values were determined in the oral fluid-buffer mixture, whereas the concentrations reported are corrected for their individual oral fluid dilution factor.

### Measurement of EtG in blood and urine

EtG was measured in blood and urine using a previously published UPLC–MS–MS method (24). All analytical details are given in this reference. For EtG results in oral fluid, blood, and urine, results above

### Table 1. Maximum Concentrations, Time-To-Maximum Concentrations, and Total Detection Times for Ethanol and EtG in Blood, Urine, and Oral Fluid

<table>
<thead>
<tr>
<th>Ethanol Dose</th>
<th>Blood Ethanol</th>
<th>Blood EtG</th>
<th>OF Ethanol</th>
<th>OF EtG</th>
<th>Urine Ethanol</th>
<th>Urine EtG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 g/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax</td>
<td>0.55 g/L</td>
<td>0.36 mg/L</td>
<td>0.61 g/L</td>
<td>0.012 mg/L</td>
<td>0.82 g/L</td>
<td>69.8 mg/L</td>
</tr>
<tr>
<td></td>
<td>(0.51–0.71)</td>
<td>(0.28–0.41)</td>
<td>(0.54–0.87)</td>
<td>(0.008–0.014)</td>
<td>(0.63–1.05)</td>
<td>(47.1–96.5)</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>1.5 (1.5–1.5)</td>
<td>3.5 (3.5–3.5)</td>
<td>1.5 (1.5–1.5)</td>
<td>3.5 (3.5–3.5)</td>
<td>1.5 (1.5–3.5)</td>
<td>5.5 (3.5–5.5)</td>
</tr>
<tr>
<td>Detection time (h)</td>
<td>3.5 (3.5–5.5)</td>
<td>11.5 (11.5–11.5)</td>
<td>3.5 (3.5–3.5)</td>
<td>3.5 (3.5–8.5)</td>
<td>3.5 (3.5–5.5)</td>
<td>24 (12–24)</td>
</tr>
<tr>
<td>1.0 g/kg</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax</td>
<td>1.25 g/L</td>
<td>1.06 mg/L</td>
<td>1.25 g/L</td>
<td>0.032 mg/L</td>
<td>1.53 g/L</td>
<td>159.9 mg/L</td>
</tr>
<tr>
<td></td>
<td>(1.11–1.52)</td>
<td>(0.8–1.22)</td>
<td>(1.06–1.46)</td>
<td>(0.013–0.059)</td>
<td>(1.28–1.80)</td>
<td>(97.2–225.5)</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>1.5 (1.5–1.5)</td>
<td>5.5 (5.5–5.5)</td>
<td>1.5 (1.5–1.5)</td>
<td>3.5 (3.5–5.5)</td>
<td>1.5 (1.5–3.5)</td>
<td>5.5 (3.5–5.5)</td>
</tr>
<tr>
<td>Detection time (h)</td>
<td>8.5 (5.5–8.5)</td>
<td>11.5 (11.5–11.5)</td>
<td>5.5 (5.5–8.5)</td>
<td>11.5 (3.5–11.5)</td>
<td>8.5 (5.5–8.5)</td>
<td>24 (24–24)</td>
</tr>
</tbody>
</table>

* After ingestion of low (n = 11) and high (n = 10) doses of ethanol. Times are expressed in hours from start of alcohol intake.

**Median values are given, with the range in parentheses.**

† Last urine sample was collected at 24 h.
LOQ are reported by their concentration; results between LOD and LOQ are reported as “trace”, whereas results below LOD are reported as negative. In the figures, concentrations above LOQ are plotted by their values, concentrations between LOQ and LOD are set to LOD, and those below are set to 0.00 mg/L. Reported values of EtG in urine are normalized to a creatinine concentration of 1000 mg/L.

Statistics
All data were handled using the Kinetica (version 4.4) pharmacokinetic program. Statistic parameters were calculated using SPSS (version 14.0, Chicago, IL). All results are presented as median followed by the range in parentheses.

Results
All samples of oral fluid, blood, and urine were negative for ethanol and EtG before start of ethanol intake.

The maximum concentrations (Cmax), time to maximum concentrations (Tmax), and total detection times for ethanol and EtG in blood, urine, and oral fluid after ingestion of both low and high doses of ethanol are summarized in Table I.

Results after ingestion of 0.5 g ethanol per kg body weight \((n = 11)\)
The median Cmax for EtG in blood was 0.36 mg/L (range 0.28–0.41 mg/L) (Figure 1). The concentrations of EtG in oral fluid were considerably lower compared to blood. Two subjects showed no EtG in oral fluid above the LOD, and only three subjects showed concentrations of EtG in oral fluid above the LOQ (0.012, 0.014, and 0.008 mg/L, respectively). In these subjects, the Cmax oral fluid/blood ratio was 0.035, 0.044, and 0.020, respectively. The Tmax for EtG was 3.5 h for all subjects in both blood and oral fluid. Concentrations of ethanol in oral fluid and blood were similar, and the median Cmax oral fluid/blood ratio was 1.09 (range 0.96–1.23).

EtG was eliminated with a terminal half-life of median 2.83 h (range 2.32–3.59 h) in blood, and the area under the curve (AUC) was median 2.43 mg/L h (range 2.04–2.77 mg/L h). In oral fluid, terminal half-lives and AUC could not be calculated because of an insufficient number of positive samples.

In urine, the EtG Cmax values were 194 times higher than in blood (range 168–247). Nine of the 11 subjects still showed EtG (above LOQ) in urine at the time of last sample collection (24 h after start of drinking) at a median concentration of 1.1 mg/L (range 0.6–2.6 mg/L) (normalized to 1000 mg/L creatinine).

Results after ingestion of 1.0 g ethanol per kg body weight \((n = 10)\)
The Cmax for EtG in blood was 1.06 mg/L (range 0.8–1.22) (Figure 1). Two subjects did not exceed LOQ for EtG in oral fluid, but the remaining eight subjects showed a median Cmax of 0.032 mg/L (0.013–0.059 mg/L) and a median oral fluid/blood ratio of 0.029 (range 0.012–0.054) for EtG (Figure 2). The Tmax for EtG was 5.5 h for all subjects in blood and median 3.5 h (range 3.5–5.5 h) in oral fluid. The median Cmax oral fluid/blood ratio was 1.0 (range 0.89–1.12) for ethanol.
EtG was eliminated with a terminal half-life of median 2.5 h (range 2.2–3.2 h) in blood and the AUC was median 8.58 mg/L h (range 6.92–11.02 mg/L h). In oral fluid (n = 8), AUC was median 0.14 mg/L h (range 0.10–0.24 mg/L h). Only one subject had enough concentration measurements of EtG in oral fluid above LOQ after Cmax to calculate terminal half-life, and this value was 2.9 h (no. 10).

In urine, the EtG Cmax values were 160 times higher than in blood (range 97–225). All subjects showed EtG in the last sample (collected 24 h after start of drinking), and the median concentration was 8.6 mg/L (range 1.5–18.6 mg/L) (normalized to 1000 mg/L creatinine).

**Discussion**

This study focused on the kinetics of EtG and its parent drug ethanol in oral fluid, blood, and urine after controlled dosing of both low and high doses of ethanol. Our main finding was that the concentrations of EtG were considerably lower in oral fluid compared to blood with shorter detection times. This controlled study could therefore not confirm the previous publication, which indicated a longer detection time for EtG (23). Secondly, this study showed a dose-dependent relationship for EtG kinetics in blood, urine, and oral fluid. EtS was not included in the oral fluid method. An earlier study showed that this compound could not be detected in samples from the presently used or other tested collecting devices (23). To our knowledge, detection of EtS in oral fluid has not been published.

The strength of this study was the measurement of EtG in three different media sampled nearly simultaneously, the use of both low and high doses of ethanol to each subject, and the controlled conditions regarding administration of ethanol and ingestion of food and drink. The main weakness of the present study was the limited number of subjects. Secondly, the relatively homogenous study population (similar age, all moderate drinkers, no somatic or psychiatric illness or regular use of medication) could question the generalization to larger populations. Thirdly, the sampling frequency was not higher in the first hours following alcohol ingestion, leading to a possible underestimation of the maximum ethanol concentrations. There was also too long of period between the samples collected 11.5 and 24 h after alcohol ingestion. This was during the nighttime, and sampling was therefore difficult. Also, the ethanol concentrations were measured using a less sensitive alcohol dehydrogenase method. This might have resulted in an underestimation of the detection times for ethanol. On the other hand, the elimination of ethanol is fast, and the detection time would probably increase only marginally if a more sensitive method were used.

There are many factors that determine the level of EtG in oral fluid. For the transfer of drugs from blood into saliva, the pK_a value is the most important factor, as saliva is acidic compared to blood and tends to trap basic drugs. This is the main reason why basic drugs like amphetamines (27) and opiates (28) show higher concentrations in oral fluid compared to blood, whereas more acidic drugs like diazepam show the opposite situation (29,30), although other factors like protein binding are also important. Considering the pK_a value of 3.21 for EtG (31), which is comparable to the one for diazepam, the low concentrations of EtG in oral fluid compared to blood are not surprising, and the oral fluid/blood ratio was comparable to those reported for diazepam (29,30).

Variations in the pH of saliva would lead to changed concentrations of EtG, and this is one factor making oral fluid a less controlled medium than blood.

Also, the amount of saliva produced at the sampling time is an important factor, as this is important for the volume of oral fluid and hence possible dilution. Molecules like amylase and IgG have been mentioned as possible ways to normalize for this, but no scientific evidence of their usefulness exists (32). If a collector that stimulates production of saliva is used, analyte concentrations might be affected (14). Such a collector was, however, not used in the present study. We also corrected the results for the dilution of oral fluid with buffer in the sampling kit, but this could be a source of error when comparing our results with other publications. Some researchers have also discussed the importance of bacteria in the oral cavity (33), and the possibility of local production of EtG from ethanol or degradation has not been studied. For all these reasons, oral fluid must be considered a less controlled medium compared to blood. This is also seen in Figures 1 and 2, which show larger variations in oral fluid EtG compared to blood EtG.

In the previous publication of the analytical method for EtG in oral fluid (23), EtG concentrations were measured after an uncontrolled ingestion of 0.5–1 L of wine, and the maximum concentration of EtG was seen 14 h after start of drinking in two out of three subjects. This was not consistent with the present results, which show Cmax was seen after 3.5–5.5 h, and the median detection time was 11.5 h (range 3.5–11.5 h) after the highest dose of ethanol. For the first hours after alcohol ingestion, the results were in accordance with the present study. We have no explanation for the differences between the previous publication and the present one, but the results from the latter study represent a larger number of subjects and drinking under more controlled conditions.

In this study, the dose-response relationship was evident for the EtG kinetics in all the media investigated: blood (Figure 1), urine, and oral fluid. The blood results from the low dose study was in accordance with previously published results (7–9) with similar terminal half lives in blood and relatively minor interindividual variations. Unlike our previous study, the majority of subjects in the present work were women, but although there are reports in the literature of decreased glucuronidation in women (34,35), we found no differences between the sexes.

This study was evidence, together with previous works (3,6–38), that the enzymes responsible for EtG formation, uridine diphosphate glucuronosyl transferase (UGT) 1A1 and 2B7, are not saturated in the area of ethanol concentrations tested. One could also ask if the opposite is the situation: that a higher proportion of ethanol is converted to EtG at
higher concentrations possibly because of other pathways being saturated. This is indicated in the present study, where the median AUC value for EtG in blood was 3.5 times higher after the high dose compared to the low dose, even if the dose only doubled. An alternative explanation that probably explains at least some of this is the increased bioavailability of ethanol after high doses (39,40) as partly indicated in the present study, but the concentrations of EtG increased more than those of ethanol from the low to high dose study. On the other hand, samples were not collected during the first hour after start of drinking, and the very highest concentrations of ethanol could therefore be missed.

The kinetics of ethanol in oral fluid has previously been investigated (22,41), and our results were similar with oral fluid/blood ratio close to one. This is as expected, considering the molecular properties of ethanol and its possibility to cross membranes freely.

In conclusion, this study showed that the concentrations of EtG in oral fluid are considerably lower than those in blood, and the detection times are shorter. For instance, in workplace drug testing, there would therefore be limited value in using EtG in oral fluid as a marker for recent alcohol intake compared to ethanol itself, at least for the doses of ethanol tested in the present study.

**Acknowledgments**

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**References**


