Pharmacokinetics of Ethanol in Saliva: Comparison with Blood and Breath Alcohol Profiles, Subjective Feelings of Intoxication, and Diminished Performance

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The concentration–time profiles of ethanol were determined for capillary blood, end-expired breath, and saliva after 21 healthy men ingested ethanol at 0.68 g/kg body weight. Near the time of obtaining body fluids, the volunteers estimated their feelings of intoxication, and body sway (with open and closed eyes), hand tremor, positional alcohol nystagmus (PAN), and roving ocular movements (ROM) were quantitatively recorded. The concentration–time profiles of ethanol in blood, breath, and saliva agreed well within individuals but there were large variations between subjects. The mean saliva–ethanol profiles ran slightly above those for blood and breath. Subjective ratings of intoxication and impairment of body function (standing steadiness and hand steadiness) were highest at the time of reaching the peak concentrations of ethanol in body fluids. PAN was evident in most subjects between 60 and 120 min after the start of drinking, whereas ROM appeared mainly during the postabsorptive phase of ethanol kinetics (120–420 min). The blood ethanol concentration thresholds were between 500 and 700 mg/L (50–70 mg/dL) when the diminished performance had recovered to baseline values.

Indexing Terms: toxicology · forensic medicine

Before ethanol exerts its untoward effects on a person's performance and behavior, the drug must cross the blood–brain barrier and interact with nerve cell membranes (1). The concentration of ethanol in the bloodstream that reaches the brain should therefore provide an indirect way to monitor ethanol-induced impairment and the associated decrease in performance. However, obtaining specimens of blood entering or leaving the brain is precluded for forensic purposes. Instead, peripheral sampling sites such as an antecubital vein or a fingertip capillary have been used as the source of blood for quantitative analysis of ethanol in clinical and medicolegal practice (2, 3).

Measuring the concentrations of ethanol in breath, urine, or saliva has certain practical advantages compared with drawing blood samples (4–7). However, translating the concentrations of ethanol in these alternative body fluids into the presumed blood alcohol concentration (BAC) has met with strong objections from some quarters.  

To overcome this problem, many legislative bodies have defined threshold limits of alcohol concentration for motorists according to the particular biological specimen analyzed, for example, 0.1 g/100 mL (1 g/L) of blood, 0.1 g/210 L of breath, or 0.1 g/67 mL of urine (8). However, the critics of this approach have pointed to the lack of empirical studies relating the concentration of ethanol in breath, saliva, and urine to the resulting impairment of body function.

This paper deals with the pharmacokinetics of ethanol in specimens of capillary blood, mixed salivary secretions, and end-expired breath. I have compared the concentrations of ethanol in these body fluids with subjective intoxication ratings, body sway, hand tremor, and eye movements.

Materials and Methods

Subjects and Conditions

Twenty-one healthy men, mean age 42 years (range 30–55 years) and mean body weight 84 kg (range 74–97 kg), were recruited for this study as paid volunteers. They were all accustomed to moderate drinking and some smoked cigarettes, but not during the experiments. Three subjects were tested in each experimental session. The subjects arrived at the laboratory at about 0730 without having eaten breakfast. Starting at 0900, 0910, and 0920, each subject in turn drank 0.68 g of ethanol per kilogram of body weight as neat whisky (ethanol 400 m/mL) within exactly 20 min. Drinks were served in glasses of different colors and sizes to mask the volumes dispensed to each subject. A meal was served 5 h after the drinking started.

Sampling of Body Fluids and Determination of Ethanol

Triplicate samples of capillary blood were taken from a fingertip before the subjects began drinking and then at 30, 60, 90, 120, 180, 240, 300, 360, and 420 min thereafter. The aliquots of blood (10 µL) were measured with capillary microcaps and diluted immediately with 0.9 mL of sodium fluoride solution (0.05 g/L) and stored in AutoAnalyzer (Technicon, Tarrytown, NY) cups for 1–3 days at 4 °C until analyzed. Ethanol was determined by an enzymatic method with yeast alcohol dehydrogenase and a Technicon AutoAnalyzer. The standard deviation (SD) of this method was higher at higher concentrations of ethanol in the samples. At a mean BAC of 530 mg/L (53 mg/dL), the SD for a single determination was 16 mg/L, corresponding to a coefficient of variation (CV) of 3% (9).

Just before the samples of capillary blood were obtained, each subject provided a mixed salivary secretion by tongue and lip movements, ejecting the specimen
into a small glass vial that was then closed with a screw cap. The samples were stored at 4 °C until analyzed 1–3 days later. Duplicate 10-μL aliquots were removed with capillary microscops and diluted with 0.9 mL of sodium fluoride solution in the same way as for blood samples. The SD of this method was 7 mg/L at a mean concentration of 650 mg/L (65 mg/dL), corresponding to a CV of 1.4% (10).

The concentration of ethanol in breath was determined in duplicate by using a Mk II Gas Chromatograph Intoximeter (Intoximeters Inc., St. Louis, MO). Some of the subjects provided duplicate breaths before and after the blood was drawn. The Intoximeter was calibrated with air–alcohol standards generated from a wet-bath simulator device. The results are presented here as milligrams of ethanol per 210 L of breath. The precision of breath alcohol analysis was 2.3 mg/210 L when the mean concentration of ethanol was 51 mg/210 L, which corresponds to a CV of 4.5% (11).

The time interval between collecting blood, breath, and saliva was always <10 min, and no corrections were made for any metabolism of ethanol occurring. Separate studies have shown that the concentration of ethanol in blood and saliva did not change during the period of storage before analysis (9, 10).

Subjective Feelings of Intoxication

The volunteers estimated their feelings of intoxication with reference to an arbitrary scale wherein the number 10 meant tipsy or a little high (12). Scores >10 therefore indicate successively greater subjective feelings of intoxication and scores <10 reflect feelings closer to the subject's normal state. The subjective ratings of intoxication were made just before or after blood samples were drawn.

Objective Tests of Impairment

Before the drinking started, various performance tests were made to establish baseline values. These tests were repeated at ~40–60-min intervals for 7 h after drinking. Body sway (with open and closed eyes), hand tremor, positional alcohol nystagmus (PAN), and roving ocular movements (ROM) were recorded by using methods described in more detail elsewhere (13–15). Body sway was measured with the subject standing on a special platform in an upright position. The amount of swaying in lateral and sagittal directions was recorded for two 70-s periods. The first recording was made when the subject's eyes were open; after a 30-s rest period, recordings were made without visual cues (eyes closed). Disruption of the ability to stand upright after consuming a moderate dose of alcohol is more pronounced in the sagittal plane, so these are the values reported here (16). Hand tremor was recorded with the subject sitting in a chair with one arm outstretched holding a metal stylus (1 mm diameter) as steady as possible for 60 s. The time spent in contact between the metal stylus and the walls of a 3.5-mm-diameter hole was measured electronically and expressed as a time-error score (seconds).

Eye movements were recorded from the resulting electro-oculograms. Displacement of the cornea–retina potentials were monitored by methods described elsewhere (13). Electrodes were placed at the outer corners of the eyes to register horizontal movements; the reference electrode was on the forehead. Electrodes were placed over and under the eyes to record vertical movements, with the eyes acting as dipoles. Spontaneous ocular movements were recorded with subjects in a supine position and with the head placed alternately in the right and left lateral positions. The occurrence of PAN and the velocity of the eye movements were derived from tracings made on a chart recorder (13).

Pharmacokinetics of Ethanol

Blood ethanol, saliva ethanol, and breath ethanol profiles were plotted for each subject. The peak concentration as well as the concentration of ethanol at 40 min postdrinking were noted. Because body fluids were collected exactly 10 min after the end of drinking, several subjects had residual alcohol in the mouth so that the peak concentrations of ethanol in breath and saliva were artificially too high. The elimination rates of ethanol from blood, saliva, and breath were established from the slopes of the postabsorptive descending parts of the ethanol profiles as described by Widmark (17). The pharmacokinetic parameters $C_p$ and $t_e$ correspond to the $y$-intercept and $x$-intercept, respectively; the apparent volume of distribution of ethanol ($V_d$) was derived from the ratio of the dose (0.68 g/kg) divided by $C_p$. The overall rate of disposal of alcohol was calculated from the dose (0.68 g/kg) divided by $t_e$.

Statistical Methods

The experiments furnished serial measurements of several variables in 21 subjects, e.g., concentrations of ethanol in blood, saliva, and breath as well as subjective and objective tests. The data were evaluated as recommended by Matthews et al. (18), with use of summary measures to depict the concentration–time profile for each subject. Blood–breath and blood–saliva ethanol relationships were compared by linear regression analysis and also by calculating the mean difference (bias) and the 95% limits of agreement as recommended by Bland and Altman (19). Impairment scores at various times after drinking were compared with each subject's baseline (predrinking) score. The differences in the impairment score from zero change was evaluated by Student's $t$-test for paired observations. Because of large variability in these measurements of impairment, logarithms (base 10) were taken, e.g., log(40 min) – log(baseline), and the statistical analysis was calculated in terms of logarithms. The slopes of regression lines were compared by analysis of covariance (20).

Results

Concentration–time profiles of ethanol. Figure 1 shows changes of ethanol concentration over time for capillary blood, saliva, and end-expired breath for each of the 21 subjects. The general shapes of these curves agreed well,
but large interindividual variations were evident. At 40 min after the end of drinking, the spread of values was 500–1090 mg/L (50–109 mg/dL, CV 17%) for blood alcohol, 570–1350 mg/L (57–135 mg/dL, CV 20%) for saliva alcohol, and 52–108 mg/210 L (CV 17%) for breath alcohol. The average concentration–time curves for blood, breath, and saliva after ingesting ethanol at 0.68 g/kg are shown in Figure 2.

**Blood ethanol parameters.** Table 1 reports the kinetic parameters of ethanol derived from the individual concentration–time profiles for blood, saliva, and breath.

The peak concentration of ethanol in saliva, the value at 40 min postdrinking, and the theoretical $C_0$ value were higher than the corresponding values for kinetics in blood and breath ($P < 0.05$). Otherwise, no differences in the pharmacokinetic parameters of ethanol were statistically significant. The peak BAC was reached within 10, 40, 70, and 110 min after the end of drinking for 9, 6, 4, and 2 subjects, respectively.

**Correlations between ethanol concentrations in blood, breath, and saliva.** Figure 3 shows scatter plots of the concentrations of ethanol in blood and breath (lower plot) and in blood and saliva (upper plot) for the specimens collected between 40 and 400 min after the end of drinking. The regression statistics are included within the same diagrams. As expected, the correlation coefficients were highly significant: $r = 0.97$ (blood–saliva) and $r = 0.98$ (blood–breath). The slope of the blood–breath scatter plot (1.039 ± 0.0134) was significantly greater than unity ($t = 2.9, P < 0.05$), and the y-intercept ($-2.89 ± 0.799$) differed significantly from zero ($t = 3.6, P < 0.05$). The slope of the saliva–blood regression line (1.095 ± 0.0188) was also significantly greater than unity ($t = 5.05, P < 0.001$), but the y-intercept (0.23 ± 1.12) was not significantly different from zero ($t = 0.21, P > 0.05$). The residual standard deviation ($S_{yw}$) was slightly less for the breath alcohol–blood alcohol data, being 4.2 mg/210 L compared with 59 mg/L (5.9 mg/dL) for saliva alcohol ($F = 1.97, P < 0.05$). Looking at these scatter plots, one notes a tendency towards higher residual variance at higher concentrations of ethanol in the specimens analyzed. Table 2 therefore gives the mean difference between the logarithms of the breath–blood and saliva–blood paired values and the 95% limits of agreement. The mean breath–blood and saliva–blood differences in alcohol concentration were therefore −4.2% and +9.4%, respectively. The 95% limits of agreement suggest that breath alcohol might range from 23% below to 19% above the corresponding BAC. Similarly, saliva alcohol might range from 12% below to 36% above the coexisting BAC.
Table 1. Pharmacokinetics of Ethanol Derived from Measurements in Blood, Breath, and Saliva

<table>
<thead>
<tr>
<th>Variable</th>
<th>Blood</th>
<th>Breath</th>
<th>Saliva</th>
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<tbody>
<tr>
<td>Peak EtOH conc&lt;sup&gt;a&lt;/sup&gt;</td>
<td>910 ± 160 (91 ± 16)</td>
<td>97 ± 7</td>
<td>1090 ± 240 (109 ± 24)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>EtOH conc at 40 min postdrinking&lt;sup&gt;b&lt;/sup&gt;</td>
<td>820 ± 140 (82 ± 14)</td>
<td>84 ± 15</td>
<td>890 ± 190 (89 ± 19)</td>
</tr>
<tr>
<td>Theoretical EtOH (C&lt;sub&gt;0&lt;/sub&gt;)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1000 ± 60 (100 ± 6)</td>
<td>99 ± 7</td>
<td>1100 ± 100 (110 ± 10)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Time to peak EtOH, min&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38; 10-100</td>
<td>36; 10-100</td>
<td>44; 10-100</td>
</tr>
<tr>
<td>Time to zero EtOH, min</td>
<td>494 ± 38</td>
<td>488 ± 38</td>
<td>499 ± 36</td>
</tr>
<tr>
<td>Disappearance rate of EtOH, units&lt;sup&gt;b&lt;/sup&gt;/h</td>
<td>120 ± 11 (12 ± 1.1)</td>
<td>12 ± 1.2</td>
<td>130 ± 25 (13 ± 2.5)</td>
</tr>
<tr>
<td>Apparent volume of distribution, L/kg</td>
<td>0.69 ± 0.038</td>
<td>0.69 ± 0.050</td>
<td>0.66 ± 0.058</td>
</tr>
<tr>
<td>Rate of disposal of EtOH, mg/kg per hour</td>
<td>83 ± 6.4</td>
<td>84 ± 6.7</td>
<td>86.8 ± 6.4</td>
</tr>
</tbody>
</table>

<sup>a</sup> From healthy men who drank ethanol, 0.68 g/kg body weight, in 20 min after an overnight fast.  
<sup>b</sup> mg/L (and mg/dL for breath and saliva), mg/210 L for breath.  
<sup>c</sup> *P < 0.01 according to analysis of variance.  
<sup>d</sup> Timed from end of drinking.  
<sup>e</sup> Mean; and range.

Subjective and objective tests of impairment. Figure 4 shows the time-to-time changes in mean body sway with open and closed eyes, hand tremor, and subjective feelings of intoxication. Shortly after the drinking ended, impairment of body function was apparent in all the performance tests. The maximum impairment seemed to coincide with the time of reaching the peak concentrations of ethanol in body fluids. However, the subjects quickly recovered from this acute state of impairment, with the rate of recovery depending on the particular test made. Body sway with open eyes recovered first and hand tremor remained impaired longest. A large between-subject variability was observed even before the ingestion of alcohol: CV = 36% for body sway with open eyes, 41% for body sway with closed eyes, and 49% for hand tremor.

Alcohol-induced effects on eye movements. Figure 5 (upper part) shows the number of subjects showing PAN and ROM after ingestion of ethanol, 0.68 g/kg. PAN occurred more often than ROM during the early phase of intoxication (60-120 min). By contrast, ROM was more evident between 120 and 420 min after drinking, when the acute effects of alcohol had worn off. The velocity of eye movements expressed as degrees/second for both PAN and ROM are shown in the lower part of Figure 5. These results support the qualitative observations made regarding the frequency of occurrence of PAN and ROM. One subject did not show any measurable PAN after ingesting the ethanol, 0.68 g/kg body weight.

Ethanol concentration thresholds for recovery from impairment. Figure 6 plots ethanol-induced impairment as a function of the concentration of ethanol in blood, breath, and saliva in tests made >70 min after the end of drinking. At this time, most subjects had already reached their peak alcohol concentration in blood. The ethanol concentration threshold for recovery from impairment was highest for body sway with open eyes and lowest for hand tremor. The feelings of being tipsy had vanished in most subjects when the BAC was ~700 mg/L (70 mg/dL) on average. Moreover, the ethanol

![Graph](image-url)

Fig. 3. Scatter plots and regression statistics for saliva alcohol vs blood alcohol (top), and breath alcohol vs blood alcohol (bottom).  
N = number of correlations; r = correlation coefficient; S<sub>xy</sub> = standard error estimate (given as a percentage of r)

Table 2. Mean Differences in Concentrations of Ethanol and 95% Range of Individual Differences for Breath vs Blood and Saliva vs Blood

<table>
<thead>
<tr>
<th>Comparison</th>
<th>n</th>
<th>Conc ratio&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breath vs blood</td>
<td>168</td>
<td>0.959&lt;sup&gt;a&lt;/sup&gt; (0.77-1.18)</td>
<td>-4.2 (-23 to 19)</td>
</tr>
<tr>
<td>Saliva vs blood</td>
<td>168</td>
<td>1.004&lt;sup&gt;a&lt;/sup&gt; (0.89-1.36)</td>
<td>+9.4 (-12 to 36)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Calculated after making logarithmic transformation. The antilogarithm of the mean log difference gives the geometric mean of the breath/blood and saliva/blood ratios.  
<sup>b</sup> Numerical values for mg/210 L vs mg/dL (breath vs blood) or mg/dL vs mg/dL (saliva vs blood).  
<sup>c</sup> Difference from zero statistically highly significant (P < 0.001).
Fig. 4. Mean (±SE) changes in body sway with open and closed eyes (sagittal direction), hand tremor, and subjective feelings of intoxication at various times after 21 healthy men drank ethanol, 0.68 g/kg, on an empty stomach.

* P < 0.05, ** P < 0.01, *** P < 0.001: significance of difference from baseline measurements.

concentration threshold depended on the body fluid analyzed, being about the same for blood and breath but slightly higher for saliva. Interestingly, the slopes of the impairment-concentration relationships ran parallel regardless of the body fluid analyzed, as shown by analysis of covariance (P > 0.05).

**Discussion**

More than 70 years ago, on the basis of experiments in animals and humans, Sir Edward Mellanby (21) made the following statement:

Now, although there is an unquestionable relationship between alcohol in the blood and intoxication, it is not certain that the relation between the two is close enough to allow the latter to be strictly determined from the former. This fact would not be of much importance if there were as accurate a measure of intoxication as there is of alcohol in the blood, but this is not the case.

This dilemma noted by Mellanby has been neatly sidestepped by introducing threshold limits of alcohol concentration in body fluids as evidence of impairment (3, 8). The suspect's blood or breath alcohol concentration is the sole deciding factor when a “per se” law operates; this kind of legal framework, therefore, makes it unnecessary to show that a person's performance and behavior or ability to operate safely a motor vehicle were impaired by alcohol. The magnitude of behavioral impairment after drinking depends on the nature of the performance task, the pharmacokinetics of ethanol (rising or falling BAC), and the individual's development of tolerance to the effects of ethanol on the central nervous system (22–24). Some investigators advocate and sup-

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**Fig. 5.** Occurrence of positional alcohol nystagmus (PAN) and roving ocular movements (ROM) after subjects drank ethanol, 0.68 g/kg (upper graph), and the intensity of PAN and ROM (degrees/s) at various times after the start of drinking (lower graph).
port the use of behavioral tests of impairment or "under the influence" as opposed to the use of chemistry-based enforcement. The results reported here lead me to conclude that the enforcement of driving under the influence laws based on behavioral tests of impairment as a means of detection would prove much less effective and hinder the prosecution of drunk drivers. This problem will become increasingly evident with the trend toward lowering the prohibited blood or breath alcohol limits for driving, thus making sobriety testing in the field ineffective. Nevertheless, a dose–response relationship between the concentration of ethanol in body fluids (blood and breath) and the risk of involvement in a traffic accident is well established (25). Moreover, a multitude of epidemiological surveys confirm the overrepresentation of drunk drivers in fatal traffic accidents (26).

The way that ethanol becomes absorbed, distributed, and eliminated from the body can be monitored from serial measurements in specimens of blood, breath, or saliva. However, the saliva ethanol content is about 9% higher than that of capillary blood, presumably because of the differences in water content: 850 g/L in whole blood and 990 g/L in saliva (27). The ratio of blood flow to tissue mass for the salivary glands is very large, and any differences in the concentrations of ethanol attributable to nonequilibrium distribution of ethanol should be small or negligible (28, 29). The passage of alcohol into saliva and the good correlation with BAC was demonstrated during the 1930s (30). However, except for certain research projects, saliva has not been used much as a body fluid for measuring alcohol (8). The early chemical methods of analysis required fairly large volumes of specimen, which might explain this lack of enthusiasm for saliva. Newman and Abramson (31) measured the concentrations of ethanol in serial samples of blood and saliva and found a close agreement between the results. They also saw a rapid development of tolerance to the effects of ethanol, as reflected in target-shooting scores quickly recovering to baseline values even though the concentrations of alcohol in blood and saliva still exceeded 1000 mg/L (100 mg/dL). Their classic study supports the use of saliva as a substitute for blood in pharmacokinetic studies and therefore also as an indirect measure of ethanol-induced impairment.

In the experiments reported here, in which the volunteers drank neat whisky on an empty stomach, the rate of absorption of ethanol occurred so fast that a concentration threshold for onset of impairment could not be established with certainty. However, it was strikingly obvious that the maximum impairment coincided with the time of reaching the peak concentrations of ethanol in blood, breath, and saliva. On reaching the postpeak phase of ethanol kinetics, the degree of impairment rapidly diminished. This held for subjective estimates of intoxication as well as the various objective tests made. The threshold limits of ethanol concentration for when a person's test performance recovered to baseline values were about the same for blood and breath alcohol, but was slightly higher for saliva. More importantly, the rates of recovery from ethanol-induced impairment, as reflected in the slopes of the alcohol concentration–impairment relationships, were about the same regardless of the body fluid analyzed.

The battery of performance tests used in these exper-
The document discusses the effects of ethanol on driving ability. It mentions that the rate of ethanol in the body can be determined through various methods, such as breath tests or blood tests. The relationship between BAC and driving ability is also explored, with emphasis on the importance of understanding the interindividual differences in how alcohol affects different individuals.

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References: