Differences between Capillary and Venous Blood-Alcohol Concentrations as a Function of Time after Drinking, with Emphasis on Sampling Variations in Left vs Right Arm

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Twelve healthy men drank 0.8 g of ethanol per kilogram of body weight during 30 min after an overnight (10 h) fast. At nine exactly timed intervals (30–390 min after the start of drinking), blood was sampled through indwelling catheters in cubital veins on the left and right arms. Immediately thereafter, capillary blood was sampled from fingertips on the left and right hands. The blood ethanol concentration (BAC) was determined by headspace gas chromatography. The SD for alcohol determinations in venous blood, including the left vs right arm sampling variation, was 30 mg/L (range 8.3–83 mg/L), whereas for capillary blood the SD was 35 mg/L (range 11–60 mg/L). This difference much exceeded the purely analytical errors: SD = 2.67 mg/L for venous blood and 14.2 mg/L for capillary blood. During the first 60 min after the subjects started to drink, capillary BAC exceeded venous BAC, the mean difference at 30 min being 136 mg/L (range 36–216 mg/L). In the postabsorptive state later than 60 min after drinking, venous BAC exceeded capillary BAC [mean difference 58 mg/L (range 0.0–170 mg/L)], the values for venous and capillary BAC crossing 37 min (range 6–77 min) after the end of drinking. Apparently, the source of blood analyzed, venous or capillary, must be considered in clinical pharmacokinetic studies of ethanol.

Most clinical biochemistry tests involve the use of whole blood, plasma, or serum as the biological matrix. For some substances, such as glucose and lactate, the results depend in part on whether venous blood or capillary blood is analyzed (1, 2). The concentration of ethanol in blood is rarely reported with reference to the source of blood analyzed, i.e., artery, capillary, or vein. Ethanol distributes into the total body water without binding to plasma proteins and is cleared from the body chiefly by metabolism in the liver (3). In routine analytical work, scant attention has hitherto been given to possible differences in concentration of ethanol in different vascular compartments, or to the significance this might have in medicolegal practice. The precision of blood-ethanol analysis is usually well defined, being derived from the variance of replicate determinations, with aliquots taken from the same pool of blood in one single collection tube submitted to the laboratory for analysis (4).

Automated methods of forensic blood-ethanol analysis have coefficients of variation (CV) of <1%, so instead of attempting to reduce analytical errors of the method even further, investigators should turn their attention to the importance of sampling variations (5). Statutory limits for blood-alcohol concentration (BAC) in motorists are rigorously enforced, regardless of the way in which the forensic blood specimens were obtained (6). The components of variation introduced during sampling are generally considered to be negligible or zero. The widespread use of per se statutory

BAC limits as evidence of impairment means that small increments in BAC make the difference between punishment or acquittal in borderline cases (6). The significance of variations in blood source and sampling site, in relation to the concentration of alcohol, must be carefully documented.

The aim of the present work was to establish the time course of alcohol concentrations in specimens of venous and capillary whole blood during absorption, distribution, and elimination stages of ethanol metabolism. We obtained near-simultaneous specimens of cubital-vein blood from both arms and capillary blood from fingertips on both hands. Thus we could resolve the magnitude of variation caused by the sampling procedure from that of the purely analytical errors.

Materials and Methods

Subjects and Conditions

The 12 healthy men who took part in this study as paid volunteers were all medical students accustomed to moderate drinking: mean age 27.8 y (range 21–43), mean body weight 74.8 kg (range 71–81), and mean height 183 cm (range 180–193). The experiments started at about 0815 hours after an overnight fast (10 h). Indwelling catheters were inserted in a proximal direction into medial cubital veins on the right and left arms. The catheters were kept patent by flushing with isotonic saline containing a few units of heparin. Specimens of venous blood were collected into 5-mL Vacutainer Tubes (Becton Dickinson, Rutherford, NJ 07070) containing 20 mg of NaF and 75 int. units of sodium heparin.

The subjects drank 0.8 g of ethanol per kilogram of body weight during 30 min. The drink was prepared from 950 mL/L ethanol reagent, which we diluted fourfold with an orange-flavored drink to make a "cocktail." Two or three subjects took part in each experimental session. Drinking was begun by successive subjects at 15-min intervals, starting at about 0900 hours.

Procedures

Blood sampling. Specimens of venous blood were obtained at exactly 30, 60, 90, 120, 150, 180, 210, 270, 300, and 390 min, timed from the start of drinking, with the subjects lying quietly on a bed. Two assistants drew venous blood at exactly the same time from left- and right-arm cubital veins. Immediately thereafter (within about a minute), capillary blood was obtained from a fingertip on the left and right hands. Getting samples of capillary blood took slightly longer than venous-blood sampling. After the fingertips were pricked with sterile mini-lancets, the free-flowing blood (100 mg) filled Widmark S-shaped capillary tubes, the open ends of which were then closed with small rubber stoppers. The inside walls of these glass tubes are coated with fluoride and oxalate as preservative and anticoagulant.

This sampling protocol gave us 10 specimens of venous blood and 10 capillary blood specimens from the left and right arms of each test subject.

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**Determination of ethanol in blood.** The concentration of ethanol in venous whole blood was determined by headspace gas chromatography (HS-GC) after 11-fold dilution with an aqueous solution of n-propanol (80 mg/L), which served as internal standard. The contents of the Vacutainer Tubes were mixed for about 15 min on a Rotamix mixer before we removed 100-μL aliquots for dilution with internal standard, using a fixed-volume dilutor dispenser. The diluted blood was dispensed directly into 22-mL headspace vials, which we then closed with a rubber septum and made airtight with a crimped-on aluminum cap, in preparation for analysis.

Before analysis of capillary blood, we removed the rubber stoppers from each Widmark tube and recorded the weight of the tube plus blood to the nearest milligram. The blood was then ejected from the Widmark tube into a small test tube containing exactly 200 μL of distilled water. We then reweighed the empty Widmark tube and calculated the amount of blood taken for analysis and the dilution factor. After this initial dilution, we again diluted the hemolyzed blood specimen 11-fold with n-propanol, exactly the same as for venous blood specimens. The capillary blood specimens were stored at 4 °C until analysis the next day. The 11-fold dilution of whole blood before analysis helps to abolish any effects of ethanol on the matrix during the headspace equilibration procedure, so that aqueous ethanol standards can be used for calibration.

**Gas-chromatographic analysis.** We used a Sigma 2000 gas chromatograph with flame ionization detector and HS-100 headspace analyzer (all from Perkin-Elmer Corp., Norwalk, NJ) for measuring blood ethanol. The results were recorded by an LCI-100 electronic integrator (Perkin-Elmer). The stationary phase was Carbopack C (80–100 mesh) coated with 0.2% Carbowax 1500 and held in a 2 m × 3 mm (i.d.) glass column maintained at 110 °C. Hydrogen (30 mL/min) and air (300 mL/min) were supplied to the detector; nitrogen was the carrier gas (flow rate, 20 mL/min). Under these conditions the retention times of ethanol and n-propanol were 0.78 min and 1.62 min, respectively; the peak height ratios for ethanol/n-propanol were used for quantitative analysis.

All specimens of blood collected in this project were analyzed with the same HS-GC unit. The instrument was not switched off during the period of the study, which lasted several weeks, and recalibration was unnecessary between runs. The GC unit was first calibrated by single-point calibration based on the average result of six replicate determinations of a 100 mg/dL ethanol standard to calculate a calibration factor. This was subsequently used to derive a response factor to calculate unknown blood ethanol concentrations and standards of known concentration (50, 100, or 150 mg/dL) analyzed after every tenth unknown blood specimen. These solutions (Merck Ltd., Darmstadt, F.R.G.) were supplied already diluted to the stated concentrations in flame-sealed borosilicate glass ampules. The difference in concentration between the known concentrations of the standard (target value) and the results determined by analysis was defined as the "residual," with an expected value of zero. The average residual was taken as an index of accuracy (systematic error) of the blood-alcohol measurements. The response of the detector was linear over a wide concentration range, and the regression line passed through zero.

**Control analysis of capillary blood.** To check the results obtained from analysis of capillary blood against those of venous blood and to assess the precision of the method without the influence of left vs right arm sampling differences, we designed a small experiment. Empty Widmark capillary tubes were filled in duplicate with blood taken from 20 different specimens in Vacutainer Tubes, gathered during this study. These duplicate determinations were used to get a measure of the analytical error inherent in capillary blood assays by eliminating biological sampling variations. The venous blood specimens used to fill the capillaries were also analyzed in duplicate within the same run.

**Statistical analysis.** The time course of changes in venous and capillary BAC was plotted for each subject, and the peak BAC and the time elapsed from end of drinking to the peak concentration was read from the curves. The times at which the mean capillary BAC (left and right hands) exceeded that of the mean venous BAC (left and right arms) were noted for each subject. Differences between left arm and right arm, left hand and right hand, and venous vs capillary BAC were evaluated by Student's t-test for individual comparisons. Analytical variations were calculated, as usual, from differences between duplicate determinations on the assumption of a gaussian distribution of random errors. The SD of a single determination was worked out from the variance of this mean difference between duplicates. Variances were compared by F-test (7).

**Results**

**Accuracy and Precision**

**Accuracy of the gas-chromatographic method.** The mean difference (residual) between concentrations of ethanol determined in the control standards and the target values of 50, 100, and 150 mg/dL was -0.137 (SD 0.625) mg/dL. The difference from zero was not statistically significant (P < 0.05). These results were derived from 72 determinations made during the entire time that blood samples were being analyzed. Systematic errors of the HS-GC method are therefore negligible.

**Precision of analyzing alcohol in venous and capillary blood.** The mean difference between duplicate determinations of venous blood was 0.11 (SD 0.379) mg/dL, and therefore a single assay has an SD of 0.267 mg/dL. The mean difference between duplicate capillaries filled with venous blood from 20 Vacutainer Tubes was 0.49 (SD 2.02) mg/dL. The SD of a single determination was therefore 1.42 mg/dL. The precision of analyzing venous blood is considerably higher than for capillary blood (F = 18.9, P < 0.001).

**Precision of alcohol analysis, including sampling variations.** The differences in concentration of alcohol in blood from left and right arms were not significant between 30 and 390 min after drinking. This held for both venous and capillary specimens, as shown in Figure 1. The SD of the analysis was highest during the absorption phase 30–90 min after drinking. The 30-min venous blood specimen had an SD of 8.3 mg/dL compared with 1.6 mg/dL at 90 min, when BAC was similar, and 1.0 mg/dL at 390 min, when the mean BAC was lower. The difference in variance between 30-min and 90-min samples was statistically significant (F = 26.9, P < 0.001). The maximum SD for capillary blood was 6.0 mg/dL, which occurred at 60 min after drinking, compared with 3.6 mg/dL at 90 min and 1.1 mg/dL at 390 min. The difference in variance between the 30-min and 90-min samples was not significant (P > 0.05).
Concentration–Time Profiles

Alcohol in venous blood. Figure 2 (left) shows the mean concentration–time profiles of ethanol for venous blood specimens obtained from left- and right-arm veins. These measurements agree well on average, although five of the 12 subjects had small but systematic differences in concentration between both arms. During the postabsorptive state, the mean left-right arm difference for these five subjects was 1.4 (SD 0.89) mg/dL (P <0.01).

Alcohol in capillary blood. Figure 2 (right) shows the mean concentration–time profiles of ethanol for fingertip capillary blood obtained from the left and right hands. In some individuals there were marked left vs right hand differences in capillary BAC at certain sampling times, but this might be associated with problems in getting adequate blood for analysis. The mean-value curves agreed well.

Capillary vs venous blood-alcohol. Figure 3 shows mean venous BAC (left and right arms) and mean capillary BAC (left and right hands) plotted as a function of time after drinking. These time profiles differ, depending in part on sampling time after drinking. During rapid absorption of alcohol from the gut, i.e., 30–60 min after the start of drinking, the capillary BAC exceeded the venous BAC. The capillary–venous difference was 13.6 (SD 7.8) mg/dL at 30 min and 3.3 (SD 6.9) mg/dL at 60 min after the start of drinking. Later than 60 min, the curves for venous/capillary samples crossed, and for the remainder of the elimination phase the venous BAC exceeded the capillary BAC; between 90 and 390 min, the mean difference was 5.8 (SD 3.4) mg/dL. The differences between capillary and venous BAC were significantly different from zero except at the 60- and 90-min sampling intervals. The mean inter-individual differences are plotted as a function of time in Figure 4. The mean crossover point for capillary and venous BAC was 37 min after the end of drinking (range 6–87 min), which corresponds to 67 min (range 36–117 min) from the start of drinking (Figure 4).

Discussion

Impairment by alcohol is a common cause of accidents at work and on the highway (8), and reliability of chemical tests for intoxication is often the subject of much controversy and debate (9, 10). Statutory BAC limits for drivers refer to whole blood as the biological specimen, but the source of blood—i.e., artery, capillary, or vein—is usually not defined precisely (6).

Looking at the forensic alcohol literature, one finds that the source of blood was recognized early as a complicating factor that might account for discrepancies in reported BAC results (11). Specifically, large differences were observed if venous BAC was compared with arterial BAC or with the results obtained indirectly from analysis of breath when alcohol was being absorbed from the gut (12). But this important forensic science issue has received scant attention in recent years, and it is widely understood that, in the postabsorptive state, the capillary, venous, and arterial BAC's are approximately equal and that any differences are negligible or zero. A paper by Sedman et al. (13) questioned...
this notion, but their findings have failed to influence blood sampling routines or the interpretation of BAC results in forensic science practice. More recently, Martin et al. (14) compared venous and arterial BAC after subjects drank a bolus dose of ethanol 5 h after their last meal. Clear-cut arteriovenous differences in BAC were reported during absorption of alcohol from the gut, but later on, during the elimination phase, fairly good agreement was found between the source of blood and the alcohol concentration.

Ethanol is completely miscible with water and distributes into the water compartment of body fluids and tissue (2). During absorption of alcohol from the gut, the arterial BAC equilibrates with the water fraction of all body fluids and tissue according to an exponential function. The speed of the distribution process is determined in part by the blood flow (F) and the size of the distribution volume (V), and the ratio V/F expresses the equilibration time constant. For the whole body, F/V is about 0.07 L/(min × L of tissue), which corresponds to a time constant of 14 min and a half-time of 10 min for the equilibrium process (15–17). This suggests that if BAC changes rapidly as compared with the speed of equilibration of alcohol between blood and tissue water, a significant arterial–venous concentration gradient should exist. Furthermore, the arteriovenous difference will depend on the rate of blood flow and the vascularity and mass of tissue involved. The significance of nonequilibrium between blood and tissue and fluctuations in blood flow on the magnitude of arteriovenous differences were analyzed in detail by Zierler (15). Except for situations with a restricted skin perfusion, capillary fingertip blood will be more representative of arterial BAC. The different time profiles for ethanol in capillary and venous BAC can be attributed, at least in part, to the distribution kinetics of ethanol.

The large superficial cubital veins drain blood from surface tissue as well as deep tissue in the forearm and hand. The venous anatomy is not necessarily identical on both sides, and the relative contributions of blood from surface and deep tissue might differ, depending on the sampling site on each arm. We found that five of 12 subjects had small but systematic differences in BAC between samples from their right and left-arm cubital veins. These differences ranged from 0.8 to 2.4 mg/dL in the postabsorptive phase. One explanation for this might be different perfusion-distribution volume ratios for the vascular areas being drained into the particular veins catheterized. We found a significantly higher variance in left vs right arm differences 30 min after drinking compared with 90 min, presumably because the BAC changes much faster shortly after drinking (30-min specimen) when alcohol is still being rapidly absorbed from the gut. A different perfusion-distribution volume ratio on each side will exert a greater influence at 30 min after drinking than at 90 min.

The BAC differences in various parts of the venous system complicate the interpretation of results for legal purposes. The contribution of sampling errors to uncertainties in reported BAC has been touched upon by others but never clearly established. Several studies from Germany have focused on left vs right arm differences in concentrations of ethanol (18–21). One study (18) reported that there were no physiological BAC differences between left and right arms and that the maximum deviation was only 1 mg/dL. In contrast, Naeve et al. (19) reported significant left vs right arm differences, which were most pronounced when alcohol was being rapidly absorbed from the gut.

During the absorption phase of the BAC time profile the concentration of alcohol in the arterial blood is higher than in the venous blood and the magnitude of the arteriovenous difference depends in part on the rate of absorption of alcohol from the gut: rapid absorption exaggerates the difference; slow absorption minimizes it. During the postabsorptive state the alcohol concentration gradients between blood in artery and vein are reversed and venous BAC now exceeds arterial and capillary BAC. The onset of the postabsorption period, as reflected in the time of crossover for capillary and venous BAC, began at 36 to 107 min (mean, 67 min) after subjects began drinking and therefore 6 to 77 min (mean, 37 min) after the drink was finished (drinking time, 30 min). A difference in the water content of venous and capillary blood might provide an explanation for the different BAC in postabsorptive samples. However, on the basis of blood hematocrit values and hemoglobin measurements, the differences in water were considered negligible under proper conditions of sampling (12, 13). A recent paper, however, reports a 3% higher hematocrit for capillary blood as compared with venous blood (22).

When BAC measurements are used for legal purposes, it seems advisable to make an adjustment to the average result of several determinations to compensate for inherent analytical and sampling variations. The amount subtracted will depend on the accuracy and precision of the analytical techniques used and the experience of the analysts involved. The degree of confidence in the final prosecution BAC—whether set at 95%, 99%, or 99.9%—will also determine the size of the subtraction term. These levels of probability imply that a person with a true BAC just below the statutory limit for driving runs a 1 in 20, 1 in 100, or 1 in 1000 chance, respectively, that the reported BAC exceeds the true value. Results of BAC measurements for legal purposes in Sweden are reported as not less than the prosecution value with 99.9% confidence. Litigation in driving-while-intoxicated trials in Sweden and defense challenges directed at the reliability of forensic alcohol analysis are virtually nonexistent.

The systematic differences observed between venous and capillary BAC is a more difficult problem to tackle, because this depends in part on the phase of alcohol metabolism when the samples are taken. This will require some careful consideration by forensic experts called upon to evaluate blood-alcohol measurements for legal purposes. We conclude that both analytical precision and sampling-site variations should be defined precisely and the source of blood obtained specified on the analytical report.

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References

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