*Amanda J. Jenkins,*[1] *Barry S. Levine,*[1] *and John E. Smialek*[1]

# Distribution of Ethanol in Postmortem Liver

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**ABSTRACT:** The analysis of multiple specimens for ethanol has become a necessary and accepted practice in postmortem forensic toxicology. The correlation between blood and various body fluids has been well documented. However, there is little data on the distribution of ethanol in specimens such as the liver. In postmortem cases where blood is unavailable or contaminated, liver may be used for alcohol and drug analyses. This study reports the analysis, by head space gas chromatography, of heart blood and liver specimens for ethanol from 103 postmortem cases. The average liver/heart blood ratio in cases with a blood alcohol level (BAC) ≥0.04 g/dL was 0.56, SD = 0.30, with a range of 0–1.40.

**KEYWORDS:** toxicology, ethanol, chromatographic analysis

The analysis of multiple specimens for ethanol has become a necessary and accepted practice in postmortem forensic toxicology laboratories [*1*]. The analysis of heart blood for ethanol should be conducted on all postmortem cases, whether or not an autopsy is performed. If the blood ethanol concentration (BAC) is greater than a pre-determined cut-off, such as 0.01 or 0.02 g/dL, then the analysis of additional specimens such as peripheral blood, vitreous humor and urine would be required. There are several reasons for this practice. The analysis of these specimens can provide information about the absorptive status of the individual. This may be important when an estimate of a blood ethanol concentration some time prior to death is required. In addition, vitreous humor and urine, two specimens resistant to the putrefaction process [*2*], can provide information as to whether the measured BAC resulted from antemortem consumption or postmortem formation. A positive BAC in combination with a negative vitreous humor and urine ethanol concentration would strongly suggest postmortem ethanol formation in the blood [*3*]. In addition, the analysis of multiple specimens enables the identification of contaminated samples.

One specimen often analyzed in postmortem forensic toxicological analysis is liver. Recent work has indicated that the analysis of tricyclic antidepressants in liver can assist in the interpretation of blood postmortem tricyclic antidepressant concentrations [*4*]. Surprisingly, little work has been published on the distribution of ethanol in liver. The following is a compilation of data collected to ascertain the distribution of ethanol in liver specimens.

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[1]Office of the Chief Medical Examiner, State of Maryland, Baltimore, MD.

## Experimental

### *Specimen Collection*

Blood and liver specimens were collected from autopsies performed by pathologists of the Office of the Chief Medical Examiner, State of Maryland. Specimens were collected in 140 mL plastic containers without preservative. The site of blood collection was the heart, but the specific anatomical region was unknown. Similarly, the specific site of liver sampling was not provided by the pathologists. Specimens were refrigerated at 4°C between autopsy and analysis.

### *Specimen Preparation*

0.5 mL of heart blood was diluted with 4.5 mL of a 0.02% internal standard solution (n-propanol) and pipetted into a 23 mL head-space vial. 2 g of liver were homogenized in 18 mL of 0.02% n-propanol and 5 mL of the homogenate pipetted into a head space vial. This resulted in a liver ethanol concentration in units of grams of ethanol per deciliter of homogenate (g/dL). All specimens were analyzed in duplicate. Duplicate analysis of liver homogenates in this laboratory routinely show variation of <10%.

### *Instrumentation and Chromatographic Conditions*

A Perkin-Elmer 8500 gas chromatograph with a flame ionization detector interfaced with a HS-101 head space analyzer was used for ethanol analysis. Specimens were equilibrated for 35 minutes prior to gas chromatographic analysis. The stainless steel column (10 ft × $^1/_8$ inch internal diameter) was packed with 0.2% Carbowax 1500 on 80/100 Carbopak C. The instrument parameters were as follows: needle temperature, 90°C; transfer line temperature, 90°C; oven temperature, 120°C; detector temperature, 250°C; pressurization time, 0.5 min; injection time, 0.08 min; withdrawal time, 0.2 min, carrier gas, helium; flow rate, 30 mL/min. For each run, the instrument was calibrated utilizing an aqueous ethanol standard prepared in-house and validated by a U.S. National Institute of Standards and Technology Alcohol Reference Standard Solution.

### *Quantitation*

The area ratio of ethanol to internal standard of the specimen was compared to aqueous ethanol standards. Standards were diluted with internal standard in the same manner as the specimens. Values less than 0.01 g/dL were reported as negative; values above 0.01 g/dL were reported to 2 significant figures.

## Results

The heart blood and corresponding liver specimens from 103 postmortem cases were analyzed. Ethanol concentrations in blood

ranged from 0.01–0.54 g/dL, and in liver ranged from 0–0.41 g/dL. The results were divided into two groups: those cases with a BAC <0.04 g/dL and those with a BAC ≥0.04 g/dL. It was felt that a BAC <0.04 g/dL could be attributed to decomposition [3]. In this study there were 32 cases with a BAC <0.04 g/dL; in 21 (66%) of these cases, the liver ethanol concentration was <0.01 g/dL, the limit of quantitation of the assay. In this group, the highest liver ethanol concentration was 0.06 g/dL, with a BAC of 0.03 g/dL.

The distribution of liver to heart blood ratios for the 71 cases with a BAC greater than or equal to 0.04 g/dL is shown in Fig. 1. The average liver/heart blood ratio was 0.56 with a standard deviation of 0.30. The ratios ranged from 0 to 1.40, with a median of 0.53. Seventy-six percent of the cases had liver to blood ratios between 0.26 and 0.86. Figure 2 illustrates the correlation between liver and heart blood ethanol concentrations for the 103 cases. The data is described by the straight line, y = 3.8246e-2 + 1.2693x, $r^2$ = 0.716. The standard error of the regression [a measure of the amount of error in predicting a concentration of ethanol in blood, (y) given an individual liver ethanol concentration, (x)] was determined to be 0.06 g/dL. Results from all 103 cases were utilized in this calculation, including possible outliers and those cases in which the BAC was ≥0.01 g/dL and the corresponding liver specimen was negative.

Six cases were studied in which the cause of death was reported as Acute Ethanol Intoxication. The ethanol concentrations in heart blood and liver are shown in Table 1. The ethanol concentration in heart blood and liver in these cases ranged from 0.3–0.54 g/dL and 0.14–0.41 g/dL, respectively. The average liver/heart blood ratio was 0.65, with a range of 0.47–0.85.

## Discussion

Numerous studies have documented the correlation between ethanol concentrations in blood and other body fluids such as vitreous humor, urine, saliva and cerebrospinal fluid. The body fluid/blood ratios of these specimens have been reported as 1.17 [3], 1.29 [5], 1.10 [6] and 1.19 [7], respectively. However, few studies have investigated the distribution of ethanol in organs such as the brain, liver and kidney. Ethanol distributes according to the water content of the body fluid or tissue. Harger et al. [8] examined the distribution of ethanol in dogs in various organs and biological fluids. These investigators determined that the water content of whole blood was 78.6%, and that of the liver was 73.2%. In addition, they empirically determined a liver/blood distribution ratio of 0.797 after equilibrium was reached. This is higher than the average ratio of 0.56 determined in the present study. One possible explanation is a species difference. Alternatively, the ethanol distribution in the postmortem specimens may not have been at equilibrium. However, the liver is assumed to reach equilibrium with the blood rapidly after ethanol absorption due to its rich blood supply [9]. Harger et al. [8] found no change in liver/blood ratios in dogs from 5 min to 12 h after ethanol administration. In addition, the liver is the site of the majority of ethanol metabolism (95%) and this could result in a reduction of the liver ethanol concentration and thus decrease the ratio. Also, storage conditions may have resulted in the loss of ethanol from the liver. The liver specimens were refrigerated at 4°C until analysis. The maximum time interval between collection and analysis was one month. The headspace of the plastic specimen containers was not determined, the specimens were sampled for other toxicological analyses a maximum of once and the majority of the containers were full, with approximately 120 g of specimen. The authors are unaware of any published studies in which the loss of ethanol over time in the liver due to

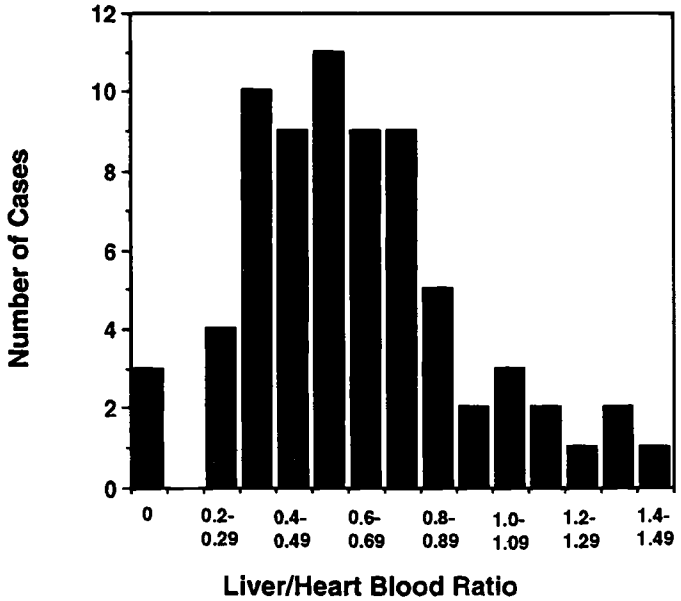


FIG. 1—*Distribution of liver/heart blood ratios from cases with a BAC ≥0.04 g/dL* (N = 71).

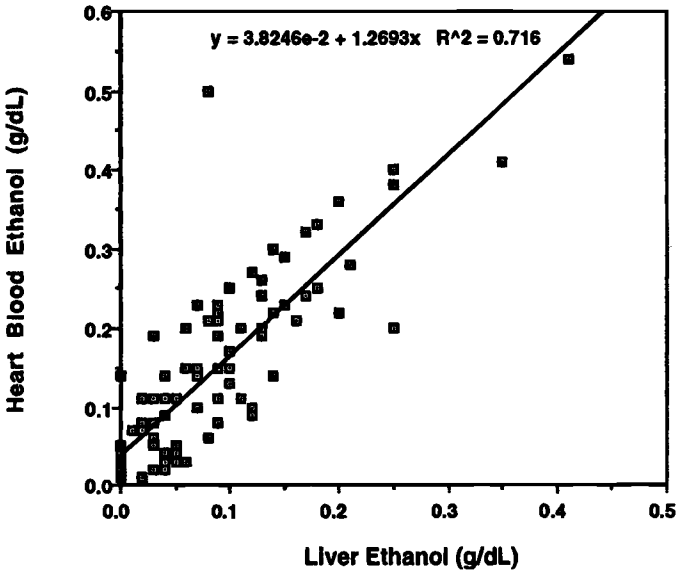


FIG. 2—*Correlation between liver and heart blood ethanol concentrations* (N = 103).

TABLE 1—*Heart blood/liver ethanol ratios in six cases of acute ethanol intoxication.*

| Sample No. | Blood Ethanol (g/dL) | Liver Ethanol (g/dL) | Liver/Blood Ratio |
|---|---|---|---|
| 1 | 0.30 | 0.14 | 0.47 |
| 2 | 0.32 | 0.17 | 0.53 |
| 3 | 0.38 | 0.25 | 0.66 |
| 4 | 0.40 | 0.25 | 0.63 |
| 5 | 0.41 | 0.35 | 0.85 |
| 6 | 0.54 | 0.41 | 0.76 |

storage conditions has been evaluated. The loss of ethanol in blood samples has been previously reported [*10*] and may occur from physical loss, and chemical or microbial mechanisms. In the present study all heart blood samples were analyzed within 24 h of collection.

In the present study, the mean liver/blood ratio in six cases of acute ethanol intoxication was 0.65. This is in agreement with the work of Christopoulos et al. [*11*] in humans, who reported an average liver/blood ratio of 0.60 ± 0.056 in 10 cases of acute ethanol intoxication (BAC >0.49%) with a range of 0.54–0.72. In the same study, liver/blood ratios from 13 cases of violent death averaged 0.63 ± 0.10, with a range of 0.51–0.83.

Liver is a specimen usually readily available at autopsy. In cases of trauma or when blood samples are contaminated, it may be the only specimen available for toxicological analysis. This underscores the importance of conducting postmortem studies of drug (including ethanol) disposition in this organ.

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Address requests for reprints or additional information to
Barry S. Levine, Ph.D.
Office of the Chief Medical Examiner
111 Penn St.
Baltimore, MD 21201-1020