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Concentrations of Ethanol in Two Segments of the Vascular System

The determination of ethanol is probably one of the most frequently requested tests in the forensic or clinical toxicology laboratory [*1*]. Large differences in ethanol distribution between whole blood and various human tissue have been reported [*2*] as well as ethanol concentration gradients within human and animal vascular systems [*3–8*]. Forney [*9*] reported that during the absorption-distribution phase, after the oral administration of ethanol, the "concentration of alcohol in arterial blood may be 50 to 100 percent higher than it (is) in venous blood." During this time blood from an arm vein will not truly reflect the concentration of ethanol supplied to the brain, where its effects are produced. These vascular ethanol concentration gradients generally have been ignored, possibly because the reports of other authors [*3–6*] have indicated that the arteriovenous (A/V) equilibrium of ethanol is attained rapidly in man when ethanol is administered by rapid intravenous infusion. Dundee et al [*4*] stated that after oral administration the A/V ratio reaches unity much more slowly than when alcohol is given intravenously. Harger [*10*] pointed to errors from using venous blood during the absorption phase after oral administration of ethanol. This work substantiates and extends the capillary-venous ethanol concentration differences reported in the literature and illustrates that equal capillary and venous ethanol concentration may not be achieved readily after the oral administration of ethanol to man.

Experimental

Assay Procedure

Preparation of Biological Samples—Venous blood samples were collected in heparinized Vacutainers® (Becton, Dickinson and Co., Rutherford, N.J.), or capillary blood samples were collected in precalibrated 20-μl capillary tubes (Corning Glass Works, Corning, N.Y.), or both. An internal standard solution of *n*-propanol, 1 mg/ml, and sodium heparin (sodium heparin U.S.P., Riker Laboratories, Northridge, Calif.), 40 units/ml, was prepared by diluting 1.25 ml of *n*-propanol and 40 000 units of sodium heparin to 100 ml with deionized water in a volumetric flask, transferring 5 ml of this solution to a 50-ml volumetric flask, and diluting to volume with deionized water. Twenty

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microlitres of each blood sample was transferred to a 400-μl Eppendorf tube (Scientific Products, Romulus, Mich.) containing 20 μl of internal standard solution. The contents of each tube were thoroughly mixed on a vortex mixer and stored at 4°C (40°F) until assayed. All blood samples were assayed the same day that they were drawn by the gas-liquid chromatography (GLC) method. One to seven microliters of each sample was injected onto the gas chromatographic column.

Preparation of Ethanol Reference Standards—Reference standard solutions of ethanol were prepared by the serial dilution of absolute ethanol (reagent grade; U.S. Industrial Chemicals Co., New York, N.Y.) with blood-bank whole blood. Concentrations of 0.0040 to 1.6 mg/ml ethanol were prepared. Reference samples were analyzed as described above.

Gas Chromatography—The assay was performed on a gas chromatograph (Varian model 2100; Varian Aerograph, Walnut Creek, Calif.) equipped with a flame ionization detector. Two 6 ft (1.8 m) by 3.5-mm inside diameter, U-shaped columns were packed with 80 to 100 mesh styrene divinyl benzene polymer (Porapak Q; Waters Associates Inc., Framingham, Mass.) and conditioned at 250°C (482°F). Two inches of silanized glass wool were packed at the injection port ends of the columns to prevent column contamination by whole blood. Operating conditions were as follows: column temperature, 150°C (302°F); detector temperature, 200°C (392°F); injection port temperature, 160°C (320°F); nitrogen (carrier gas) flow, 30 cm^3/min; air flow, 225 cm^3/min; and hydrogen flow, 30 cm^3/min.

Oral Administration of Ethyl Alcohol to Man

Two adult male subjects with no known disease who weighed 155 and 173 lb (69 and 78 kg) and who were both 28 years of age were given 30 ml of absolute ethanol orally (0.34 and 0.30 g/kg) in 6 fl oz (0.17 litre) of orange juice. Each subject had fasted from 10:00 p.m. the night before the alcohol was administered. Neither subject had received any barbiturates or other known enzyme-inducing agents for a period of 30 days preceding the study. They had received no other medication nor alcoholic beverages for a period of 3 days before the study. The doses of ethanol were swallowed as rapidly as possible by the subjects, and an electronic digital clock was started at the midpoint of alcohol ingestion. No food or beverages were taken until 3 h after the ingestion of the alcohol in the orange juice. Starting from 3 h after drinking the alcohol, food and non-alcoholic beverages were taken ad libitum. Capillary and venous blood samples were collected simultaneously at 0, 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 120, 150, 180, 210, and 240 min. Blood sampling was performed as follows.

Collection of Whole Capillary Blood Samples—For each sample a finger was wiped with 70% isopropanol in water, then wiped twice with dry cotton, and then lanced. The first drop of blood was wiped off with cotton. The sample of blood was collected rapidly in a precalibrated, 20-μl capillary tube.

Collection of Whole Venous Blood Samples—Five-millilitre samples of whole venous blood were withdrawn from a forearm vein into a 10-ml Vacutainer® tube containing 143 U.S.P. units of sodium heparin. The blood samples were analyzed as previously discussed.

Results and Discussion

Table 1 and Figs. 1 and 2 give the results of these studies. These data are supplemental to those reported by Forney et al [*7,8*], Dundee et al [*3,4*], and Gostomyzyk et al [*5,6*] and, although in general agreement with their findings, illustrate some important trends not seen in previous work.

TABLE 1—*Capillary and venous ethanol concentrations after the oral administration of 30 ml of absolute ethanol to two adult male human subjects.*

	Concentration of Ethanol, mg/ml			
	Subject 1		Subject 2	
Time,[a] min	Capillary	Venous	Capillary	Venous
0[b]	0	0	0	0
5	0.10	0.040	0.12	0.094
10	0.19	0.11	0.15	0.11
15	0.23	0.15	0.14	0.13
20	0.42	0.30	0.17	0.15
25	0.47	0.42	0.19	0.18
30	0.55	0.42	0.20	0.21
45	0.51	0.43	0.30	0.28
60	0.47	0.44	0.33	0.36
75	0.37	0.41	0.32	0.33
90	0.30	0.37	0.26	0.28
120	0.23	0.29	0.16	0.18
150	0.16	0.21	0.093	0.12
180	0.12	0.16	0.053	0.059
210	0.052	0.087	0.026	0.035
240	0.027	0.040	0.0074	0.011
Area under curves, 0–240 min	56.82	59.83	36.02	38.26

[a] Time was measured from the midpoint of the rapid ingestion of the alcohol and orange juice.
[b] Zero time was about 8 a.m.

Initially, capillary ethanol concentrations rise much faster than venous ethanol concentrations and tend to peak sooner than venous ethanol concentrations. These trends are particularly well illustrated by the subject who absorbed alcohol rapidly (Fig. 1). This initial concentration gradient eventually reverses, and later, capillary ethanol concentrations become significantly less than corresponding venous concentrations. Figures 3 and 4 show plots of capillary/venous concentration ratio versus time and demonstrate the change in the concentration gradient with time.

Many authors have reported that capillary and arterial ethanol blood concentrations do not differ. Moreover, most authors agree [3–6] that arteriovenous equilibrium of ethanol is attained rapidly in man. These authors define arteriovenous equilibrium as zero arteriovenous concentration difference. The results presented in Figs. 1–4 are not in agreement with their conclusions based on this definition. Equal capillary and venous concentrations were not achieved in either subject at 4 h after administration.

Interpretation of the data presented in Figs. 1–4 requires knowledge of the origin or composition of the blood samples. Blood samples obtained by venipuncture are composed of venous blood, but the composition of capillary blood samples obtained from fingertip blood is not quite as obvious. Investigators [7] have expressed the concern that capillary blood may become diluted by serous fluids located near the capillaries when fingertip samples are collected. Forney et al [7] have shown that this hemodilution phenomenon is of no importance. Hemoglobin determinations made on capillary and venous bloods, drawn simultaneously, showed no significant differences. Therefore, fingertip blood samples would accurately reflect conditions prevailing in the capillaries.

Use of the data presented above enables the formulation of a model (Fig. 5) capable of describing the interaction of capillary and venous ethanol concentrations. The model presented in Fig. 5 would predict the following data.

1. An ethanol concentration gradient would be present in the capillaries. Con-

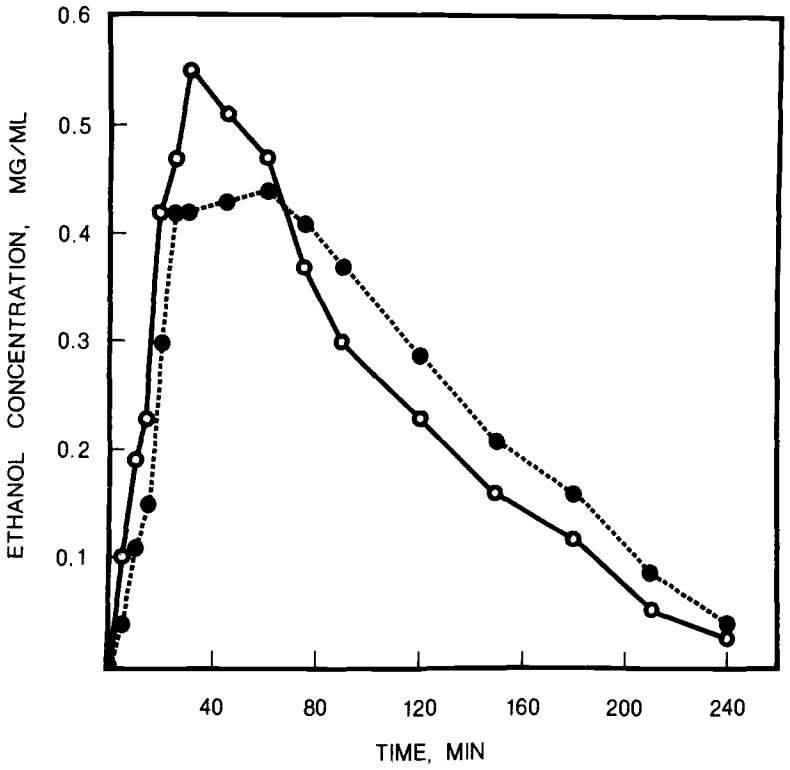

FIG. 1—*Capillary and venous ethanol concentrations measured simultaneously in the same subject after the oral administration of 30 ml of absolute ethanol. Subject 1,* O—O *capillary concentration;* ●----● *venous concentration.*

centrations of ethanol determined from capillary samples probably reflect the average ethanol concentration in the capillary.

2. Capillary ethanol concentrations would initially be higher than venous concentrations, then lower at later times.

3. Arterial, venous, and capillary concentrations would be related as described in Table 2.

TABLE 2—*Relationship of arterial, capillary, and venous ethanol concentrations according to model presented in Fig. 5.*

Portion of Ethanol Blood Concentration Curve	Relationship of Arterial, Capillary, and Venous Ethanol Concentrations
Upslope—before the intersection of the capillary and venous curves	arterial > capillary > venous
Intersection of capillary and venous curves	arterial = capillary = venous
Downslope—after the intersection of the capillary and venous curves	venous > capillary > arterial

4. Since ethanol elimination only occurs from the central compartment, this model would predict equal areas under the capillary and venous ethanol concentrations curves from time 0 to ∞.

Data collected in these studies and by other authors are consistent with the trends

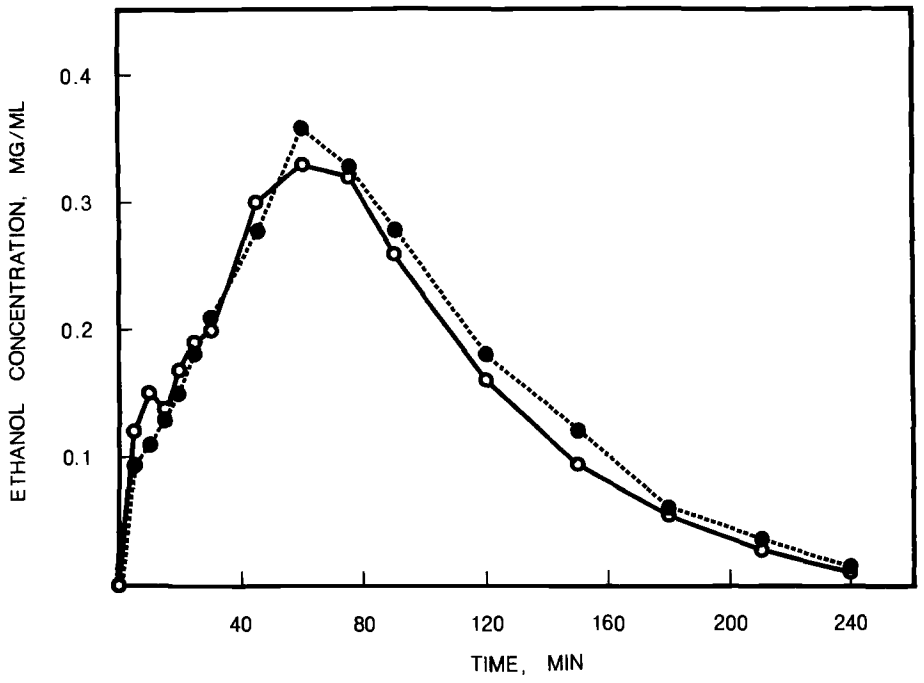

FIG. 2—*Capillary and venous ethanol concentrations measured simultaneously in the same subject after the oral administration of 30 ml of absolute ethanol. Subject 2,* O—O *capillary concentration;* ●----● *venous concentration.*

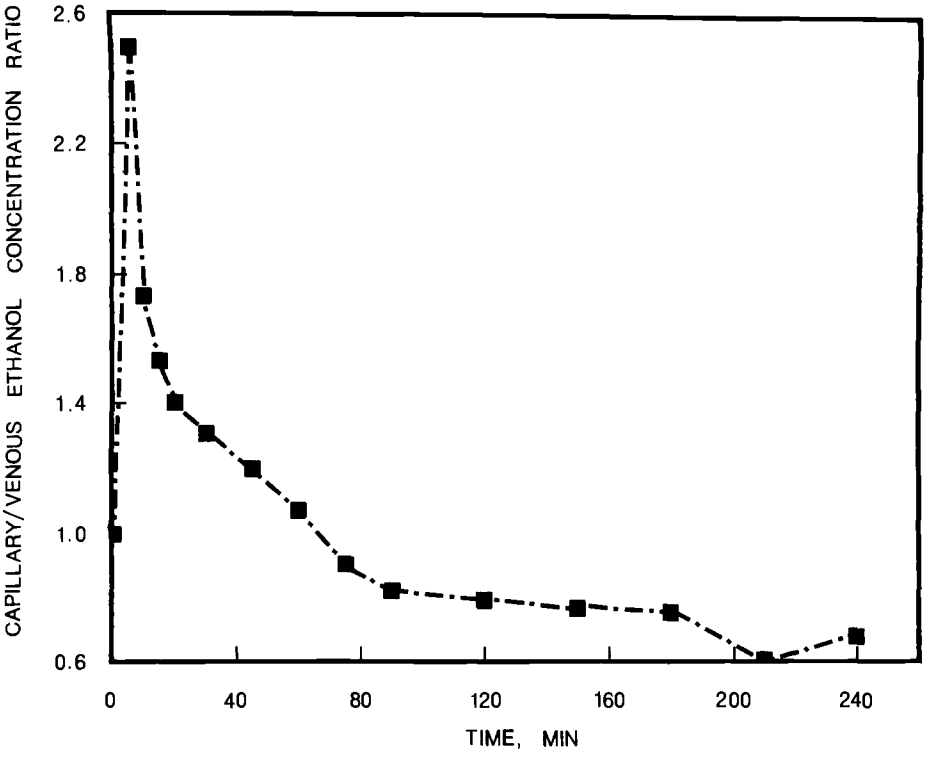

FIG. 3—*Plot of capillary/venous ethanol concentration ratio versus time for Subject 1.*

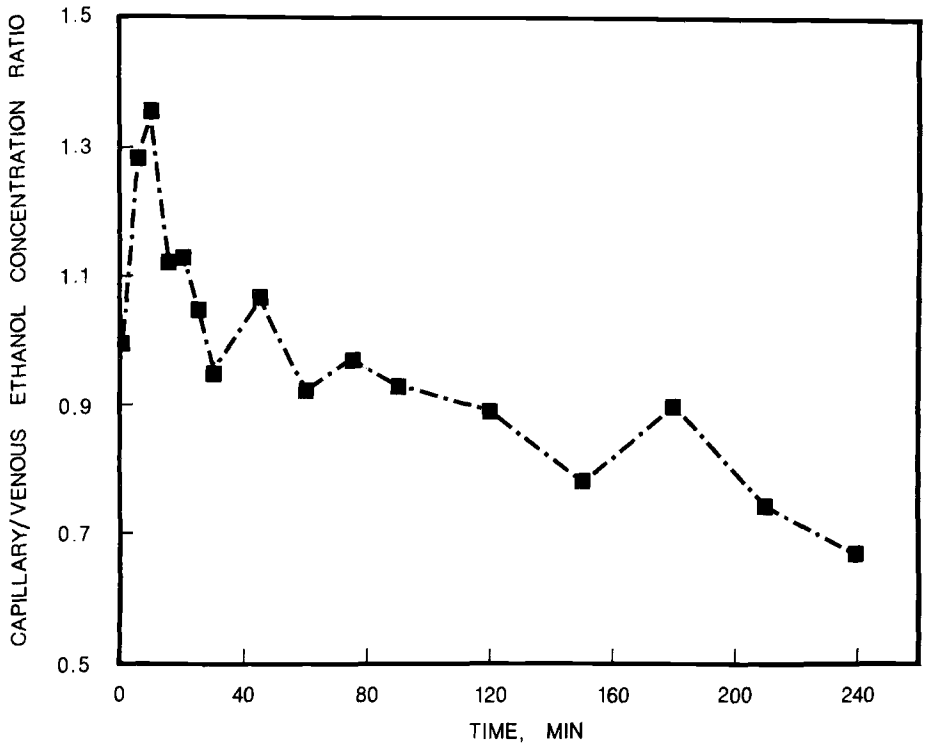

FIG. 4—*Plot of capillary/venous ethanol concentration ratio versus time for Subject 2.*

characteristic of the model presented in Fig. 5. This is a pharmacokinetic model and is not intended to be an anatomically exact one. For example, Table 1 gives the areas under the capillary and venous curves from time 0 to 240 min for the two human subjects. The areas vary by less than 5% within each subject, providing additional support for this model (see point 4).

In the use of blood concentrations for predicting the alcohol level in the blood supplied to the brain, it is reasonable to assume that capillary blood concentrations would be more reliable than venous blood concentrations. However, capillary ethanol blood concentrations may not accurately reflect ethanol concentrations in the blood supplied to the brain because of the possible multicompartmental nature of ethyl-alcohol distribution. Harger [*10*] showed an excellent correlation of brain alcohol level with heart blood alcohol concentrations in the dog based on data obtained by Forney et al [*8*]. With respect to Fig. 5, heart blood would be equivalent to arterial blood.

Summary

The time courses of capillary and venous blood concentrations of ethanol after oral administration of 0.30 and 0.34 g/kg doses of ethanol to two normal human subjects indicated that, by interpolation, equal capillary and venous concentrations are reached at only one time, and this time varied with the subject. The capillary/venous blood concentration ratios varied markedly as a function of time in both subjects over the entire observation period of 4 h.

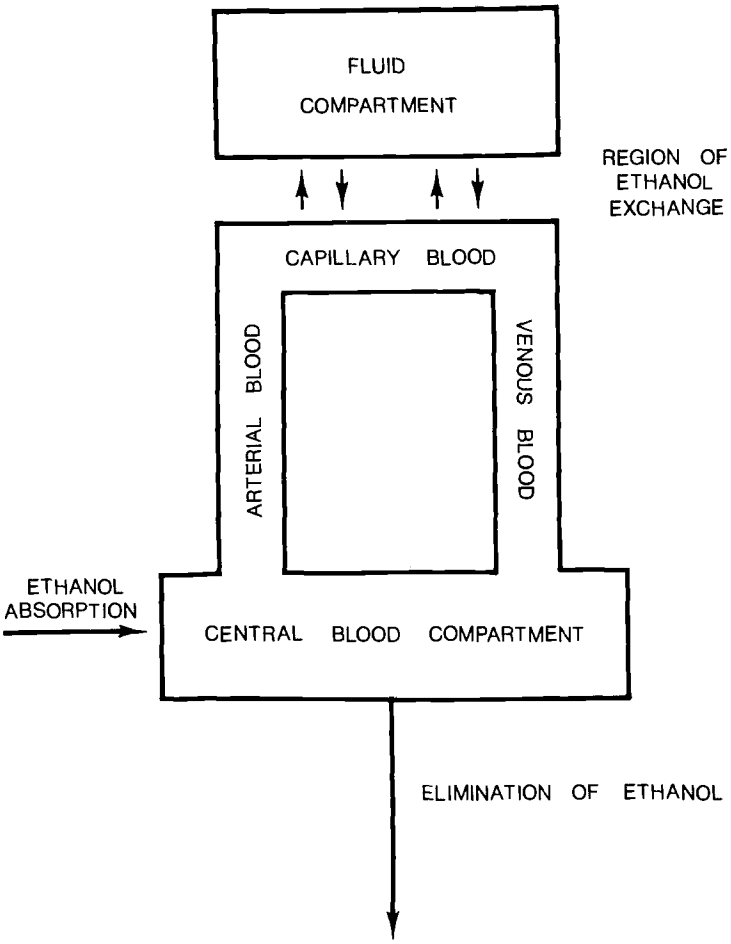

FIG. 5—*Schematic model describing the relationship between capillary and venous ethanol concentrations in humans.*

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