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The Distribution of Ethanol in Postmortem Blood Specimens

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ABSTRACT: Ethanol was determined by gas chromatography in a variety of tissues and body fluids secured at autopsy in 61 cases. The specimens tested included right and left heart blood, femoral blood, pericardial fluid, cerebrospinal fluid, vitreous humor, urine, stomach contents, and brain.

Statistical analysis of the cases revealed no significant differences among the various blood sites tested. However, the variations in blood ethanol concentrations among the various sampling sites within each case were as follows: 40 cases showed differences of less than 25%; 16 cases revealed variability between 25% and 50%, 4 cases had differences exceeding 50%. In one case, satisfactory blood analyses could not be accomplished. The larger variances occurred especially in those instances in which stomach alcohol concentration was 0.50% or greater. In one case, the variability amongst the different blood sites exceeded 400% (femoral blood—0.043%, right atrium—0.070%, root of aorta—0.156%); the brain was 0.050%, and the stomach contents was 1.2%. For all 61 cases, variances in blood alcohol content among the different sampling sites in a single cadaver ranged from 1.8 to 428%.

KEYWORDS: toxicology, ethanol, postmortem blood specimens

The distribution of ethanol in postmortem tissues and body fluids has been previously investigated by a number of authors [*1–5*]. The results of their work are essentially consistent with the fact that ethanol, because of its hydrophilic nature, distributes with body water.

A rather extensive investigation addressing the suitability of postmortem blood for alcohol determination was conducted by Plueckhahn [*6*]. He concluded that postmortem blood, whether secured from a peripheral site, such as femoral vein, or from a central area, that is, heart blood, manifests no statistically measurable differences in alcohol concentration.

More recently, Prouty, et al. determined ethanol concentrations in both heart and femoral blood in 100 postmortem cases [*7*]. Their conclusions are consistent with those of Plueckhahn in that no statistical difference in alcohol concentration was noted between femoral and heart blood.

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Results of blood ethanol determinations of specimens secured from various sites in routine Suffolk County, New York Medical Examiner cases over the years led to the suspicion that differences existed among various blood sites in the same cadaver in some cases[4] [*9–11*]. Other very recent reports may support this contention[5,6] [*8*].

These findings raise a question as to the validity of postmortem alcohol estimations, especially when the site of the test specimen is unknown. Alcohol determinations, whether antemortem or postmortem, can and do have enormous legal and social implications. It is therefore imperative to have a better understanding of the distribution of this drug in the human.

Experimental

Criteria for selection of the 61 cases included a presence of ethanol at a mean blood concentration of 0.05% or greater; absence of gross trauma to the body; absence of significant decompositional changes; and complete autopsy.

All autopsies were performed by a qualified forensic pathologist with specimen acquisition supervised by the principal author. After the initial "Y" incision, the chest plate is removed by sharp incision through the sternoclavicular joints and mechanical transection of the ribs. The initial incision is extended into the right groin and the proximal femoral vein is exposed by sharp and blunt dissection.

The blood samples and pericardial fluid are secured via syringe in the following sequence and manner: under direct visualization an aliquot (1 to 10 mL) of femoral venous blood is aspirated; the pericardial sac is incised and its entire content aspirated; the right atrium is punctured and a sample of blood is aspirated; and lastly, the root of the aorta is punctured and a sample of blood is secured. All blood samples are placed in 15 cc plastic centrifuge tubes containing 14 mg potassium oxalate (anticoagulant) and 17.5 mg sodium fluoride (preservative).

Other specimens are then secured: the sclera is punctured at the lateral canthus and a portion of vitreous fluid is aspirated; a spinal needle is inserted into the anterior subarachnoid space at the upper lumbar level and CSF is removed; the stomach is incised and the entire contents is obtained; prior to evisceration, the bladder is punctured under direct visualization and a portion of urine is aspirated; and finally, a section of brain is secured by collecting a combination of gray matter and white matter from the frontal lobe.

A total of 61 cases were examined by gas-chromatography using a direct injection technique. Samples were prepared for analysis by diluting 1 part body fluid with 10 parts of 0.02% (w/v) aqueous n-propanol, as internal standard. Tissues were treated in similar manner by homogenization with the diluent. All samples were then centrifuged at 2000 rpm for 5 min and 1 μL of supernatant fluid was analyzed using a 6 feet by 2 mm i.d. Carbopack B coated with 5% Carbowax 20M column at 85°C with flame ionization detection. Helium served as the carrier gas at a flow rate of 30 mL/min. Detector and injector temperatures were 200°C.

Concentrations of ethanol were determined from a previously constructed aqueous calibration curve prepared in a manner identical to the unknowns.

[4]Unpublished data, Division of Medical-Legal Investigations and Forensic Sciences, Suffolk County, New York.

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Results and Discussion

The chromatographic parameters used provided retention times of 1.4 and 3 minutes for ethanol and n-propanol, respectively. The standard curve exhibited a rectilinear relationship between concentration and detector response over a range of 0 to 0.789% with a coefficient of correlation exceeding 0.99.

Data for the 61 cases are summarized in Table 1. Although case 18 appears in the table, results of blood specimens were not available due to a laboratory accident. The mean values for each body fluid and tissue are presented in Fig. 1. It also shows the mean concentration for the three different blood sites sampled. The maximum percent differences in site-to-site blood ethanol concentrations in each case appear in Table 2.

The mean alcohol concentration for all blood samples was 0.194 g%. Mean values for the various sites were 0.190 g% for right atrial blood, 0.197 g% for femoral blood, and 0.195 g% for root of aorta. Analysis of variance demonstrated no statistically significant difference between these sites at $P < 0.05$. This is in agreement with reports previously cited.

However, when viewed individually, wide variations in alcohol concentration among the various blood samples secured from the same cadaver were noted in a number of cases. Twenty (33.3%) of the 60 cases investigated had within blood differences greater than 25%. Four manifested variabilities greater than 50% with one case exceeding 400%.

Excluding stomach content as a sample, no statistical differences were found to exist between the other sites, that is, blood, brain, vitreous, CSF, and pericardial fluid. An examination of the data does reveal, however, that certain trends may exist. For instance, pericardial fluid was noted to be 30.2% higher than brain on average. Regarding alcohol concentration: brain < blood < vitreous fluid < CSF < pericardial fluid. These results are consistent with the well-established fact that alcohol distributes with body water. Brain, being the most lipoid in nature, would be expected to manifest the lowest concentration while specimens such as vitreous fluid, CSF, and pericardial fluid would be anticipated to have higher values.

High gastric alcohol content may play a role in blood variability. Of the cases studied, 39 had stomach alcohol concentrations less than 0.50 g%, 22 were between 0.50 g% and 1.0 g%, and 11 were above 1.0 g%. Although no definitive correlation was found to exist between elevated gastric alcohol concentration and within blood variability, every one of the four cases that had site-to-site differences greater than 50% had gastric content concentrations of 0.50 g% or above. Three of the four cases had gastric values between 1.0 and 5.1 g%.

A concentration of alcohol in stomach contents of 0.50 g% or greater has been reported to be that value, which implies active absorption at the time of death [*4*]. Previous work has indicated that variances may occur between arterial and venous blood during the absorption-distribution phase [*9–11*]. The blood variabilities noted in this study cannot be due to this phenomenon since no one site manifested consistently higher values.

Marraccini, et al. [*8*] have suggested that alcohol concentrations may be substantially higher in aortic blood than other sites, such as femoral blood, due to perimortem aspiration of stomach contents into lung, with subsequent redistribution into central blood. This is an attractive hypothesis but we find it not to be consistent with our observations that, in some cases, femoral blood values exceed those of central origin.

Corry [*12*] describes both biochemical and microbiological processes that result in artificially elevated levels of alcohol in postmortem blood. Although this is well known to occur, localized ethanol production at a specific site seems improbable. Thus, the observation of within blood variability is not explained by this mechanism.

Another proposed explanation for these variabilities involves the effects of salt concentration and volume or both of sample. In this regard, we conducted an experiment

TABLE 1—*Ethanol concentration[a] (g%) of tissues and body fluids.*

Case	Femoral Vein	Right Atrium	Root Aorta	Brain	CSF	Vitreous Fluid	Urine	Stomach Contents	Pericar Fluid
1	0.223	0.186	0.231	0.159	0.242	0.210	0.170	0.741	0.292
2	0.191	0.217	0.161	0.197	0.271	0.279	0.357	0.259	0.254
3	0.137	0.133	0.133	0.108	0.171	0.148	*	0.156	0.148
4	0.115	0.104	0.095	0.105	0.128	*	0.131	0.114	*
5	0.194	0.171	0.197	0.168	*	0.223	*	0.263	0.204
6	0.062	0.059	0.056	0.040	*	*	0.063	0.449	0.062
7	0.119	0.120	0.113	0.106	0.145	0.148	0.185	0.132	0.133
8	0.234	0.201	0.204	0.175	*	0.254	0.290	*	0.240
9	0.297	0.302	0.309	0.237	0.285	0.242	*	3.056	0.363
10	*	0.182	0.199	*	*	*	*	*	0.193
11	0.214	0.223	0.214	0.190	0.243	0.221	*	0.554	0.240
12	0.304	0.311	0.269	0.237	0.376	0.382	*	0.369	0.322
13	0.276	0.286	0.306	0.268	*	0.334	0.353	1.556	0.376
14	0.206	0.084	0.272	0.179	0.197	0.225	0.285	1.489	0.240
15	0.260	0.272	0.247	0.235	0.344	0.308	0.369	0.498	0.312
16	0.107	0.105	0.101	0.087	0.127	0.142	0.203	0.117	0.122
17	0.043	0.070	0.156	0.050	*	0.038	0.048	1.204	0.149
18	*	*	*	0.076	*	0.058	0.086	0.587	*
19	0.072	0.064	*	0.069	*	0.071	0.095	0.602	*
20	0.074	0.064	0.083	0.063	0.083	0.078	0.072	0.466	0.088
21	0.155	0.181	0.172	0.154	0.212	0.220	*	0.146	0.192
22	0.166	0.114	0.150	0.098	*	0.122	0.297	1.342	0.254
23	0.254	0.255	0.228	0.205	0.277	0.286	0.277	0.398	*
24	*	0.233	0.212	0.104	0.141	0.115	0.151	1.216	0.231
25	0.249	0.211	0.217	0.184	0.259	0.218	0.283	0.212	0.226
26	0.220	0.220	0.230	*	0.225	0.257	2.401	0.397	
27	0.217	0.205	0.224	0.148	0.246	0.198	0.267	0.822	0.257
28	0.181	0.215	0.212	0.157	0.259	0.271	0.269	0.250	0.221
29	0.271	0.284	0.260	0.259	*	0.353	*	0.274	0.326
30	0.211	0.177	0.195	0.152	0.245	*	0.264	*	0.226

31	0.388	0.339	0.324	0.300	0.433	0.379	0.474	0.330	0.395
32	0.297	0.268	0.274	0.217	*	*	0.375	1.062	*
33	0.187	0.160	0.142	0.131	*	0.221	*	0.183	0.163
34	0.107	0.092	0.088	0.096	0.117	0.119	0.126	0.105	0.107
35	0.230	0.197	0.215	*	0.285	0.291	0.322	0.259	0.257
36	0.139	0.103	*	*	0.168	0.107	*	0.368	*
37	0.092	0.147	0.364	0.137	*	0.105	0.066	5.085	0.521
38	0.105	0.083	0.107	0.068	*	0.118	0.144	0.230	0.120
39	0.202	0.175	0.157	0.210	0.282	0.281	0.325	0.178	0.243
40	*	0.233	0.313	0.270	0.914	0.324	0.261	0.261	0.304
41	0.173	0.125	0.138	0.206	0.108	0.053	0.191	0.540	0.224
42	0.078	0.080	0.068	*	0.107	0.094	*	0.097	0.101
43	0.071	0.062	0.049	0.072	0.065	0.086	0.114	0.073	0.068
44	0.130	0.138	0.126	0.120	0.139	0.153	*	0.155	0.154
45	0.206	0.176	0.161	0.080	*	0.159	*	0.787	0.200
46	0.219	0.187	0.168	0.181	*	*	0.316	*	0.244
47	0.159	0.133	0.131	0.134	0.204	0.196	0.178	0.291	0.168
48	0.387	0.349	0.344	0.326	*	0.397	0.404	0.919	0.375
49	0.087	0.460	*	0.098	*	0.087	0.141	3.027	0.351
50	0.309	0.289	0.277	0.260	*	0.338	0.403	0.883	*
51	0.227	0.204	0.229	0.204	*	0.255	0.297	0.273	0.254
52	0.140	0.123	0.143	0.129	*	0.127	0.173	0.464	0.222
53	0.106	0.111	0.135	0.123	0.158	0.156	0.169	0.621	0.147
54	0.225	0.228	0.201	0.202	*	0.250	*	0.438	*
55	0.373	*	0.343	0.287	*	0.384	0.436	*	0.429
56	0.180	0.131	0.120	0.181	0.219	0.201	0.264	*	0.166
57	0.332	0.364	0.275	0.388	0.464	0.429	0.477	0.749	0.436
58	0.099	0.083	0.076	0.085	*	0.113	0.117	0.104	0.104
59	0.282	0.277	*	0.258	*	0.315	0.281	*	0.369
60	0.075	0.066	0.064	0.072	0.095	0.083	0.104	0.111	0.081
61	0.564	0.584	0.572	0.538	0.660	0.631	0.546	2.445	0.722

*Specimens not available or not analyzed.
[a]Mean of duplicate analysis.

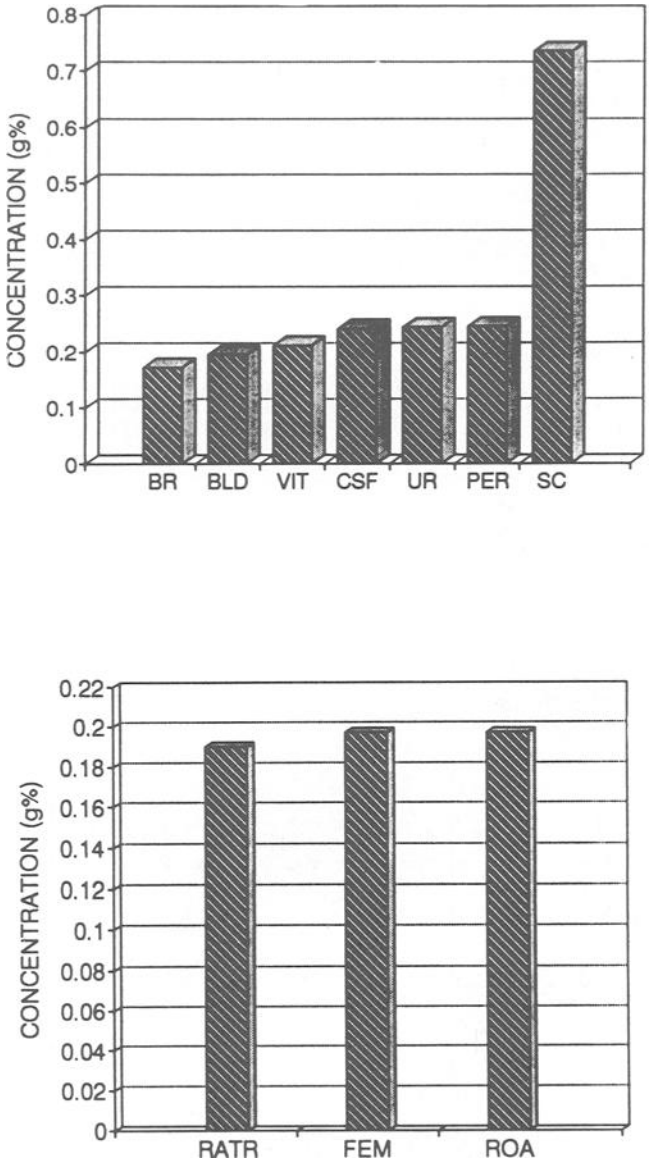

FIG. 1—*Mean alcohol concentration for tissues and body fluids. (BR = Brain; BLD = Blood; VIT = Vitreous humor; CSF = Cerebrospinal fluid; UR = Urine; PER = Pericardial fluid; SC = Stomach contents)* (top) *mean alcohol concentration for three blood sites (RATR = Right atrium; FEM = Femoral vein; ROA = Root of aorta)* (bottom).

that shows that neither salts nor sample size is responsible. Aliquots of a blood of known alcohol concentration were transferred, in volumes ranging from 0.5 mL to 10.0 mL, to 12 15 mL centrifuge cones, six of which contained 14 mg potassium oxalate and 17.5 mg sodium fluoride and six of which were devoid of any salt. After an equilibration interval of 2 h at room temperature, each specimen was analyzed for ethanol as described in the experimental section. No variability was observed among any of the test samples.

TABLE 2—*Maximum percent differences in site-to-site blood ethanol concentrations in each case.*[a]

Case #	Variability	Case #	Variability
59	1.8%	60	17.2%
3	3.0%	42	17.6%
61	3.5%	25	18.0%
9	4.0%	28	18.8%
11	4.2%	30	19.2%
26	4.5%	31	19.8%
16	5.9%	4	21.1%
7	6.2%	47	21.4%
55	8.5%	34	21.6%
29	9.2%	1	24.2%
27	9.3%	53	27.4%
10	9.3%	45	28.0%
44	9.5%	39	28.7%
24	9.9%	38	28.9%
15	10.1%	20	29.7%
6	10.7%	58	30.3%
32	10.8%	46	30.4%
13	10.9%	33	31.7%
50	11.6%	57	32.4%
23	11.8%	40	34.3%
51	12.3%	2	34.8%
19	12.5%	36	35.0%
48	12.5%	41	38.4%
54	13.4%	43	44.9%
5	15.2%	22	45.6%
12	15.6%	56	50.0%
52	16.3%	14	223.8%
8	16.4%	17	262.8%
35	16.8%	37	295.7%
21	16.8%	49	428.7%

[a]Computed as: % difference = ((high concentration/low concentration) − 1) × 100.

Postmortem absorption of ethanol from the stomach with subsequent "postmortem circulation," as demonstrated by Fallani [*13*], may play a role in this phenomenon. Alcohol has been shown to diffuse from the stomach when instilled in cadavers [*14*]. Mechanical manipulation of the body may be expected to enhance postmortem blood movement. The observation that no single blood site consistently shows the highest alcohol concentration is consonant with the notion of alterations in postmortem blood circulation patterns, perhaps as a result of postmortem clotting or other physical phenomena, collapsed vessels, for example.

Complete results of another study, involving alcohol and drug analyses of an even wider variety of tissues and body fluids from each of 20 cases, are to be reported elsewhere. However, to summarize them briefly, the results of this study are consistent with those reported above as they relate to alcohol distribution at different blood sites. Various areas of the brain differ significantly in their alcohol content. For example, frontal lobe or gray matter exceeded medulla or white matter in alcohol. Interestingly, liver-alcohol concentrations were substantially and consistently lower than any other site. Perhaps the most constant finding was the high degree of correlation between bile and mean blood values, corroborating the earlier work of Backer, et al. [*4*].

It is evident that further work needs to be done to resolve the questions raised by all these findings. Until then, the forensic toxicologist must exercise extreme caution in interpreting postmortem alcohol results.

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