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REBREATHED AIR AS A REFERENCE FOR BREATH-ALCOHOL TESTERS

A. L. Flores

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INTERIM REPORT

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16. Abstract

A technique has been devised for a reference measurement of the performance of breath-alcohol measuring instruments directly from the respiratory system. It is shown that this technique is superior and simpler than comparison measurements based on blood-alcohol analysis.

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PREFACE

The Department of Transportation Standard for Devices to Measure Breath Alcohol[1] requires the evaluation of "deep lung" sampling performance of a particular device by direct comparison of breath alcohol with blood alcohol using intoxicated human subjects. The alcohol content in the deep lung air and the alcohol content in the blood are related, but the relationship can be quantitatively used only under specific optimum conditions, as discussed below. It is therefore desirable to devise a reference measurement obtained directly from the respiratory system for use under the standard. The standard allows for the incorporation of an alternate reference measurement involving breath if a suitable method can be demonstrated. This report provides the basis for the use of rebreathed air as a suitable alternate reference measurement.

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1. BACKGROUND

Since, by current law, intoxication is specified in terms of blood-alcohol concentration, the DOT standard validates, through "deep lung" sampling performance tests, the accuracy with which an instrument measures blood alcohol by measurement of breath alcohol.

After ingestion by an individual, alcohol passes from the stomach into the small intestine from where it diffuses through the intestinal wall into the blood stream. From this point, it is then carried to the heart. From the heart it is pumped through the pulmonary capillary network, then back to the heart; it is then distributed directly to the brain and other parts of the body through the system of arteries. Absorption of alcohol into the tissues at various locations proceeds nonuniformly so that only after several hours after ingestion have elapsed is the blood-alcohol concentration uniform throughout the body.[2] * Diffusion of alcohol from the pulmonary capillary blood into the alveolar air sacs deep in the lungs proceeds rapidly and efficiently because of the very large gas exchange surface area of the lungs. However, measurement of the alcohol concentration in the alveoli is not straightforward since the concentration in the exhaled breath is modified in passage through the airways linking the alveoli with the external environment. As will be seen below, the use of rebreathing techniques allows for the collection of samples which are more representative of alveolar air than collection by simple exhalation techniques.

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* In the lungs and in the brain the blood supply is abundant and blood tissue equilibrium is established very quickly.

2. PROCEDURES

Alcohol measurements were made with a Perkin-Elmer model 900 Gas Chromatograph* equipped with a heated (140°C) precision gas-sampling valve with a 0.1cc sampling tube. A six-foot Carbowax 20M (20%) on Chromasorb WAW 80/100 mesh analytical column was used at a temperature of 110°C. Flame ionization detectors were used. All tubing was 1/8 inch O.D. stainless steel. Adaptors were attached to the gas-sampling valve according to whether breath or blood was to be analyzed.

* Gas Chromatograph is hereinafter abreviated to G.C.

3. BREATH ANALYSIS

The sampling apparatus used is illustrated in Figure 1. To sample the last portion of the subject's exhalation, the lower leg of a 1/2 inch I.D. insulated tygon "Y" connector was connected directly to the inlet of the gas-sampling valve and a plastic bag of about 1 liter inflated volume was connected to one of the upper legs of the tygon "Y". The other end of the plastic bag was equipped with a whistle. The subject providing the breath sample blew into the other leg of the tygon "Y" through a sanitary mouthpiece. All air flow restrictions in this sampling system were minimized to make it as easy as possible for the subject to provide a maximal exhalation volume within a few seconds. The greatest restriction to flow other than the sampling valve tubing was the whistle which served to indicate continuous, uniform exhalation and to provide a sufficient pressure gradient to force breath through the sampling tube. Once the whistle began to sound, air passed through the sample tube at 0.3 to 0.7cc/sec, ensuring that after the subject had delivered the breath sample, the sample tube (being only 0.1cc in volume) contained only air from the very last portion of the breath.

The G.C. output was standardized with a 0.100 BAC (breath)* air-alcohol mixture obtained by bubbling air through a commercial breath simulator (Decatur Electronics). The simulator contained an alcohol in water solution (1.21 mg alcohol per ml), thermostated at 34°C. Air emerging from the simulator solution contained 0.480 mg/L,[3] which is defined by the Standard as the breath equivalent of 0.100 BAC. Reproducibility was ±1 percent.

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* Alcohol units used in this report are as follows:
BAC = Blood-Alcohol Concentration = grams alcohol/100 ml blood
BAC(BREATH) = BAC derived from breath measurement (grams alcohol/210 liters air)

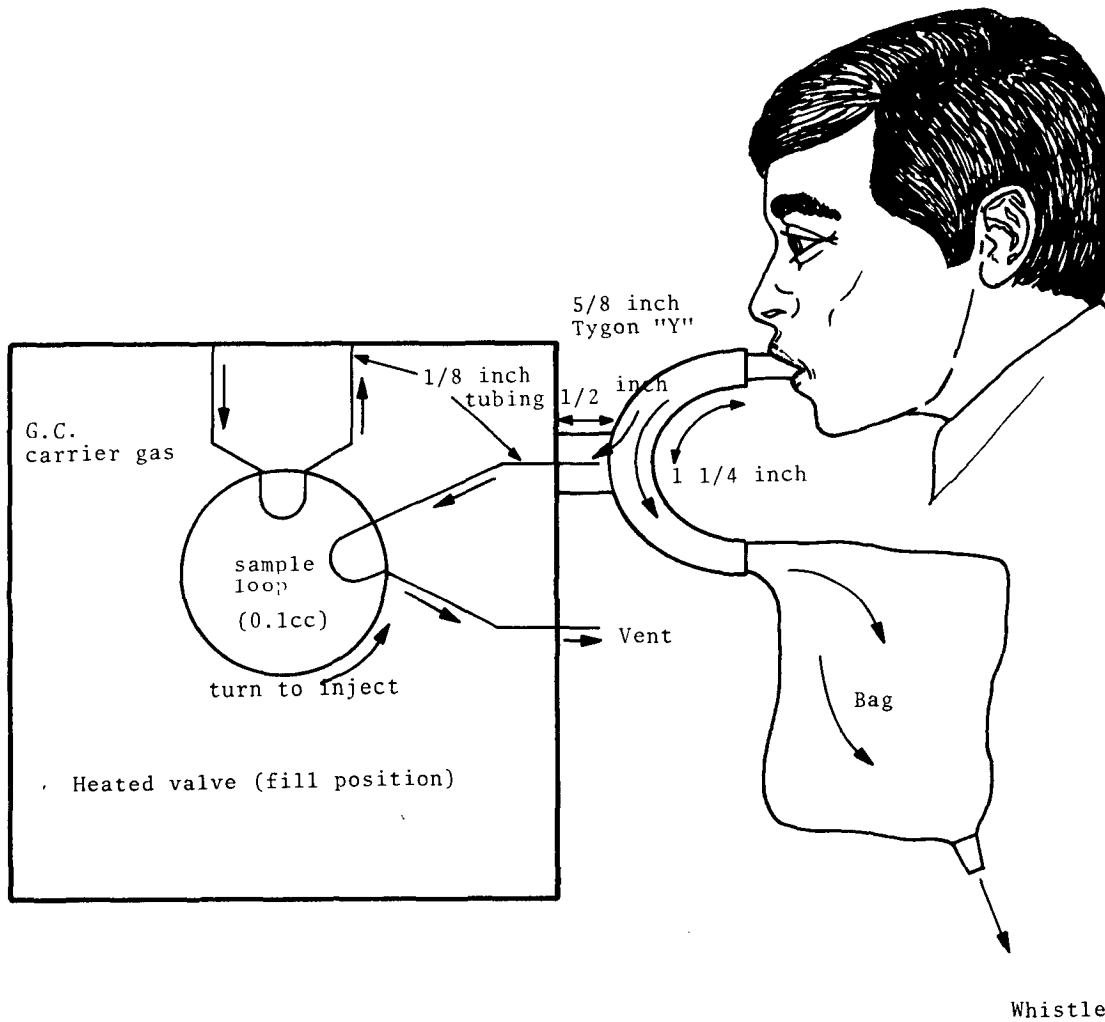

Figure 1. Breath Sampler

4. BREATH SAMPLES

The following types of breath samples were delivered into the G.C. sampling system, where R denotes the number of rebreathing cycles.

a. Simple exhalation sample (no rebreathing, R=0): the subject inhaled, then forcefully exhaled into the sampling system continuously until the lungs were exhausted.

b. Rebreathing sample: With the nose closed with the fingers, the subject exhaled into and re-inhaled deeply the same air from a plastic bag (about 4 liters inflated volume) the specified number of times while excluding fresh air from his respiratory system. After performing the required number of rebreathing cycles, the subject removed the bag from his mouth and quickly exhaled into the sampling system for as long as he could. The types of rebreathed samples measured were: 3 rebreathing cycles (R=3), 5 rebreathing cycles (R=5), and 7 rebreathing cycles (R=7). Preliminary tests showed that rebreathing more than 7 times did not result in significantly different results from 7 rebreathings. In later experiments only R=0 and R=7 samples were taken.

   The rebreathing procedure used was similar to that used by Harger and co-workers[4] except that the rebreathing bag was not pre-warmed and direct sampling of rebreathed air from the subject's mouth rather than from the rebreathing bag was used. Preliminary work at this laboratory showed that pre-warming the bag had little effect; this observation is borne out by calculation.[5]

Blood samples were obtained from the cubital arm vein concurrently with breath sampling. Samples were withdrawn directly into Becton-Dickenson Vacutainer tubes containing preservative and anti-coagulant. They were stored under refrigeration for later analysis. Storage periods never exceeded 72 hours. BAC was determined by the G.C. headspace technique which is described in detail in the appendix.

5. SUBJECTS

Male and female subjects in good health were used to acquire the data. Most subjects were used for more than one day of testing. Two-hundred and forty-two pairs of blood and breath data points were collected.

6. RESULTS

The data obtained are listed in Table 1. The statistical summary is given in Table 2, grouped according to number of rebreathings (R) and also according to whether or not the samples were taken early in the post-absorption period (two hours after drinking had stopped when the alcohol in the entire vascular system was presumed to have become uniformily distributed), or well into the post-absorption period (three to four hours after drinking had stopped). Correlation of blood vs. breath results (1 volume of blood equivalent to 2100 volumes of breath) is good throughout. The highest correlation (correlation coefficient) with slopes closest to unity and least scatter (standard error of estimate) is obtained with the higher rebreathings taken well into the post-absorption.

Correlation coefficients obtained for linear regression analysis of breath-alcohol levels for R=0, 3, 5, 7 vs. blood regardless of whether for the early or late period were above 0.980 (a correlation coefficient of unity corresponding to perfect linear correlation; i.e.: all points would lie on a single straight line). Deviation of regression coefficients (i.e., slope of best fit straight line) from unity ranged from $\pm$ 10 percent and intercepts ranged about zero from -.009 to +.004 BAC. Mean deviations for breath values from the corresponding blood value ranged from +.013 BAC (breath .013 BAC lower than blood) to -.002 BAC higher than blood). Again better results (least deviation, least scatter in deviation) were obtained for higher R, well into the post-absorption.

TABLE 1. BREATH VS. BLOOD DATA

SUBJ	P	R	BAC (Breath)	BAC
3	0	0.	.112	.126
3	1	0	.075	.083
45	0	0	.100	.131
45	1	0	.093	.111
46	0	0	.104	.128
46	1	0	.074	.098
47	0	0	.065	.075
22	0	0	.135	.166
22	1	0	.083	.099
34	0	0	.134	.154
34	1	0	.066	.084
40	0	0	.119	.136
48	0	0	.064	.076
3	0	0	.114	.129
3	1	0	.074	.082
27	1	0	.102	.109
50	0	0	.109	.121
50	1	0	.060	.071
51	0	0	.096	.113
51	1	0	.045	.055
2	0	0	.219	.230
2	1	0	.187	.211
18	0	0	.100	.111
18	1	0	.075	.088
52	0	0	.078	.079
52	1	0	.044	.053
1	0	0	.151	.159
1	0	0	.148	.154
1	1	0	.109	.125
1	1	0	.105	.120
3	0	0	.101	.116
3	1	0	.078	.087
50	0	0	.088	.104
50	1	0	.072	.085
51	0	0	.072	.080
51	1	0	.047	.056
2	0	0	.129	.143
35	0	0	.087	.098
52	0	0	.082	.090
16	0	0	.094	.110
16	1	0	.049	.056
43	0	0	.052	.057
43	1	0	.024	.027
52	0	0	.078	.083
52	1	0	.050	.062
53	0	0	.122	.128
53	1	0	.067	.070
26	0	0	.095	.108
26	1	0	.052	.064
47	0	0	.078	.090
47	1	0	.038	.046
57	0	0	.029	.039
57	1	0	.002	.005
61	0	0	.065	.076
51	1	0	.051	.065
56	1	0	.074	.089
62	1	0	.037	.053
7	0	0	.095	.107
7	1	0	.074	.086
26	0	0	.057	.071
26	1	0	.030	.043
47	0	0	.076	.096
47	1	0	.037	.054
57	0	0	.069	.083
57	1	0	.032	.044
54	0	0	.161	.163
59	1	0	.082	.096
60	1	0	.091	.099
2	0	0	.075	.086
35	0	0	.056	.066
26	0	0	.072	.083
33	0	0	.054	.076
64	0	0	.093	.114
59	0	0	.069	.078
60	0	0	.064	.075
18	0	0	.102	.116
66	0	0	.091	.105
3	0	0	.099	.115
52	0	0	.068	.084
54	0	0	.090	.101

SUBJ	P	R	BAC (Breath)	BAC
3	0	3	.122	.126
3	1	3	.085	.083
45	0	3	.125	.131
45	1	3	.106	.111
46	0	3	.128	.128
46	1	3	.085	.098
47	0	3	.073	.075
22	0	3	.161	.166
22	1	3	.095	.099
34	0	3	.152	.154
34	1	3	.079	.084
40	0	3	.129	.136
48	0	3	.067	.076
3	0	3	.135	.129
3	1	3	.083	.082
27	1	3	.114	.109
50	0	3	.121	.121
50	1	3	.066	.071
51	0	3	.106	.113
51	1	3	.053	.055
2	1	3	.192	.211
18	0	3	.113	.111
18	1	3	.083	.088
52	0	3	.075	.079
52	1	3	.059	.053
1	0	3	.160	.159
1	0	3	.155	.154
1	1	3	.123	.125
1	1	3	.113	.120
3	0	3	.118	.116
3	1	3	.095	.087
50	0	3	.102	.104
50	1	3	.082	.085
51	0	3	.091	.080
51	1	3	.053	.056
2	0	3	.136	.143
35	0	3	.092	.098
52	0	3	.089	.090
3	0	5	.130	.126
3	1	5	.087	.083
45	0	5	.135	.131
45	1	5	.113	.111
46	0	5	.131	.128
46	1	5	.093	.098
47	0	5	.076	.075
22	0	5	.164	.166
22	1	5	.101	.099
34	0	5	.160	.154
34	1	5	.081	.084
40	0	5	.118	.136
48	0	5	.073	.076
3	0	5	.137	.129
3	1	5	.087	.082
27	1	5	.118	.109
50	0	5	.125	.121
50	1	5	.068	.071
51	0	5	.106	.113
51	1	5	.055	.055
2	0	5	.243	.230
2	1	5	.216	.211
18	0	5	.116	.111
18	1	5	.087	.088
52	0	5	.076	.079
1	0	5	.163	.159
1	0	5	.160	.154
1	1	5	.122	.125
1	1	5	.119	.120
3	0	5	.120	.116
3	1	5	.099	.087
50	0	5	.105	.104
50	1	5	.085	.085
51	0	5	.083	.080
51	1	5	.056	.056
2	0	5	.139	.143
35	0	5	.098	.098
52	0	5	.098	.090

SUBJ	P	R	BAC (Breath)	BAC
3	0	7	.128	.126
3	1	7	.088	.083
45	0	7	.139	.131
45	1	7	.114	.111
46	0	7	.134	.128
46	1	7	.094	.098
47	0	7	.076	.075
22	0	7	.164	.166
22	1	7	.101	.099
34	0	7	.165	.154
34	1	7	.081	.084
40	0	7	.132	.136
48	0	7	.069	.076
3	0	7	.141	.129
3	1	7	.088	.082
27	1	7	.119	.109
50	0	7	.129	.121
50	1	7	.071	.071
51	0	7	.118	.113
51	1	7	.053	.055
2	0	7	.259	.230
2	1	7	.211	.211
18	0	7	.117	.111
18	1	7	.089	.088
52	0	7	.078	.079
52	1	7	.055	.053
1	0	7	.169	.159
1	0	7	.165	.154
1	1	7	.122	.125
1	1	7	.125	.116
3	0	7	.099	.087
3	1	7	.098	.104
50	0	7	.098	.085
50	1	7	.087	.080
51	0	7	.095	.056
51	1	7	.056	.143
2	0	7	.139	.098
35	0	7	.101	.090
52	0	7	.093	.094
3	0	7	.097	.080
22	0	7	.076	.106
51	0	7	.096	.132
36	0	7	.127	.094
57	0	7	.131	.134
58	0	7	.134	.062
59	0	7	.057	.075
60	0	7	.081	.110
16	0	7	.122	.056
16	1	7	.053	.057
43	0	7	.059	.027
43	1	7	.030	.053
52	0	7	.090	.128
52	1	7	.059	.070
53	0	7	.128	.108
53	1	7	.076	.064
26	0	7	.111	.096
26	1	7	.060	.046
47	0	7	.086	.054
47	1	7	.037	.083
57	0	7	.086	.044
61	0	7	.078	.163
51	1	7	.059	.096
56	1	7	.085	.099
62	1	7	.049	.086
7	0	7	.107	.066
7	1	7	.084	.080
26	0	7	.071	.076
26	1	7	.040	.096
47	0	7	.089	.054
47	1	7	.045	.083
57	0	7	.077	.044
57	1	7	.039	.163
54	0	7	.165	.106
59	1	7	.091	.099
60	1	7	.099	.086
2	0	7	.081	.066
35	0	7	.062	.080
26	0	7	.086	.076
33	0	7	.080	.114
64	0	7	.104	.075
60	0	7	.072	.116
18	0	7	.121	.105
66	0	7	.107	.115
3	0	7	.111	.084
52	0	7	.082	.101
54	0	7	.102	.078
59	0	7	.071	

Notes P: Stage in post-absorption period.
0 = early, 1 = late.
R: Number of absorptions.

TABLE 2. DATA SUMMARY

R	P	Dμ	Dσ	D%	Regression Data			
					Intercept	Slope	S.E.	C.C
0	0,1	0.013	0.006	15.1	-0.007	0.942	0.005	0.989
3		0.003	0.005	3.16	0.000	0.969	0.005	0.988
5		-0.001	0.005	-1.03	-0.004	1.044	0.005	0.992
7		-0.001	0.006	-0.052	-0.006	1.071	0.006	0.990
0	0	0.013	0.006	13.1	-0.009	0.957	0.006	0.986
3		0.003	0.005	2.37	-0.004	1.017	0.005	0.985
5		-0.002	0.007	-1.31	-0.005	1.056	0.006	0.987
7		-0.001	0.007	-0.69	-0.009	1.104	0.006	0.988
0	1	0.012	0.006	18.0	-0.006	0.951	0.005	0.990
3		0.004	0.005	4.14	0.004	0.910	0.005	0.992
5		-0.001	0.004	-0.65	-0.002	1.029	0.004	0.996
7		0.000	0.004	-0.09	-0.001	1.016	0.004	0.992

NOTES:

R = Number of rebreathings
P = Stage in post-absorption period; 0=early, 1=late
Dμ = BAC minus BAC(Breath), mean
Dσ = BAC minus BAC(Breath), standard deviation
D% = Average percentage difference, BAC(Breath) from BAC
S.E. = Standard error of estimate
C.C. = Correlation coefficient

7. DISCUSSION

The data for R=0 show that alveolar air is not obtained in a single exhalation even though only the last portion of the breath is sampled, the mean difference (D_μ) between blood-alcohol level and breath-alcohol level being about 0.013 BAC unit. For R=3, the proportion of alveolar air is greatly increased although correlation of breath with blood is not as good as the R=5 and R=7 data. Of the data for R=5 and R=7 the uncertainty associated with the mean difference (D_μ) and regression line (S.E.) blurs the distinction between rebreathing 5 times vs. rebreathing 7 times. However, it is clear that for R=5 and R=7, data obtained late in the post-absorption correlate somewhat better with blood than the data obtained early in the post-absorption period with less scatter and higher correlation coefficient.

Thus, the data presented show the rebreathed air is essentially equivalent to alveolar air (that portion of the pulmonary air which is in equilibrium with the pulmonary capillary blood) to within an error of about 0.008 BAC unit (2-sigma level, R=7, late data).

In previous work at this laboratory, alcohol concentration profiles in exhaled breath have been obtained using a mass spectrometer with a specially designed heated inlet which allowed real-time measurement. A typical example of the R=0 and R=3 samples (rebreathings beyond R=3 were not performed in these experiments) is shown in Figure 2. The initial rise of the R=0 profile corresponds to dead-space air; i.e., upper respiratory air. After passage of the dead-space air, the alcohol concentration continues to rise at a constant rate up to termination of the breath. This continued rise corresponds to a gradual loading of the unsaturated moist surfaces of the upper respiratory tract with alcohol. The rebreathed sample profile is more nearly a step function because the surface moisture has been previously loaded with alcohol by rebreathing. These mass spectrometer results demonstrate that alveolar alcohol cannot be obtained from a single breath sample and that the single breath measurement will be too low by about 15 percent according to Table 2.

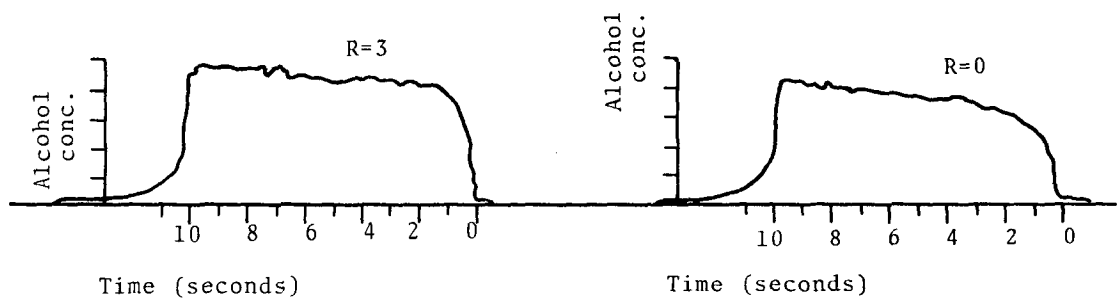

Figure 2. Mass Spectrometer Output. Real-time monitoring of alcoholic breath. Subject delivered single breath sample into instrument followed by R=3 sample 1-1/2 minutes later.

8. EVALUATION OF "DEEP LUNG" SAMPLING

When the DOT standard was formulated, no reliable respiratory reference for deep lung sampling was available. It was therefore decided that blood samples obtained from the cubital vein or fingertip would be used as an independent reference. This introduced a variability since samples from different physiological systems in a subject obtained from sites remote to each other within the body were compared.

According to the data obtained using venous blood as an independent reference, in order to minimize variability, testing should not begin until about three hours after drinking had stopped. However, such a requirement would result in additional strain on human subjects since in order to obtain a range of BAC's from 0.05 to 0.20 three hours after drinking had stoppped, large quantities of alcohol approaching lethal dosages would have to be administered.

The data show that rebreathed air is a reliable direct reference for deep lung sampling. The data for R=7 late in the post-absorption show that venous blood and hence pulmonary capillary blood correlate almost exactly with rebreathed air. Since the pulmonary gas exchange mechanism which determines the partitioning of alcohol between pulmonary blood and the alveoli spaces does not depend on stage of absorption, this means that rebreathed air correlates almost exactly with pulmonary capillary blood regardless of whether sampling is performed late in the post-absorption or not.

The use of rebreathed air thus provides a better reference for deep lung sampling than the use of blood obtained form the extremities. The use of rebreathed air as a reference has several other important advantages over blood as a reference. First, the number of reference samples which a subject can give by rebreathing is much larger than the number by blood sampling. Consequently, a better statistical base can be designed into

the evaluation. In the DOT standard, the evaluation rests on only eight blood samples from eight subjects. The amount of information that can be obtained by rebreathing vs. that which can be obtained by blood sampling on a practical basis is graphically displayed in Figure 3 in which the BAC decline of an intoxicated subject is monitored. Sixteen R=7 samples were provided by the subject over a five-hour period with ease compared to the two blood samples taken with discomfort to the subject. Second, using rebreathing, the "quality" or cooperativeness of the subject can be monitored throughout the test period. Figure 3 depicts this. The smooth decline in BAC (breath) with time attests to the complete cooperation of the subject in providing the breath samples. Also, the instability of BAC until about 90 minutes after drinking has stopped is evident. A third important advantage is that the testing can begin earlier since there is no need to wait for alcohol distribution in the two physiological systems to equalize. Thus, it would be easier to obtain the required spread in BAC form 0.05 to 0.20. Further, the range of BAC's experienced by each test subject over several hours could be incorporated into the evaluation; hence, a range of BAC's obtained from each of several subjects might be used. This would incorporate into the evaluation more individual variabilty in breathing patterns which would make for a more thorough test.

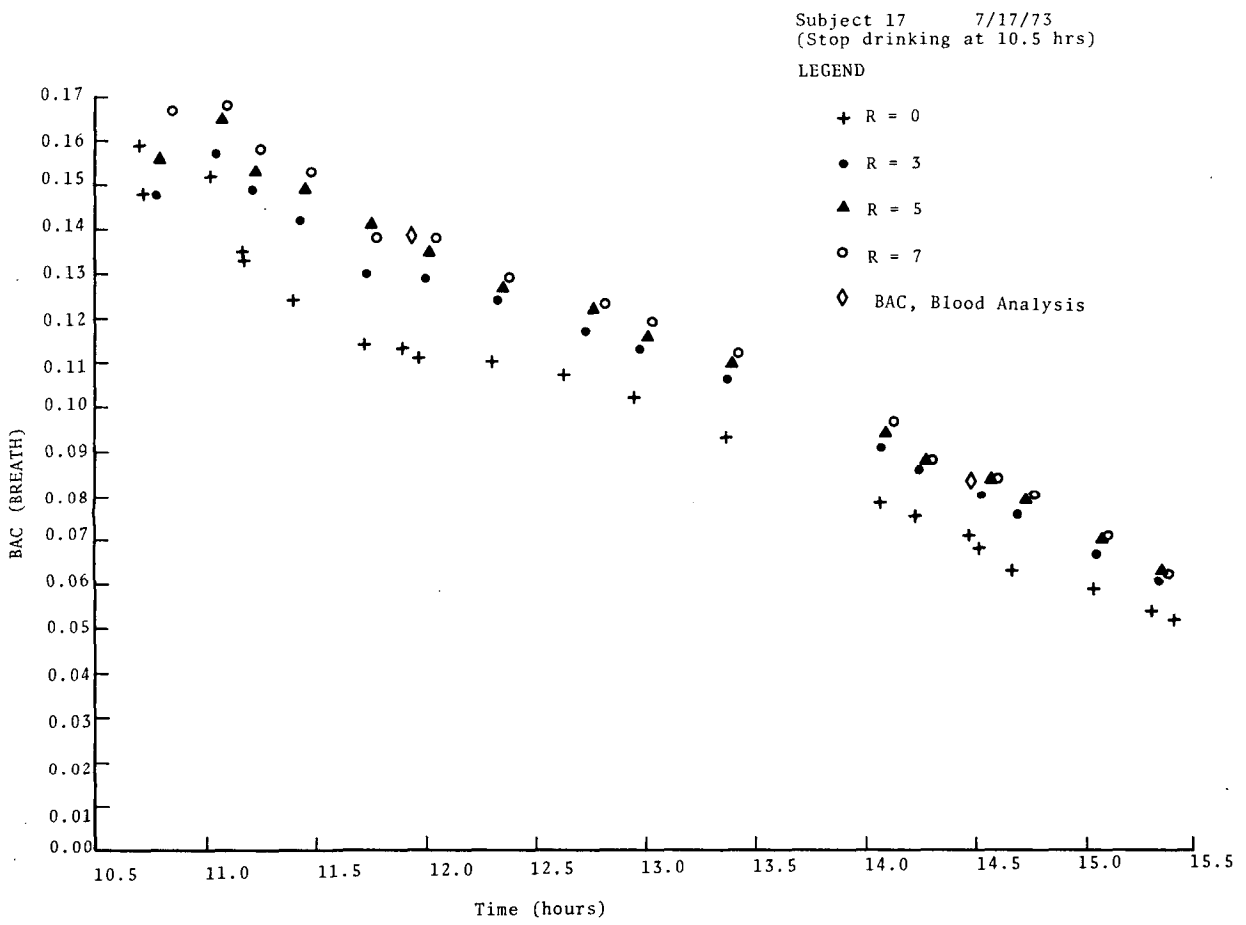

Figure 3. Alcohol Disappearance

9. LEGAL IMPLICATIONS

The legal indicator of intoxication is the blood-alcohol concentration (BAC). The BAC can be measured by testing the amount of alcohol in the blood. However, a common method is the "Headspace Technique" which measures the alcohol concentration of air which is in equilibrium with the blood. The measurement of the alcohol concentration of deep lung air by rebreathing is equivalent to the Headspace Technique for the measurement of the BAC. It is suggested that the term "Blood-Alcohol Concentration" be defined as follows: "The BAC can be measured by testing the concentration of alcohol in the blood or by testing the alcohol concentration of air that is in equilibrium with the blood, including the testing of deep lung air. Therefore, the breath-alcohol test by rebreathing is a measurement of the BAC which does not need any further validation."

10. CONCLUSIONS

A rebreathing procedure similar to one used by Hager, Forney and their co-workers has been tested for use as a reference method for deep lung sampling performance evaluation in the DOT Standard for Devices to Measure Breath Alcohol. Correlation of rebreathed air samples with venous blood samples is near unity when sufficient time is allowed for venous blood to approach equilibrium with pulmonary blood. Even when venous blood is far from equilibrium with pulmonary blood, correlation of rebreathed air with pulmonary blood is near unity.

Rebreathed samples provide a direct reference for deep lung sampling and are therefore more meaningful than indirect reference by blood sample analysis. Because the number of rebreathing samples which a subject can provide is far greater than the number of blood samples that is reasonable to obtain within a given period, rebreathing allows a larger statistical base for the evaluation. This makes possible an evaluation of the cooperation of the human subject in the testing and hence of the validity of the data obtained. The use of rebreathing samples does not require as long a waiting period as is required when blood reference is used before testing can start, which is an important practical consideration.

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APPENDIX

TSC BLOOD-ALCOHOL ANALYSIS
GAS CHROMATOGRAPH HEADSPACE TECHNIQUE

In the headspace technique, the equilibrated vapors above a solution are analyzed rather than the liquid itself. The specific method used at TSC has been designed to minimize operator error arising from:

1. Preparation of test and reference specimens prior to analysis, and
2. Injection of vapors from the headspace above these samples into the gas chromatograph.

An internal reference (Solution A) and an external reference (Solution B) are prepared in a way that allows the operator to only add a predetermined volume of internal reference (Solution A) to a predetermined volume of test specimen. Hence, once solutions A and B are prepared, only two volumes are metered: the test sample and the internal reference. The volume of external reference or work standard needs only be approximated since the concentration of internal reference in it is already equal to that added to the test specimen (blood).

Injection of headspace vapors into the gas chromatograph is accomplished by use of a heated injection valve rather than by syringe since this requires no operator skill.

PROCEDURE

Solution A (Internal Reference): Prepare a 20% (v/v) acetonitrile solution in distilled water.

Solution B (External Reference): Prepare a standard aqueous alcohol solution by pipeting 1 ml of absolute alcohol into a 500 ml volumetric flask containing the following: preservatives and coagulants to yield, upon dilution to volume, concentrations of these stabilizing additives present in the blood to be analyzed.

Dilute to volume with distilled water. Pipet 2 ml of Solution A into this solution. Record temperature of absolute alcohol for volume to weight conversion. Solutions A and B are to be used together and discarded together.

Blood samples (duplicate analysis): Using an Ostwald-Folin pipet (Class B or better), pipet 2 ml of blood to be analyzed into 60 cc serum bottle. Using a precision microliter syringe, dispense 8 microliters of solution A into serum bottle, cap and seal.

Work standard: Pour about 2 ml of solution B into a 60 cc serum bottle, cap and seal.

Thermostat prepared aliquots of unknown blood and working standards at 29°C (or other temperature close to but above room temperature) for 45 minutes.

Sample analysis: Position rubber septum on 1/8-inch sample valve inlet fitting and seal with 1/8-inch mating nut. Insert a 1-inch long No. 23 syringe needle through septum. Before injection of headspace sample, flush sampling lines with 50 cc of fresh air using a clean 50 cc syringe. Using a 20 cc plastic syringe equipped with a 1-inch long No. 22 needle (previously well flushed with fresh air), quickly obtain a 10 cc sample of headspace vapors as follows:

Insert needle through serum bottle stopper, connect syringe (plunger depressed) and pump in and out to 10 cc mark five times, retract plunger to 10 cc mark and remove. Connect syring to needle on sample valve and inject headspace vapors into sample valve. Switch sample valve to inject position.

CALCULATION:

where $$BAC = R_u \times 1/R_{st} \times C_{st},$$

R_u = (peak height ethanol/peak height acetonitrile) test specimen

R_{st} = (peak height ethanol/peak height acenonitrile) work standard

C_{st} = BAC of working standard.