

Studies in Breath-Alcohol Analysis: Biological Factors¹

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Summary: Various biological factors affecting breath-alcohol analysis were studied experimentally. End-expiratory temperatures in 55 healthy subjects were found to range from 32.41 to 35.69°C with a mean of 34.53°C. Forced vital capacity in the same subjects ranged from 1825 to 6550 ml with a mean of 4038 ml, and maximum exhalation after normal inhalation ranged from 1180 to 4550 ml with a mean of 2730 ml. It was found that 65-70% of available breath must be discarded before the alveolar plateau is reached during expiration. End-expiratory (alveolar) carbon dioxide in 155 healthy subjects was 3.5-8.3% by volume (mean = 6.52). After oral alcohol intake, retained mouth-alcohol in 8 subjects had disappeared after 11 minutes without subsequent water-rinsing of the mouth, and after 8 minutes with rinsing. Water condensation in plastic mouthpieces/saliva traps during breath sampling yielded mean weight gains of 13.0, 8.6, and 4.6 mg, respectively, at initial mouthpiece temperatures of 3°C, 22.5°C, and 34.7°C, respectively.

Zusammenfassung: Einige der Grundlagen, die für die Atemalkoholanalyse von Bedeutung sind, wurden experimentell untersucht.

Bei 55 Versuchspersonen wurde die Temperatur der expiratorischen Luft gemessen und im Mittel Werte von 34,53°C mit Schwankungen im Bereich von 32,41 bis 35,69°C erhalten. Die Vitalkapazität betrug bei den gleichen Personen im Mittel 4038 ml mit Schwankungen im Bereich von 1825 bis 6550 ml, das Maximum des ausgeatmeten Luftvolumens lag nach normalem Einatmen bei 2730 ml im Mittel bei Schwankungen von 1180 bis 4550 ml. Es konnte festgestellt werden, daß ca. 65 bis 70% der gesamten ausgeatmeten Luft verworfen werden muß, bevor echte Alveolarluft vorliegt, bzw. ein konstanter Atemalkoholwert erhalten wird. Die CO₂-Konzentration der Alveolarluft lag bei 3,5-8,3% (v/v), im Mittel bei 6,52%, getestet an 155 Versuchspersonen.

Nach oraler Alkoholaufnahme war der noch in den Mundschleimhäuten verbliebene Alkohol nach 11 Minuten ohne besondere Mundspülung verschwunden, hingegen nach 8 Minuten, wenn die Mundhöhle mit Wasser gespült wurde. Die Wasserkondensation in den Mundstücken aus Plastikmaterial, durch die die Atemluft in das Prüfgerät eingeblasen wurde, zeigte eine Abhängigkeit von der Temperatur des Mundstücks: bei 3°C kondensierten im Mittel 13,0 mg, bei 22,5°C 8,6 mg und bei 34,7°C 4,6 mg Wasser.

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Key words: Breath alcohol analysis, expiratory volume - alcohol retention in the mouth

Recent developments in breath-alcohol analysis have been focused nearly exclusively on techniques of alcohol² detection and quantitation, which have consequently attained a high state of technical sophistication. Much less attention has been devoted to the equally important problems of breath sampling. Early breath-alcohol instruments (16, 24, 28) utilized static breath specimens, generally of large volume and collected in rubber balloons or fixed-volume cylinders, while recent devices have generally followed a trend toward much smaller breath volumes and dynamic breath-sampling, e.g., use of flow-through chambers or alcohol sensing of flowing breath streams. All breath-alcohol methods depend on fundamental information with respect to the nature, composition, and properties of breath samples. The dynamic sampling schemes, because of generally small sample volumes and transitory sensor exposure, are also particularly affected by key breath-sample characteristics, such as temperature, volume, pressure, duration and time-composition of single expirations, etc. Appropriate experimental data applicable to these matters are scarce.

Although it was generally recognized that only alveolar air is in alcohol equilibrium with the pulmonary circulation, some pioneer methods for breath-alcohol analysis employed mixed expired breath samples (24, 28). More than 35 years ago, the assumed constancy and supposed fixed value in all persons of the carbon dioxide content of the alveolar air became the basis, in several procedures, for determining the quantity of alveolar air in mixed expired breath samples analyzed for alcohol. Despite numerous indications from pulmonary function measurements and other medical sources of wide fluctuations in alveolar carbon dioxide (11) the measurement of breath-derived CO₂ continues to be used for quantitation of the alveolar air in some breath-alcohol methods (4). Additional data regarding the variability of the carbon dioxide content of alveolar air thus seem pertinent.

Sporadically, such alleged sources of error of biological origin in breath-alcohol analyses as prolonged retention of mouth-alcohol following ingestion of alcoholic beverages (33, 38) are mentioned in the literature. The validity of such assertions is best evaluated by rigorous, pertinent, contemporaneous experimental studies of the issues involved.

² Terms and nonstandard abbreviations used:

The unmodified word *alcohol* in this article refers to ethanol.

BAC, blood-alcohol concentration.

BrAC, Breath-alcohol concentration.

BTPS, body temperature and pressure and saturated with water vapor.

The studies reported here were carried out in order to obtain forensically valid data applicable to the foregoing and related problems. While considerable information regarding respiratory phenomena in alcohol-free subjects is available in the literature, few data exist on the effect of alcohol on respiration. HITCHCOCK (26) has suggested that alcohol ingestion may significantly affect respiration, and JOHNSTONE and REIER (29) reported various effects on respiratory patterns and ventilation of intravenous alcohol infusions which produced low or moderately elevated blood alcohol concentrations. Forensic breath-alcohol analysis is commonly performed on highly intoxicated subjects, and therefore certain of these studies were conducted with subjects under the influence of alcohol to the extent commonly encountered in clinical and law enforcement situations, and during actual breath-alcohol analysis with typical fourth-generation instruments.

EXPERIMENTAL

Apparatus, Methods, and Procedures

Concentrations of alcohol in blood and alcohol reference solutions were measured by automated gas chromatographic headspace analysis with a Multifract F40 instrument (Perkin-Elmer Corp., Norwalk, Conn. 06852) according to the method of MACHATA (35) modified by adding 1 g of sodium chloride to 1 ml of blood or other liquid and equilibrating at 65°C for 45 minutes prior to headspace analysis.

Breath-alcohol concentrations were determined by infrared absorbance measurement at 3.39 μm with a Model 4011 Intoxilyzer (Omicron Systems Corp., Palo Alto, Calif. 94303) and by a catalytic oxidation technique with a prototype Model P-7 HALTTM breath-alcohol apparatus (Borg-Warner Corp., Alcohol Countermeasure Systems, Des Plaines, Ill. 60018).

Known concentrations of alcohol in air were produced by controlled temperature equilibration at 34°C \pm 0.2 with Mark II Simulators (Smith & Wesson Electronics Co., Eatontown, N.J. 07724), operated with compressed gas (79% N₂ + 21% O₂) at 15 inches of H₂O delivery pressure. Ethanol reference solutions for equilibration with air at 34°C were prepared by appropriate dilution from a 60.5 g/liter stock solution of ethanol³, and their alcohol concentrations were individually verified, prior to use, by gas chromatographic analysis as noted above. These alcohol-in-air reference mixtures were used to calibrate the HALTTM breath-alcohol apparatus and periodically to check the calibration and performance of that device and of the Intoxilyzer apparatus.

The carbon dioxide content of expired breath was measured in physiological samples (BTPS, at body temperature, without removal of water vapor) and under ambient conditions⁴ by two methods which were calibrated to yield identical results on reference gases. Laboratory determinations of carbon dioxide were per-

³ Equilibration of air with a 1.21 g/liter ethanol solution maintained at 34°C will yield a gas mixture containing 0.476 mg of ethanol per liter, or 100 mg per 210 liters. The alcohol content of this mixture is equal to that of expired alveolar air from a subject with a BAC of 100 mg/dl (=0.100 %w/v), according to the accepted blood/breath alcohol relation that "2.1 liters of expired alveolar air contain approximately the same quantity of alcohol as 1 milliliter of blood" (7, 22), and corresponds to a BrAC of 0.100 g/210 liters.

formed on 55 subjects (32♂, age 17-53; 23♀, age 18-54) by non-dispersive infrared absorbance measurement with a Model KK146 Godart Capnograph (Instrumentation Associates, Inc., New York, N.Y. 10023). The capnograph response for each complete expiration was recorded on a Model 194 ten-inch potentiometric strip-chart recorder (Honeywell, Inc., Ft. Washington, Pa. 19034), and the end-expiratory carbon dioxide concentration was determined from the maximum alveolar plateau indication. Field measurements of carbon dioxide were performed on 100 adult subjects (59♂, 41♀) by an alkali absorption technique (15, 36) with a direct-reading Fridericia apparatus of 100 ml capacity (SGA Scientific, Inc., Bloomfield, N.J. 07003). Calibrations were accomplished with certified reference gases (4.97±0.01 mol% CO₂ + 95.03 mol% N₂, Cat. No. 477542; 9.74 ± 0.01 mol% CO₂ + 11.73 mol% O₂ + 78.53 mol% N₂, Cat. No. 47753, Corning Scientific Instruments, Medfield, Mass. 02052). All breath CO₂ measurements were carried out on non-fasting subjects. After a period of resting-state normal respiration, the standing subjects performed a maximum exhalation following a normal inspiration. The terminal 100 ml of end-expiratory breath were collected for the Fridericia analyses, or the entire exhalation was analyzed for CO₂ with the flow-through cell of the capnograph (0.44 ml sample-cell volume, 80 milliseconds full-scale response), using a by-pass mouthpiece.

Breath pressure was measured with a Model 2050C Magnehelic® direct-reading differential pressure gauge (Dwyer Instruments, Inc., Michigan City, Ind. 46360). Breath temperature was measured with a Heath/Schlumberger Model EU-200-41/EU-200-62 digital thermometer (Heath Co., Benton Harbor, Mich. 49022) and a Model No. 705 thermolinear probe (Yellow Springs Instrument Co., Yellow Springs, Ohio 45387). Breath volumes were measured with a Dräger Model 2207001 Volumeter® (North American Dräger, Telford, Pa. 18969) or a Model MK12 Wright Respirometer (British Oxygen Co., Ltd., Pinnacles, Harlow, Essex, U.K.).

The effect of residual alcohol in the mouth (after oral alcohol retention) on breath-alcohol analysis results was studied in 8 subjects (1♂, age 52; 7♀, age 23-50) by sequential breath-alcohol measurements with a Model 4011 Intoxilyzer, in the 3-digit indication mode. Following several baseline breath-alcohol determinations, each initially alcohol-free subject held 30 ml of a room-temperature (24.4°C) 11.4 %v/v alcohol solution (48 ml of 80-proof vodka + 120 ml water) in his mouth for 1 min without swallowing and then rapidly expelled it completely. Beginning immediately after expectoration of the alcohol solution, the end-expiratory breath was analyzed for alcohol content at intervals of 1 min for 20 min. After an interval of several hours, the procedure was repeated with the same subjects, with the addition of thorough mouth-rinsing. The mouth was rinsed twice for 30-second periods with 30-ml portions of distilled water at room temperature after expectoration of the alcohol solution; breath-alcohol analysis began immediately after expectoration of the final rinse solution. Each subject refrained from talking during the entire experimental period, keeping the mouth closed and breathing through the nose only, except for oral exhalation during breath-sampling. All subjects remained seated and inactive during the experiment.

Water condensation from breath onto the interior surfaces of the standard commercial plastic mouthpiece/saliva trap commonly employed in breath-alcohol analysis was studied in 5 alcohol-free subjects (2♂, age 31, 32; 3♀, age 23-42) at 3 mouthpiece temperatures. Each subject, after a normal inhalation, exhaled completely through a factory-new plastic mouthpiece/saliva trap (5.7 x 1.9 x 0.95 cm OD; Smith & Wesson Electronics Co., Eatontown, N.J. 07724) into a Breathalyzer® Collection Unit (Smith & Wesson Electronics Co.) maintained at 50°C operating temperature. Prior to use, mouthpieces were allowed to reach temperature equi-

⁴ The ambient barometric pressure over the duration of this set of measurements varied from 734.7 to 740.7 torr (corrected for temperature and gravity) and room temperatures varied between 24.0 and 24.2°C. The instruments were frequently recalibrated at ambient conditions at the time breath samples were analyzed.

librium (for 30 min) at 3^o C, 22.5^o C, and 34.7^u C, respectively. Each mouthpiece was weighed immediately before and immediately after an exhalation on a standard single-pan semimicro balance, with precautions to minimize temperature changes. End-expiratory breath temperature and maximum exhalation volume after normal inhalation were measured for each subject as outlined above, as was sampling time.

The composition of expired breath in a healthy man was measured by continuous mass-spectrometric analysis, in the single-breath test mode, with a Model 1100 Medical Gas Analyzer (Perkin-Elmer Corp., Medical Instruments, Pomona, Calif. 91767). The CO₂ and O₂ of the expired breath were recorded, in percent by volume, against time, with precautions to assure constant rates of breath flow.

In vivo studies of breath sampling during breath-alcohol analysis and correlation studies of BACs and BrACs were conducted on 55 subjects (40 ♂, age 21-50; 15 ♀, age 23-46). Following appropriate briefing, preparation, and establishment of alcohol-free status, the subjects consumed doses of alcoholic beverages calculated to yield maximum BACs ranging up to 0.20% w/v. Breath volume, pressure, and temperature were measured in replicate following alcohol consumption. After the subjects had reached the post-absorptive state, as evidenced by frequent breath-alcohol monitoring, paired samples of breath and antecubital venous whole blood were collected as nearly simultaneously as practicable, and always within 2 min. Blood specimens of approximately 10 ml were collected, with sterile precautions, into Vacutainer[®] tubes (Cat. No. 4726; Becton, Dickinson & Co., Rutherford, N.J. 07070) containing 20 mg of potassium oxalate and 25 mg of sodium fluoride. After numerical coding, the blood specimens were stored at 4^o C, and were analyzed for alcohol within 48 hours, in replicate, by analysts who had no knowledge of the results of the breath-alcohol tests.

All human subject studies were conducted on healthy volunteer subjects in accordance with national standards for investigations involving human subjects (39) and pursuant to approval of full experimental protocols by an institutional Human Experimentation Committee.

RESULTS

Performance Characteristics of Methods

Key characteristics of the principal methods used in the studies are summarized in Table 1, which includes data on the precision, in non-biological reference systems, of the methods used for breath measurements. The two procedures employed for measurement of the carbon dioxide content of breath yielded identical results with comparable precision in both static gas analyses and in vivo subject studies, as did the two instruments used for breath volume measurements.

Breath-Sample Characteristics in Human Subjects After Alcohol Consumption

The results of the studies of breath temperature and breath volume in 55 healthy adult human subjects are summarized in Tables 2 and 3. All temperatures shown were recorded at the end of an expiratory vital capacity maneuver⁵ and are, therefore, end-expiratory temperatures. The breath-volume data were obtained in standing subjects and include both the maximum expiratory volume measured by an expiratory forced vital capacity maneuver⁵, and the maximum expiratory volume in the same subjects after a normal inhalation. All volumes shown are for breath at

Table 1. *Characteristics of Methods Employed*

Blood-Alcohol Determination by Automated GC Headspace Analysis with Perkin-Elmer Multifrac F-40 Instrument				
Precision of Replicate Determinations (N = 29, 28, 27, 24):				
At 50 mg/dl BAC	Mean = 50.0 mg/dl	SD = 0.27 mg/dl	CV = 0.55%	
100	100.0	1.05	1.05	
150	150.0	0.95	0.63	
200	200.0	1.77	0.89	
Gas CO ₂ Content Measurement by Godart Infrared Capnograph				
Precision of Replicate Measurements under Ambient Conditions ^a (N = 42):				
At 5.3 % v/v (Nominal)	Mean = 5.31 % v/v	SD = 0.030 % v/v	CV = 0.56%	
Gas Pressure Measurement by Direct-Reading Magnehelic [®] Pressure Gauge				
Precision of Replicate Measurements under Ambient Conditions ^a (N = 11):				
At 15 in H ₂ O	Mean = 15.0 in H ₂ O	SD = 0.08 in H ₂ O	CV = 0.53%	
Gas Temperature Measurement by Heath-Schlumberger Digital Thermometer + YSI Thermoliner Probe No. 705				
Precision of Replicate Measurements under Ambient Conditions ^a (N = 21):				
At 34.5° C	Mean = 34.50° C	SD = 0.007° C	CV = 0.02%	
Gas Volume Measurement by Direct-Reading Dräger Volumeter [®]				
Precision of Replicate Measurements of Gas Syringe Output under Ambient Conditions ^a (N = 10):				
At 470 ml Volume	Mean = 470.9 ml	SD = 1.94 ml	CV = 0.41%	
Simulator Solution Measurement by Automated GC Headspace Analysis with Perkin-Elmer Multifrac F-40 Instrument				
Validation of Ethanol Solution Concentration for Nominal 100 mg/210 L BrAC Simulator Output at 34° C				
For 14 Solutions on 14 Days (Expressed as Corresponding BrAC) (N = 14)				
	Mean = 100.3 mg/210 L	SD = 1.64 mg/210 L	CV = 1.64%	
^a Ambient Conditions: Barometric Pressure 738.3 Torr, Room Temperature 22.3° C				

Table 2. *End-Expiratory Breath Temperatures in Human Subjects, after Alcohol Consumption, Measured at the Mouth*

Subjects	No	End-Expiratory Temperature, °C	
		Range	Mean
Males	40	32.41-35.57	34.48
Females	15	33.53-35.69	34.68
Total	55	32.41-35.69	34.53

Table 3. *End-Expiratory Breath Volumes in Human Subjects after Alcohol Consumption*

Subjects	No	Forced Vital Capacity, ml		Maximum Exhalation After Normal Inhalation, ml	
		Range	Mean	Range	Mean
Males	40	2245-6550	4502	1180-4550	2951
Females	15	1825-3200	2800	1480-3000	2141
Total	55	1825-6550	4038	1180-4550	2730

physiological temperature, essentially saturated with water vapor, at ambient barometric pressure⁶ (BTPS).

Table 4 summarizes the breath pressure and breath volume data for 19 subjects during breath-alcohol analysis with a prototype version of the Borg-Warner P-7 HALTTM apparatus. The corresponding data for 23 subjects during breath-alcohol analysis with the Intoxilyzer are given in Table 5. The breath pressures shown are peak values attained (above the ambient pressure) during the sampling period, measured at the breath sample inlets of the respective instruments. The volumes, for the P-7 apparatus, are shown as the absolute volumes delivered to the device during the fixed 4.2 seconds sampling period, and as the fraction of the maximum expiratory volume after a normal inhalation in the same subjects. The data for the Intoxilyzer represent absolute volumes delivered to the device during its 12.0 seconds sampling period and the fraction of the vital capacity⁵, since the

⁵ Vital capacity (VC) is the volume of air that can be expelled during a maximal exhalation after a maximum inspiration. Forced vital capacity (FVC) is the air volume obtained by a vital capacity maneuver performed with an expiration as forceful and rapid as possible. In *normal* subjects, VC equals FVC.

⁶ The observed barometric pressure extremes over the duration of these human subject studies were 716.7 - 747.1 torr (corrected for temperature and gravity); and room temperatures varied between 22.0 and 25.8°C. Calibration of breath-alcohol instruments was carried out at the respective atmospheric pressures and temperatures existing at the time of breath sampling or reference sample analysis.

time, volume, and pressure breath-sampling characteristics of this instrument require a preceding maximal inhalation in most persons.

Table 4. Characteristics of Breath Samples from Human Subjects, after Alcohol Consumption, and during Breath-Sampling with a Prototype Model P-7 Borg-Warner Breath-Alcohol Apparatus

Subjects	No	Breath Pressure, in H ₂ O		Breath Sample Volume, ml ^a		P-7 Sample Volume, ml	
		Range	Mean	Range	Mean	Range	Mean
Males	13	14-50	23.4	200-1025	647	0.06-0.51	0.24
Females	6	8-25	16.8	195-760	427	0.08-0.51	0.24
Total	19	8-50	21.3	195-1025	577	0.06-0.51	0.24

^aSampling period = 4.2 seconds

Table 5. Characteristics of Breath Samples from Human Subjects, after Alcohol Consumption, and during Breath-Sampling with an Intoxilyzer Breath-Alcohol Apparatus

Subjects	No	Breath Pressure, in H ₂ O		Breath Sample Volume, ml ^a		Intoxilyzer Sample Volume, ml	
		Range	Mean	Range	Mean	Range	Mean
Males	17	8-33	15.3	1725-4100	3055	0.51-0.94	0.70
Females	6	6-17	12.0	1160-2480	1890	0.45-0.95	0.66
Total	23	6-33	14.4	1160-4100	2751	0.45-0.95	0.69

^aSampling Period 12.0 Seconds; Nominal Breath Sample Cell Volume 600 ml

Carbon Dioxide Content of Breath

Results of the measurements of the carbon dioxide concentration of breath in 155 healthy subjects, in the resting state, standing, are given in Table 6. The data represent combined results of the CO₂ measurements by the alkali absorption technique (100 subjects) and by means of the capnograph (55 subjects), since the two separate sets of data statistically do not differ significantly. The data shown are for terminal portions of end-expiratory breath, corresponding to the recorded alveolar plateaux, as illustrated in Fig. 1, and thus represent the CO₂ content of expired "true alveolar" air, at physiological conditions (BTPS, i.e., essentially saturated with water vapor and at a mean end-expiratory temperature of 34.53° C). The observed CO₂ values measured by both the alkali absorption method and capnography were found by us to be unaffected by presence or absence of water vapor and the observed values as stated, in percent v/v, do not require corrections for water vapor.

Table 6. *End-Expiratory Carbon Dioxide Content in Human Subjects*

Subjects	No	End-Expiratory Carbon Dioxide			
		Range % v/v	Mean % v/v	SD % v/v	CV
Males	91	4.2-8.3	6.86	0.75	10.9%
Females	64	3.5-8.0	6.03	0.85	14.1%
Total	155	3.5-8.3	6.52	0.89	13.7%

The values shown in Table 6 can be converted to partial pressure of carbon dioxide, P_{CO₂}, in dry gas by the following equation:

$$P_{CO_2}, \text{ torr} = \frac{CO_2, \% \text{ v/v}}{100} (P_B - P_{H_2O})$$

where P_B is the observed barometric pressure and P_{H₂O} is the partial pressure of water vapor in breath. With corrections for physiological water vapor pressure of breath (41.02 torr at 34.5° C) and at the mean barometric pressure of 737.7 torr during collection of these data and at the mean end-expiratory breath temperature of 34.5° C, the end-expiratory CO₂ data for all 155 subjects shown in Table 6, expressed in P_{CO₂} terms, are as follows:

Range:	24.38-57.83	torr
Mean:	45.42	torr
SD:	6.20	torr
CV:	13.7	%

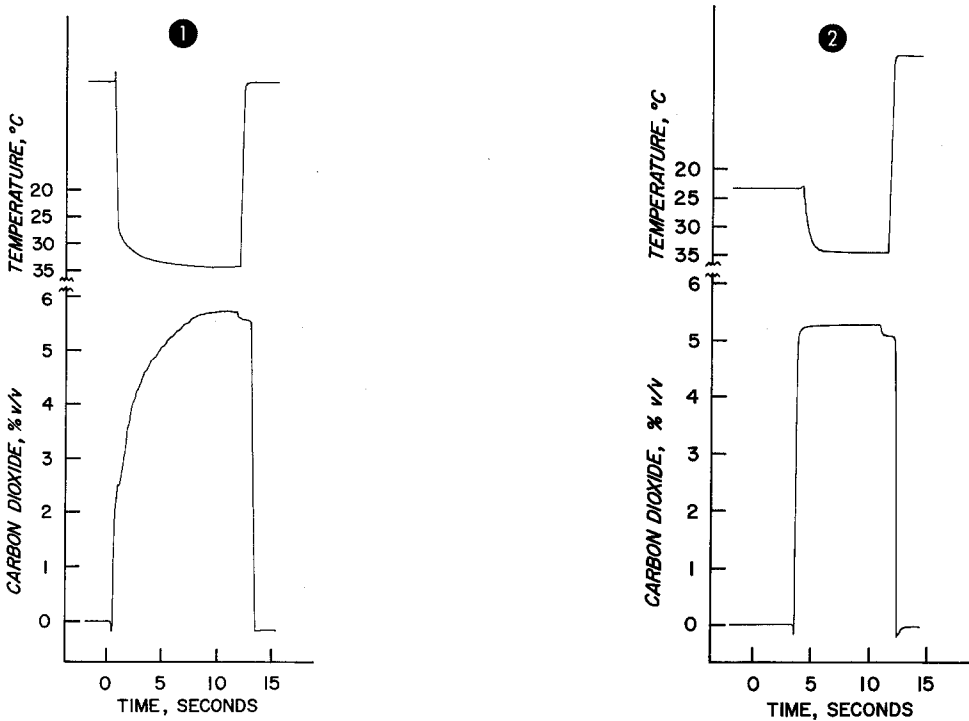


Fig. 1. Typical simultaneous in-vivo recording of temperature (upper curve) and CO₂ (lower curve) of breath during a single continuous full expiration after a normal inhalation, demonstrating the simultaneous alveolar plateau indications by both procedures

Fig. 2. Typical simultaneous recording of temperature (upper curve) and CO₂ (lower curve) for an in-vitro system, illustrating the rapid response to nearly instantaneous changes in the measured variables

Fig. 1 illustrates a typical simultaneous in-vivo recording of end-expiratory temperature and end-expiratory CO₂ (measured by capnograph) during a single continuous full expiration after a normal inspiration. The same simultaneous measurements for an in-vitro system are shown in Fig. 2, illustrating the thermistor response to an instantaneous transfer from a 23.3° C to a 34.5° C medium and capnograph response to instantaneous change in gas flow from CO₂-free air to medical gas of nominal 5.3% v/v CO₂ content flowing at 2 liters/min.

Mass Spectrometric Expirogram

A typical single-breath mass spectrometric expirogram for CO₂ and O₂ is shown in Fig. 3, with the concentration of both gases shown, in percent v/v, during a continuous full expiration after normal inspiration, at constant breath flow rate.

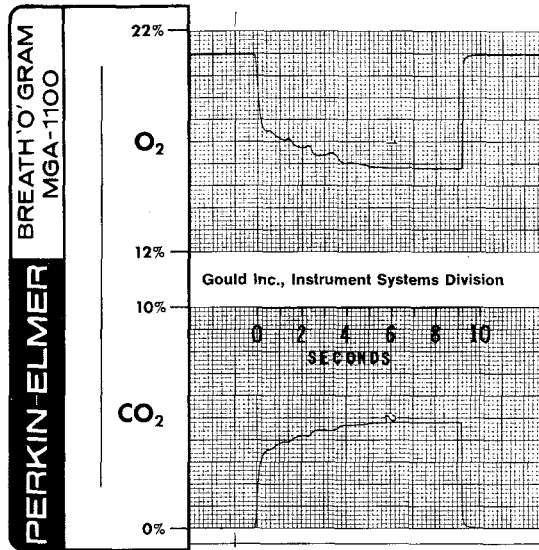


Fig. 3. Typical single-breath mass spectrometric expirogram obtained with a Model 1100 Perkin-Elmer Medical Gas Analyzer. Upper and lower curves show the breath O_2 and CO_2 content, respectively, (in vol. per 100 volumes) during a single continuous full expiration at constant breath flow rate

Performance of Breath-Alcohol Apparatus

The accuracy of analysis of Simulator-produced alcohol vapor reference specimens with the Borg-Warner prototype P-7 apparatus is shown in Fig. 4. Illustrative of the typical precision of this device in the analysis of such reference specimens, the results (indicated as the BrAC displayed by the digital readout of the P-7 device) of 51 consecutive measurements of Simulator-produced reference specimens of nominal 100 mg/210 L BrAC value³ were: Range, 99-104 mg/210 L; Mean, 100.7 mg/210 L; SD, 1.19 mg/210 L; CV, 1.19%. In Figure 5, performance of the same apparatus is illustrated in indicating the coexisting BAC by analysis of the breath. Precision of these measurements in breath is illustrated by the mean difference of +0.3 mg/210 L BrAC between sequential results for 19 sets of 3 consecutive breath specimens over a BrAC range to 113 mg/210 liters. The P-7 apparatus calibration for all measurements was carried out with alcohol vapor reference specimens based on the accepted blood/breath alcohol relation for expired alveolar air³.

Performance of the Intoxilyzer is illustrated in Fig. 6 and 7. The accuracy of analysis of Simulator-produced alcohol vapor reference specimens is shown in Fig. 6; and typical precision of these measurements is illustrated by the data for 30 consecutive measurements of alcohol vapor reference specimens of nominal 100 mg/210 L BrAC value: Range, 0.98-103 mg/210 L; Mean, 101.1 mg/210 L; SD 1.46

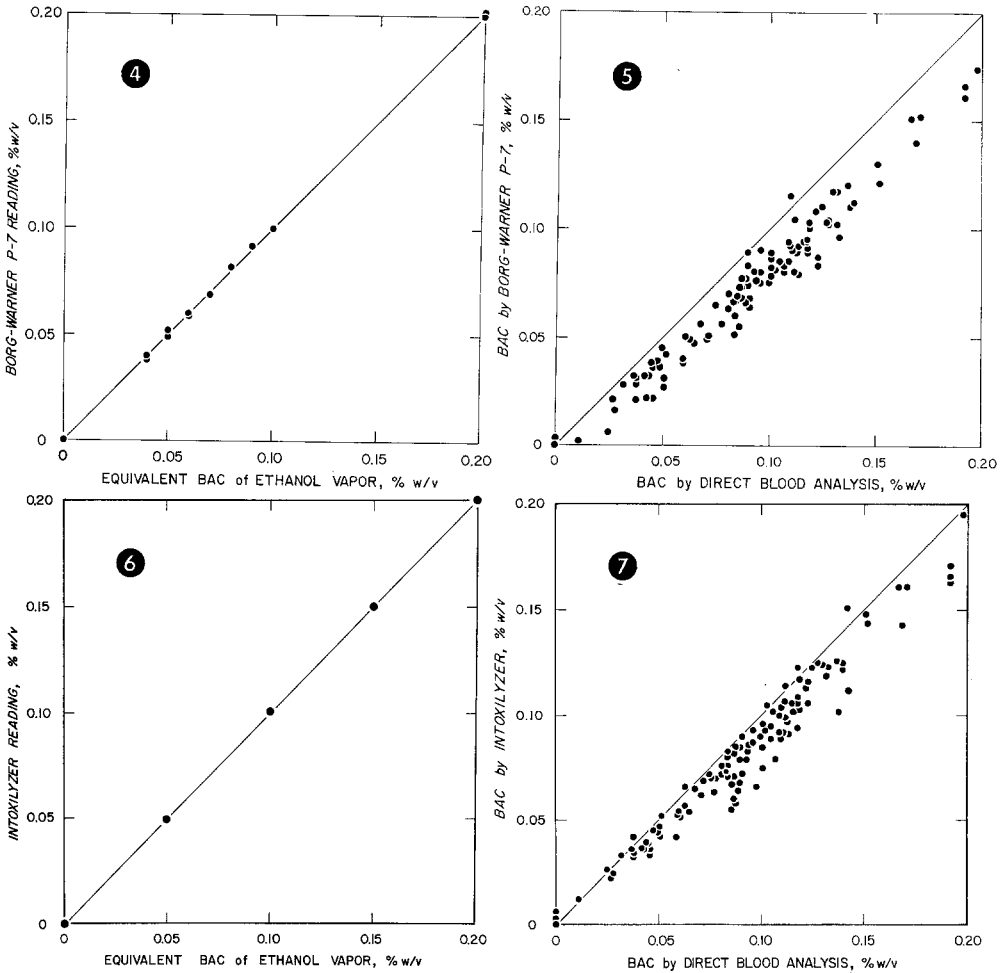


Fig. 4. Correlation between expected values and results obtained with a prototype Borg-Warner Model P-7 breath-alcohol apparatus in the analysis of 220 Simulator-produced alcohol-vapor reference specimens. The points represent the means of replicate results. The perfect correlation line is shown; the least squares linear regression line equation for these data is $y = 1.018x - 0.001$, with a correlation coefficient, $r = 0.998$ (Vapor-alcohol concentrations are expressed in terms of the corresponding BACs³ to conform to the instrument digital BAC readout)

Fig. 5. Correlation between results of analyses for alcohol in 121 pairs of simultaneously collected blood and breath specimens. Some points represent replicate results. The perfect correlation line is shown; the least squares linear regression line equation for these data is $y = 0.862x - 0.005$, with a correlation coefficient, $r = 0.979$. (Breath analyses were performed with a prototype Borg-Warner Model P-7 breath-alcohol apparatus with direct digital BAC readout; blood analyses were performed, in duplicate, by an automated gas chromatographic headspace procedure (35))

Fig. 6. Correlation between expected values and results obtained with an Intoxilyzer apparatus in the analysis of 180 Simulator-produced alcohol-vapor reference specimens. The points represent the means of replicate results. The perfect correlation line is shown; the least squares linear regression line equation for these data is $y = 1.002x - 0.0004$, with a correlation coefficient, $r = 0.998$. (Vapor-alcohol concentrations are expressed in terms of the corresponding BACs³ to conform to the instrument digital BAC readout)

Fig. 7. Correlation between results of analyses for alcohol in 128 pairs of simultaneously collected blood and breath specimens. Some points represent replicate results. The perfect correlation line is shown; the least squares linear regression line equation for these data is $y = 0.902x - 0.001$ with a correlation coefficient, $r = 0.980$. (Breath analyses were performed with an Intoxilyzer apparatus with direct digital BAC readout; blood analyses were performed, in duplicate, by an automated gas chromatographic headspace procedure (35).

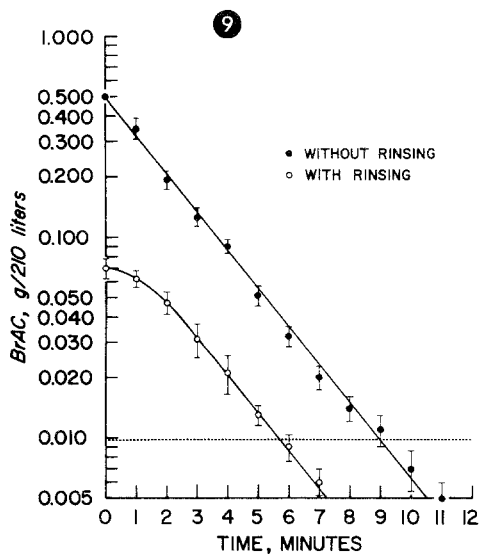
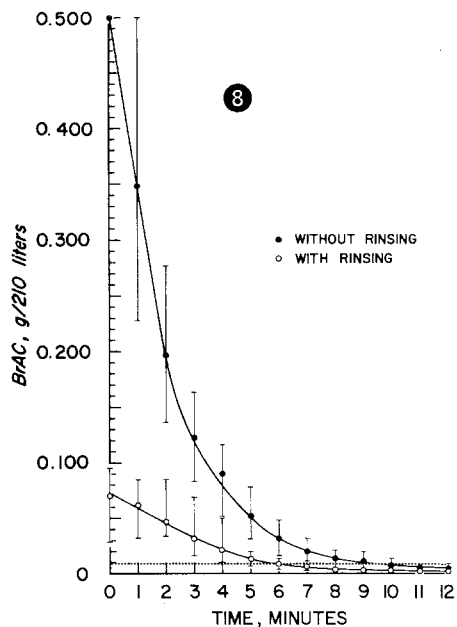


Fig. 8. Duration of residual mouth-alcohol effect on breath-alcohol analysis results, with and without water rinsing of the mouth after oral alcohol contact. Mean values (circles) and total ranges (brackets) for eight subjects are shown; time represents the deprivation period. (Breath analyses were performed with an Intoxilyzer apparatus with direct digital readout)

Fig. 9. Duration of residual mouth-alcohol effect on breath-alcohol analysis results, with and without water rinsing of the mouth after oral alcohol contact, illustrating the exponential nature of the phenomenon. Mean values (circles) and standard errors of the means (brackets) for eight subjects are shown; time represents the deprivation period. (Breath analyses were performed with an Intoxilyzer apparatus with direct digital readout)

mg/210 L; CV, 1.44%. Figure 7 illustrates performance of the same apparatus, with factory calibration, in indicating the coexisting BAC by breath analysis. Precision of the breath-alcohol measurements is shown by the mean difference of +0.5 mg/210 L BrAC between sequential results for 14 sets of 3 consecutive breath specimens over a BrAC range to 134 mg/210 liters.

Duration of Mouth-Alcohol Presence after Oral Alcohol Intake

The effect upon breath-alcohol analysis results of residual alcohol in the mouth, at various intervals after oral alcohol intake, is shown in Fig. 8 and 9. The data in these figures represent the Intoxilyzer BrAC indications in the 3-digit display mode, corrected by subtraction of each subject's initial "blank" value. Fig. 8 gives the results after a 1-min oral retention of the 11.4 % v/v alcohol solution *without* rinsing, while the data in Fig. 9 are from identical experiments *with* rinsing of the mouth. Fig. 8 illustrates the mean and extremes of the observed values in rectangular coordinate presentation, while the data are shown in Fig. 9 in semilogarithmic presentation suggested by the evident exponential form of the data with time (deprivation period).

Water Condensation in Mouthpieces/Saliva Traps During Breath Sampling

Table 7 summarizes the results of weight gain measurements of standard rectangular plastic mouthpieces/saliva traps during breath sampling in 5 subjects, at three different initial mouthpiece temperatures from 3.0 to 34.7° C. The results shown are the mean values of two consecutive tests by each subject at each mouthpiece temperature, using factory-fresh mouthpieces for every breath sample. Breath-sampling time for a complete expiration, after normal inhalation, through the mouth

Table 7. *Water Condensation in Mouthpieces/Saliva Traps during Breath Sampling*

<u>Subjects</u>	<u>Initial Mouthpiece Temperature, ° C</u>	<u>Mouthpiece Weight Gain, mg^a</u>	
		Range	Mean
5	3.0	9.7-18.0	13.0
5	22.5	7.9-9.5	8.6
5	34.7	3.3-6.4	4.6

^aMean of duplicate tests

piece into the Breathalyzer Collection Unit varied from 7.4 to 18.2 seconds; breath volumes ranged between 2,123 and 2,430 ml, and breath delivery pressures at the plastic inlet tube of the apparatus varied between 9.3 and 21.7 inches of water. Mean values for the 5 subjects for breath sampling time, breath volume, and breath pressure were 12.2 seconds, 2,301 ml, and 14.9 in H₂O, respectively.

DISCUSSION

The several methods employed in this study have good precision, as shown in Table 1, and the differences found in the human subject studies are, thus, not attributable to the methods of measurement.

The basic breath sample characteristics found in these studies are of considerable theoretical and practical significance for general breath sampling and breath-alcohol analysis. The breath temperature data in Table 2 are of significance in several respects. The literature concerning breath temperatures is inconsistent, and breath temperatures as low as 31° C (leaving the mouth) have been reported in the literature dealing with breath-alcohol analysis (8, 34). Information regarding the temperature of the breath collected for alcohol analysis is required to determine the minimal constant temperature at which the breath collection and storage systems must be maintained to prevent condensation of water vapor and consequent alcohol loss, and to apply proper Charles' law corrections to the breath volumes for calibration and analysis. Further, the vapor curve of alcohol is quite steep over the physiologically significant range; as illustrated in Figure 10 based on the data of HARGER *et al.* (20), the air/blood and air/water partition ratios of alcohol increase about 7% per degree of temperature increase between 30° and 40° C. The end-expiratory air employed in breath-alcohol analysis can thus be expected to have a significantly lower ethanol vapor pressure at a mean temperature of 34.5° C than the air in the alveoli at 37.5° C, and the alcohol vapor pressure in breath will clearly fluctuate somewhat with changes in deep body temperature and in breath pressure. However, the final ethanol vapor pressure attained is not solely a function of the temperature differences and the consequent changes in ethanol vapor pressure and in breath volumes. Air/blood partition data for ethanol are thus not directly applicable to theoretical calculations of breath-alcohol concentrations or the partial pressure of alcohol, P_{ALC}, in breath.

The end-expiratory temperatures and the mean value of 34.5° C found in this study and shown in Table 2 agree well with the data of HARGER and FORNEY (21) who reported a mean temperature of 34.4° C in a plastic mouthpiece at the end of an exhalation, for a small series of subjects.

We have observed that the rise in expiratory breath temperature, when measured with a rapidly-responding device, approximately parallels that in the alcohol content of exhaled air as indicated by a continuously-indicating instrument such as the Intoxilyzer, in a manner similar to the phenomena illustrated in Fig. 1 and 2. Since there are substantial analogies between the clearance of alcohol and of carbon dioxide from the circulation into the breath, we simultaneously monitored the electrical output signals from a capnograph and from a rapidly-responding thermistor thermometer by means of digital voltmeters, while measuring breath CO_2 and temperature during vital capacity maneuvers and during continuous exhalation after normal inhalation with precautions to assure constant airflow, and also recorded these signals simultaneously against time on a potentiometric strip-chart recorder. As illustrated in Fig. 1 which is typical of the findings, both end-expiratory temperature and end-expiratory CO_2 attain their respective alveolar plateaux at or near the same time. The slightly slower initial rise of the breath temperature curve in Fig. 1, compared with the time-course of CO_2 , is probably largely attributable to the differences in response time of the respective sensors. The capnograph has a response time of 80 milliseconds full-scale (in the 0-10 % v/v range), while the "time constant"⁷ of YSI Model 705 thermolinear probe designed for air temperature measurements is given as 600 milliseconds. The rapid rise to final values for the same measurements in the in vitro system shown in Fig. 2 indicates that the slower responses in Fig. 1 are biologically based and not instrumental artefacts.

It thus appears feasible to use breath temperature measurement by means of a sufficiently rapidly responding device to indicate when the alveolar breath-alcohol plateau has been reached and the sample for analysis should be taken. The regular and predictable nature of the breath temperature-time curve would allow, by means of relatively simple electronic circuitry, the rate of temperature change or a function thereof to trigger valving for automated collection of end-expiratory, substantially alveolar breath samples without knowledge of the available breath volume and independent of technician judgment or skill, or of complete subject cooperation. A coincident end-expiratory breath temperature indication could be used, if desired, to signal the possibility of undetected abnormalities in deep-body temperature which might impair or vitiate the validity

⁷ "Time Constant", the standard measure of probe response time is the time required for a probe to read 63% of a newly impressed temperature. While approximately 63% of the final response is attained in 1 time constant, approximately 5 time constants are required for a probe to indicate 99% of the total change (42).

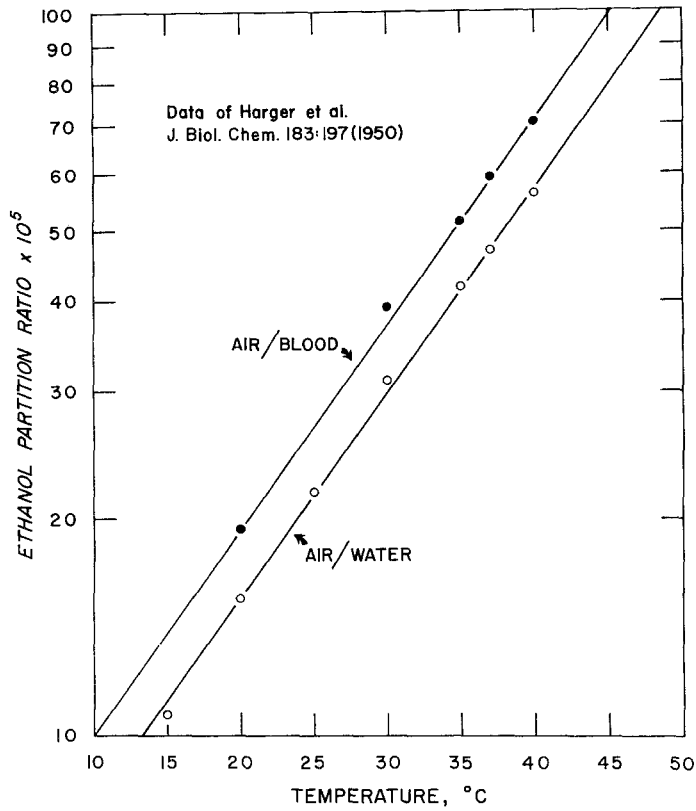


Fig. 10. In-vitro ethanol partition ratios for air/blood and air/water systems (data of HARGER *et al.* (20))

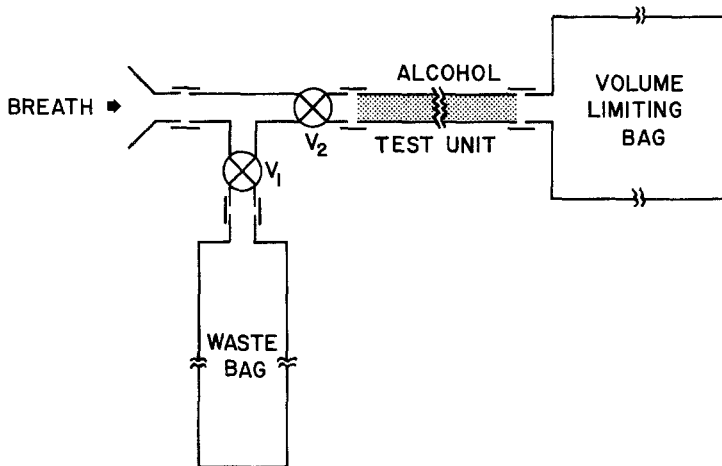


Fig. 11. Breath-sampling scheme for alcohol analysis, commonly employed in commercial breath-alcohol devices, and intended to procure expired alveolar air by initial discard of dead-space air into a waste bag

of an instrument calibration or result conversion dependent on biological factors.

On the same basis, a sufficient fast continuous alcohol sensor could obviously activate an automatic sample selection or breath-alcohol peak result retention feature in an instrument with flow-through sampling. The asymptotes of the partial pressures of water vapor or of carbon dioxide or of oxygen in breath against time could probably be used analogously by means of appropriate sensors.

Procurement of expired true alveolar breath, which is considered to be in alcohol equilibrium with the pulmonary arterial blood (7, 22, 23), is one of the most difficult problems encountered in breath-alcohol analysis. This is particularly true in law-enforcement situations where untrained and sometimes only marginally-cooperative subjects are involved, and only a single sampling may be permitted. Illustrative of one facet of this problem is the breath sampling arrangement schematically shown in Fig. 11, intended for procurement of expired alveolar air from untrained subjects and commonly employed in commercial breath-alcohol testing devices. The sampling design, derived from that of WRIGHT (41), is intended to accomplish discard of the dead space air by diversion of the initial expired breath to the waste bag, on the assumption that the remaining sample to be collected is alveolar air. While WRIGHT noted the need for varying the discard bag volume, the versions currently employed in breath-alcohol analysis practice (1, 2, 3, 6, 13, 14, 31) use discard bags of fixed volume, which presupposes that the respiratory dead-space volume is identical in all persons, and that the discard bag capacity chosen is adequate. However, as WRIGHT (41) has noted, "there is a strong suggestion that much more than the conventional physiological dead-space requires to be discarded before a true sample a alcoholic breath can be obtained".

It is a reasonable hypothesis that the alcohol content of breath, during continuous complete exhalation, parallels the time course of the breath CO_2 and O_2 contents, especially the former. As illustrated in Fig. 3 by means of a rapid response single-breath mass spectrometric expirogram⁸, the CO_2 and O_2 concentrations of breath become asymptotic at approximately 6 seconds during a continuous expiration period of 9.2 seconds, or after 65-70% of the total breath has been exhaled, assuming a constant breath flow rate during the expiration. Hence, two-thirds or more of the available breath needs to be discarded before the highest consistent breath-alcohol concentration (or partial pressure) plateau is attained in the expired specimen. From the data in Table 3, this requires discarding a

⁸ The response time of the Model 1100 Medical Gas Analyzer is given as less than 100 milliseconds to 90% response.

mean of at least 1818 ml after a normal inhalation, whereas the discard bag volumes of current commercial systems of the type illustrated in Fig. 11 commonly have capacities of only 500 to 750 ml or less. The larger (two-thirds) discard requirement is in agreement with our other studies which indicate that end-expiratory breath-samples are in substantially better postabsorptive alcohol equilibrium with the blood than so-called "alveolar-air" specimens obtained after discard of the dead space air. JONES and JONES (30), in a recent study of the alcohol concentration of breath as a function of the expired breath-volume in seven subjects, also concluded that "it is necessary to discard more than 70% of the lung vital capacity prior to breath sampling."

Forensic breath-alcohol analysts and breath-alcohol apparatus instruction manuals, unfortunately, commonly suggest that tested subjects provide a breath specimen after a maximal or near-maximal inhalation. In such situations, the data in Table 3 indicate that a mean of 2690 ml must be discarded prior to sampling to reach the highest breath-alcohol concentration plateau. These findings can, in substantial part, explain the documented inadequacy of performance of breath-alcohol screening test devices which employ breath collection bags of limited volume, reported by PROUTY and O'NEILL (37). Similar considerations with respect to breath volumes apply to other schemes for breath sampling and collection for alcohol analysis.

The influence of breath sampling on breath-alcohol analysis results is illustrated in Fig. 4, 5, 6 and 7. As shown in Fig. 4 and 6, both the prototype Borg-Warner P-7 breath-alcohol apparatus and the Intoxilyzer are obviously capable of comparably highly accurate and precise alcohol analyses in gas specimens. However, as illustrated in Fig. 5, the correlation is not as good between the alcohol content of 121 specimen pairs of blood and breath, when the latter were analyzed with the prototype Borg-Warner P-7 apparatus. The mean difference between the direct blood analysis results and the BACs indicated by the P-7 apparatus was -0.0178 %w/v. These differences in results are probably attributable to the fact that, typically, a mean of only 24% of the available breath (and never more than 51%) was supplied to the instrument, as shown in Table 4, whereas the instrument calibration was based on the accepted blood/breath ratio³, which applies only to expired true alveolar air, or rebreathed air. Accordingly, the prototype instrument is undergoing further design modifications. The mean alcohol concentration difference between direct blood analysis results and the BACs indicated by the Intoxilyzer was -0.0095 %w/v for 128 pairs of blood and breath specimens, as illustrated in Fig. 7. As shown in Table 5, a mean of 69% of the available breath was typically delivered to this instrument, with a range of 45 to 95%.

It is evident that there is direct correspondence between the extent to which a breath-alcohol apparatus is capable of consistently obtaining expired alveolar breath, and the closeness of correlation between the results of analyses of simultaneously collected specimens of blood and breath for alcohol.

Breath (delivery) pressure is another significant variable in at least two respects. With fixed breath sample path geometry, fixed flow resistance, and fixed sampling time (as employed in several newer breath-alcohol devices), the breath flow rate and hence the breath volume throughput are functions of the breath pressure. As shown in Tables 4 and 5, the breath delivery pressure can fluctuate widely among different subjects, and these differences are evidently related to the variation in delivered breath volumes. Further, the typical breath delivery pressures shown in Tables 4 and 5 are presumably reflected in comparable increases (above atmospheric) in the intrathoracic pressure gradients. When breath samples are reduced from these pressures to atmospheric pressure, the concentration of alcohol is proportionately decreased. If equilibration or full or partial re-equilibration of alcohol occurs in the respiratory tract at the higher pressures, the subsequent expansion could account for decreases in gas alcohol concentration of the order of 2 to 12 percent. The resistance to breath flow in breath-alcohol instruments and such accessories as mouthpieces should, therefore, be kept to a minimum. Further, when breath-alcohol simulators or equilibrators are used to calibrate breath-alcohol instruments, the reference vapor mixtures should be produced at total pressures corresponding to those to be expected during breath sampling with the instrument being calibrated.

The end-expiratory carbon dioxide concentrations found in this study and summarized in Table 6 are of special pertinence with respect to those breath-alcohol analysis methods and devices which rely upon determination of the alcohol-carbon dioxide ratio for estimation of the BAC. It was first suggested by HARGER (19) in 1931 and HARGER *et al.* (24) in 1938 that the fraction of alveolar air in a mixed expired breath specimen could be determined from its carbon dioxide content, based on their interpretation of a 1905 report by FITZGERALD and HALDANE (12) as establishing that "the alveolar air of normal subjects always contains close to 5.5 per cent of carbon dioxide by volume," and on the assumption that the diffusion ratio for alcohol and carbon dioxide into the air is the same for all parts of the respiratory tract. JETTER and FORRESTER (27) acknowledged in 1941 that "for the alcohol-carbon dioxide ratio to be a generally accurate measure of the quantity of alcohol in a given volume of alveolar air, and hence indirectly of the blood alcohol, the pressure of carbon dioxide in the alveolar air must be the same in all persons it must be concluded that a variation

up to ± 15 per cent from the expected alveolar carbon dioxide tension may occur". They gave the maximum experimental variations observed between 79 pairs of BACs (from 32 men), determined by direct analysis of blood and calculated from the breath-alcohol carbon dioxide ratio, as ± 16 %. HARGER *et al.* (23) subsequently reported correlations between the alcohol per 190 mg of CO₂ from mixed expired breath and alcohol per cubic centimeter of peripheral venous blood, in 100 consecutive determinations with 33 subjects, finding a mean deviation of ± 9.7 % and maximum deviations of -28 % and +32 %; and HARGER in 1956 (17) and again in 1960 (18) stated "as regards the possibility of high calculated blood alcohol figures in these two breath methods, due to normal deviation from the 5.5 % levels, the FITZGERALD-HALDANE results for men indicate that the maximum positive error is 7 to 11 %, and will occur in one case out of seven".

In the experiments reported here, we recorded the carbon dioxide concentration of the entire exhalation and determined the end-expiratory CO₂ value from the alveolar plateau. The BTPS data summarized in Table 6 can therefore be confidently considered to represent expired alveolar breath CO₂ content. It is of interest that the mean values found in this study differ significantly from the long-assumed 5.5 %v/v for both males and females, and that the extremes found in this series of 155 normal subjects differed by -46.3 % and +27.3 % from the mean end-expiratory CO₂. From the data in Table 6, it can be estimated that the end-expiratory CO₂ concentrations of 99.7 % of the healthy population will range between 3.85 %v/v and 9.19 %v/v. The corresponding errors in estimating BACs from the actual mean alveolar breath CO₂ concentration of 6.52 %v/v would range from +69 % to -29 % of the actual BACs. Errors of comparable magnitude would result from BAC estimates based on an assumed mean of 5.5 %v/v for the alveolar CO₂ content.

It is well established (9, 11) that additional large deviations from alveolar breath CO₂ values for normal, healthy subjects occur from respiratory diseases and pulmonary dysfunction, as the result of such temporary respiratory abnormalities as hyperventilation, and from metabolic acid-base disturbances. It is consequently evident that the BAC cannot validly be determined from breath CO₂ measurements.

The need to take suitable precautions, in breath-alcohol analysis, against possible specimen contamination from oral alcohol retention soon after ingestion of alcoholic beverages was recognized by BOGEN (5), who first suggested, in 1927, use of breath-alcohol analysis for assessment of alcoholic influence, and noted that the effects of retained mouth alcohol disappear "usually within 15 minutes after imbibition". A so-called deprivation period of at least 15 minutes after the end of alcohol ingestion, or from the time of first observation of a

tested subject, prior to breath-alcohol analysis has been universally employed in all forensic and well-controlled experimental applications of breath-alcohol analysis (10). Nevertheless, articles in the scientific literature periodically "rediscover" mouth-alcohol interference with breath-alcohol analysis and claim that such effects are of long duration and substantial magnitude. Thus, SPECTOR (38) reported breath-alcohol experiments (apparently with only 2 subjects) and concluded that "determinations of the concentration of ethanol in so-called alveolar gas are highly inaccurate for at least the first 20 minutes after exposure to ethanol", and that further "rinsing the mouth with water does not eliminate these false readings".

The data in Figures 8 and 9 reflect the results of our most recent studies of the effect of retained mouth-alcohol on analyses of end-expiratory breath for alcohol. In law enforcement practice in the United States, blood and breath-alcohol analysis results are reported in per cent w/v to 2 decimal places, truncated; so that BAC values less than 0.01 %w/v (or BrAC value less than 0.01 g/210 liters) are reported as completely negative. The data in Figures 8 and 9 demonstrate that, without rinsing of the mouth after alcohol intake, no effects of oral alcohol retention on breath-alcohol analyses so reported were found after a deprivation period of 11 minutes or longer, and that all such effects had ceased after a deprivation period of 8 minutes or longer with rinsing of the mouth. The respective mean times for disappearance of the residual mouth-alcohol effects were approximately 9 and 6 minutes. As illustrated in Fig. 9, the disappearance of mouth-alcohol with time is an exponential phenomenon. Further, in our studies, mouth-rinsing after oral alcohol contact significantly and consistently reduced the interval to disappearance of discernible effects on breath-alcohol analysis, compared with the non-rinsing situation. Since these experiments incorporated contrived conditions most adverse to mouth-alcohol clearance, even more rapid disappearance of oral alcohol effects can be anticipated under normal research or law enforcement conditions. These findings agree fully with those of KEMPE (32), and again confirm the adequacy of the accepted 15-minute deprivation period prior to breath-alcohol analysis for traffic law enforcement purposes⁹, and the efficacy of rinsing the mouth with water (e.g., after vomiting)

⁹ "Procedures for breath alcohol analysis...should include the following controls in conjunction with the testing of each subject: (a) Continuous observation of the subject for at least fifteen (15) minutes prior to collection of the breath specimen, during which period the subject must not have ingested alcohol, regurgitated, or vomited..." (25).

in reducing the effects of residual mouth alcohol and in accelerating their disappearance.

Breath leaves the mouth at a temperature of approximately 34°C and has a water vapor content corresponding to saturation or near saturation at the exit temperature. It is, accordingly, common experience to note visible moisture condensation in plastic combination mouthpieces-saliva traps when these are interposed, at ambient temperatures, between the subjects' mouth and the inlet of a breath-alcohol apparatus. Since alcohol is completely miscible with water at all temperatures and since the air/water partition ratio of alcohol decreases sharply with decreasing temperatures (Fig. 10), it is a matter of interest to estimate the possible loss of alcohol from breath specimens as a result of this moisture condensation.

The results of our studies on water condensation in mouthpieces, during typical breath-sampling for alcohol analysis, are summarized in Table 7. As might be anticipated, there is essentially an inverse linear relation between initial mouthpiece temperature and weight gain from breath-moisture condensation. From the data in Table 7, the mean breath volume of 2,301 ml involved, and the accepted ethanol partition ratio data for the air/water system (20) illustrated in Fig. 10, 0.51 %, 0.96 %, and 1.45 % of the breath-alcohol content is lost into the condensate in the mouthpieces at initial mouthpiece temperatures of 34.7° , 22.5° , and 3.0°C , respectively, assuming that air/water alcohol equilibrium occurs at 34°C . Greater alcohol losses result if the air/water alcohol equilibrium in the mouthpiece occurs at lower temperatures than 34°C . In either event, the alcohol loss occurs prior to measurement and/or analysis of the sample for alcohol and, while advantageous to a tested subject in law enforcement situations, can in part explain the generally noted tendency for breath-alcohol analyses to underestimate the coexisting BACs. The commonly employed transparent plastic mouthpieces used in this condensate study have inlet and outlet orifices of only 3.1 mm diameter. The prolonged time required for complete expiration through these mouthpieces reflects the effect of this undesirable constriction in the breath path. In contrast, the disposable mouthpieces most commonly used in pulmonary function testing have unrestricted flow paths of 18 mm or 30 mm. Radical redesign of the mouthpiece/saliva trap intended for use in breath-alcohol analysis is clearly needed.

It is hoped that the results of these studies will facilitate further advances in breath-alcohol analysis, including improvements in apparatus design to meet and exceed the specifications incorporated in the U.S. Department of Transportation "Standard For Devices to Measure Breath Alcohol" (40).

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