

# Accurate Measurement of Blood Alcohol Concentration with Isothermal Rebreathing

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**ABSTRACT.** The importance of interaction of exhaled air with the airway surface was evaluated by comparing the effects of different breathing maneuvers and inhaled air temperature on the relationship between breath alcohol concentration (BRAC) and blood alcohol concentration (BAC). Breath alcohol was measured with an infrared absorption unit. Blood and simulator liquid alcohol concentrations were measured by gas chromatography. Breath samples were measured after both low and high exhaled volumes and after rebreathing. Breathing maneuvers were performed after either hyperventilation, breathhold or normal breathing. Inspired air temperature was varied between 0°C and 40°C. The rebreathing method for sampling alveolar alcohol

samples was evaluated with a new isothermal rebreather that was designed to provide a substantial amount of heat to the rebreathed air in order to heat the airway surfaces. Using a single breath test, the indicated BAC values vary from 14% above the actual BAC to as low as 55% below the actual BAC. Hyperventilation caused a significant decrease in BRAC and breathhold caused a significant increase in BRAC. When isothermal rebreathing is applied to such tests, the breath test results were always within  $\pm 10\%$  of the true BAC, even with an altered breathing pattern. Isothermal rebreathing provided an accurate sample of alveolar air that was not affected by altered breathing pattern or air temperature. (*J. Stud. Alcohol* 51: 6-13, 1990)

**S**INGLE BREATH testing for estimation of blood alcohol concentration (BAC) is a method used widely in both scientific and legal environments. Confidence in the accuracy of this method has been shaken with the recent knowledge regarding soluble gas interaction with the airways during breathing. Such interaction leaves doubt as to the relationship between true alveolar and exhaled alcohol concentrations. Since the early description of breath alcohol testing (Bogen, 1927; Liljestrand and Linde, 1930), it has been assumed that the concentration of alcohol in end-exhaled breath is constant and the same as the alcohol concentration in the alveolar air. However, several studies (Aharonson et al., 1974; Jones, 1982a; Slemeyer, 1981; Wright, 1962) have shown that highly soluble gases, such as alcohol, have a considerable amount of interaction with the airway surface during exhalation that precludes an equilibrium between end-tidal and alveolar gas concentrations. The magnitude of this interaction depends principally upon tissue solubility of the gas and ambient temperature and humidity (Jones, 1982b). In addition, breathing pattern prior to the delivery of the breath sample influences the breath alcohol concentra-

tion (BRAC). Hyperventilation reduces alcohol concentration in the breath, while a breathhold period prior to breath sample delivery increases BRAC (Jones, 1982c). BRAC increases with the amount of air exhaled, and a flat alcohol plateau is never reached if the exhaled flow rate is maintained (Jones, 1982a; Slemeyer, 1981). Other factors that contribute to the interindividual variability are differences in body temperature (Harger et al., 1950; Jones, 1983a) and hematocrit (Jones, 1983a; Payne et al., 1968).

An approach commonly used to obtain alveolar gas samples is to rebreathe into a bag, which provides for the mixing of gas from different regions of the lung. The rebreathing approach has been used by several previous investigators (Forney et al., 1964; Harger and Forney, 1970; Harger et al., 1956; Pinkwart et al., 1981; Schwarz et al., 1982) to obtain an alveolar sample for estimation of BAC. Rebreathing of heated air prior to the delivery of a breath sample would theoretically decrease the influence of temperature and humidity disequilibrium and assist in prevention of airway condensation and subsequent alcohol loss due to cooling (Jones, 1983b). In a test of the use of a heated rebreathing bag, Jones (1983b) found higher alcohol concentrations in the breath after rebreathing compared to measurements after a single exhalation. Since rebreathing produces a breath sample that is closer to

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the true mixed alveolar air, the findings of Jones suggest that there is a normal loss of alcohol to the airway surface during exhalation and that rebreathing produces a better sample of alveolar air. A drawback of many of the previous rebreathing studies is that the rebreathing bag and airway surfaces were not heated enough to decrease the airway alcohol deposition.

Minimizing the interaction of alcohol with the airways by using a heated rebreathing apparatus should lead to an improved correlation between BRAC and BAC. In this study, a rebreathing device was constructed that maintains air near body temperature during the entire rebreathing maneuver. BACs estimated with this rebreathing technique were compared to breath alcohol values, obtained with a conventional single breath technique after different ventilation patterns performed prior to sample delivery. Breath values were compared to BAC analyzed by gas chromatography. The purpose of this study was to test the hypothesis that breathing pattern and air temperature affect the single breath test but have minimal effect on the rebreathing test for breath alcohol measurement.

## Method

### Subjects

Seven female and seven male volunteers without history of cardiac or pulmonary disease and with normal findings on physical examination participated in the study. The study was approved by the University of Washington Human Subjects Review Committee. Written informed consent was obtained from each subject. Characteristics of the subjects are summarized in Table 1. The subjects arrived at the laboratory at 9 AM with no food consumption during the previous 12 hours. Body temperature, hematocrit and vital capacity were measured. Alcohol was administered in the form of liquor either with ice or water in a dose calculated according to the Widmark (1932) formula to give a BAC of approximately 0.12 g/100 ml at the time of the initial testing. After completion of drinking, the subjects waited for a minimum of 45 minutes for absorption to take place.

### Materials

**Rebreathing apparatus.** A rebreathing bag (nylon, 4

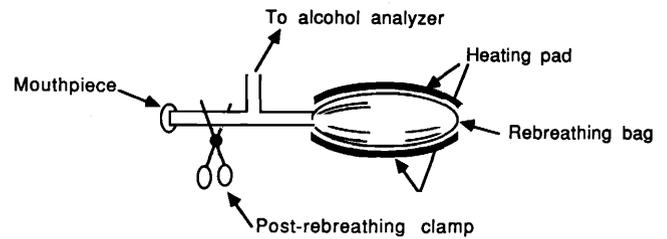


FIGURE 1. Schematic of isothermal rebreathing apparatus

liter volume) was contained in an insulated bag containing two heating pads, one on each side of the bag (Figure 1). A temperature control system sensed the temperature in the rebreathing bag and provided heat to warm the bag to preset temperatures up to 70° C. The rebreathing bag was connected through a plastic tube and a stopcock to a mouthpiece. A thermocouple (Chromel-Alumel) was inserted into the plastic tube for continuous measurement of gas temperature during the rebreathing maneuver. The output signal from the thermocouple was AD-converted and stored in a computer. The partial pressure of oxygen was measured with a mass spectrometer (Perkin Elmer MGA-1100), which sampled gas (50 ml/min) from a side port in the connecting tube.

**Breath alcohol analysis.** Alcohol concentration in the exhaled breath and the rebreathing bag was determined with an infrared absorption analyzer using two wavelengths: 3.44  $\mu$  as a primary wavelength for alcohol and 3.39  $\mu$  as a secondary wavelength to correct for any contaminating acetone present. A zero reference point was established by measuring the amount of infrared energy striking the detector when the sample chamber was filled with room air. Alcohol present in a breath sample absorbs infrared energy. The difference in absorbed energy between the reference and the breath test measurement is directly proportional to the alcohol concentration in the breath sample. The minimum exhalation sample was taken as the reading from the infrared unit (IRU) after fulfilling a minimum criteria consistent with commercially available breath alcohol analyzers: the airway (IRU inlet) pressure was required to exceed 15 cm H<sub>2</sub>O for a minimum time period of 4 seconds. The maximum exhalation sample was taken as the reading from the IRU after the subject had exhaled completely (to residual volume).

Two inlet ports were provided for delivery of air to

TABLE 1. Characteristics of the subjects (mean  $\pm$  SD)

Sex	n	Age (yrs)	Weight (kg)	Body temp. (°C)	Vital capacity (l)	Hematocrit (%)	Alcohol dose (oz)
Female	7	32 $\pm$ 8	63 $\pm$ 7	36.2 $\pm$ 0.6	3.7 $\pm$ 0.6	37.7 $\pm$ 1.0	6.3 $\pm$ 0.6
Male	7	40 $\pm$ 5	88 $\pm$ 7	35.9 $\pm$ 0.3	5.1 $\pm$ 0.7	42.6 $\pm$ 1.8	9.0 $\pm$ 1.2

the IRU sample chamber. One of these had a heated connection tube fitted to a mouthpiece, through which breath samples were delivered. Reference samples of room air were also drawn through this port with a vacuum pump. The second inlet port was for sampling of air from the simulator or from the rebreathing bag by drawing a sample with the vacuum pump.

**Calibration procedures.** The IRU was calibrated with a breath-alcohol simulator (Smith & Wesson Mark IIA) via air drawn by the vacuum pump through a 0.12 gm/100ml alcohol-water solution maintained at a constant temperature ( $34 \pm 0.2^\circ\text{C}$ ). Repeated measurements with the simulator were done during each series of experiments. Samples from the water-alcohol mixture were also analyzed by gas chromatography for cross-calibration of the blood samples and the IRU.

**Calculations.** The within-day variation of simulator measurements on the IRU was always within  $\pm 3\%$  of the mean value for the day. The average value from the simulator measurements each day ( $\text{Cal}_{\text{sim}}$ ) was therefore compared to results from the gas chromatographic (GC) analysis of the simulator fluid ( $\text{Cal}_{\text{GC}}$ ). Assuming a water-gas partition coefficient for alcohol at  $34^\circ\text{C}$  of 2586 (Jones, 1983a), an instrument factor ( $F_{\text{IRU}}$ ) can be calculated as:

$$F_{\text{IRU}} = (\text{Cal}_{\text{sim}}/\text{Cal}_{\text{GC}}) * 2586 \quad (1)$$

A blood-breath ratio (BBR) for each experimental measurement can be calculated as:

$$\text{BBR} = (R_{\text{GC}}/R_{\text{IRU}}) * F_{\text{IRU}} \quad (2)$$

where  $R_{\text{GC}}$  is the BAC reading determined with gas chromatographic technique and  $R_{\text{IRU}}$  is the reading from the IRU.

### Procedure

**Experimental protocol.** Each experiment consisted of five different breathing maneuvers, repeated two or three times on each subject. For each maneuver, the breath alcohol was measured first by a single breath maneuver directly into the IRU. Values were read from the digital display when they first fulfilled the minimum criteria stated above ( $\text{SB}_{\text{min}}$ ) and again after a full exhalation to residual volume ( $\text{SB}_{\text{max}}$ ). The rebreathing maneuver was performed with six exhalations into the bag and six inhalations from the bag. The seventh exhalation was trapped in the bag and subsequently analyzed by the IRU via the simulator inlet port. Subjects used nose clips during the rebreathing procedure. The test series were always started with the single breath into the IRU, followed by the rebreathing maneuver. The test sequence was as follows:

1. Normal expiration: A single inhalation to total lung capacity (TLC) followed by a complete exhalation into the IRU sample port to residual volume (RV). Repeat using rebreathing maneuver.
2. Hyperventilation: Three deep breaths through the mouth before inhalation to TLC followed by an exhalation into the IRU sample port to RV. Repeat using rebreathing maneuver.
3. Breath hold: A single inhalation to TLC, breath hold of 15 seconds followed by an exhalation into the IRU sample port to RV. Repeat using rebreathing maneuver.
4. Cold, dry air: Three deep breaths of cold, dry air ( $0^\circ\text{C}$ , 0% relative humidity) before inhalation to TLC, followed by an exhalation into the IRU sample port to RV. Repeat using rebreathing maneuver.
5. Warm, dry air: Three deep breaths of warm, dry air ( $40^\circ\text{C}$ , 0% relative humidity) before inhalation to TLC, followed by an exhalation into the IRU sample port to RV. Repeat using rebreathing maneuver.

**Blood alcohol analysis.** Blood samples were taken from an antecubital vein into Vacutainer tubes (sodium fluoride and potassium oxalate) at three or four points in time after the estimated start (at least 1 hour after termination of drinking) of the postabsorptive phase. Samples were placed in refrigerator at  $+4^\circ\text{C}$  for subsequent analysis by gas chromatography. Blood alcohol samples were analyzed on a gas chromatograph (Perkin Elmer Model 3920) equipped with a Poropak S column and a flame ionization detector. The GC was calibrated daily with known water-alcohol mixtures.

A total of 45 BAC values were obtained (3 values in 11 subjects and 4 values in 3 subjects). BAC values were plotted against time for each subject. The first BAC value and associated breath values were discarded if this value clearly differed from a straight line fitted through the rest of the data points, indicating that the postabsorptive phase was not reached at the time for the first sample. In total, six BAC values (and related breath values) were excluded for this reason. BAC values plotted against time for Subjects 1-4 are shown in Figure 2. The first BAC value and related single

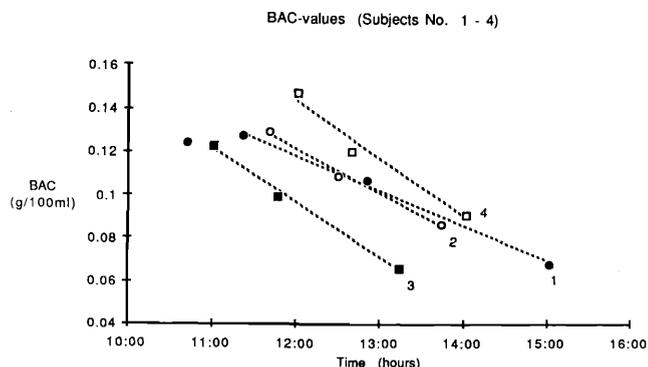


FIGURE 2. BAC versus time for Subjects 1-4. Regression lines for each subject are shown. The first data point for Subject 1 is not included in the regression.

breath values were rejected in Subject 1. By the method of least squares, a line was fitted through remaining data points for each subject, and time-dependent BAC values were calculated by linear interpolation for comparison with the breath samples.

### Results

The five different breathing patterns were repeated 32 times (2 times in 10 of the subjects and 3 times in 4 of the subjects). Seven of these sets of measurements were excluded because the BAC values indicated that the subject was not in the postabsorptive phase. The remaining 25 sets of measurements had the following distribution among the subjects: 1 set of measurements was obtained in 5 subjects, 2 in 7 subjects and 3 in 2 subjects. The mean ( $\pm$  SD) "burn off rate" (rate of decline of BAC in the postabsorptive phase) for the men was  $0.018 \pm 0.004$  g/100ml/hour and for the women  $0.022 \pm 0.005$  g/100ml/hour. The average Widmark (1932)  $r$  was  $0.628 \pm 0.084$  for the men and  $0.536 \pm 0.056$  for the women.

The BRAC always increased during the single breath exhalation into the IRU. Figure 3 shows an example of such a curve for a male subject with a large (6.3 liter) vital capacity. This subject took nearly 45 seconds for exhalation of the entire breath. In the process, the BRAC increased to over 50% above the concentration at the time of the minimum breath criteria. In the example shown, a BAC of less than 0.10 gm/100ml would be determined if the subject stopped with less than 20 seconds of exhalation. However, the subject exhaled beyond 20 seconds and a reading of more than 0.10 gm/100ml was obtained. This qualitative change occurred in all of the subjects, although the magnitude of the difference between the minimum and maximum breath samples varied from subject to subject.

**Simulator data.** The average IRU reading ( $Cal_{sim}$ ) from the simulator measurements each day was compared to the gas chromatographic analysis ( $Cal_{GC}$ ) of the simulator fluid the same day. Mean values of

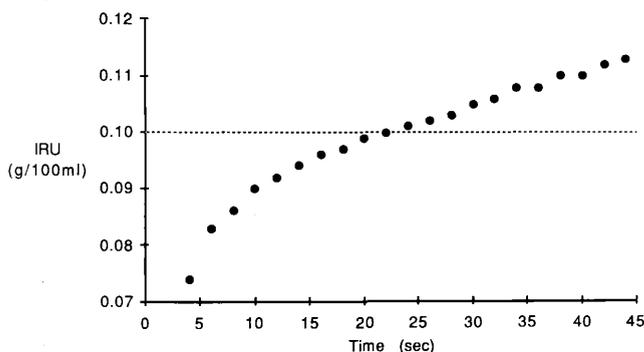


FIGURE 3. Experimental data showing breath alcohol versus time during a prolonged exhalation. The horizontal dashed line indicates the 0.10 gm/100ml level.

$Cal_{sim}$  and  $Cal_{GC}$  for the whole experimental period were  $0.101 \pm 0.002$  g/100 ml and  $0.118 \pm 0.002$  g/100 ml, respectively. The average instrument factor ( $F_{IRU}$ ) calculated according to Equation 1 was  $2,226 \pm 66$ .

**BAC data:** A comparison of BAC values and single breath values (minimum and maximum) and rebreathing values are shown in Figure 4 for maneuver

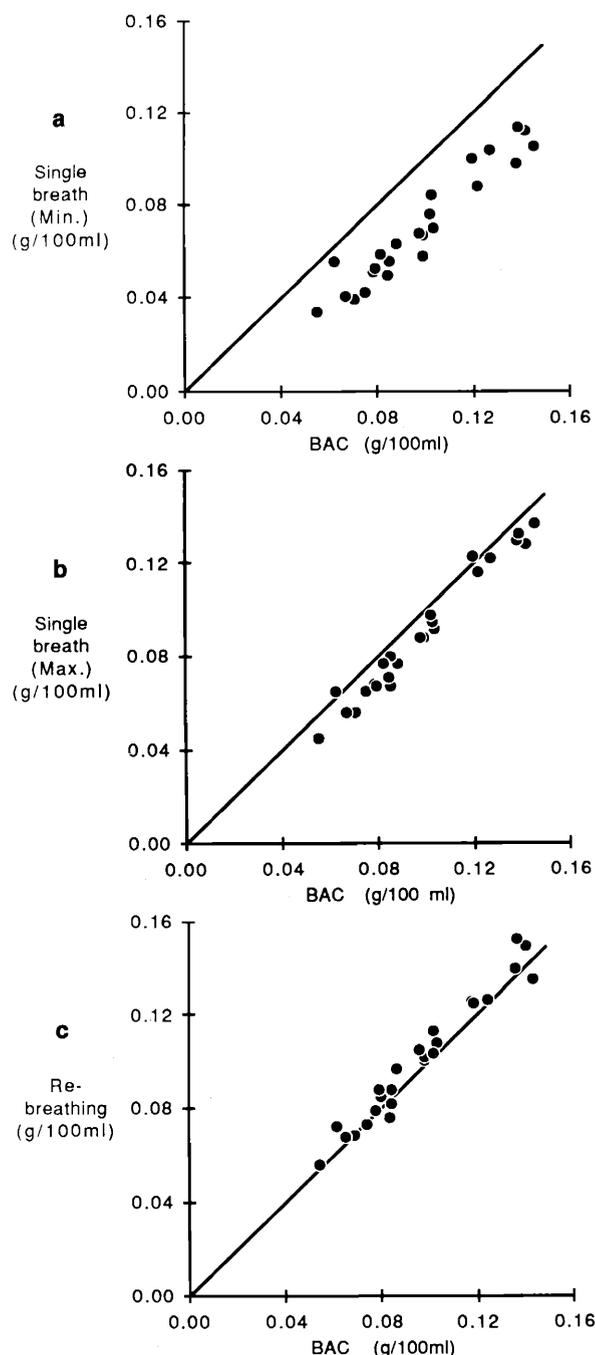


FIGURE 4. Comparison of BAC values and minimum single breath values (a), maximum single breath values (b) and rebreathing values (c) after a normal ventilatory pattern preceding the breath sample deliveries.

1 (normal ventilatory pattern before delivery of breath samples). The minimum values were all considerably lower than the true BAC values. The maximum values were closer, but also, on the average, lower than the true BAC. The rebreathing values were slightly higher than the BAC values. The data for maneuvers 2 and 3 (hyperventilation and breath hold periods preceding breath sample deliveries) are shown in Figure 5. The

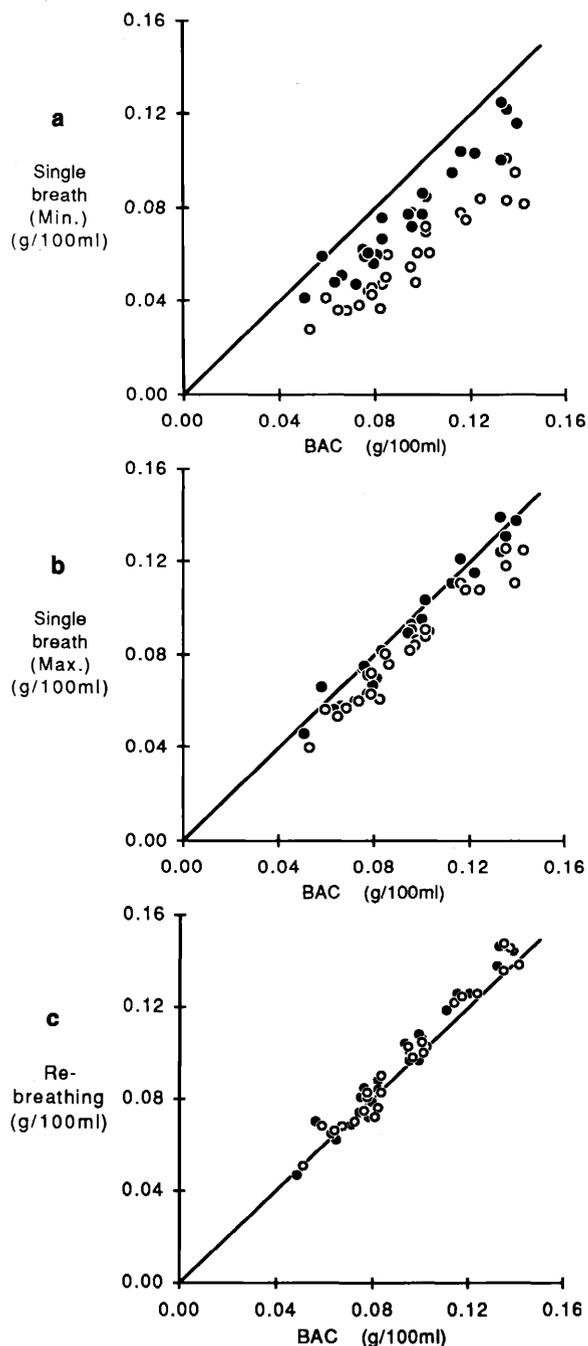


FIGURE 5. Comparison of BAC values and minimum single breath values (a), maximum single breath values (b) and re-breathing values (c) after a hyperventilation (open circles) and breath holding (closed circles) period preceding the breath sample deliveries.

TABLE 2: Mean ( $\pm$  SD) difference in percent between indirectly and directly measured BAC

Condition	n	Single breath		Rebreathing
		Min.	Max.	
Normal	25	-30 $\pm$ 9	-10 $\pm$ 7	4 $\pm$ 6
Hyperventilation	25	-39 $\pm$ 8	-14 $\pm$ 6	3 $\pm$ 6
Breath hold	25	-18 $\pm$ 8	-4 $\pm$ 7	5 $\pm$ 7
Cold air	25	-39 $\pm$ 8	-15 $\pm$ 6	0 $\pm$ 5
Warm air	25	-38 $\pm$ 7	-15 $\pm$ 6	1 $\pm$ 7
All conditions	125	-33 $\pm$ 11	-12 $\pm$ 8	2 $\pm$ 6

trends are similar for the relationship between the minimum reading, maximum reading and rebreathing. For the single breath values, the hyperventilation values are lower than the breathhold values. For the rebreathing data, there is no difference between the two different prebreathing patterns. Table 2 summarizes the average differences in percent between indirectly and directly measured BAC during the five different conditions.

**BBR and temperature data.** Figure 6 shows average values and SDs for experimental BBR values (calculated according to Equation 2), measured during the five different conditions. The trend of increasing alcohol values going from minimum to maximum to rebreathing, within any specified breathing pattern, results in a progressive decrease in BBR values. The effect of prebreath hyperventilation in increasing BBR or breathhold in decreasing BBR is apparent in the minimum values but is also statistically significant ( $p < 0.05$ ) for the maximum values. Table 3 shows the average air temperature at end exhalation of the last breath into the rebreathing bag. There are no statistically significant differences ( $p > 0.05$ ) among the various temperature values. Figure 7 shows experimental BBR values plotted against the air temperature during end exhalation of the last

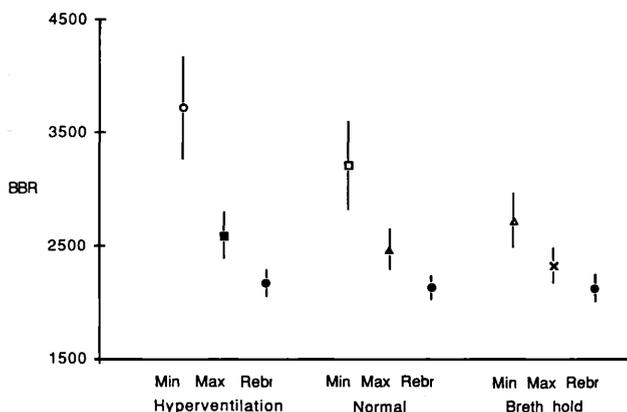


FIGURE 6. BBR values measured during each of the conditions. Mean values  $\pm$  1 standard deviation are shown. Values indicated with different symbols are significantly different from each other ( $p < 0.05$ ).

TABLE 3: Mean ( $\pm$  SD) breath temperature at end exhalation of the last breath during rebreathing

Condition	Temp. ( $^{\circ}$ C)
Normal	36.3 $\pm$ 1.0
Hyperventilation	36.4 $\pm$ 0.8
Breath hold	36.8 $\pm$ 0.8
Cold air	36.2 $\pm$ 1.0
Warm air	36.5 $\pm$ 0.8

breath during the rebreathing. There is a significant ( $p < 0.05$ ) relationship between BBR values and end-rebreathing breath temperature.

### Discussion

The data in Figure 3 indicate that during a single exhalation, the alcohol concentration is always increasing. When these data are combined with the data in Figure 6, it is clear that the breath alcohol increases during exhalation never reaching the true alcohol concentration in the alveolus. The isothermal rebreathing process provides a breath sample that is much closer to the true alveolar alcohol concentration. If flow is stopped during a single exhalation, the alcohol in the sample chamber will not change, and an apparent plateau will be seen, but at a value that may well be different from the true alveolar alcohol concentration. Because exhalation to residual volume cannot be assured during the single breath test, there is a great potential for variability in the breath alcohol sample. Using isothermal rebreathing, the thermal and alcohol disequilibrium between the respired air and the airway surface is minimized, providing a breath sample that closely reflects the true alveolar sample provided. The isothermal rebreathing maneuver negates the impact of altered pretest breathing or altered inspired air temperature, thus allowing for a breath alcohol test that will be within  $\pm 10\%$  of the true blood value.

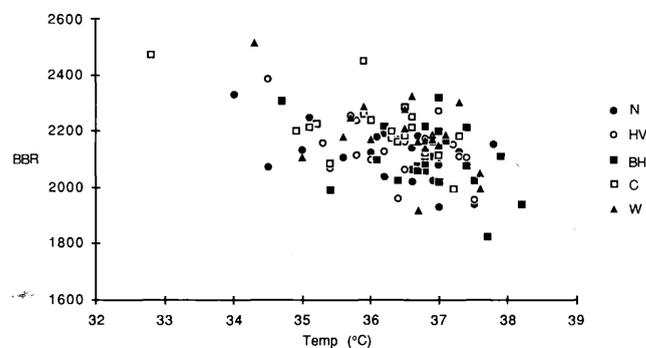


FIGURE 7. Individual experimental BBR values plotted against end-rebreathing breath temperature. Data are shown for normal ventilatory pattern (N), hyperventilation (HV), breath holding (BH), cold air inhalation (C) and warm air inhalation (W) preceding the rebreathing maneuver.

The results of this study are consistent with the earlier studies (i.e., Jones, 1982b,c) that showed the effect of various breathing maneuvers on BRAC. Pretest hyperventilation decreases the BRAC and pretest breathhold increases the BRAC. In addition, pretest hyperventilation with either cold air or warm air decreases the BRAC.

The change in BRAC can be explained by the interaction of exhaled air with the airway surface mucosa. During exhalation, some of the alcohol from the warm alveolar air deposits on the surface of the cooler airways, decreasing the amount of alcohol in the exhaled air which leaves the mouth. With hyperventilation, there is additional airway surface cooling, causing a greater alcohol solubility in the airway tissue and a greater loss of alcohol during exhalation, resulting in a lower exhaled breath alcohol. With breathholding, there is a warming of the airway tissue, causing a lower alcohol solubility in the airway tissue and a lesser loss of alcohol during exhalation, resulting in a higher exhaled breath alcohol. For both the warm dry air and cold dry air prebreathing, the humidification of the dry air causes an evaporative cooling due to the latent heat of vaporization lost from the airway surface. The net cooling causes an increased solubility of alcohol and a greater loss of alcohol from the exhaled breath.

The effects of prebreathing (hyperventilation versus breathhold) can be demonstrated by showing the average BBRs calculated for each maneuver (Figure 6). The BBR is derived by dividing the true BAC by the measured concentration of alcohol in the breath sample. The minimum BBR values are greater than the maximum end-exhaled BBR values, which, in turn, are greater than the rebreathing BBR values. This is true for all three maneuvers. For each data set (minimum, maximum and rebreathing), the pretest hyperventilation BBR values were greater than the normal breathing BBR values, which were greater than the pretest breathholding BBR values. There was no statistical difference, however, among the normal-rebreathing BBR, breathhold-rebreathing BBR and hyperventilation-rebreathing BBR values. Altering the breathing pattern before delivery of the sample has a significant effect on the BBR values for the single exhalation. The rebreathing maneuver eliminates the effect of the altered breathing patterns by ensuring that the airways are warmed.

Directly measured partition ratio values for human blood at  $37^{\circ}$ C (Jones, 1983a) indicate that the expected blood breath ratio when true alveolar air is sampled should be approximately 1,756, a value that is 20% lower than the accepted value of 2,100 used to calibrate most breath-testing instruments. This corresponds to an alcohol value after a single exhalation which is 20% lower than the alcohol value likely to be in the alveoli.

The difference can be explained by the overall loss of alcohol to the airway surface which is apparent from the physiological studies described earlier. The process of rebreathing yields *in vivo* BBR values that are closer to the value expected from the *in vitro* measurements of Jones (1983a). Figure 7 shows that the BBR value depends on the temperature of the exhaled breath. As temperature increases, the rebreathed BBR approaches the expected value of 1,756. This trend is consistent with the previous measurements of Jones (1983b) who showed mean ( $\pm$  SD) BBR values of  $1,947 \pm 110$  after rebreathing. The fact that our rebreathing values approached 2,075 (normal rebreathing, solid points in Figure 7) at 37°C rather than the expected 1,756 may be explained by a slight amount of condensation that occurred in the tubing leading from the Isothermal Rebreather to the IRU. This condensation may have resulted in a small loss of alcohol before measurement in the IRU.

The likely partition ratio of alcohol in the lung alveolar space is 1,756 as measured by the *in vitro* measurements of Jones (1983a). An empirical measurement of a BBR of 2,100 would indicate an effective 20% loss of alcohol during exhalation; for the same BAC, a breath alcohol concentration corresponding to a BBR of 2,100 is 20% lower than a BRAC corresponding to a BBR of 1,756. The effect of different breathing maneuvers is to alter the loss of alcohol during exhalation, changing the amount of alcohol in the exhaled breath sample. The use of isothermal rebreathing to negate the effect of this loss of alcohol due to interaction with the airway surface will result in breath samples that are substantially closer to the true alveolar air. Calculation of a true BAC from such a rebreathing breath sample will require use of a partition ratio which is closer to the true blood alcohol partition ratio at body temperature. Such an approach will decrease the variability of BBR values that have been reported and improve the accuracy of breath alcohol tests.

The accuracy of any breath testing instrument depends on its calibration. In most circumstances, an attempt is made to calibrate the testing instrument to have an instrument factor of 2,100. In this study the IRU had an instrument factor of 2,226. If an instrument with a factor of 2,100 had been used, the breath values would have been 6% ( $= 2,226/2,100$ ) higher and the average blood-breath ratios shown in Figures 6 and 7 would have been 6% lower.

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New York, NY 10010

### ***21st Annual Medical-Scientific Conference***

The American Society of Addiction Medicine (ASAM—formerly the American Medical Society on Alcoholism and Other Drug Dependencies) will hold its 21st Annual Medical-Scientific Conference in Phoenix, Arizona, April 26-29, 1990.

The program will include major symposia on such topics as "Pharmacological Treatment of Cocaine Abuse," "Cross-Cultural Issues in Addictions," and "Assessment of Severity and Outcome in the Addictions." Day-long symposia are being organized by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) and the National Institute on Drug Abuse (NIDA).

ASAM is a national/international society of over 3600 physicians from all areas of medicine. ASAM's Conference attracts a large representative group of members as well as non-member physicians, nurses, counselors, and other professionals, many of whom are knowledgeable about addictions and others who are new to the field.

Society members will receive registration materials in the mail. Interested non-members should call the Conference Manager, Louisa Macpherson, at 203-227-7084, for information.