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Synthesis Review

Alcohol breath test: gas exchange issues

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Hlastala MP, Anderson JC. Alcohol breath test: gas exchange issues. *J Appl Physiol* 121: 367–375, 2016. First published May 19, 2016; doi:10.1152/japplphysiol.00548.2015.—The alcohol breath test is reviewed with a focus on gas exchange factors affecting its accuracy. The basis of the alcohol breath test is the assumption that alveolar air reaches the mouth during exhalation with no change in alcohol concentration. Recent investigations have shown that alcohol concentration is altered during its transit to the mouth. The exhaled alcohol concentration is modified by interaction with the mucosa of the pulmonary airways. Exhaled alcohol concentration is not an accurate indicator of alveolar alcohol concentration. Measuring alcohol concentration in the breath is very different process than measuring a blood level from air equilibrated with a blood sample. Airway exchange of alcohol leads to a bias against certain individuals depending on the anatomic and physiologic characteristics. Methodological modifications are proposed to improve the accuracy of the alcohol breath test to become fair to all.

alcohol breath test; ethanol; airway gas exchange; lung volume; bias

THIS REVIEW IS AN UPDATE OF a prior review of the alcohol breath test (ABT) by one of us (M. P. Hlastala) (21).[1] The earlier review focused on inconsistencies and data anomalies in the ABT. This review deals with mechanisms of alcohol exchange in the airways of the lungs and explanations for the inconsistencies. For a review of alcohol pharmacokinetics and other aspects of the ABT, the reader is referred to reviews by Jones (23, 27). The accuracy of the ABT is eroded by the fact that alcohol exchanges in the airways rather than the alveoli of the lungs. Relative to alveolar exchange, airway exchange is incomplete. The deviation of breath alcohol concentration (BrAC) from alveolar alcohol concentration depends on several physiological factors that are not controlled in the present usage of the ABT. We propose methodological modifications designed to improve the accuracy of the ABT by measuring and controlling for factors contributing to biological uncertainty.

Alveolar gas exchange has been studied since the early work of Rahn (41) and Riley and Cournand (42). Their studies focused on the exchange of the primary respiratory gases: oxygen and carbon dioxide. The next level of study used the elimination of trace inert gases as a tool for assessing ventilation-perfusion heterogeneity in normal lungs by Farhi (9) and Farhi and Yokoyama (10), who found that the relative alveolar exchange of an inert gas depends on that gas's solubility in blood. Examination of inert gas elimination evolved into the Multiple Inert Gas Elimination Technique (MIGET), a method using the elimination of six gases of varying solubility to describe ventilation-perfusion relationships in the lungs developed by Wagner et al. (52).

In the decades from 1950 through 1980, investigators observed that the appearance of highly blood-soluble gases (e.g., ethanol) in the exhaled breath deviated from lower blood-soluble gases that exchanged in the alveolus. Instead of reaching a plateau, BrAC increased with increasing exhaled volume (55). The zero concentration portion (phase I) of the expirogram, which indicates airway dead space, diminished as blood solubility of the gas increased (45, 46). During a single exhalation breath test, the BrAC was ~20% less than the corresponding blood alcohol concentration (BAC) (20). The BrAC changed throughout exhalation and never stabilized (56). Breathing pattern before and during the test affected the alcohol concentration in exhaled breath (25, 37, 38). These observations indicated that highly blood soluble gases must exchange in a different manner than the alveolar mechanism used by low blood soluble gases.

Theoretical Considerations

Following on these observations, investigations on highly soluble gas exchange demonstrated the significant role played by the conducting airways. Highly soluble gases are absorbed from the airway wall during inhalation and desorbed back to the airway wall during exhalation (6, 15, 16, 46). Experimental measurements and mathematical modeling have shown that highly soluble gases exchange through the airway tissue between the bronchial circulation and the respired air (47, 48). Of the highly soluble gases, ethanol is, by far, the most studied because of the social and legal implications. Until recently, there have been few hypothesis-driven studies as to the mechanisms of exchange of ethanol by the lungs.

The general impact of the airways on ethanol exchange (both absorption and deposition) was recognized over 50 yr ago (55),

[1] The terms "ethanol" and "alcohol" will be used interchangeably in this paper.

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However, the specific physical mechanisms were only understood more recently [i.e., starting with mathematical modeling by Tsu et al. (49)]. Alcohol easily diffuses through airway tissue, due to its very high solubility in water. In fact, passive diffusion is the primary mechanism governing gas exchange in the lungs (48).

Fick's first law of diffusion describes the role of solubility and diffusivity in the medium through which gases diffuse. The equation describing the diffusive flow between lung air and capillary blood (or vice versa) follows from Fick's first law of diffusion:

$$\frac{dV}{dt} = \frac{\beta_m DA}{l}(P_a - P_b)$$

where dV/dt is volumetric flow of gas across the membrane; β_m is the solubility of gas in the membrane (diffusive barrier); D is the molecular diffusivity, which is proportional to the velocity of the diffusing molecules (depends on temperature, viscosity of the medium and molecular mass and size); A is the cross-sectional area of the membrane; l is the distance across the diffusive barrier; P_a is the partial pressure of gas in the air (alveolar or airway air in the case of alveolar or airway gas exchange, respectively); P_b is the partial pressure of gas in the blood; and t is time.

Inert gases of low ($\lambda_{b:a} < 0.10$), intermediate ($0.10 < \lambda_{b:a} < 10$), and high ($\lambda_{b:a} > 10$) solubility move easily between the alveolar air and blood because of the thinness of the alveolocapillary membrane (containing the capillary endothelial cell, basement membrane, and alveolar epithelial cell).[2,3] The alveolocapillary membrane is ~0.10-μm thick. This relative thinness allows all gases to diffuse across the alveolocapillary membrane quite rapidly (51).

The highly soluble gases can exchange across the airway tissue between the airspace and bronchial circulation. Thus highly soluble gases interact with the inspired air before it reaches the alveolus. The increased tissue thickness and reduced capillarity limits diffusional transport relative to the alveolocapillary membrane. Airway tissue thickness has been estimated to range from 160 μm in the trachea to 30 μm in the bronchioles (4). Gas transport across this thick membrane takes longer. Thus diffusivity and solubility within the membrane are more important than that in the alveolus. Additionally, the cross-sectional area is reduced because capillarity of the airway tissue is ~30% of that in the alveolus (4). As a result of this significant diffusion barrier, gases of small molecular size and high blood solubility have the greatest interaction with the airways.

Human lungs have two circulations that play a role in gas exchange. The pulmonary circulation feeds the alveoli with blood containing low oxygen and high carbon dioxide. The bronchial circulation is located within the airway tissue and follows the branching airway tree. The bronchial circulation brings blood with high oxygen content and nutrients to the cells of the airways (7). In addition, it has been calculated that alcohol in exhaled breath originates almost completely from the bronchial blood that perfuses the blood vessels within the airway tissue (4–6) rather than the pulmonary blood that supplies the alveoli.

The critical assumption of the ABT is that alveolar (deep lung) air, containing alcohol vapor in equilibrium with venous BAC (VBAC) reaches the mouth unchanged.[4] This "alveolar air" assumption is based on our early understanding of oxygen and carbon dioxide exchange and is implicit in the practice of using a single "correction factor" to correlate BrAC to BAC.[5,6] To our knowledge, this alveolar air assumption has never been confirmed experimentally. While this alveolar air assumption seemed reasonable in the 1950s, viewing the process through modern eyes, it is hard to imagine how a gas with such a very high solubility [partition coefficient, $\lambda_{b:a}$ of ~1,810 and $\lambda_{w:a}$ (w is water) of ~2,130 at body temperature (24)] could pass through the 17 generations of narrow airways without interacting with the moist mucus lining those airways.[7] The most misunderstood tool in forensic research is the ABT, an indirect estimate of ethanol concentration in the blood. The central thesis of this synthesis is that "alveolar alcohol concentration can never be measured in the exhaled breath using a single exhalation maneuver."[8] This synthesis reviews the published literature supporting that thesis.

Airway Gas Exchange: Experimental and Theoretical Models

Studies by Wanner and colleagues (33, 53) with human subjects and studies from our laboratory and others (3, 15, 16, 43, 45–48) with humans and animals have shown that gases exchange across the airway tissue between the bronchial circulation and the respired air. The relative ease of that exchange depends on the solubility of the gas in water and tissue, molecular weight of the gas, and magnitude of bronchial blood

[2] Not reactive with blood protein. Inert gases have a linear content-pressure relationship.

[3] $\lambda_{b:a}$ is the blood to air partition coefficient and the ratio of solubility in the two media: blood (β_b) to air (β_a).

[4] The term "deep lung air" is often misinterpreted as air coming from the bottom of the lungs in the erect subject. Deep lung air is considered to be a synonym for "alveolar air." The alveoli and air they contain are located in all regions (spatially) of the lung.

[5] The leveling off of breath alcohol concentration at the end of expiration has been interpreted by forensic scientists as indicating that alveolar air is expired. However, this is false interpretation. The leveling off of breath alcohol concentration is simply due to the stopping of expiration by the subject and a continual plotting of BrAC that is constant and not replenished with new expirate.

[6] The term, Partition Ratio, is used by forensic scientists to describe the relationship of BrAC to BAC despite the lack of equilibrium conditions and the temporally and spatially distributed nature of airway exchange.

[7] The $\lambda_{b:a}$ of 1,756, reported by Jones (24) and often referenced in other papers, was determined from the experiment using a single blood sample with varying temperature. The average $\lambda_{b:a}$ of alcohol averaged from the 10 subjects was 1,810.

[8] Obtaining an alveolar alcohol sample is essential to the alcohol breath test in order to assume a fixed equilibrium relationship between alcohol concentration in alveolar air and blood perfusing the alveolus. Most breath test instrument manufacturers indicate in their manuals that alveolar air is obtained if the minimum sampling criteria (e.g., air volume = 1.1 liter) are fulfilled. In addition, most states require that a valid sample must be "essentially alveolar in concentration." As an example, Title 17 of the California Code of Regulations, Section 1219.3-Breath Collection states "A breath sample shall be expired breath which is essentially alveolar in composition." Another example is Massachusetts Regulation 501 CMR 2.05, which requires analysis of "samples of alveolar or deep lung air."

flow (for most gases).[9] However, the dominant factor is solubility of the gas in water because the gas diffuses through the airway tissue, which is mostly water.

The exchange of gases between the respired air and bronchial blood has been studied in humans and animals using mathematical models validated with human data for a variety of gases (3–5, 14, 32, 39), including ethanol (4, 6, 15, 16, 49, 57). All authors found that alcohol exchange between the blood and the respired air is very different than that for oxygen (O_2) and carbon dioxide (CO_2), which exchange within the alveoli with the pulmonary blood. Alcohol and other gases with very high solubility, on the other hand, exchange in the airways between the inspired air and the bronchial circulation blood perfusing the airways. These gases easily exchange across the airway tissues despite the low bronchial blood flow (~1% of cardiac output) relative to pulmonary blood flow (100% of cardiac output) and thick airway tissue (~30 to 160 μm) relative to the alveolocapillary membrane (0.1 μm). Alcohol's extremely high solubility minimizes the diffusion barrier imposed by the airway tissue, allowing alcohol to pass easily through this longer diffusion distance.

During inhalation, respired air absorbs ethanol from the surface of the airway tree and equilibrates with the water of the airway just before entering the alveoli [as calculated by math models (4, 6) using Weibel's (54) standard airway anatomy]. Once in the alveolus, the alcohol in the air can freely move into the pulmonary blood and vice versa. For an average airway path, the partial pressures of alcohol are equal or nearly equal between the blood and the alveolar air and there is no or very little net flux of alcohol in the alveoli. For those airway pathways that are shorter in length, the air entering the alveolus may not be fully equilibrated with bronchial blood due to the shorter transit time. In such a case, the alveolar air will equilibrate with pulmonary capillary blood due to the ease for diffusion through the alveolocapillary membrane. During exhalation, the air deposits some of the alcohol onto the airway tissue (from which alcohol was partially taken during inhalation and partially replaced by the bronchial circulation). Calculations show that the ethanol in expired gas comes almost completely from the bronchial blood (4, 6). Because all (or nearly all) of the alcohol exchange occurs in the airways (4, 6), the alcohol concentration difference between prepulmonary capillary and postpulmonary capillary blood is negligible.[10] Even still, a small portion of this airway exchange comes from the pulmonary blood in the more distal airways. The exchange of alcohol in the lungs is shown schematically in Fig. 1. The airway exchange pathway is not available to O_2 and CO_2 because the water solubility for each is so much smaller than alcohol (~180,000- and 600-fold for O_2 and CO_2, respectively).

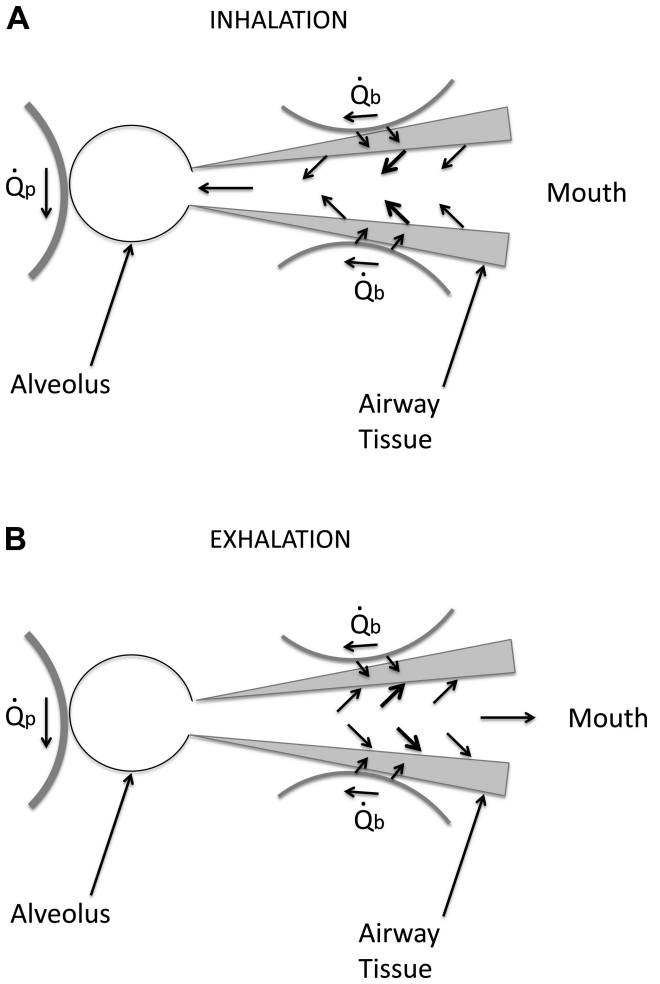

Fig. 1. Schematic showing airway exchange of alcohol during both: *A*: inhalation. *B*: exhalation. Airway tissue volume (gray), perfused by the bronchial circulation (Qb) decreases from the mouth to the alveoli. Inspired air picks up alcohol from the airway tissue.

The exchange process affects the appearance of ethanol in the exhaled breath. As exhaled breath leaves the mouth, the alcohol concentration in the breath increases with exhaled volume. The concentration rises quickly at first and then at a slower rate. The first volume of breath contains a small amount of alcohol that originates from the airway tissue. The initial exhaled breath is air that picked up some alcohol in the airways during inhalation, never reaching the alveolus, and then picked up more alcohol during the initial part of exhalation. While this initial expired breath may appear to resemble an exponential, it is likely a more linear process that is blurred due to mixing in the airways. After the air within the airway space has been exhaled (~1 ml per pound of body weight and named "dead space" for respiratory gas exchange), the next volume of exhalate originates from the alveolar region. The first portion of the alveolar air that reaches the mouth loses most of its alcohol due to deposition onto the airway tissue. With continued exhalation, the air coming from the alveolus deposits less alcohol onto the airway surface because of an increased airway surface alcohol concentration due to deposition from the earlier exhalate and, to a lesser degree, bronchial circulation replenishment. This exchange resembles an exponential process; the

[9] Normally, it would be expected that changes in blood flow would have little effect because the bronchial perfusion of the airways is quite low (~1% of cardiac output) and the relatively large tissue thickness creates a significant diffusion barrier. However, alcohol is a very highly diffusible gas due to its large water solubility. Thus availability of alcohol in the airways is affected by bronchial blood flow.

[10] Modeling shows that the uptake of ethanol by the inspired air is complete (equal to alveolar alcohol concentration) when the air reaches the 17th airway generation. Therefore, there is little net exchange in the alveolus.

amount of exchange depends on the difference in partial pressure of alcohol between the exhaled gas passing through the airways and the partial pressure of alcohol in the airway wall (tissue and mucus). The exhaled alcohol expirogram shows the changing BrAC as a function of volume (Fig. 2), never reaching a plateau (with positive expiratory flow). After air from the conducting airways has been exhaled, the expirogram is essentially an exponential process, in which the exhaled BrAC slowly approaches alveolar alcohol concentration as alcohol continues to exchange with the airway tissue. BrAC never gets close to alveolar alcohol concentration during a single maximal exhalation. By assuming that BrAC is equal to alveolar alcohol concentration, breath test manufacturers are essentially overcorrecting BrAC.

The effects of airway exchange on the appearance of BrAC can be further understood by examining the effects of inhaled volume size. During inhalation, alcohol absorbs into the passing airstream from the airway wall diminishing the airway wall alcohol concentration. Larger inhaled volumes cause a greater depletion of the airway wall alcohol concentration (22). This depletion is greater in the proximal than distal airways. As air leaves the alveolus during exhalation, alcohol is deposited onto the airway surface. Deposition of alcohol is greater earlier vs. later in exhalation, after large vs. small inhalations, and in proximal vs. distal airways. Throughout exhalation, the concentration of ethanol in the airway wall is increasing due to the deposition of ethanol and resupply of ethanol from the bronchial circulation. As exhalation continues, the rate of increase in the airway wall concentration diminishes. A more complete exhalation builds up the ethanol concentration in the airway tissue, diminishing the deposition and resulting in a greater end-exhaled BrAC.

For a full inhalation to total lung capacity followed by a full exhalation to residual volume, the exhaled BrAC can reach ~80% of the alveolar value. For alcohol with its extremely high solubility, there is not enough air volume in the alveoli relative to the airway tree volume to reach equilibrium with the airway tissue during exhalation. To determine the alveolar volume needed to overcome the depleting effects of the airway tissue and provide an alveolar breath sample, our previously described mathematical model was used (4). For a person with a 5-liter vital capacity to deliver a breath sample with alveolar alcohol concentration, it would take an exhaled volume of ~12, 25, and 50 liters to reach 90, 95, and 99% of equilibrium (Fig. 3), respectively. There is not enough air in the lungs to allow alveolar alcohol concentration to equilibrate with airway tissue alcohol concentration so that the exhaled alcohol concentration can reach alveolar alcohol concentration. In the human, end-exhaled alcohol concentration is, therefore, always lower than true alveolar alcohol concentration but to varying degrees.

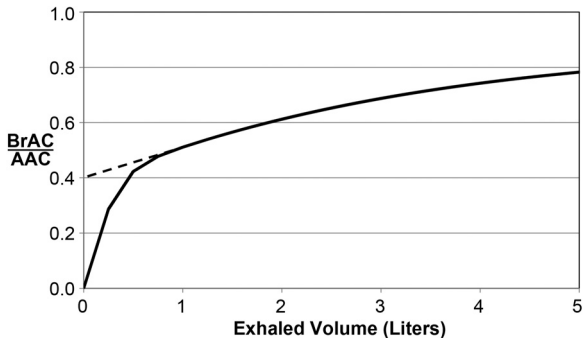

Fig. 2. Typical exhaled alcohol expirogram after a full inhalation followed by a complete exhalation at a constant flow rate. The dashed line is an extrapolation of the airway exchange exponential for gas originating in the alveolus if it were not preceded by airway exhalate.

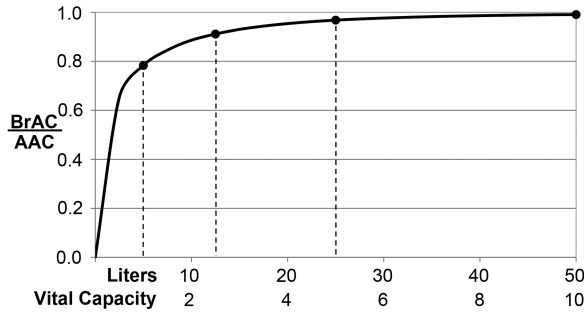

Fig. 3. Breath alcohol concentration normalized by alveolar alcohol concentration vs. exhaled volume for a theoretical 50 liter exhalation. Breath alcohol concentration (BrAC) of 1.0 is when BrAC equals alveolar alcohol concentration (AAA). This is like Fig. 2 (BrAC vs. exhaled volume) collapsed to the left by expanding the abscissa scale. Breath alcohol approaches alveolar alcohol concentrations only after impossibly large exhalation volumes are exhaled (using a math model). The solid dots show relative equilibrium of 80, 90, 95, and 99% at exhaled volumes of 5 (1 vital capacity), 12, 25, and 50 liters, respectively.

The optimal BrAC occurs only under perfect conditions: full inspiration (to total lung capacity) followed by a full expiration (to residual volume). Both full inspiration and full expiration require considerable effort with inspiratory and expiratory muscles, respectively. Maximum effort is rarely used by subjects having little experience in performing respiratory maneuvers. Individuals who exhaled less than maximal will deliver a BrAC sample that is lower than 80% of equilibrium. After a period of breath hold, a complete exhalation will yield an even greater BrAC (28, 39) than following normal breathing. Subjects who comply with maximal effort are therefore disfavored by having a greater BrAC than a person with less effort, both having the same BAC.

Methods to Measure Rather than Predict Alveolar Alcohol Concentration

It is now clear that it is not possible to measure alveolar alcohol with a single-exhalation technique.[11] Indirect methods must be used. One approach to estimating alveolar alcohol concentration is to use isothermal (temperature controlled) rebreathing. Early attempts at estimating alveolar alcohol concentration via rebreathing (without heating) had difficulties due to the condensation of water vapor in the rebreathing bag with subsequent solvation of ethanol in the condensed water (18, 40). The use of isothermal rebreathing has been shown to obtain samples of alveolar alcohol concentration without the airway interaction (minimized by the rebreathing) or conden-

[11] Alcohol concentration always decreases in passing from the alveoli through the airways to the mouth because of the loss of alcohol from the air to the airway tissue. The leveling-off of BrAC at the end of expiration has been misinterpreted as an indication that alveolar air has been obtained. However, the leveling off is actually due to the stopping of expired flow with a constant BrAC plotted against time.

sation (eliminated by temperature control at greater than body temperature). When using isothermal rebreathing, both Jones (28) and Ohlsson et al. (39) found that alveolar alcohol concentration was ~16–20% greater than the end-exhaled alcohol concentration from a single exhalation. Unfortunately, use of rebreathing to obtain an alveolar sample can be difficult for intoxicated individuals to perform.

Another method for estimating alveolar alcohol concentration has been proposed by Lindberg et al. (34). These authors used a free exhalation method in which dilution of breath alcohol by ambient air was assessed by measuring the dilution of water vapor by ambient air. By correcting to an assumed water vapor concentration of air saturated at 37°C, this approach neglects the airway exchange of both water vapor and alcohol with the airway tissue. With a full inhalation followed by a full exhalation, airway deposition accounts for ~8 and 20% for water vapor and alcohol, respectively (19). These relative amounts of airway exchange change with less than complete exhalations. Therefore, the method used by Lindberg et al. (34) results in an underestimation, on average, of alveolar alcohol concentration.

In contrast to the free breathing method, the rebreathing findings are consistent with direct measurements of the blood:air partition coefficient ($\lambda_{b:a}$). The most robust and reliable study that directly measured the ethanol partition coefficient at body temperature was carried out by Jones (24), who determined $\lambda_{b:a}$ = 1,810, which is about 16% less than the 2,100 correction value used in breath test machines in the United States. This determination of alveolar alcohol concentration is consistent with the isothermal rebreathing determination of alveolar alcohol concentration. Estimates of alveolar alcohol concentration using the above indirect methods are also consistent with predictions of the modeling approach (see above). All of these indirect methods estimate that alveolar alcohol concentration is ~16 to 20% greater than end-exhaled alcohol concentration indicating that alveolar alcohol concentration cannot be measured using breath from a full single exhalation. If the exhalation is less than maximal, then the BrAC will be >20% below alveolar alcohol concentration. This may occur under circumstances of either incomplete inhalation or incomplete exhalation. Both require significant effort beyond normal breathing. Reduction of BrAC can also occur after hyperventilation or lower than normal body temperature.

During exhalation, the alcohol concentration in the respired air changes as the air transits from the alveolus to the mouth. The extent of the change is impacted by a variety of factors alluded to above. BrAC changes with the volume of air exhaled (Fig. 2), lung size (e.g., path length effects), prior breathing pattern, and breath temperature (e.g., through the partition coefficient and airway thermoregulation). When these impacting factors are not measured and, thus, not used to adjust BrAC, inequalities exist. The volume of air exhaled affects BrAC. Its effect is largely a result of breath test specifications and human demographics. For a breath sample to be accepted, most breath test instruments require the breath sample to meet minimum requirements. In one ABT instrument, a subject must produce and hold a minimum flow rate (~66 ml/s) for a minimum time (~5 s) and a minimum exhaled volume of 1.5 liters (some machines require a smaller minimum volume, either 1.1 or 1.3 liters). These minimum sample criteria differ among different instrument brands.

Minimum Exhalation Requirement

These minimum exhalation thresholds were instituted to ensure the dead space (airway volume) of the lung was cleared before measuring ethanol in the exhaled breath. Dead space is the region of the lung that does not participate in gas exchange. Classically, dead space is defined as the volume of air in the airways because it does not participate in the exchange of respiratory gases, which occurs in the alveolus. It was presumed that once the dead space volume was exhaled, alveolar air would reach the mouth. As mentioned earlier, alcohol exchanges with the airway tissue and the bronchial blood because of alcohol's very high solubility in the airway tissue. Thus air in the airways is an "exchange" space and not "dead" space because it is actively participating in ethanol exchange with the airway wall. For alcohol exchange, dead space volume is nearly zero.

It is not clear why the minimum volume requirement is still used. It was developed with the assumption that, after dead space volume was exhaled, alveolar air came next. However, it is now clear that dead space is nearly nonexistent for ethanol exchange and BrAC continues to increase all the way through exhalation resulting from dynamic airway exchange during exhalation. It is not possible to get alveolar air to the mouth with a single exhalation. Since alveolar air never reaches the mouth, it no longer makes sense to require a minimum exhalation volume.

The presence of a minimum exhaled volume is problematic for subjects with smaller lung volumes or for those with respiratory disease. The probability of not being able to provide a minimum exhaled volume goes up with age due to a decreased lung volume. In addition, female subjects are more likely than males to be unable to provide a minimum sample due to their smaller lung volumes (17, 29). Schoknecht and Stock (44) have proposed adjusting the minimum exhalation volume to make it possible to provide a minimum volume for individuals of all ages. These authors recognized the decrease in lung volume with age in adults. However, lung volume in healthy humans changes with age, height, gender, and race (1). Given today's analytical capabilities, minimal lung volume should be adjusted for age, height, gender, and race to minimize lung volume related bias in the ABT.

An important rationale for retention of the minimum exhaled volume is to ensure that subjects are exhaling rather than putting their lips on the mouthpiece and holding their breath. A minimal exhalation volume is important for indicating cooperation. Therefore, it would be appropriate to reduce the minimum volume to a smaller fraction of lung volume (~10% of the predicted lung volume for a person's age, height, gender, and race) to allow for assurance of exhalation. For subjects with predicted lung volumes of 2.0 and 5.0 liters, the minimum volume requirements would be set at 200 and 500 ml, respectively.

Lack of Fairness in the ABT

The breathing pattern just before a breath test affects the measured BrAC. The effects of breathing rapidly (hyperventilation), shallow breathing (hypoventilation), or breath holding before the breath test were compared with a resting breathing pattern before the breath test in human subjects. The breath test consisted of a deep inhalation of room air followed by a forced

full exhalation. These studies showed hyperventilation decreased BrAC and hypoventilation increased BrAC relative to the resting breathing pattern (25, 37–39). Hyperventilation via rapid breathing for 20 s (25) or 45 s (38) before exhalation decreased the ethanol concentration by 11 to 12%. Hypoventilation can increase BrAC from 7 to 15% over the control maneuver. An exhalation following a five-min period of shallow breathing caused the BrAC to increase by 7%. Breath holds of 30 s (25) or 45 s (38) before exhalation increased BrAC by 15%. While it is unlikely that a subject will hold his/her breath for these durations, it is important to note that these data are inconsistent with the assumption that alveolar air reaches the mouth unchanged. These changes in BrAC relative to the resting breathing pattern were thought by Jones (25) to be driven by changes in exhaled breath temperature. But actually, there is little change in the airway tissue temperatures with changes in ventilation. Changes in BrAC are primarily caused by changes in the amount of alcohol dissolved in the airway tissue (15). For more details regarding the details of these physiological factors, the reader is referred to earlier reviews by Hlastala (21) and Jones (26).

Uncontrolled body and breath temperature can bias the measured BrAC using a single exhalation. The subject with a higher body temperature has an increased BrAC compared with a subject with a lower body temperature (assuming the same BAC) (12, 13) Similarly, if two subjects have the same BAC and the same body temperature, the subject with the higher end-exhaled breath temperature will have a greater BrAC than the person with a lower end-exhaled breath temperature, assuming that they exhale the same volume (24).

There is some interaction among body, environment, and breath temperatures. During inhalation inspired air is warmed as it passes through the branching airway tree. In addition, water vapor and alcohol (if BAC is non-zero) are added to the air during inhalation (36). During exhalation, the opposite occurs; the exhaled air is cooled and water vapor and alcohol are deposited onto the airway surface. By the time the air reaches the mouth during exhalation, the alcohol concentration has decreased at least 20% (20), water vapor concentration has decreased by ~8% (19), and the air has cooled to 35°C (44). The relevant temperature is the temperature of the airways where the gas exchange is occurring. This exchange is distributed among the airways (4, 20) and the temperature changes dynamically during the breath (36). Thus the measurement of BrAC should be corrected to account for the thermodynamic state of the exhaled breath. Such corrections are commonly performed for other exhaled breath measurements (e.g., correction to BTPS for spirometry).

Bias

ABTs have been presumed to be accurate because of a significant correlation between BrAC and BAC when data from many subjects are grouped together [some of these correlation studies are referenced in the prior review (21)]. In a population of subjects, the effects of these exchange mechanisms are averaged out. For an individual subject's test, the effects of these gas exchange factors can be significant. Some individuals have higher BrAC and others have lower BrAC with the same BAC. The individual differences are due to both random measurement uncertainty (50) and systematic biological uncertainty. For example, a person who exhales all of their available breath volume is more likely to have a greater BrAC than a person with the same BAC who exhaled only half of his/her available breath volume assuming both have the same BAC, gender, lung volume, and body temperature.

Our synthesis aims at reducing the biological uncertainty that affects individual breath tests. Many jurisdictions assign the biologic uncertainty found from population studies to every single individual's breath test. However, the biologic uncertainty for a population does not necessarily represent the biologic uncertainty of the individual due to, at a minimum, statistical heterogeneity and outlier effects (8). True understanding of biologic uncertainty for a single subject would require many ($n >> 2$) breath samples from that subject to determine a mean and standard deviation (uncertainty). We believe a more appropriate path to addressing biologic uncertainty is to minimize the effect of the gas exchange factors, described here, on the individual breath test.

Imagine two individuals: one male and one female, both with VBAC values of 0.083 g/dl. Both are arrested and take breath tests. The male exhales only 3 liters (60%) of his 5-liter vital capacity and is on the low side of normothermic (temperature = 97.9°F) and has a BrAC of 0.075 g/210 liters.[12] The female exhales 3 liters of her 3.5-liter lung (83%) vital capacity after 15 s of hyperventilation and is on the high side of normothermic (temperature = 98.9°F) and has a BrAC of 0.090 g/210 liters. Which one is more likely to be prosecuted? There are, of course, several other factors. However, the one with a BrAC >0.08 is more likely to face a more difficult legal challenge ahead.

Partition ratio. In the context of the ABT, the term "Partition Ratio" (PR = BAC/BrAC, where BAC is blood alcohol concentration) no longer applies to the ABT. In chemical physics, partition describes the distribution of a molecular species between two media upon achieving equilibration. For example, it can be used to describe the distribution of alcohol between blood and air in an alveolus if full equilibrium is achieved. This is the case with oxygen (during normoxia) and poorly soluble inert gases, such as nitrogen and argon exchanging in the alveolus (51). Airway alcohol exchange is distributed both spatially and temporally (4, 6). This exchange is incomplete (i.e., equilibration is not achieved) within a normal respiratory cycle (both tidal and prolonged exhalation). Use of the PR term implies an equilibrium process that does not exist either spatially or temporally for alcohol exchange in the lungs except between pulmonary capillary blood and alveolar gas. As soon as air begins to pass through the smaller airways during exhalation, disequilibrium begins as alcohol deposits onto the distal airway tissue as it travels to the mouth.

Correlation of blood and BrAC. The VBAC is not the appropriate blood to compare with BrAC. Studies have shown a closer correlation of arterial blood alcohol concentration (ABAC) with BrAC than that of VBAC with BrAC (30, 34, 35). VBAC drawn from the antecubital vein is not representative of mixed venous blood that perfuses the alveoli because during the absorption phase alcohol is added via the splanchnic

[12] In the forensic community, the gram/210 liter unit is used for breath alcohol concentration. Using this unit presumes that there is fixed relationship between BAC and BrAC and that the average ratio of experimentally measured values is 2,100.

circulation (perfusing the intestines) and removed from the circulation by the peripheral tissues causing mixed venous BAC (MVBAC) to be greater than VBAC. During the postabsorptive (elimination) phase, alcohol is added to the circulation by the peripheral tissues and removed from the hepatic circulation due to metabolism in the liver causing MVBAC to be lower than VBAC. Because the solubility of ethanol is so great in blood, very little alcohol diffuses into the breath and MVBAC is nearly identical to ABAC. As the blood passes from pulmonary arterioles through capillaries in the alveolus to become pulmonary venous blood, the BAC does not change as little or no alcohol exchange occurs in the alveolus. Pulmonary venous blood passes through the left heart becoming systemic arterial blood. The bronchial blood perfusing the bronchial circulation comes from the arterial blood via the bronchial arteries. The exchange of alcohol occurs in the airways with bronchial arterial blood. Hence the stronger correlation of BrAC with ABAC over the BAC absorption and elimination cycle (30, 34, 35). Unfortunately, an arterial blood sample is more difficult to obtain because the depth of the arterial system from the surface of the skin. Thus all blood-to-breath alcohol comparisons using venous blood are technically flawed.

Improving the Accuracy of the ABT

In the current implementation of the ABT, important physiologic factors are not measured to adjust BrAC for their effects on measured breath alcohol. The lack of making these measurements leads to an increase the uncertainty (biological) of the measured BrAC. To improve the accuracy and reliability of the breath test, these variables should be measured and used to correct BrAC in an effort to control the important variables. Subjects can breathe through a mouthpiece that is connected through one-way valves to two tubes allowing inhalation through one tube and exhalation through the other tube. One tube, the inspiratory line, contains a one-way valve and flow meter allowing the flow of air entering the mouthpiece to be measured (11) and integrated to calculate inspired volume. Increasing inspired volume decreases BrAC by sweeping away alcohol from the airway tissue (22). The other tube, the expiratory line, contains a flow meter, a capnometer for carbon dioxide measurement (to correct for altered prebreath test breathing pattern variation), an inline thermocouple, and a one-way valve allowing air to exit the mouthpiece. This mouthpiece manifold would allow the total inhaled and exhaled volume to be measured. Increasing expired volume increases BrAC. These volume measurements could be combined with a subject's predicted vital capacity to control for the influence of volume on BrAC. Vital capacity would be predicted by entering demographic information (age, height, gender, and race) into correlations outlined in the ATS/ERS guidelines (1). Such an approach has been recommended by Schoknect and Stock (44). Measurement of carbon dioxide throughout the observation period and then immediately before the breath test could assist in determining breathing pattern and whether the subject hypoventilated (breathhold) or hyperventilated (rapid breathing) before the breath test (31). Increasing ventilation (decreased exhaled P_{CO_2}) decreases BrAC. Measured exhaled breath temperature would be combined with body temperature (e.g., infrared measurement of forehead) to adjust the BrAC. Increased airway tissue temperature increases BrAC. Mouth alcohol can be reduced by requiring all subjects to rinse out their mouths before the start of the observation period. Standardizing the administration of the ABT decreases biological uncertainty.

The lung volume bias could be neutralized by significant reduction of the minimum exhalation volume used by all ABT machines. A small minimum exhalation volume (adjusted for predicted lung volume) will be required to ensure a sample can even be provided by older, shorter subjects or those with lung disease.

Limitations of Physiological Factor Measurement and Correction

Correcting for the numerous physiological variables would substantially improve the accuracy of the ABT. It might not be possible to correct for all variations. Some physiological factors will be present that cannot be easily measured or controlled. Elevated bronchial blood flow, increased mucous volume, and lung disease cannot be accurately quantified using measurements that are both simple and repeatable in the forensic setting. Measurements to quantify these conditions require tools that although complex are used in today's physiology laboratories.

Summary

There is now substantial evidence to demonstrate that it is not possible, with a single exhalation, to obtain air representing the alveolar alcohol concentration in the exhaled breath. End-exhaled alcohol concentration is always lower than alveolar alcohol concentration because of the alcohol deposition onto the airway tissue during the exhalation process. This observation invalidates a critical assumption of the ABT in its current implementation, namely, that end exhaled BrAC is equal to alveolar air alcohol concentration. Measuring alcohol concentration in the breath is a very different process than measuring a blood level from air equilibrated a blood sample. This inappropriate assumption has been the basis for development of currently used breath alcohol testing devices with no scientific substantiation. As a result, the ABT is inherently biased against certain individuals: those who inhale less or exhale further have a greater body temperature or greater breath temperature, and those with smaller lung volumes. Individuals with less favorable physiological factors are more likely to be prosecuted as they will have higher than appropriate BrAC. Measurement of a few physiological factors can improve the biological uncertainty and reduce the biasing factors. Until these changes are applied, the bias makes it more likely for some people to be prosecuted for alcohol-related offences than others regardless of the standard, breath or blood. Therefore, it is incumbent upon forensic scientists to modify the ABT so that it is fair for everyone.

DISCLOSURES

Both authors served as expert witness in legal cases.

AUTHOR CONTRIBUTIONS

M.P.H. and J.C.A. conception and design of research; M.P.H. drafted manuscript; M.P.H. and J.C.A. edited and revised manuscript; M.P.H. and

J.C.A. approved final version of manuscript; J.C.A. interpreted results of experiments; J.C.A. prepared figures.

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