The promise and perils of exhaled breath condensates

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The promise and perils of exhaled breath condensates. Am J Physiol Lung Cell Mol Physiol 287: L1073–L1080, 2004; doi:10.1152/ajplung.00069.2004.—The exhaled breath condensate (EBC) approach provides a convenient and noninvasive approach for sampling the pulmonary epithelial lining fluid (ELF). Increased EBC concentrations of more than a dozen inflammatory markers and hydrogen ions have been reported in lung diseases associated with inflammation. However, the usefulness of EBC is compromised by uncertainties concerning the sources of the EBC droplets and by the extreme and variable dilution of ELF droplets with condensed water vapor (~20,000-fold). Reported increases in EBC concentrations may reflect proportionate increases in the total volume rather than the concentration of ELF droplets in the collected samples. Conclusions regarding ELF concentrations can only be made if this dilution is estimated with a dilutional indicator (e.g., conductivity of lyophilized EBC). In normal EBC samples, pH is effectively set by oral contamination with NH3, and EBC pH cannot provide reliable information regarding ELF pH in normal subjects. Acidification of EBC observed in asthma and other conditions may reflect acidification of ELF, decreases in NH3 added to the EBC, and/or the presence of gastric droplets in the EBC.

conductivity; urea; cations; ammonium; dilution

OVER THE PAST SEVERAL YEARS there has been a dramatic surge in the number of papers published concerning exhaled breath condensates (EBC), and this increase shows no signs of slackening (see Fig. 1). The remarkable popularity of EBC collections is readily understandable: it promises access to the fluids lining the pulmonary surfaces (epithelial lining fluid, ELF) without sputum induction or bronchoalveolar lavage (BAL). Sputum induction is frequently difficult to accomplish, involves some discomfort, and may not produce secretions that are representative of the lungs as a whole. BAL is associated with a variety of risks from sedation, impaired gas exchange, inflammation, and infection. BAL is particularly risky in patients with lung disease. It is costly and can result in artifacts associated with the instillation of saline into the lungs. In contrast, collection of EBC is based on the collection of exhaled air in cooled condensers and is not associated with any appreciable discomfort or risk to the subject. EBC can be collected as often as needed, it is relatively inexpensive, and it is not associated with the specific artifacts associated with BAL. Enthusiasm concerning the EBC has been heightened by reports that concentrations of inflammatory indicators are increased in asthma and other lung diseases associated with pulmonary inflammation (partial listing shown in Table 1).

Unfortunately, there are a number of serious problems associated with the analysis and interpretation of EBC that deserve careful consideration. Unless these issues are properly addressed, the EBC approach may be doomed to becoming an interesting, although by no means unique, historical phenomenon. The literature concerning the EBC has been extensively summarized in two recent reviews (16, 18) and will be considered in a consensus report due from the American Thoracic Society and European Respiratory Society. Rather than repeat much of this material, this critique will focus on several fundamental problems associated with analysis of samples of EBC and consider some possible solutions.

METHODOLOGY

The procedures used for collecting the EBC are considered in some depth in the consensus paper, and only a few comments concerning these techniques will be made here. EBC collection is accomplished by exhaling into a cooled condenser and collecting the fluid that is deposited on the walls of the condenser. A variety of devices are available that differ significantly in cost and may also differ in efficiency. Criteria for selecting a specific device may be dictated by some of the following factors. Typical glass laboratory condensers (we use a 66-cm commercial Pyrex Allihn condenser) are inexpensive and allow fluid to drip out continuously. Our condensers are cooled with recirculating ice water, but even colder refrigerators are sometimes used to increase the efficiency with which the EBC is collected. Using this apparatus, we are able to collect ~10 ml of EBC per hour. The condensers can be autoclaved and are separated from the patient by 64 cm of respiratory tubing. The length of the tubing and its position relative to the condenser decrease the likelihood of salivary contamination, but a trap between the mouth and condenser is also helpful. Filters cannot be used at this site to decrease salivary contamination, since they remove the respiratory droplets as well as saliva. The concentration of amylase in the condensate relative to oral amylase has been used to check for salivary contamination.

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nation, but this approach may not be reliable for those EBC samples that contain relatively few droplets from the respiratory tract. Under these circumstances, even miniscule contamination by saliva may represent a serious problem. Commercial devices are available that have the advantage that small volumes of fluid can be recovered from the walls of the condenser with a plunger. These condensers are more convenient for home application. The temperature in these condensers gradually increases during collection, potentially limiting the time of collection and the total volume collected.

Some uncertainty persists regarding nasal contamination of the collected samples (see Fig. 2). Because nasal secretions frequently contain inflammatory mediators, this can be a significant problem. Nasal clips may reduce droplets from the nose, but they are uncomfortable for longer intervals, and drainage into the mouth from the nose may represent another route by which nasal droplets enter the exhaled air. Patients are instructed to discard samples if they belch, but gastric contamination is difficult to rule out.

Respiratory droplets can be collected by procedures other than condensation on cold surfaces: e.g., filtration, impaction, and electrostatic precipitation on devices at body temperature. After collection with these devices, the solutes can be washed off with small volumes of water. These alternative methods may reduce the amount of unwanted water vapor and volatile buffers, such as ammonium (see below), but may not be suitable for thermally labile or volatile mediators.

The surface of the collecting device may be of some importance, particularly when hydrophobic mediators are collected. Adherence of solutes to condenser surfaces can be particularly troublesome at the low concentrations that prevail for most molecules present in the EBC. Tests should be made for these artifacts before selection of a specific device. Meticulous attention must be given to the purity of the water used, and all tubes, tubing, and collection surfaces must be thoroughly washed with this water before use.

**DILUTION OF ELF BY WATER VAPOR**

Approximately 350 ml of water are lost from the lungs each day. Until recently, it had been assumed that virtually all of this water is derived from water vapor, which is generated as a gas from the lung surfaces. Water vapor cannot act as a vehicle for electrolytes and other nonvolatile solutes. Evaporation occurs from the entire surface of the lungs and may be enhanced by aquaporins in the endothelial and epithelial cells of the lungs (1). Because the saturation of the exhaled air is nearly complete, the rate of ventilation (Ve) effectively determines the amount of water lost from the lungs. Full saturation of the exhaled air is essential because it minimizes airway drying and local increases in the concentrations of solutes in the fluid lining the airway that could damage the pulmonary epithelium and interfere with gas exchange.

The unexpected detection of nonvolatile inflammatory molecules in the EBC indicates that the condensate also contains droplets of ELF generated from membrane surfaces. These droplets could be formed at a variety of sites, including the airways, upper respiratory tract, and even the upper gastrointestinal tract (see Fig. 2). It is likely that most of the respiratory droplets are produced in the airways, where air turbulence is greatest, but alveolar fluid may be transported into the airways and then become aerosolized. Both the volume and number of respiratory droplets can be augmented by increased airway secretions, cough, and other undefined processes. Unlike water vapor, the formation of droplets of respiratory fluid serves no known function other than spreading infections and there is no reason for believing that droplet formation is kept constant or is related in a constant fashion to the production of water vapor.

Although respiratory or other droplets containing nonvolatile solutes are present in the condensate, concentrations of these solutes are extraordinarily low (see Fig. 3) (4, 10). NH4+ represents the most abundant ion found in condensates, and there is reason to believe that there are similar concentrations of HCO3- in the condensate (see below). HCO3- and NH4+ in the condensates are the ionized forms of volatile buffers, and nearly all of these molecules are delivered to the condensates as gases (CO2 and NH3) rather than in the respiratory droplets. These volatile substances will, therefore, be considered below. In contrast to these volatile species, only trace concentrations of nonvolatile substances (electrolytes and urea) can be found in the condensates, and extremely sensitive procedures are needed to detect them. What is worse, concentrations of these substances are quite variable in many studies, even when they are collected sequentially in the same individuals (Fig. 4) (4).

**Table 1. EBC publications (through January, 2004)**

<table>
<thead>
<tr>
<th>Markers</th>
<th>Number</th>
<th>Disorders</th>
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<tbody>
<tr>
<td>H2O2</td>
<td>30</td>
<td>Asthma</td>
<td>21</td>
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<tr>
<td>Leukotrienes</td>
<td>11</td>
<td>Chronic obstructive lung disease</td>
<td>6</td>
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<tr>
<td>8-Isoprostane</td>
<td>6</td>
<td>Smokers</td>
<td>5</td>
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<tr>
<td>Nitrothiols/nitrotyrosine</td>
<td>5</td>
<td>Acute lung injury</td>
<td>4</td>
</tr>
<tr>
<td>Nitrite/nitrate</td>
<td>5</td>
<td>Cystic fibrosis</td>
<td>4</td>
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<td>Interleukin-6</td>
<td>5</td>
<td>Obstructive sleep apnea</td>
<td>3</td>
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<td>Acid</td>
<td>4</td>
<td>Surgery</td>
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<td>Aldehydes</td>
<td>3</td>
<td>Idiopathic pulmonary fibrosis</td>
<td>2</td>
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<tr>
<td>Isoprostanes</td>
<td>3</td>
<td>Dry air</td>
<td>1</td>
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<tr>
<td>Adenosine</td>
<td>2</td>
<td>Ozone exposure</td>
<td>1</td>
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<tr>
<td>Endothelin</td>
<td>1</td>
<td>Carcinoma</td>
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<tr>
<td>Vibronectin</td>
<td>1</td>
<td>Bronchiectasis</td>
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<tr>
<td>Glutathione</td>
<td>1</td>
<td>Ciliary dyskinesia</td>
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<tr>
<td>Interleukin-4</td>
<td>1</td>
<td>Pneumonia</td>
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<td>Hepatocyte growth factor</td>
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EBC, exhaled breath condensate.
Much of the variability observed in solute concentrations of EBC is presumably due to differences in the dilution of ELF droplets by water vapor. Reported increases in EBC concentrations of inflammatory mediators could be due, at least in part, to increases in the total volume of droplets of ELF relative to the much larger volumes of water vapor that were converted from the gaseous to the liquid phase by cooling in the condenser (Fig. 5). Such increases might be expected if secretions in the airway increased and were associated with increased turbulence. It is impossible to make any conclusions regarding changes in ELF concentrations from changes in EBC concentrations unless information is also available concerning the dilution of the ELF droplets by water vapor.

Concentrations of nonvolatile solutes in the EBC ([X]_{EBC}) are determined by two factors: the concentration of these solutes in the ELF droplets ([X]_{ELF}) and the ratio of the volume of these droplets (V_{ELF}) to the volume of the condensate (V_{EBC})

$$[X]_{EBC} = \frac{[X]_{ELF} V_{ELF}}{V_{EBC} + n \bar{v}_{ELF}}$$

where n designates the number of these respiratory droplets, \(\bar{v}_{ELF}\) is the average volume of these droplets, and V_{vapor} is the volume of water generated when the water vapor is turned into liquid droplets in the condenser. Any increase observed in the mediator concentrations could be due to increases in ELF concentrations, increases in the volume or number of ELF droplets, or a decrease in the V_{vapor} that is collected.

Fig. 2. Aerosol droplets can be derived from multiple sites when collected from the mouth. Some of the principal ingredients that are derived from each site are indicated.

Fig. 3. Ionic concentrations in EBC samples obtained from normal subjects. Note that the concentrations of NH₄⁺ far exceed those of any other ion or buffer.

Fig. 4. Total EBC molality as judged by both ion chromatography of cations (Na⁺ + K⁺ + Ca²⁺ + Mg²⁺) and conductivity of lyophilized EBC obtained in 2 consecutive 30-min collections of EBC. Both indicators of dilution show that EBC concentrations are quite variable in some subjects. [From Effros et al. (4).]
The dilution (D) of the ELF droplets in the EBC is

\[ D = \frac{V_{EBC}}{V_{ELF}} \]  

(2)

Because the principal objective of EBC studies is to determine solute concentrations in the ELF, some measure of the dilution of ELF by water vapor is essential. Because the dilution of all nonvolatile ELF solutes in the condensates by water vapor should be the same, it is likely that a variety of solutes could be used to calculate D. Ideally, the concentration of the dilutional indicator in the ELF should be equal to that in plasma. We suggested that the total molar concentration of cations could be used for this purpose (4, 10)

\[ D = \frac{[\text{total cations}]_{\text{plasma}}}{[\text{total cations}]_{\text{ELF}}} \frac{[\text{total cations}]_{\text{EBC}}}{[\text{total cations}]_{\text{EBC}}} \]  

(3)

Although the relative concentrations of individual cations in the condensates differ from those found in the plasma, there is some reason to believe that the total osmolality of the plasma and respiratory fluid are the same, justifying the third term of Eq. 3. The inorganic cations and associated anions represent the predominant osmotic constituents of extracellular fluids such as plasma, ELF, and the EBC. We originally used the sum of Na\(^+\) and K\(^+\) (measured by flame photometry) in Eq. 3 but now measure the inorganic cations by ion chromatography and use the sum of Na\(^+\), K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\) for this purpose. Ion chromatography increases the sensitivity of these measurements by an order of magnitude. Evidence that this calculation is appropriate is provided by the observation that the average value of D calculated with Eq. 3 was very similar to that obtained with urea (Fig. 6)

\[ D = \frac{[\text{urea}]_{\text{plasma}}}{[\text{urea}]_{\text{EBC}}} \]  

(4)

Justification for the use of urea as an indicator of dilution is based on the physiological properties of this molecule, i.e., urea is neither produced nor destroyed in significant amounts by intact lungs, it is not volatile, and it rapidly equilibrates across the barriers separating the blood from the air spaces (8). It is therefore likely that plasma and ELF concentrations of urea are very similar. Urea has also been used to measure dilution of respiratory fluid during BAL, but urea rapidly enters the instilled fluid during lavage (5, 11). Because no fluid is instilled during collections of condensates, this is not a problem with EBC.

As indicated in Fig. 6, D averaged \(\sim 20,000\), regardless of whether it was measured by total cations or urea. (Dwyer reported dilution by 10,000 for urea, Ref. 3). This implies that there are <0.1 \(\mu\)l of ELF in every milliliter of EBC in our normal subjects. Investigators should be aware of the extreme
degree of dilution present in the condensate before embarking on analyses of EBC samples. The concentrations of nonvolatile compounds in the EBC average only ∼1% of those found in BAL samples, and BAL concentrations are only ∼1% of those in ELF (11).

We had originally hoped it would be possible to calculate D from the ratio of plasma to ELF conductivity but were frustrated by the presence of high concentrations of NH₃⁺, which contributes to the conductivity in the ELF (4, 10). We found it was possible to remove nearly all of the NH₃⁺ from the condensates by lyophilizing (freeze-drying) the EBC at −100°C and <2 mmHg for 24 h, after which the conductivity (in units of mol of saline per liter) closely matched the total nonvolatile cation concentration (Fig. 7). Because total plasma cation concentrations remain within a very narrow range in normal subjects (149 ± 1.8 milliequivalents/liter, with a coefficient of variation of 2.7%), it was not necessary to measure plasma cationic concentrations in these subjects and

\[
D = \frac{150,000 \ \mu M}{\text{conductivity (\mu M)_lyophilized EBC}} \tag{5}
\]

Eq. 5 is probably the most practical way to estimate D since lyophilizers are available in most institutions. Lyophilization also permits concentration of the samples and is used to preserve unstable compounds. Conductivity meters are relatively inexpensive and do not consume samples.

It has been suggested that increases in the concentrations of inflammatory mediators in EBC with disease could be documented if concentrations of other mediators failed to increase (7). This is a risky approach since an increase in the ratio A:B of the concentrations of one mediator (A) to another mediator (B) could reflect an increase in A or a decrease in B. Unless there is an independent calculation of dilution, it is impossible to tell whether an increase in the concentration of any EBC mediator is associated with an increase, decrease, or no change at all in the corresponding concentration in the ELF.

The usefulness of EBC mediators in detecting inflammation depends, in part, on whether differences between patients and normal subjects significantly exceed the variation in each of these populations. In many studies, including our studies of electrolytes, these concentrations are normally quite variable, even when repeated in individuals (Fig. 4). In other studies, significant variation is found between individuals, but they remain relatively unchanged in repeat studies of individuals. This is not very reassuring, because it may indicate that either EBC concentrations or droplet formation differ between individuals. Any change that is observed between normal subjects and patients may, therefore, reflect alterations in either of these variables. In a recent study, concentrations of interleukin-6 remained very similar among all normal subjects (2). This suggests that both ELF concentrations and the ratio between the volume of droplets released from the ELF and the quantity of water vapor formed within the lungs that is converted to liquid in the condenser remained the same among these individuals. As discussed above, it would be difficult to explain this relationship, but it could be postulated that the narrow variation in these data reflect more reproducible collection and analysis. However, what was even more surprising in this study was the observation that the concentrations of interleukin-6 were elevated to nearly the same level in patients with presumably variable degrees of chronic obstructive pulmonary disease, as judged from pulmonary function. A more graded effect on interleukin-6 concentrations might have been expected.

Use of dilutional references has an additional advantage. It permits calculation of the volume of ELF exhaled from the mouth each minute

\[
V_{\text{ELF}} = \frac{[\text{total cations}]_{\text{EBC}}}{[\text{total cations}]_{\text{plasma}}} V_{\text{EBC}} \tag{6}
\]

where \(V_{\text{ELF}}\) and \(V_{\text{EBC}}\) designate rate of production of ELF and EBC. This equation is analogous to that used for calculating glomerular filtration rate from the clearance of inulin.

The development of a “perfect” dilutional indicator may prove difficult, but it must be kept in mind that if dilution is not estimated, it will be impossible to tell whether increases in the concentrations of inflammatory mediators in EBC are merely due to increased droplet formation or are caused by true increases in their concentrations in the ELF.

**ACID-BASE BALANCE IN THE EBC**

Certainly, one of the most provocative findings to emerge from EBC studies has been the observation of Hunt et al. (15) that the EBC is acidified in patients with bronchial asthma, a phenomenon they have designated as “acidopnea.” Acidopnea has also been described in other illnesses associated with airway inflammation (acute lung injury, chronic obstructive lung disease, bronchiectasis, and cystic fibrosis) (12, 17, 22). It is quite possible that the ELF as well as the EBC is acidic in these conditions, but there is reason to doubt whether the pH of the ELF can be used to estimate the pH of the EBC. Measurements of the ionic constituents of normal EBC indicate that the pH of this fluid is normally regulated by oral and atmospheric contaminants rather than the buffers in the ELF.
As indicated in Fig. 3, NH$_4^+$ represents the overwhelmingly predominant cation and buffer in normal EBC. This statement can be made with confidence because NH$_3$ (and associated anions, discussed below) accounts for most of the conductivity of unlyophilized condensates. There is general agreement in both the EBC and dental research communities that most of the NH$_3$ released from the mouth is derived from the oral cavity, much of it by bacterial degradation of urea. Concentrations of NH$_4^+$ are significantly reduced in patients who have endotracheal or tracheostomy tubes, which allow the oral cavity to be bypassed (4, 10, 23). Furthermore, exhalation of NH$_3$ can be reduced by a factor of 10 by rinsing the mouth with acidic solutions, which tend to trap oral NH$_3$ as NH$_4^+$ (19).

The efficiency with which NH$_4^+$ is trapped in the condensate is related to the high water:air partition coefficient of NH$_4^+$, which is ~50,000:1 at a pH of 7.4. As indicated in Fig. 7, accumulation of NH$_4^+$ can be significantly reduced by collecting respiratory droplets on warm, dry filters that retain little water. Concentrations of NH$_4^+$ in condensates are affected by the manner in which the samples are collected. We found that if the tubing that connects the mouthpiece to the condenser is shortened, the concentrations of NH$_4^+$ in the condensate are increased, but those of Na$^+$ are unaffected (10). This reflects the fact that NH$_3$ gas in the exhaled air is trapped in the small amount of aqueous droplets lining the tubing. It implies that the amount of NH$_3$ reaching the condenser can be reduced when the tidal volume is decreased relative to the volume of the dead space tubing. Furthermore, the efficiency with which NH$_3$ is trapped in the condenser as NH$_4^+$ can be enhanced by increasing the PCO$_2$ in the air traversing the condenser, thereby acidifying the condensate and promoting conversion of NH$_3$ to NH$_4^+$ (10). End-tidal PCO$_2$ tends to be decreased in patients with increased dead space, and this may contribute to the observation that NH$_4^+$ concentrations are also reduced in patients with airway disease (12, 14). Because the solubility of gases in water is influenced by temperature, it is likely that the temperature of the condensate also affects trapping of NH$_3$. The design of the condenser may be of some importance, since some units retain the condensate throughout the collection period, which may enhance recovery of NH$_4^+$.

As a primarily oral contaminant, it is unlikely that NH$_4^+$ concentrations in the EBC collected from the mouth can provide useful information regarding NH$_4^+$ formation in the lungs. Conclusions regarding the status of NH$_4^+$ metabolism in the lungs based on oral collections would therefore seem premature (14).

The requirement for electroneutrality implies that one or more anions are also present in high concentrations that match those of NH$_4^+$ in the EBC. A clue to the nature of the anions is provided by the observation that NH$_4^+$ can be removed by lyophilization if it is added to water as NH$_4$OH or NH$_4$HCO$_3$. NH$_4^+$ cannot be removed by lyophilization if it is added as NH$_4$Cl. This suggests that the unidentified EBC anion, like NH$_4^+$, is volatile. The most obvious candidate would be HCO$_3^-$.

Although we could not measure HCO$_3^-$ (a component of the eluent used in the ion chromatograph), it is a relatively simple matter to calculate that if the samples are exposed to room air (0.03% CO$_2$), bicarbonate is the principal anion. Because NH$_4^+$ represents virtually the entire cationic buffer present in normal condensates, a calculation can be made of the HCO$_3^-$ present in an aqueous solution after addition of NH$_4$OH in room air (Fig. 8). The strong ion difference approach of Stewart (21) was used for this purpose. It is noted in Fig. 8 that even low partial pressures of CO$_2$ in the ambient air have a dramatic effect on the pH of solutions of NH$_4$OH. Lactic acid (pKa = 3) has little effect on pH unless concentrations approach those of NH$_4^+$.

Hunt et al. (15) have recommended that 1-ml samples of condensate should be purged for 10 min with 350 ml/min of argon to release CO$_2$ and minimize HCO$_3^-$ in the samples. Because the pH stabilized after this period, the investigators concluded that all of the CO$_2$ had been released. An isolated reduction in CO$_2$ to ambient or even lower concentrations should make the EBC more alkaline than that in the ELF (Fig. 8), and EBC pH should therefore be an unsatisfactory surrogate for ELF pH. However, it is not at all clear that purging the EBC with argon actually removes all of the CO$_2$ from the EBC samples. Stabilization of the pH would occur if the ambient CO$_2$ were constant. Hunt et al. (15) did not report any measurements of P CO$_2$ in their samples, but when a similar approach was used by Gessner et al. (12), sufficient CO$_2$ was still present in the samples to allow measurements of P CO$_2$ and HCO$_3^-$ with a routine arterial blood gas electrode. Because these devices cannot detect ambient P CO$_2$, it is likely that argon did not reduce P CO$_2$ below ambient levels. More than an hour might be needed to fully purge P CO$_2$ from the solutions (20), and the samples and pH electrode must be kept strictly anaerobic.

Vaughan et al. (24) have reported that the pH of EBC recovered from the mouth is very similar in most subjects to the pH of EBC collected after intubation. Although no measurements of NH$_4^+$ were indicated in this study, NH$_4^+$ in EBC samples obtained from endotracheal or tracheostomy samples are lower than those obtained from the mouth. Oral samples should have consequently been more alkaline than those obtained with nonoral sampling. The pH of EBC might not have changed if there were proportionately less anionic buffer of some sort (e.g., HCO$_3^-$ or other anions) in the nonoral samples. Regardless of the nature of the oral buffers, NH$_4^+$ and associated anions produced in the mouth or atmosphere (HCO$_3^-$), rather than ELF buffers, set the EBC pH in normal subjects. It can be concluded that EBC pH cannot be used to calculate ELF pH in normal individuals. This is consistent with the observation that surface pH in the bronchi was much higher than EBC pH of patients with acute lung injury (12).
In theory, prior removal of $\text{NH}_4^+$, $\text{HCO}_3^-$, and other volatile buffers from condensates by lyophilization might yield an index of ELF pH that would be independent of the effects of volatile buffers such as $\text{CO}_2$ and $\text{NH}_3$. Although some $\text{NH}_3$ is released from the lungs, it does not significantly contribute to pulmonary pH for two reasons. Concentrations of $\text{HCO}_3^-$ are at least two orders of magnitude greater in respiratory fluid than $\text{NH}_4^+$, and any $\text{NH}_3$ produced in the lungs would readily equilibrate between the vasculature and the pulmonary epithelium (9). Measurement of pH of lyophilized samples should be performed anaerobically to avoid ambient $\text{CO}_2$, and special electrodes may be needed for measuring pH in these extremely dilute solutions. The buffers used to standardize the electrodes must be diluted to levels comparable to those of the EBC samples.

Although the pH values of normal condensates appear to be set by the prevailing $\text{NH}_4^+$ and $\text{HCO}_3^-$ concentrations, this cannot be true for more acidic samples obtained from patients with bronchial asthma. At least three factors could contribute to acidification of the condensates: 1) acidification of the ELF and respiratory droplets released from the ELF (15), 2) reductions in $\text{NH}_4^+$ that could be related to decreased exchange of $\text{NH}_4^+$ in the mouth and condensers (6), and 3) the presence of gastric droplets in the condensate. Acid reflux is very common in patients with severe asthma and other obstructive lung diseases (13), in part because of increases in intra-abdominal pressures during exhalation and the tendency of bronchodilators to relax the distal esophageal sphincter. Aerosolization of even small amounts of gastric juice, which may have a pH between 1 and 2, from the stomach or pharynx could have a profound effect on ELF pH. Because direct measurements of bronchial pH in patients with acute lung injury and acid EBC failed to demonstrate acidic mucosal pH (12), acidification of samples obtained from the mouth may have occurred outside of the lungs in those asthmatic patients who have very acidic condensates. It would be difficult to rule out the presence of gastric droplets in acidic EBC, but the presence of pepsin and reversal of acidopenia with proton pump inhibitors would be helpful. It has been suggested that exacerbations of asthma can be caused by acid reflux, and the discovery of a low condensate pH could alert the physician that acid secretion and/or reflux should be inhibited.

**OPPORTUNITIES AND LIMITATIONS OF THE EBC APPROACH**

EBC collection is best suited for the collection of nonvolatile, hydrophilic solutes. These solutes are introduced into the exhaled air in respiratory droplets. If a dilutional indicator is used, the concentrations of hydrophilic solutes in the ELF can be calculated from the EBC concentrations, regardless of the efficiency with which ELF droplets are produced in the airways or are captured in the condensate. This is not true for volatile indicators because the distribution of volatile indicators between air and water can have a profound effect on the concentrations that are reached in the condensate. This is well illustrated for EBC concentrations of $\text{NH}_4^+$, which depend on multiple factors, including temperature, time of exposure, pH at the site of generation, collection, tidal volumes, and oral concentrations, etc. It is difficult to imagine how a reference indicator could be developed for these indicators, and direct measurements in gas would seem more appropriate than condensates, which are influenced by the partition coefficients between the gas and liquid phases. For many volatile mediators, the total rate of production of the mediator may be more informative than the concentration in the air phase or its distribution into the water that becomes deposited in the condenser. It may also be difficult to interpret EBC concentrations of highly lipophilic solutes, even if these are not volatile, since they tend to remain associated with lipid membranes and surfactant in the lungs and condensate and collection surfaces.

Proper use of dilutional indicators with EBC studies should provide important new information regarding ELF concentrations of suitable indicators. These studies may eventually be supplemented by alternative “dry” techniques for collecting droplets of ELF, in which the same dilutional indicators can be used. Furthermore, these indicators can be used to investigate a variable that has been virtually overlooked in the past: the rate of production of respiratory droplets, $V_{\text{ELF}}$. This variable may be augmented in diseases and various respiratory maneuvers, including cough, external chest vibration, and PEEP, etc. These procedures may also make it possible to collect from specific regions of the airways, e.g., the laryngeal droplets may be selected during cough or phonation. The future of the EBC approach will depend on, in part, the success with which droplet formation can be enhanced, thereby improving the chances of measuring trace quantities of respiratory solutes in the EBC. It will also depend on the development of procedures that can detect and analyze the contribution of droplets from the stomach, mouth, and nose to the EBC.

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**REFERENCES**


