# The Effects of Volatile Salivary Acids and Bases on Exhaled Breath Condensate pH

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***Rationale:*** **Recent studies have reported acidification of exhaled breath condensate (EBC) in inflammatory lung diseases. This phenomenon, designated "acidopnea," has been attributed to airway inflammation.**

***Objectives:*** **To determine whether salivary acids and bases can influence EBC pH in chronic obstructive pulmonary disease (COPD).**

***Methods:*** **Measurements were made of pH, electrolytes, and volatile bases and acids in saliva and EBC equilibrated with air in 10 healthy subjects and 10 patients.**

***Results:*** **The average EBC pH in COPD was reduced (normal, 7.24 ± 0.24 SEM; range, 6.11–8.34; COPD, 6.67 ± 0.18; range, 5.74–7.64; $p = 0.079$). EBCs were well buffered by $NH_4^+/NH_3$ and $CO_2/HCO_3^-$ in all but four patients, who had $NH_4^+$ concentrations under 60 μmol/L, and acetate concentrations that approached or exceeded those of $NH_4^+$. Saliva contained high concentrations of acetate (~ 6,000 μmol/L) and $NH_4^+$ (~ 12,000 μmol/L). EBC acetate increased and EBC $NH_4^+$ decreased when salivary pH was low, consistent with a salivary source for these volatile constituents. Nonvolatile acids did not play a significant role in determining pH of condensates because of extreme dilution of respiratory droplets by water vapor (~ 1:12,000). Transfer of both acetic acid and $NH_3$ from the saliva to the EBC was in the gas phase rather than droplets.**

***Conclusions:*** **EBC acidification in COPD can be affected by the balance of volatile salivary acids and bases, suggesting that EBC pH may not be a reliable marker of airway acidification. Salivary acidification may play an important role in acidopnea.**

**Keywords:** acetate; ammonium; bicarbonate; buffer; exhaled breath condensate

In 2000, Hunt and colleagues reported that exhaled breath condensates (EBCs) are acidified during asthmatic exacerbations (1). They referred to this phenomenon as "acidopnea" and suggested that it reflected excess acid produced in the airways by inflammation. These observations were confirmed in studies of chronic obstructive pulmonary disease (COPD) and other inflammatory lung disorders (2–10). Subsequently, Hunt and colleagues found that EBC $NH_4^+$ concentrations were reduced in many patients with asthma (11). They postulated that production of $NH_3$ in the airways was reduced because of impaired glutaminase activity, and suggested that reductions in airway $NH_3$ production reduced local buffering, thereby promoting airway acidification. They also argued that concentrations of $NH_4^+$ in EBCs did not influence the pH of the EBC samples, which represented an accurate marker of airway acidification (12, 13).

This study has analyzed the acid and base concentrations in saliva and EBC from 10 healthy subjects and 10 patients with COPD to determine whether EBC pH is influenced by volatile or nonvolatile constituents in the saliva.

## METHODS

Eleven healthy subjects and 10 subjects with COPD were initially selected but condensate from one healthy subject was deleted because high amylase concentrations indicated salivary contamination (Table 1). Spirometry was performed in all subjects (Sensormedics, Yorba Linda, CA). The patients had COPD ($FEV_1 < 75\%$ predicted, $FEV_1/FVC < 70\%$) (14) and an average smoking history of 59 ± 43 (SD) pack-yr. None smoked within an hour of the study. All were taking prescribed maintenance bronchodilators, which were not used during the hour before collections.

Patients exhaled for 1 h into an insulated 66-cm polycarbonate condenser cooled with recirculated ice water. One-way valves were used to ensure that subjects inhaled fresh air and exhaled into the condenser. Nose clips were not used. The mouthpiece and condenser were connected by a 450- × 22-mm ID ventilator tubing (Corr-a-Flex 2; Hudson RCI, Temecula, CA) inclined upward to minimize salivary contamination. Condensate was collected using polycarbonate tubes. Collection and analysis of capillary plasma and saliva are described in the online supplement.

All samples were stored at −80°C. Before analysis, all samples were allowed to thaw in room air for about 30 min. To minimize potential losses of volatile acids and bases, no attempt was made to remove ambient $CO_2$ from the EBC or saliva with inert gas (*see* the online supplement). The buffering capacity of the condensates was determined by sequentially measuring the pH after adding 5-μl aliquots of 0.4 mmol/L NaOH and then 0.4 mmol/L HCl to 0.5 ml samples at room temperature:

$$\text{Buffering Capacity} = \Delta\text{molality}/\Delta\text{pH} \quad (1)$$

where Δmolality designates the change in molality of NaOH or HCl needed to alter condensate pH one unit in the range between 5 and 7.

From 1 to 8 ml of the EBC samples were lyophilized (freeze-dried) at –55°C to dryness to remove volatile solutes such as $NH_3$, $CO_2$, and acetic acid. These samples were reconstituted in 2.1 ml of deionized water and measurements were repeated of conductivity and pH. Corrections were made for the differences in volumes used in lyophilization. EBC pH, conductivity, and amylase and ionic concentrations (by ion chromatography) were determined before and/or after they were lyophilized, as described in the online supplement.

Dilution (D) of respiratory droplets (epithelial lining fluid) by water was then calculated from conductivity measurements:

$$D_{\text{conductivity}} = \text{Volume}_{\text{EBC}}/\text{Volume}_{\text{ELF}} = (\text{conductivity})_{\text{plasma}}/(\text{conductivity})^*_{\text{EBC}} \quad (2)$$

The asterisk indicates lyophilized samples (15, 16).

The coefficient of variance of repeated (5 to 10) measurements of electrolyte concentrations in the same sample at 5 μmol/L was less than 2%. The lower limits of detection were 0.5 μmol/L for each of the ions, 2.5 μmol/L NaCl for conductivity, and 0.2 mU/ml for amylase.

Statistical analyses were conducted with SigmaStat version 2 software (Jandel, San Rafael, CA). Statistical differences between mean values of normal and COPD parameters were compared by unpaired

(*Received in original form July 10, 2005; accepted in final form November 9, 2005*)

Supported in part by National Institutes of Health grants HL55268, HL074407, and DK25731.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

**Am J Respir Crit Care Med Vol 173. pp 386–392, 2006**
**Originally Published in Press as DOI: 10.1164/rccm.200507-1059OC on November 10, 2005**
**Internet address: www.atsjournals.org**

**TABLE 1. DEMOGRAPHICS AND PULMONARY FUNCTION STUDIES**

| | No. | Age (*yr*) | Sex | FVC* | $FEV_1$* | $FEV_1/FVC$* |
|---|---|---|---|---|---|---|
| Normal | 10 | 53 ± 24 | 1 M, 9 F | 95 ± 14 | 90 ± 16 | 95 ± 8 |
| COPD | 10 | 59 ± 13 | 4 M, 6 F | 68 ± 19 | 40 ± 15 | 59 ± 16 |

*Definition of abbreviations*: COPD = chronic obstructive pulmonary disease; F = female; M = male.
* Percent predicted based on standard values corrected for age, height, and race (1), mean and SD.

*t* test. Linear regression and correlation coefficients were used to compare EBC parameters. Electrolyte concentrations were compared by ranked analysis of variance repeated measurements and a Student-Newman-Keuls test of differences in mean values.

These studies were approved by the institutional human research review committees, and consent was obtained from each subject before each study. More details on the methods used in this study are available on the online supplement.

## RESULTS

### pH, Buffer, $NH_4^+$, and Volatile Acids in Unlyophilized Condensates (Equilibrated in Room Air)

EBC pH averaged 7.24 ± 0.24 SEM in normal subjects, and 6.67 ± 0.18 in patients who had COPD (p = 0.079; Figure 1). Differences in EBC $NH_4^+$ were less obvious, but it will be noted that all four of the EBC samples with $NH_4^+$ concentrations that were below 60 μmol/L were collected from patients with COPD.

Distinctly different degrees of buffering were observed among individual EBC samples (Figures 2A and 2B), but differences between buffering capacity in healthy subjects and patients with COPD were not significant (normal subjects: 0.265 ± 0.078 (mmol/L)/pH unit; COPD: 0.195 ± 0.32 (mmol/L)/pH unit; p = 0.411). When buffering was prominent, it was most pronounced at a pH of about 6.3, which is the dissociation constant of $CO_2/HCO_3^-$ at room temperature. EBC buffering curves resemble those of standard solutions containing $NH_4HCO_3$ (Figure 2C), suggesting that mixtures of $NH_4^+$ and $HCO_3^-$ ions accounted for the buffering capacity of the EBC. Buffering capacity of the EBC samples between pH 7 and 5 correlated well with $NH_4^+$ concentrations of the EBC samples ($r^2 = 0.719$, $p < 0.001$; Figure 3).

Mean concentrations of EBC acetate in COPD exceeded those in healthy subjects, but this difference did not achieve significance (p = 0.07; Figure 4A). Acetate concentrations in EBC were usually lower than those of $NH_4^+$ in most subjects. However, acetate concentrations approached or exceeded those of $NH_4^+$ in the four subjects with COPD with low EBC $NH_4^+$ (Figure 5).

Concentrations of $NH_4^+$ and acetate were more than 50 times higher in saliva than in EBC of both healthy subjects and patients with COPD, suggesting that a large portion of these volatile EBC solutes could have come from the mouth (*see* Discussion and Figure 4A vs. Figure 4B).

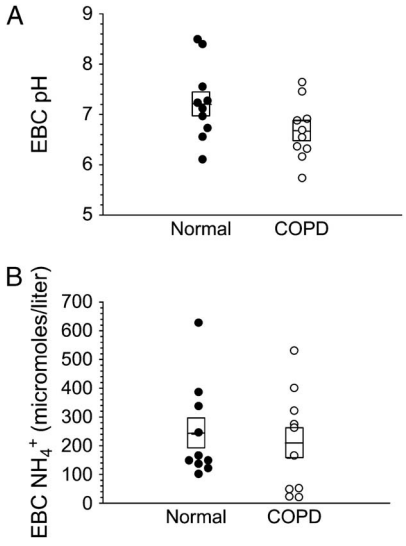


***Figure 1.*** (*A*) Mean exhaled breath condensate (EBC) pH in chronic obstructive pulmonary disease (COPD) was less than that of healthy subjects (p = 0.075). (*B*) Differences in mean EBC $NH_4^+$ concentrations were less obvious, but $NH_4^+$ concentrations below 60 μmol/L were only observed in four patients with COPD. *Rectangles* indicate mean and SEM.

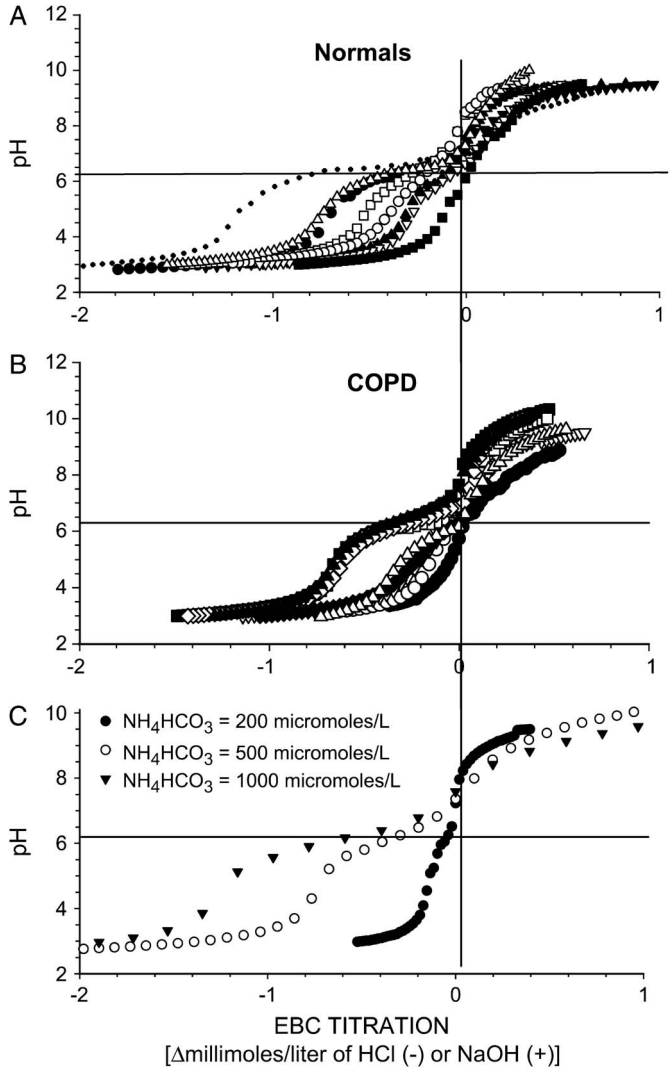


***Figure 2.*** Significant buffering was seen in some EBC samples from both healthy subjects (*A*) and patients with COPD (*B*). Buffering was maximal at about 6.3 (indicated by *horizontal line*), which is the $pK_a$ of $CO_2/HCO_3^-$ at room temperature. Note the resemblance of these titration curves to those of solutions of $NH_4HCO_3$ (*C*). No evidence was found for buffers with other $pK_a$ values.

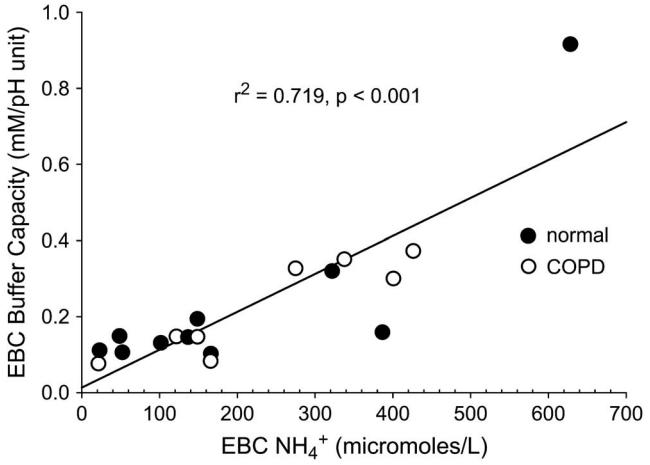


*Figure 3.* The buffer capacity of the EBC solutions between pH 5 and 7 was well correlated with the $NH_4^+$ concentrations. (The buffer capacity was calculated with Equation 1). This reflects the fact that the concentration of $NH_4^+$ in the solution effectively determines the concentration of ambient $CO_2$ that is trapped in the condensate. mM designates mmol/L.

A correlation was found between salivary pH and the concentration of $NH_4^+$ in the condensate ($r^2 = 0.383$, $p = 0.004$; Figure 6A), suggesting that salivary acidification reduces the release of $NH_3$ from the mouth and the amount of $NH_4^+$ recovered in the EBC. In contrast, EBC concentrations of acetate were increased when saliva was acidic ($r^2 = 0.509$, $p < 0.001$; Figure 6B).

### Lyophilization and Calculation of Dilution of Respiratory Droplets by Water Vapor

Lyophilization of the condensates reduced EBC $NH_4^+$ concentrations by an average of more than 99% in both the healthy subjects and patients with COPD (Figure 7A), making it possible to estimate dilution from conductivity (Equation 2). Decreases in conductivity correlated well with losses of $NH_4^+$ ($r^2 = 0.754$, $p < 0.001$; Figure 8), indicating that $NH_4^+$ and associated anions accounted for most of the solutes lost from the condensate with lyophilization. Although considerable variability was found in total EBC cation concentrations ($Na^+ + K^+ + 2\,Ca^{2+} + 2\,Mg^{2+}$), these values were well correlated with those of EBC conductivity ($r^2 = 0.930$, $p < 0.001$; Figure 9). Furthermore, concentrations of the principal nonvolatile ions in the EBC samples ($Na^+$, $Cl^-$, lactate, and $Ca^{2+}$) were well correlated with one another (*see* Table 2).

The dilution (D) calculated with Equation 2 averaged 13,402 ± 3,179 in healthy subjects and 11,615 ± 3,301 in patients with COPD ($p = 0.701$). Plasma conductivity (in units of μmol/L NaCl) averaged 157,000 ± 800 μmol/L in the healthy subjects and 154,000 ± 2,300 μmol/L in the patients with COPD.

No differences were detected between the normal and COPD EBC concentrations of individual ions or measures of total ions, cations, or anions that might influence the pH of the respiratory fluid (Figure 7). When compared with comparable plasma ratios, EBC concentrations of $K^+$, $Ca^{2+}$, lactate, and $SO_4^{2-}$ were disproportionately high relative to EBC $Na^+$ (Table 3), but no differences were found between normal and COPD samples.

Exposure of dilute solutions of $NH_4HCO_3$ to room air caused a progressive decrease in pH due to absorption of ambient $CO_2$ rather than loss of $NH_3$ (Figure 10).

No significant correlations were found between pulmonary functions and condensate parameters.

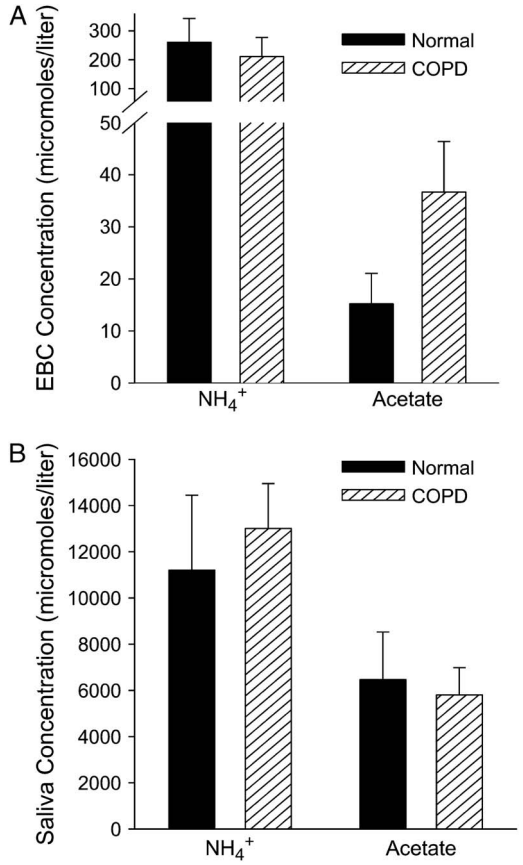


*Figure 4.* (*A*) Volatile ion concentrations in the nonlyophylized samples. Average values of $NH_4^+$ exceeded those of acetate. Average values of acetate were higher in the COPD samples than the normal samples, but significance was at $p = 0.07$. (*B*) Salivary concentrations of both $NH_4^+$ and acetate were more than 50 times greater than those in the EBC. This suggests that the oral cavity is the source for much of these constituents in the EBC.

## DISCUSSION

These observations concerning EBC pH are consistent with many others, which indicate that pH of the EBC is frequently acidic in patients with COPD and other inflammatory lung diseases (1–11). Although the suggestion of Hunt and coworkers (1) that EBC acidification reflects acidification of the airways seemed plausible, there are a number of problems associated

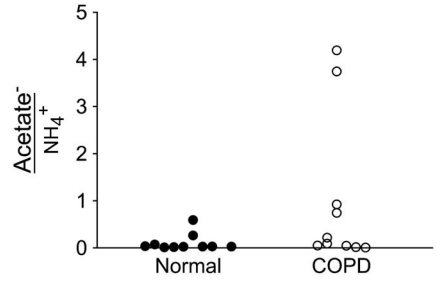


*Figure 5.* Acetate concentrations were usually well below those of $NH_4^+$. However, the ratio of acetate to $NH_4^+$ approached or exceeded 1.0 in four patients with COPD.

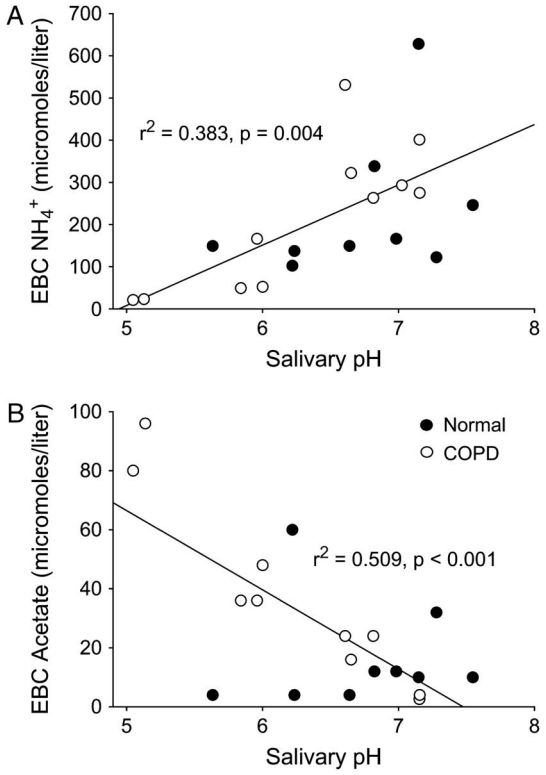


***Figure 6.*** (*A*) Acidification of the saliva increased acetate concentrations in the EBC. (*B*) Acidification of the saliva reduced $NH_4^+$ concentrations in the EBC.

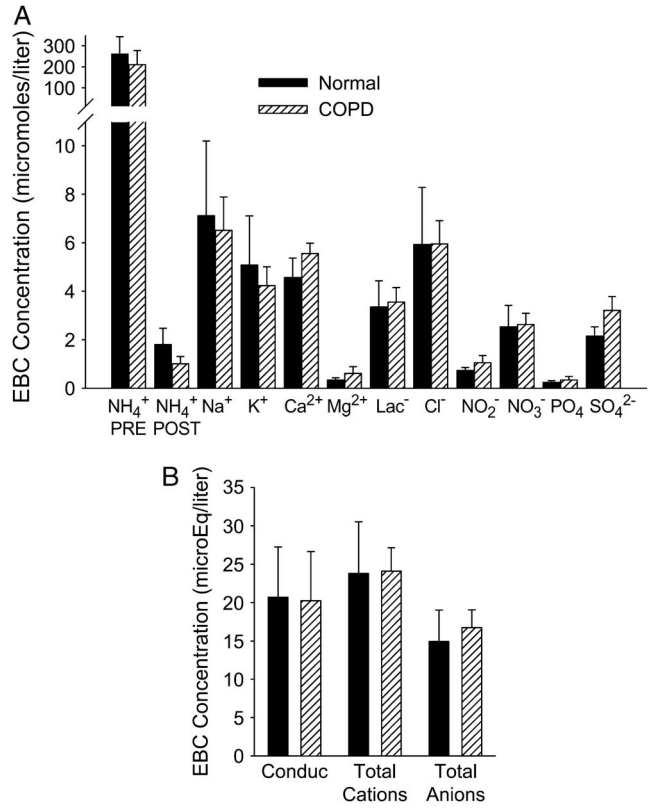


***Figure 7.*** (*A*) EBC concentrations of $NH_4^+$ and nonvolatile cations and anions. $NH_4^+$ concentrations are shown as prior to lyophilization (PRE) and after lyophilization (POST). Most of the $NH_4^+$ was removed by this procedure. All other measurements were made in the lyophylized EBC. Average values of $NH_4^+$ in the nonlyophilized samples exceeded those of any other ion by a factor of more than 20. Ion concentrations did not differ between normal and COPD samples. For the entire population, the relative molar magnitudes of the nonvolatile ionic concentrations were $Na^+ = Cl^- = Ca^{2+} > K^+ >$ lactate $> SO_4^{2-} > NO_3^- > NO_2^- = NH_4^+ >$ phosphate $> Mg^{2+}$ ($p < 0.05$). (*B*) Total cation concentrations exceeded those of the anions and the total ionic concentration estimated from conductivity (Conduc; $p < 0.05$). The total concentration of nonvolatile cations ($Na^+$, $K^+$, $Ca^{2+}$, and $Mg^{2+}$) slightly exceeded total measured by conductivity and the total anion concentrations.

with this hypothesis. It has been shown in this and previous studies that $NH_4^+$ is by far the most abundant cationic buffer in the EBC of healthy subjects and in many patients with COPD (*see* Figure 7 and References 15 and 16). Most of this $NH_4^+$ is derived from saliva, which normally contains abundant $NH_4^+$; when the mouth is "bypassed" with an endotracheal tube or by tracheostomy, EBC $NH_4^+$ concentrations fall by about 80% (12, 15–17). EBC $NH_4^+$ can also be reduced by 90% by simply washing the mouth with acidic solutions, which tend to trap $NH_3$ as $NH_4^+$ in the saliva (18). Thus, much of the $NH_4^+$ found in the EBC reflects contamination by salivary $NH_3$, some of which is derived from bacterial degradation of urea.

Although most of the $NH_4^+$ in the EBC is generated in saliva, very little of it is delivered to the EBC in salivary droplets. This conclusion is supported by several observations:

1. EBC concentrations of amylase determined with a very sensitive assay were disproportionately lower than those in the saliva.
2. Relative concentrations of nonvolatile cations in the EBC differed significantly from those in the saliva. In the EBC, $Na^+$ concentrations were greater than those of $K^+$, which were similar to those of $Ca^{2+}$. In the saliva, $Ca^{2+}$ concentrations are much lower than those of sodium, whereas sodium concentrations are much lower than those of potassium (Figure 7 and Reference 19).
3. Concentrations of $NH_4^+$ in the EBC are much greater than those of the total nonvolatile cations (Table 4). In contrast, the concentrations of $NH_4^+$ in saliva are lower than those of total nonvolatile cations. Had the $NH_4^+$ in the EBC been delivered in salivary droplets, then the total nonvolatile cation concentrations in the EBC would have been greater than the $NH_4^+$ concentrations.
4. Although $NH_3$ can be virtually eliminated from EBC samples and artificial samples containing $NH_4HCO_3$ by overnight lyophilization, it cannot be removed in this fashion from solutions of $NH_4Cl$. It must therefore be concluded that most of the $NH_4^+$ in the EBC arrived as $NH_3$ gas released from the saliva. Additional evidence that $NH_4^+$ in EBC was added in the form of $NH_3$ gas rather than droplets of saliva containing $NH_4^+$ is provided in a previous study (15).

In the absence of any acids, addition of $NH_3$ to aqueous solutions inevitably results in alkalinization:

$$NH_3(g) + H_2O \leftrightarrow NH_4^+ + OH^- \quad (3)$$

where *g* indicates the gas phase. The observation that the pH of the EBC ($\sim$7–8) is normally much lower than the dissociation constant of $NH_4^+$ (9.3 at room temperature) indicates that acid must have also been added to the EBC during collection. Because

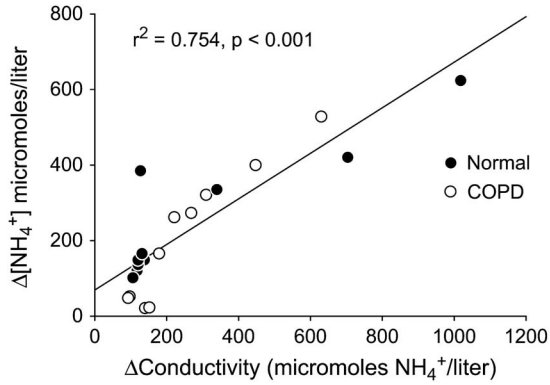


*Figure 8.* Lyophilization caused similar reductions in EBC $NH_4^+$ and conductivity, suggesting that $NH_4^+$ was the principal cation lost during lyophilization.

the concentration of acid involved would have to be comparable to that of $NH_4^+$, it must presumably be delivered to the EBC as a gas, because the concentrations of nonvolatile solutes are much lower (*see above*). The most likely source for this volatile acid is $CO_2$, which forms carbonic acid, and which is present at high concentrations in the exhaled air and low concentrations in the environment. As indicated in Figure 10, solutions containing $NH_4^+$ and $OH^-$ equilibrate with ambient $CO_2$, significantly reducing the pH of these solutions. It was impossible to directly measure $HCO_3^-$ in our solutions by ion chromatography because the anionic eluent used for this purpose contains both $HCO_3^-$ and $CO_3^{2-}$. However, we were able to titrate the EBC with NaOH and HCl to show that maximal buffering was present at a pH of about 6.3, which equals the $pK_a$ of $CO_2/HCO_3^-$ at room temperature. This observation suggests that $NH_3$ entering the EBC is neutralized by $CO_2$:

$$NH_3(g) + CO_2(g) + H_2O \leftrightarrow NH_4^+ + HCO_3^- \quad (4)$$

At the pH of the EBC samples, concentrations of $HCO_3^-$ approach those of $NH_4^+$. This explains why buffering capacities of normal EBCs are closely correlated with the $NH_4^+$ concentrations (Figure 3). These observations are consistent with the hypothesis that $NH_4^+/NH_3$ and $CO_2/HCO_3^-$ are the principal buffer

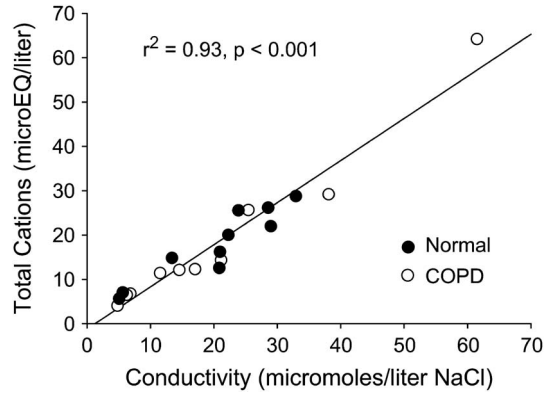


*Figure 9.* Although the values of conductivity and total cations were variable in the lyophilized samples, they were well correlated, suggesting that either could be used to calculate the dilution of respiratory droplets by water vapor.

**TABLE 2. CORRELATIONS BETWEEN CONCENTRATIONS OF $Na^+$ AND OTHER IONS IN LYOPHILIZED EXHALED BREATH CONDENSATE**

| | $r^2$ | p Value |
|---|---|---|
| Chloride | 0.946 | < 0.001 |
| Potassium | 0.812 | < 0.001 |
| Lactate | 0.731 | < 0.001 |
| Calcium | 0.682 | < 0.001 |
| Sulfate | 0.205 | < 0.001 |
| Nitrate | 0.175 | 0.066 |
| Magnesium | 0.134 | 0.112 |
| Phosphate | 0.0488 | 0.349 |
| Nitrite | 0.0414 | 0.390 |
| Ammonium | 0.0244 | 0.510 |

systems that determine the pH of the EBC in healthy subjects and many patients with COPD.

One problem associated with measurements of EBC pH is uncertainty concerning the $P_{CO_2}$ of these samples. The pH of normal EBC samples measured immediately after collection tends to be relatively acidic (pH ~ 6.0) (7, 10). This presumably reflects the presence of an end-tidal $CO_2$ of about 40 mm Hg in the exhaled air, which is equivalent to 1,200 μmol/L in the condensate. Because end-tidal $CO_2$ can be influenced by changes in arterial $P_{CO_2}$, ventilation/perfusion ratios, and dead space of the lungs, Hunt and coworkers adopted the procedure of removing $CO_2$ by flushing the samples with inert gas (argon) (1). In earlier studies, they exposed 1.0 ml to a flow of argon at 350 ml/min (1). This increases the pH of the EBC to approximately 7.65. More recently, they have suggested that 0.2 ml can be used for this purpose, increasing the EBC pH to 7.9 (12). They assumed that virtually all of the $CO_2$ was removed from the samples when the pH stabilized. However, in the absence of some other acids, removal of all of the $CO_2$ from the samples should increase the pH of solutions containing $NH_4^+$ to about 9.0 when $NH_4^+$ concentrations are as high as those reported in normal samples. This is far higher than that observed when the EBC samples are flushed with argon. The effect on EBC pH of purging the samples with argon to remove the effects of $CO_2$ on the pH would be comparable to reversing the effect of atmospheric $CO_2$ in Figure 10. Although acetic acid is found in EBC samples collected from some patients with asthma and patients with COPD (*see below*), we have been unable to detect any other acids other than $CO_2$ in concentrations comparable to those of $NH_4^+$ in healthy subjects. This suggests that flushing the EBC with inert gases for 10 min does not remove all of the $CO_2$. We have tried both of the procedures recommended to remove $CO_2$ from defined solutions of $NH_4HCO_3$ using dry nitrogen as the inert gas, but the EBC pH did not increase above 8 (*see* the online supplement), indicating that much of the $CO_2$ remained. Furthermore, there was loss of some water and $NH_3$ from these solutions. This indicates that purging EBC with inert

**TABLE 3. CONCENTRATIONS OF NONVOLATILE SOLUTES RELATIVE TO SODIUM IN EXHALED BREATH CONDENSATE AND PLASMA**

| | EBC (%) | Plasma (%) |
|---|---|---|
| $K^+$ | 76 ± 7 | ~ 3 |
| $Ca^{2+}$ | 115 ± 15 | ~ 3 |
| Lactate | 71 ± 12 | < 1 in plasma |
| Sulfate | 54 ± 8 | < 1 in plasma |

*Definition of abbreviation*: EBC = exhaled breath condensate.

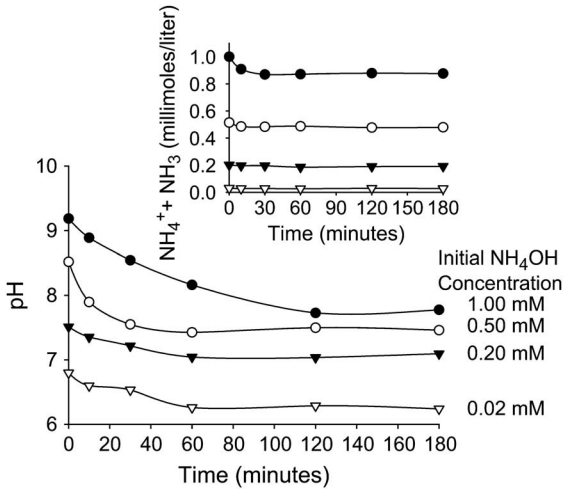


***Figure 10.*** Effect of ambient $CO_2$ on the pH of solutions of $NH_4OH$. Room air was bubbled at 20 ml/min through 30 ml of solutions of $NH_4OH$ in an open 100-ml beaker until pH stabilized. The fall in pH reflects diffusion of room air into the solution with increases in $HCO_3^-$ to more than 97% of those of $NH_4^+$ in the solutions. These curves are consistent with an ambient fractional volume of $CO_2$ ($F_{CO_2}$) of about 0.001. If $CO_2$ could be removed from these solutions by flushing with inert gases, the pH would return to that measured before exposure to air. Total concentration of $NH_3 + NH_4^+$ remains relatively unchanged (*upper panel*).

gases is nonselective and other important volatile constituents of the EBC can be lost. We reasoned that the $P_{CO_2}$ of the samples could be decreased to ambient levels and loss of other volatile acids and bases minimized by exposing our samples to room air for 30 min before measuring pH. It also would not have been practical to expose samples of saliva to argon without foaming.

Hunt and colleagues (11) also found that EBC $NH_4^+$ concentrations are decreased in subjects with asthma. Because most of the $NH_4^+$ is derived from the mouth and saliva and salivary $NH_4^+$ concentrations are unchanged in COPD (*see* Figure 4), this suggests that decreases in EBC $NH_4^+$ reflect acidification of the saliva, which decreases the concentrations of $NH_3$ relative to those of $NH_4^+$ in the saliva.

The observation that EBC pH can fall to values of 5 and below cannot be attributed to either the $CO_2/HCO_3^-$ ($pK_a$ = 6.3) or $NH_4^+/NH_3$ ($pK_a$ = 9.3) buffer systems; nor is it possible to attribute this acidification to any nonvolatile buffers that could be delivered in respiratory or salivary droplets. The extreme dilution of nonvolatile constituents in the EBC by water vapor

**TABLE 4. COMPARISON OF EBC $NH_4^+$ AND TOTAL CATIONS IN EXHALED BREATH CONDENSATE AND SALIVA (mmol/L)**

| | EBC | | Saliva | |
|---|---|---|---|---|
| | $NH_4^+$ | Total Nonvolatile Cations | $NH_4^+$ | Total Nonvolatile Cations |
| Normal | 260 ± 82 | 21 ± 6 | 11,200 ± 3,250 | 39,730 ± 3,740 |
| COPD | 210 ± 66 | 20 ± 6 | 13,000 ± 1,940 | 47,060 ± 2,930 |

*Definition of abbreviations*: COPD = chronic obstructive pulmonary disease; EBC = exhaled breath condensate.

EBC $NH_4^+$ and total cations and salivary $NH_4^+$ data are from this study (10 normal and 10 COPD samples). Salivary total cation data are from Reference 19 (9 normal and 9 COPD samples).

(~ 1:12,000) (15, 18–21) would make the contribution of even strong acids negligible. For example, the dilution of respiratory or gastric droplets containing a 0.1 M solution of HCl would result in a pH of greater than 6.0.

Preliminary evidence has been reported that acetic acid concentrations ($pK_a$ = 4.75) are elevated in the EBC of some patients with asthma (22). We also detected increased acetic acid concentrations in some of our patients with COPD. However like $NH_4^+$, acetic acid is present in the saliva in much higher concentrations than those found in the EBC or plasma, and acetic acid represents the most abundant volatile anion in the saliva (23). This suggests that at least some of this acid may be derived from the saliva rather than the lungs. Like salivary $NH_3$, much of this acetate may reflect oral bacterial metabolism (24). The role of bacterial metabolism on the concentration of acetate in the extracellular fluid is illustrated by the observation that acetate levels in the serum increase from about 70 to about 2,000 μmol/L with ingestion of bran, because of bacterial metabolism in the colon (24). The most persuasive evidence that much of the EBC acetate, like EBC $NH_4^+$, represents a salivary contaminant is based on the observation that when the saliva is acid, the concentration of acetate in the EBC increases (Figure 6). In contrast, the concentration of $NH_4^+$ decreases when the saliva is acid. This inverse relationship is due to the fact that acidification of the saliva increases the concentration of acetic acid (which is volatile) relative to acetate (which is not volatile). In contrast, acidification reduces the concentrations of $NH_3$ relative to that of $NH_4^+$. We therefore have concluded that the pH of the EBC in these individuals is affected by the relative amounts of acetic acid and ammonia that have been added to the EBC. Although addition of $NH_3$ to the EBC increases the concentration of $HCO_3^-$ in the EBC, acetic acid reduces the concentration of $HCO_3^-$ in accordance with the reaction:

$$HCO_3^- + HAc(g) \leftrightarrow CO_2(g) + Ac^- + H_2O \qquad (5)$$

If the $P_{CO_2}$ is kept constant by exposing the EBC to room air, and if losses of $NH_3$ and acetic acid are minimized, then the pH will vary in accordance with the relative amounts of acetic acid and ammonia that have been absorbed by the EBC. Because most of these constituents are in the ionic form at the pH of the EBC, the ratio of acetate to $NH_4^+$ was used to estimate the balance of volatile acids and bases in the EBC (Figure 5).

Definitive evidence that much of the EBC acetate, like EBC $NH_4^+$, is derived from saliva rather than the lungs would require collections of EBC from patients before and after intubation. This may not be practical, because high acetate concentrations are observed among patients with lung disease, in whom elective intubation might be unwise. To the extent that extrapulmonary acids and bases influence the EBC pH, acidopnea cannot provide a reliable index of airway acidification or inflammation.

The present study indicates that "acidopnea" may reflect decreases in salivary rather than respiratory pH. Acidification of the saliva could be due to gastroesophageal reflux disease, which is quite common in patients with various forms of obstructive lung disease (25, 26). It would seem prudent to measure salivary pH when measurements are made of EBC pH. Furthermore, it would be of interest to determine whether proton pump inhibitors decrease the incidence of acidopnea. Because acetic acid aerosols can cause both cough and bronchospasm (27, 28), and because acidopnea has been linked to the deleterious effects of air pollution on lung growth in children (29, 30), it can be speculated that saliva may prove to be a source of an agent that is injurious to the lungs.

It would theoretically be advantageous to lyophilize the samples and reconstitute them with deionized water before measuring pH, to minimize the effect of volatile salivary constituents,

which can be derived from the mouth. However, the low buffer capacity of the lyophilized samples complicates these measurements. Alternatively, the presence of increased concentrations of specific nonvolatile ions could be used to detect airway acidification. As indicated in Figure 7, no difference was found in the nonvolatile anion concentrations of EBC between our limited COPD and healthy populations. Nevertheless, attention to individual constituents in the EBC may prove more useful than pH, which is a dependent variable that is a function of the concentrations and dissociation constants of multiple acids and bases.

***Conflict of Interest Statement***: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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