Nitric oxide production and absorption in trachea, bronchi, bronchioles, and respiratory bronchioles of humans

ARTHUR B. DuBOIS,1,2 PATRICK M. KELLEY,1 JAMES S. DOUGLAS,1,2 AND VAHID MOHSENIN1,2

1) John B. Pierce Laboratory and 2) Yale University, New Haven, Connecticut 06519

DuBois, Arthur B., Patrick M. Kelley, James S. Douglas, and Vahid Mohsenin. Nitric oxide production and absorption in trachea, bronchi, bronchioles, and respiratory bronchioles of humans. J. Appl. Physiol. 86(1): 159–167, 1999.—Different volumes of dead-space gas were collected and analyzed for nitric oxide (NO) content, either immediately after inspiration or after a period of breath holding on clean air or NO mixtures. This allowed calculation of NO equilibrium, NO production, and NO absorption. In seven young, healthy, adult nonsmokers, the mean NO equilibrium values (in parts per billion) were 56 ± 11 (SE) in the trachea, 37 ± 6 in the bronchi, 21 ± 3 in the bronchioles, and 16 ± 2 in the respiratory bronchioles. At any given NO concentration, the NO absorption rate (in nl/min) equaled the NO concentration (in ppb) times A (the absorption coefficient in l/min). A values (in l/min) were 0.11 ± 0.01 in the trachea, 0.17 ± 0.04 in the bronchi, 0.66 ± 0.09 in the bronchioles, and 1.35 ± 0.32 in the respiratory bronchioles. NO equilibrium concentrations and production rates in one 74-yr-old subject were three to five times as high as those found in the young subjects. Mouth equilibrium NO concentrations were 3 and 6 parts per million in two subjects who had oral production rates of 6 and 23 nl/min, respectively. In conclusion, production and absorption of NO occur throughout the first 450 ml of the airways.

THE DISCOVERY of exhaled nitric oxide (NO) in humans (8) has led to considerable interest in the analysis of exhaled NO as a possible noninvasive measure of some forms of lung injury. Different techniques have been employed to collect and analyze respiratory NO. These have included measurements of peak NO concentrations (18), NO concentration in exhaled fractions (21), the NO plateau (20), and the NO concentration difference between peak and end-expiratory samples (10). Researchers have utilized collection of gas expired from the mouth, nose, conducting airways, and terminal airways (2, 14, 16, 18). Samples have also been collected via endotracheal tubes (2). Despite the lack of a standardized technique, exhaled NO has been collected in various pathological conditions including asthma (1, 13), bronchiectasis (12), liver cirrhosis (17), Kartagener’s syndrome (16), and chronic obstructive pulmonary disease (10). It is not yet known what either normal or abnormal NO concentrations actually represent, because the physiological processes underlying gaseous NO production and absorption in the respiratory tract have not been characterized.

Earlier studies indicated that a large portion of exhaled NO arose from the nose (4, 14–16). Studies have shown that the use of a noseclip does not exclude nasal gas from exhaled samples (3, 20). However, the nasopharynx and lungs can be studied separately by closing the soft palate to isolate the nasal cavity from the respiratory tract. Quantitative studies of NO production, absorption, and equilibrium in the human nose have been made in this laboratory (5). We postulated that a similar theoretical analysis could be applied to the conducting airways of humans to study NO production, absorption, and equilibrium concentrations within them.

Evidence suggests that, once the nasal component of exhaled breath has been removed, much of the remaining exhaled NO originates within the conducting airways (9, 21). Although dead-space gas is discarded during the single-breath method for measurement of pulmonary diffusing capacity (7) or for collection of alveolar CO2 samples by the Haldane method, in the present report, small aliquots of dead-space gas are collected and analyzed for their NO content either immediately after inspiration of NO-free air or NO, or after a period of breath holding. This allowed us to calculate the rates of production and absorption of NO gas expired from different depths of the conducting airway.

Theoretically, if no change in NO concentration were found during breath holding after inhalation of a NO mixture, it would indicate that an equilibrium concentration of NO gas had been reached. At this concentration, the rates of NO production and absorption would be equal, and the NO partial pressure within the tissue surface of the conducting airway would be equal to the partial pressure of NO in the lumen. A method is presented for collection of exhaled gases from the various zones of the respiratory tract to examine equilibrium concentrations, the rates of production, rates of absorption, and absorption coefficients in each zone.

METHODS

Subjects. Initially, a 74-yr-old subject and a 24-yr-old subject were studied. The 74-yr-old subject had tracheobronchial NO concentrations which turned out to be much higher than NO concentrations found in the younger subject. Data on both subjects were reproducible. Subsequently, a subject group of adults (ages 20–32) was selected on the basis of their having no reported respiratory conditions. All were nonsmokers and had prior experience in respiratory maneuvers. Because nasal gas is a potential source of NO contamination in the exhaled samples, subjects were excluded if they were unable to close their soft palate while breathing against
inspiratory and expiratory resistance to exclude nasal gas (see Procedure for determination of contamination by nasal NO).

Equipment. A large rubber mouthpiece (W. E. Collins, Boston, MA) was fitted onto a Fleisch pneumotachograph (size no. 2), which in turn was connected to an aluminum three-way stopcock (W. E. Collins). The breathing apparatus provided enough resistance to aid the subjects in closing their soft palates naturally. One stopcock arm was attached to a rubber anesthesia bag. The other arm was attached to 1-in.-diameter tubing connected to a 9-liter Spirometer (W. E. Collins). The pneumotachometer was a low-torque potentiometer that gave a voltage proportional to expired gas volume. The anesthesia bag was attached to a Monitor Laboratories gas-mixing unit which supplied either NO-free air (scrubbed through Purafil and activated charcoal) or mixtures of NO with NO-free air. Gas samples were aspirated continuously through Purafil and activated charcoal or mixtures of NO and NO-free air.

Gas flowmeter (HealthScan, Cedar Grove, NJ) was fitted onto a Fleisch pneumotachograph (size no. 2), which in turn was connected to an aluminum spincter that gave a voltage proportional to expired gas volume. The subject was comfortably seated in front of the instrument and instructed in the method. Practice tests were performed until the subject felt comfortable with the protocol. A thermistor (Yellow Springs Instruments) was inserted in the nostril to make sure that the soft palate remained closed during inspiration and expiration. Any leaks would be indicated by fluctuations of temperature inside the nostril.

The protocol was as follows. The subject twisted the handle of the three-way stopcock and inhaled a normal tidal volume of either NO-free air or a mixture of NO and NO-free air from the anesthesia bag. The subject then twisted the handle of the three-way stopcock back and expired a partial breath into the spirometer. The subject stopped the partial breath when the desired exhaled volume was reached. The volume of gas in the spirometer was maintained by closing the glottis. The mouth was kept on the mouthpiece, with the glottis closed, for ~5–10 s to allow time for sampling. The analyzer flow rate was set by a needle valve at 60 ml/min. This sampling flow was provided by the vacuum pump of the Sievers NO analyzer. The dead space of the sampling system caused a small delay in the record; this delay was taken into account in reading the data. About 10 single expirations, ranging in volume from 50 to 450 ml, were performed for each inspired gas concentration. The first 34 ml of this volume was used to form the dead space of the mouthpiece and pneumotachograph up to the gas-sampling tube. On the basis of preliminary experiments measuring the volume of the mouth, the next 40 ml were taken to be the gas that had been held in the mouth. Preliminary experiments had shown that NO gas became concentrated in the mouth during breath holding. We report measurements made on the rate of its accumulation in the mouth, and then we focus on the volumes of gas that begin after the first 75 ml (from tubing and mouth) have been expelled.

Initial experiments in the younger subject (PK) and the older subject (AD) were done by using several different inhaled mixtures of NO ranging from 0 to 1,000 parts per billion (ppb) and several different periods of breath holding ranging from 0 to 15 s. On the basis of NO analyzed from the samples exhaled as above, it was decided to collect data on a small group of young, healthy subjects by using 10 s of breath holding after inhalation of a tidal volume of either NO-free air or NO concentrations above equilibrium values (e.g., inhalation of 396 ppb of NO diluted with NO-free air).

The actual breath-holding times for the 0- and 10-s intervals were measured from the pneumotachograph record for each subject. The time of breath holding was measured from the end of inspiratory flow to the end of expiratory flow. In the 0-s breath-holding experiment, the time was called $t_1$ and averaged ~1–2 s. The time from the end of inspiration to the end of expiration after the ~10-s breath-holding period was called $t_2$ and averaged ~11–12 s. When the subject inhaled NO, breath-holding times were $t_3$ and $t_4$ for ~1–2 and 11–12 s, respectively. The interval value used in calculations was the difference between these times ($t_1 - t_2$, or $t_3 - t_4$) and equaled ~10 s.

As explained in the APPENDIX, the term NO$\infty$ is used to indicate the equilibrium concentration that is approached during breath holding. The absorption coefficient $A$, when it is multiplied by the NO concentration ([NO]), gives the rate of NO absorption, and the term $Q_\infty$ is the rate of production of NO gas and could equally well be called the NO output. The equations utilized in analyzing these data are listed below and are derived in the APPENDIX.

\begin{equation}
\text{NO}^\infty = \frac{([\text{NO}_1][\text{NO}_4] - [\text{NO}_3][\text{NO}_2])}{([\text{NO}_1 + [\text{NO}_4] - [\text{NO}_2 - [\text{NO}_3]])
\end{equation}

\begin{equation}
A = V \times \ln ([\text{NO}_1] - [\text{NO}_4])/([\text{NO}_2 - [\text{NO}_3] \times 1/(t_2 - t_1))
\end{equation}

or the equation can take this alternate form

\begin{equation}
A = V \times \ln ([\text{NO}_1] - [\text{NO}_4])/([\text{NO}_4 - [\text{NO}_3] \times 1/(t_2 - t_3)
\end{equation}

\begin{equation}
Q_\infty = A \times [\text{NO}^\infty]
\end{equation}

Mouth experiments. Two sets of experiments were performed on two subjects to measure the production and absorption of NO in the mouth. In the first set of experiments, while subjects were breathing through the nose, the gas was held in the mouth by pressing the back of the tongue against the roof of the mouth. The mouth was filled and emptied twice with NO-free air, by using the glossoaryngeal muscles, and then the mouth was filled with NO-free air, which was then held with positive pressure in the mouth for periods of 3 s and 2, 5, and 10 min. By using the cheek muscles, the subject expelled a 10-ml sample into a syringe for NO analysis. The experiment was repeated with 16.9 parts per million (ppm) NO instead of NO-free air, and the gas was held in the mouth for 3 and 2, 5, and 10 min. By using the cheek muscles, the subject expelled a 10-ml sample into a syringe for NO analysis.
used. This set of experiments used concentrations of 0, 1.96, 9.43, and 16.9 ppm NO.

Data analysis. Data were plotted on a Macintosh computer by using Cricket Graph. Expired NO was plotted against expired volume to give a smooth curve that was representative of NO levels in each section of the respiratory dead space. Tracheal, bronchial, bronchiolar, and respiratory bronchiolar volumes were taken from the data of Weibel (22). For each inhaled concentration (for example, 0 and 396 ppb), exhaled NO concentration and volume were graphed at each breath-holding time (for example, 0 and 10 s) to show the effect of time on an inhaled NO concentration.

The graphs were divided into zones corresponding to the volumes of the different sections of the respiratory tract. The mean height of the line was taken for each zone. This was considered the mean NO concentration for this zone at this time. This yielded four mean NO concentrations per zone: \([NO_1]\) at \(-1–2\) s of breath holding after inhaling NO-free air, \([NO_2]\) at \(-11–12\) s of breath holding after inhaling NO-free air, \([NO_3]\) at \(-1–2\) s of breath holding after inhaling 396 ppb NO, and \([NO_4]\) at \(-11–12\) s of breath holding after inhaling 396 ppb NO. The actual time that the breath was held in the zero breath-holding experiment was \(t_1\), and the actual time that the breath was held in the 10-s breath-holding experiment was \(t_2\) (see Fig. 1).

RESULTS

Normal subjects. Based on preliminary experiments and on the curves derived theoretically (see METHODS), it was decided to use a 10-s interval of breath holding after the inhalation of either 0 or 396 ppb NO to test young, healthy subjects, because, after inhalation of 0 ppb, the NO in the airways increased, whereas, after 396 ppb, it decreased during breath holding.

Subjects. Four male and three female subjects, all healthy nonsmokers, were studied. They ranged in age from 19 to 31 yr, and they had a mean height of 169 cm and a mean weight of 67 kg. They were all successful in performing the protocol.

Volumes of respiratory tract. Volumes for the different areas of the respiratory tract were taken from Weibel (22). These area volumes were considered to be as follows: trachea, 30.5 ml; bronchi, 31 ml; bronchiolae, 113 ml; and respiratory bronchiolae, 103 ml. The dead space of the mouthpiece and pneumotachograph until the point of sampling was 34 ml. The volume of the mouth was 40 ml and was considered dead space in this experiment, even though some production and absorption of NO occur in the mouth. This exchange was not the main part of the present investigation. The total dead space (system + mouth) was 74 ml and was discarded. This explains why the graphs of NO concentration vs. expired volume (see Fig. 2, A and B) begin at a volume of 75 ml on the horizontal axis (to ignore NO collected from the breathing tube and oropharynx).

Day-to-day variability. The experiment was repeated for 5 days in one subject (PK) to assess the day-to-day variations in NO levels. The young man was 24 yr of age, with a height of 167.6 cm and a weight of 72.7 kg. Table 1 lists the means, SD, and SE of unpaired data calculated for NO \(\equiv\), \(A\), and \(Q_p\) over these 5 days.

The experiment was also repeated on 5 days in one subject (AD) aged 74 yr. His height was 184.5 cm, and his weight was 84 kg. The subject's values were significantly different from those of the young subject (PK) and other young subjects. The experiment was repeated on 5 days to determine whether the values were consistent on a day-to-day basis. Table 2 lists the mean, SD, and SE values of unpaired data for these 5 days.

Rise and fall of NO over time. When the subject inhaled NO-free air and exhaled without breath holding, the NO levels were low in all areas of the airways. Expiration was not immediate, however, and the delay due to turning the stopcock and the time required to exhale was \(-1–2\) s. During breath-holding experiments, the delay in onset of expiration was also \(-1–2\) s. The actual breath-holding times for each subject were obtained from the pneumotachograph record and were used in the calculations. When breath holding was performed for 0, 5, 10, or 15 s after inhaling NO-free air, the NO concentration would build toward an equilibrium concentration in which the NO would not change if the breath were held longer. Figure 1 shows a model of the behavior of NO in any zone of the respiratory tract, starting from inhaling either NO-free air or a concentration above the equilibrium concentration. In the young adult, this equilibrium concentration agreed reasonably well with observations made after inhalation of 99 ppb NO. On the other hand, if the subject inhaled an even higher concentration of NO, such as

### Table 1. Results of experiments in 1 subject (age 24 yr) for 5 days

<table>
<thead>
<tr>
<th></th>
<th>Trachea</th>
<th>Bronchi</th>
<th>Bronchiolae</th>
<th>Respiratory Bronchiolae</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO, ppb</td>
<td>72</td>
<td>51</td>
<td>33</td>
<td>16</td>
</tr>
<tr>
<td>SD</td>
<td>20</td>
<td>19</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>SE</td>
<td>9</td>
<td>8</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>A, l/min</td>
<td>0.12</td>
<td>0.12</td>
<td>0.50</td>
<td>0.66</td>
</tr>
<tr>
<td>SD</td>
<td>0.10</td>
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<td>0.13</td>
<td>0.22</td>
</tr>
<tr>
<td>SE</td>
<td>0.05</td>
<td>0.01</td>
<td>0.06</td>
<td>0.10</td>
</tr>
<tr>
<td>Qp, nl/min</td>
<td>8.9</td>
<td>6.2</td>
<td>16.6</td>
<td>10.7</td>
</tr>
<tr>
<td>SD</td>
<td>6.3</td>
<td>2.6</td>
<td>7.7</td>
<td>7.1</td>
</tr>
<tr>
<td>SE</td>
<td>2.8</td>
<td>1.2</td>
<td>3.4</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Values are means, SD, and SE (unpaired) for 1 subject (age 24 yr) who repeated the experiment 5 times. NO, nitric oxide (NO) equilibrium concentration; A, absorption coefficient; Qp, rate of NO production; ppb, parts per billion.

### Table 2. Results of experiments in 1 subject (age 74 yr) over 5 days

<table>
<thead>
<tr>
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<th>Bronchi</th>
<th>Bronchiolae</th>
<th>Respiratory Bronchiolae</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO, ppb</td>
<td>266</td>
<td>178</td>
<td>92</td>
<td>41</td>
</tr>
<tr>
<td>SD</td>
<td>50</td>
<td>32</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>SE</td>
<td>22</td>
<td>14</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>A, l/min</td>
<td>0.08</td>
<td>0.11</td>
<td>0.65</td>
<td>1.0</td>
</tr>
<tr>
<td>SD</td>
<td>0.03</td>
<td>0.03</td>
<td>0.18</td>
<td>0.27</td>
</tr>
<tr>
<td>SE</td>
<td>0.01</td>
<td>0.02</td>
<td>0.08</td>
<td>0.12</td>
</tr>
<tr>
<td>Qp, nl/min</td>
<td>21.0</td>
<td>19.9</td>
<td>59.7</td>
<td>41.2</td>
</tr>
<tr>
<td>SD</td>
<td>4.2</td>
<td>4.3</td>
<td>24.3</td>
<td>9.4</td>
</tr>
<tr>
<td>SE</td>
<td>1.9</td>
<td>1.9</td>
<td>10.9</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Values are means, SD, and SE (unpaired) for 1 subject (age 74 yr) who repeated the experiment 5 times. NO, nitric oxide (NO) concentrations for this subject are significantly higher than younger subjects' values.
Similar comparisons on the absorption coefficient $A$, by using ratios, showed $P$ values for each pair of data from trachea to bronchi ($P < 0.4$), bronchi to bronchioles ($P < 0.001$, upward trend), and bronchioles to respiratory bronchioles ($P < 0.1$). $Q_p$ was not significantly different between trachea and bronchi or between bronchioles and respiratory bronchioles. $Q_p$ was greater in the bronchioles than in the bronchi ($P < 0.01$). Figure 3, B and C, shows these trends.

CO$_2$ approached normal alveolar levels in exhaled volumes of 150 ml or more, and this dead space volume decreased with time of breath holding, as has been found before.

Data from 74-yr-old subject. NO equilibrium levels in the 74-yr-old man were substantially higher than in the young subjects. In the trachea, the NO equilibrium was about five times as high as the young subjects’ (266 vs. 56 ppb, respectively; $P < 0.001$). The equilibrated concentration in the bronchi was about five times as high as the young subjects’ (178 vs. 37 ppb, respectively; $P < 0.001$). The bronchiolar equilibrated concentration was 4.4 times as high as the young subjects’ (92 vs. 21 ppb, respectively; $P < 0.001$), and the respiratory bronchiolar concentration was 2.6 times as high as the young subjects’ (41.2 vs. 15.7 ppb, respectively; $P < 0.02$). The $A$ values were not statistically different in the older subject, but the rate of NO production was significantly elevated. $Q_p$ was 21.0 vs. 5.7 nl/min in the young subjects’ tracheas, a 3.7-fold difference ($P < 0.001$). Similar differences were found in the bronchi (19.9 vs. 5.3 nl/min, respectively; $P < 0.001$), bronchioles (59.7 vs. 13.0 nl/min, respectively; $P < 0.001$), and respiratory bronchioles (41.2 vs. 21.1 nl/min, respectively; $P < 0.05$).

Mouth data. When NO-free air was held in the mouth for varying lengths of time, it was found that NO concentrations increased over time. When 16.9 ppm NO was held in the mouth for varying lengths of time, it was found that NO concentration decreased over time. Equations used in the tracheobronchial tree were utilized to calculate $[NO]_{ex}$, $A$, and $Q_p$. In subject AD, the $[NO]_{ex}$ was 2.3 ppm, $A$ was 0.0027 l/min, and $Q_p$ was 6.1 nl/min. In subject PK, $[NO]_{ex}$ was 6.8 ppm, $A$ was 0.0034 l/min, and $Q_p$ was 23.1 nl/min.

When a constant flow of gas was introduced into the mouth, and the effluent gas was sampled, steady-state NO concentrations were found. Data from two subjects are listed in Table 4. Steady-state NO was inversely related to the gas flow rate through the mouth. After NO of 16.9 ppm was introduced into the mouth at a constant gas flow for 5 min and then switched to NO-free air, it was found that effluent NO concentrations were higher than normally found, and a “washout” or desorption period was observed for up to 13 min.

**DISCUSSION**

The quantitative analysis of NO from all the regions of the respiratory tract is important, if exhaled NO is to be of clinical utility. The efficacy of using exhaled NO as a biomarker of inflammation or immune responses in humans remains undetermined.
This method of NO collection investigates NO from different zones of the respiratory tract, as determined by expired volume. Forward and backward mixing of exhaled gas is certain to occur (due to a parabolic gas front and forward and backward diffusion), and there is some exchange of NO with airway walls while the gas is passing upward through the airways. To minimize this exchange, expirations were quick compared with breath-holding times. Because the bronchioles are longer than they are wide, diffusion within the lung would favor NO gas exchange with the walls rather than by lengthwise diffusion. CO2 was found in exhaled volumes of 150 ml or greater. This indicates that NO gas exchange is occurring even beyond areas that have classically been considered dead space or despite diffusion of alveolar CO2 up the conducting airways. The reproducibility of results as seen in each subject was reasonably good, as contrasted to the large difference between one subject who was age 74 yr and all seven of the young subjects.

Table 3. Results of experiments in 7 subjects

<table>
<thead>
<tr>
<th></th>
<th>Trachea</th>
<th>Bronchi</th>
<th>Bronchioles</th>
<th>Respiratory Bronchioles</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO, ppb</td>
<td>56</td>
<td>36.9</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>SD</td>
<td>30</td>
<td>15</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>SE</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>A, l/min</td>
<td>0.11</td>
<td>0.17</td>
<td>0.66</td>
<td>1.35</td>
</tr>
<tr>
<td>SD</td>
<td>0.04</td>
<td>0.11</td>
<td>0.23</td>
<td>0.79</td>
</tr>
<tr>
<td>SE</td>
<td>0.01</td>
<td>0.04</td>
<td>0.09</td>
<td>0.32</td>
</tr>
<tr>
<td>Q˙p, nl/min</td>
<td>5.7</td>
<td>5.3</td>
<td>13.0</td>
<td>21.1</td>
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<tr>
<td>SD</td>
<td>2.8</td>
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<tr>
<td>SE</td>
<td>1.0</td>
<td>1.2</td>
<td>2.2</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Values are means, SD, and SE (unpaired) in 7 subjects. Ratios between adjacent means were all statistically significant (P < 0.05) when Student's two-tailed t-test was used on paired data.

NO concentration rises in all areas of the respiratory tract with 10 s of breath holding after inhalation of NO-free air. The smallest increase in NO level occurs within the respiratory bronchioles. But local production of NO in the respiratory bronchioles is appreciable, because this zone has a large volume (22). The NO concentration falls in all levels of the respiratory tract after inhalation of 396 ppb NO and after breath holding for 10 s. At the level of the respiratory bronchioles, the NO concentration approaches an equilibrium concentration within 10 s of breath holding. The predicted equilibrium concentration can be found experimentally in all areas of the respiratory tract by having subjects inhale different concentrations of NO. An example of this type of approach is shown in Fig. 2B. If the inhaled concentration is too high, the NO level will drop over time in this zone. If the inhaled concentration is too low, the NO level will rise over time, as in Fig. 1.

Different equilibrium concentrations apply to the different zones of the airways. The NO equilibrium concentration drops with depth into the airways. The

Fig. 2. A: NO concentrations measured in small volumes of gas quickly exhaled from conducting airways of subject PK, a young adult man. Bottom line connects values (○) that represent concentrations exhaled from different depths of airways immediately after inhalation of a tidal breath of air devoid of NO. Arrow above line points upward in direction taken by NO concentration when there was a delay between inspiration and the onset of expiration. ■. Values obtained after 10-s breath holding. After subject inhaled an air mixture that contained 396 parts per billion (ppb) NO, expired NO was much higher than it had been after inhalation of clean air. Top line passes through values (□) representing concentrations found in expired air samples delivered immediately after inhaling 396 ppm NO. Arrow below this line indicates decreasing trend of NO concentrations when breath was held between inspiration and expiration. ●. Values obtained after 10-s breath holding of 396 ppm NO. Arrow below this line indicates decreasing trend of NO concentrations when breath was held between inspiration and expiration.

B: NO concentrations measured in older adult male subject, AD, after inhalation of air devoid of NO or air containing 980 ppb NO. As in A, expiration was either immediate or after 10 s of breath holding; arrows indicate trends. Equilibrium values (2 sets of ▽) were obtained after 5 and 10 s of breath holding after inhalation of 204 ppb NO. Because there was no trend with time, it was apparent that equilibrium had been reached. Symbols and lines are same as in A. NO equilibrium concentrations reached by older subject were higher than those reached by younger subject.
rate of production increases with depth, as does the absorption coefficient. Both of these factors may be related to surface area, as well as mucosal perfusion, but this is not yet known.

The substantial difference between the 74-yr-old subject’s NO equilibrium concentration and the young subjects’ NO equilibrium concentrations is unexplained. Possible causes may be of respiratory or circulatory origin or due to differences in ciliary activity (16) or an age-related effect. The unexplained large difference between this older subject and the others demonstrates the complications involved in interpreting exhaled NO.

The data from the mouth show that the mouth is capable of producing concentrations of NO that are as high or higher than those found in other areas of the trachea and bronchi. Duncan et al. (6) propose the following: in addition to production of NO by the family of enzymes NO synthase, the salivary glands concentrate nitrate (from diet) in the saliva and excrete it into the mouth. Nitrate is rapidly reduced to nitrite by facultative anaerobic bacteria found on the rear of the tongue. NO is generated when the nitrite comes into contact with acidic conditions, such as those produced by acid-producing bacteria in the gingival margins (6). Experiments that utilize collection of expiratory gas from the mouth should take into account the effect of the mouth in conditioning exhaled gas.

Fractionating an exhaled breath for the study of NO is important. In pathological conditions, such as asthma, the source of NO may be the airway tissues that suffer the inflammation. The alveoli and other portions of the respiratory tract may not be affected. These conditions are not known, but further studies to characterize exhaled NO need to incorporate these theoretical considerations. The relationship between NO concentration (in parts per billion) in the airway and the underlying nitrate and nitrite concentrations in the adjacent tissues is unknown and remains to be determined.

Further considerations are acknowledged in the present experiments. Expiratory transit time, although <1 s, was not standardized. Conditioning of the gas

*Fig. 3. A: NO concentration ([NO]) at equilibrium vs. volume in 7 subjects. Tr, trachea; Br, bronchi; Brls, bronchioles; Resp. Brls, respiratory bronchioles. B: absorption coefficients vs. volume in same 7 subjects. C: rate of NO production vs. volume in same subjects; R. Brls, respiratory bronchioles.*

Table 4. Steady-state concentrations of NO in mouths of 2 subjects at varied flows

<table>
<thead>
<tr>
<th>Gas Flow/Subject</th>
<th>Flow, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean air</td>
<td>19 40 98</td>
</tr>
<tr>
<td>AD</td>
<td>0.19 0.13 0.033</td>
</tr>
<tr>
<td>PK</td>
<td>0.05 0.04 0.035</td>
</tr>
<tr>
<td>NO, 16.9 ppm</td>
<td>14 15 15.5</td>
</tr>
<tr>
<td>AD</td>
<td>13 14 14.5</td>
</tr>
<tr>
<td>PK</td>
<td>13 14 14.5</td>
</tr>
</tbody>
</table>

Values are steady-state concentrations of NO in parts per million (ppm).
bolus as it passes through the conducting airways cannot be discounted because of this reason. The mouth, which we find in this study to be a site of gas exchange, will alter exhaled NO concentrations slightly during expiration. Exhaled NO has been correlated with ciliary action (16), and ciliary action was not assessed in this experiment. The volumes of the zones of the respiratory tract are affected by unequal branching and unequal lengths of the bronchi as well as their being dependent on body size. But the measurement of NO concentration in midvolume of each segment allows a latitude of ±20% before crossing from one zone to another. The total test took ~2 h to complete. Some subjects became fatigued, and controlling the exhaled volume became difficult for them. Development of a single-breath fractionation device would be beneficial in future studies. This would enable researchers to perform larger studies on exhaled NO from all parts of the respiratory tract, and improvement would be expected in the precision, speed, and reproducibility of the experiment.

Although investigators have studied exhaled NO and considered this value to be the NO production value, they have neglected the effect of absorption. Increased exhaled NO in pathological conditions may be a result of altered NO absorption as well as an increase in the rate of NO production.

Studies on NO production and absorption in the nose have shown that the majority of NO uptake in the nose is due to chemical combination and not due to simple solubility in blood flow (11). The following calculation shows that this also applies to the airways. On the basis of the uptake of chemically inert dimethyl ether, Kimberly et al. (14) give a value of 6.6 ml/min for mucosal blood flow in the 50 ml of conducting airway distal to the trachea. Weibel’s data (22) for volumes of the conducting airway give a volume of 31 ml for the bronchi. Assuming homogenous perfusion for this area, we can calculate bronchial blood flow by multiplying the blood flow by 0.62 (i.e., 31/50 ml). This gives a mucosal blood flow in the bronchi of 4.1 ml/min. By neglecting diffusion limitations and basing the uptake on the solubility of NO in blood (partition coefficient of 0.05 at 37°C), the rate of NO absorption in the bronchi at the equilibrium concentration (a quasi-steady-state condition in which the rates of production and absorption are equal) can be calculated by the equation

$$\dot{Q}_a = \dot{Q}_{br} \times \lambda_{NO} \times F_{NO}$$

where $\dot{Q}_a$ is the rate of NO gas absorption in the bronchi, $\dot{Q}_{br}$ is bronchial blood flow, $\lambda_{NO}$ is the partition coefficient in blood, and $F_{NO}$ is the NO gas concentration in the bronchi. On the basis of the mean values for the seven young subjects, the calculation for the rate of absorption would be

$$\dot{Q}_a = 4.1 \text{ ml/min} \times 0.05 \text{ ml gas/ml blood} \times 36.9 \times 10^{-9}$$

or $7.6 \times 10^{-9}$ ml/min. The uptake due to solubility can be compared with the uptake computed from

$$\dot{Q}_a = A[NO] = 0.17 \times 36.9 \times 10^{-9} \text{ l/min}$$

equals 6.27 nl/min. Therefore, solubility in blood flow accounts for $(7.6 \times 10^{-9} \text{ ml/min})/(6.27 \times 10^{-9} \text{ l/min})$ or only ~1/1,000 of the total uptake. A similar comparison, based on the uptake of nitrous oxide, was made in the human nose (11), and it was found that the uptake of NO was 27 times as much as could be expected due to solubility. Although Runer and Lindberg (19) found 75% increased blood flow and 57% increase in ciliary activity after nebulizing sodium nitroprusside (an NO donor) into the nose of subjects, no attempt has been made to try to assess the vasodilatory effects of inhaled NO in the tracheobronchial tree, so the possibility exists that NO may increase its own uptake by dilating the superficial mucosal blood vessels. However, it is unlikely that the change in uptake due to vasodilation would be of the magnitude to explain the difference in predicted uptake due to solubility in blood and the experimentally observed uptake. The gas does not desorb on exhalation (except in the mouth), thus indicating nonreversible reactions. NO probably forms nitrite and S-nitrosothiols. The rapid uptake of NO (in the 1- to 2-s breath-holding period) suggests that, initially, a majority of the NO reacts at the mucosal surface.

In conclusion, the equilibrium concentrations of NO show a downward trend with depth into the respiratory tract. Absorption coefficient values did not show a significant trend from trachea to bronchi or from bronchioles to respiratory bronchioles, but the values did increase significantly from the bronchi to the bronchioles. The rate of production of NO was not significantly different from the trachea to the bronchi or from the bronchioles to the respiratory bronchioles, but the rate was greater in the bronchioles than in the bronchi. NO equilibrium concentrations and rates of production and absorption showed variability from day to day. A group of young, nonsmoking, healthy subjects had similar trends of NO rates and concentrations. An older subject had NO equilibrium concentrations and production rates that were significantly elevated from the concentrations of the younger subjects. The method of NO collection and analysis demonstrated is accurate and reproducible.

**APPENDIX**

Derivation of the equations for the rate of NO production ($\dot{Q}_p$) and absorption ($\dot{Q}_a$). During breathing, the rate at which NO concentration ([NO]) at time t changes in a volume (V) of the conducting airway is proportional to the $\dot{Q}_p$ of NO minus the $\dot{Q}_a$ of NO

$$\frac{d[NO]}{dt} = (\dot{Q}_p - \dot{Q}_a)/V$$

The $\dot{Q}_a$ is proportional to the [NO] and to an absorption coefficient (A)

$$\dot{Q}_a = A[NO]$$

Furthermore, the [NO] analyzed in small samples of expired air is found to increase during breath holding until the $\dot{Q}_a$
equals the $\dot{Q}_p$. Then, when $t = \infty$, $\dot{Q}_a = \dot{Q}_p$, and an equilibrium concentration $[\text{NO}]^\infty$ would be found.

By substitution

$$d[\text{NO}]_k/dt = (A[\text{NO}]^\infty - A[\text{NO}]_k)/V$$

Clearing for $[\text{NO}]_k$, where $t$ is time,

$$d[\text{NO}]_k/[\text{NO}]^\infty - [\text{NO}]_k) = (A/V)dt$$

Integrating

$$\ln([\text{NO}]^\infty - [\text{NO}]_k) = (A/V)t - \ln([\text{NO}]^\infty - [\text{NO}]_0)$$

or

$$([\text{NO}]^\infty - [\text{NO}]_0)\ln([\text{NO}]^\infty - [\text{NO}]_k) = 1/e^{A/V}$$

where $[\text{NO}]_k$ is the concentration of NO when $t = 0$.

If we know the values for $[\text{NO}]^\infty$ and for $V$, then by determining the values for $[\text{NO}]_k$ at two different times ($t_1$ and $t_2$), we can substitute these to calculate $A$ from the equation

$$A = \ln([\text{NO}]^\infty - [\text{NO}]_2)/([\text{NO}]^\infty - [\text{NO}]_1)\ln(t_2 - t_1)$$

Solution for $\dot{Q}_p$. At equilibrium, $\dot{Q}_a = \dot{Q}_p = A[\text{NO}]^\infty$. Substituting values calculated for $A$ and $[\text{NO}]^\infty$ into this, we solve for $\dot{Q}_p$.

Derivation of equations for $[\text{NO}]^\infty$. During breath holding, $[\text{NO}]$ in the conducting airway increases with time (due to NO production) or decreases (due to absorption). Eventually, the $[\text{NO}]$ reaches equilibrium at an asymptote level $([\text{NO}]^\infty)$, and the approach of $[\text{NO}]$ toward the asymptote follows a curve such that the difference of $[\text{NO}]$ from the equilibrium point is a logarithmic function of the time of breath holding

$$\ln([\text{NO}]^\infty - [\text{NO}]_2)/([\text{NO}]^\infty - [\text{NO}]_1)\ln(t_2 - t_1) = -A/V$$

where $A$ and $V$ are the values of the conducting airway under consideration. We can assign values to $V$ from the anatomy of the tracheobronchial tree (22), but values for $A$ or $[\text{NO}]^\infty$ are unknown.

To find $[\text{NO}]^\infty$, we experimentally obtain values of NO concentration $[\text{NO}]_1$ and $[\text{NO}]_2$ at two different times $t_1$ and $t_2$, respectively, from a breath-holding curve after inhalation of clean air

$$\ln([\text{NO}]^\infty - [\text{NO}]_2)/([\text{NO}]^\infty - [\text{NO}]_1)\ln(t_2 - t_1) = -A/V$$

We also determine another pair of $[\text{NO}]$ values $[\text{NO}]_3$ and $[\text{NO}]_4$ at times $t_1$ and $t_3$, respectively, from a breath-holding curve after inhalation of a NO

$$\ln([\text{NO}]^\infty - [\text{NO}]_3)/([\text{NO}]^\infty - [\text{NO}]_1)\ln(t_3 - t_1) = -A/V$$

After subtraction of this equation from the previous one to eliminate the right-hand term ($-A/V$), the antilogarithms are cross multiplied and solved for $[\text{NO}]^\infty$

$$[\text{NO}]^\infty = \{(\text{NO}]_4[\text{NO}]_4 - [\text{NO}]_2[\text{NO}]_2)/([\text{NO}]_1 + [\text{NO}]_4 - [\text{NO}]_2 - [\text{NO}]_3)\}$$

$$X = e^{(t_2 - t_1) - (t_4 - t_3)}$$

If the breath-holding times in the two curves are made experimentally equal ($t_4 - t_3 = t_2 - t_1$), the exponential term becomes 1. Then $[\text{NO}]^\infty$ is used to find the value of $A$.

Calculations. The process of NO production and absorption in the respiratory tract will lead to an $[\text{NO}]^\infty$ in each zone. This equilibrium is the concentration that will be maintained regardless of the time of breath holding. To calculate this value, we use the equation derived above

$$[\text{NO}]^\infty = ([\text{NO}]_4[\text{NO}]_4 - [\text{NO}]_2[\text{NO}]_2)/$$

$$([\text{NO}]_1 + [\text{NO}]_4 - [\text{NO}]_2 - [\text{NO}]_3)$$

The units of $[\text{NO}]^\infty$ are in parts per billion.

Absorption of NO is occurring in each zone of the respiratory tract studied. This makes it possible, by using the data from inhaling NO at 396 ppb, to calculate the volume of each zone is represented by $V$. The formula is

$$A = V \times \ln([\text{NO}]_1 - [\text{NO}]^\infty)/$$

$$([\text{NO}]_2 - [\text{NO}]^\infty)\ln(t_2 - t_1)$$

or the equation can take this alternate form

$$A = V \times \ln([\text{NO}]_3 - [\text{NO}]^\infty)/([\text{NO}]_4 - [\text{NO}]^\infty)\ln(t_4 - t_3)$$

The volume $V$ is converted to liters, and $t_4 - t_1$ is converted from values measured in seconds ($\sim 10$ s) to fractions of minutes ($\sim 0.17$ min), so $A$ is in liters per minute. The $\dot{Q}_p$ can now be calculated, since the $\dot{Q}_p$ and $\dot{Q}_a$ must be equal at the equilibrated concentration

$$\dot{Q}_p = A \times [\text{NO}]^\infty$$

The units of $\dot{Q}_p$ are in nanoliters per minute, because the units of $A$ are in liters per minute and the units of $[\text{NO}]^\infty$ are in parts per billion.

Sample calculation. $[\text{NO}]^\infty$, $A$, and $\dot{Q}_p$ were calculated on the bronchial gas obtained from subject PK by reading the set of curves at 2 and 15 s of breath holding of clean air and after inhalation of 396 ppb of NO in air as follows

$$[\text{NO}]_1 = 17.5, [\text{NO}]_2 = 45, [\text{NO}]_3 = 272, [\text{NO}]_4 = 148 \text{ ppb}$$

where $V = 31$ ml (converted to 0.031 liters) and time change ($\Delta t$) = 13 s, expressed as 0.22 min, or 4.6 reciprocal min.

Substituting to find $[\text{NO}]^\infty$

$$[\text{NO}]^\infty = (17.5 \text{ ppb} \times 148 \text{ ppb} - 45 \text{ ppb} \times 272 \text{ ppb})$$

$$/17.5 \text{ ppb} + 148 \text{ ppb} - 45 \text{ ppb} - 272 \text{ ppb}) = 63.7 \text{ ppb}$$

Substituting to find the value of $A$

$$A = 0.031 \times 4.6 \times \ln(272 - 63.7)/(148 - 63.7) = 0.129 \text{ l/min}$$

Substituting to find the $\dot{Q}_p$

$$\dot{Q}_p = 0.129 \times 63.7 = 8.22 \text{ nl/min}$$

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Address for reprint requests: A. B. DuBois, c/o John B. Pierce Laboratory, 290 Congress Ave., New Haven, CT 06519 (E-mail: adubois@jbpierce.org).

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