Role of nitrogen in transmucosal gas exchange rate in the rat middle ear

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Submitted 30 January 2006; accepted in final form 29 June 2006

Kania, Romain E., Philippe Herman, Patrice Tran Ba Huy, and Amos Ar. Role of nitrogen in transmucosal gas exchange rate in the rat middle ear. J Appl Physiol 101: 1281–1287, 2006.—This study investigates the role of nitrogen (N2) in transmucosal gas exchange of the middle ear (ME). We used an experimental rat model to measure gas volume variations in the ME cavity at constant pressure. We disturbed the steady-state gas composition with either air or N2 to measure resulting changes in volume at ambient pressure. Changes in gas volume over time could be characterized by three phases: a primary transient increase with time (phase I), followed by a linear decrease (phase II), and then a gradual decrease (phase III). The mean slope of phase II was −0.128 μl/min (SD 0.023) in the air group (n = 10) and −0.105 μl/min (SD 0.032) in the N2 group (n = 10), but the difference was not significant (P = 0.13), which suggests that the rate of gas loss can be attributed mainly to the same steady-state partial pressure gradient of N2 reached in this phase. Furthermore, a mathematical model was developed analyzing the transmucosal N2 exchange in phase II. The model takes gas diffusion into account, predicting that, in the absence of change in mucosal blood flow rate, gas volume in the ME should show a linear decrease with time after steady-state conditions and gas composition are established. In accordance with the experimental results, the mathematical model also suggested that transmucosal gas absorption of the rat ME during steady-state conditions is governed mainly by diffusive N2 exchange between the ME gas and its mucosal blood circulation.

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with a mathematical modeling. Experimentally, the steady-state gas composition of the ME was disturbed with air or N₂, with subsequent determination of the volume of the enclosed gas at constant pressure. Second, an additional mathematical model was developed, based on physical diffusion properties of N₂ and some experimental data (mucosa thickness and bulla volume). The rationale of this mathematical modeling was to test whether its results could match the data of the experiments to support the predominant role of N₂ in ME gas absorption during steady-state conditions.

**MATERIALS AND METHODS**

**Experimental Design**

The gas volume variations, bulla volume, and mucosa thickness were compared between the ME of male rats, filled with pure N₂ (N₂ group; n = 10) or room air (air group; n = 10).

**Animals.** Twenty male rats (Sprague-Dawley; 100–250 g; 1–2 mo old) were obtained from Elevage Janvier (France) and kept according to the European guidelines for care and use of laboratory animals (Journal Officiel des Communautés Européennes, 24 VIII 1999, L222/29–37). Water and food (A04, UAR; Epinay sur Orge, France) were given freely. The study was performed in accordance with the regulations of the Institutional Animal Care and Use Committee (A75–10–01), and the protocol was approved by the Animal Care and Research Committee.

**Experimental procedures.** Basically, the same procedures employed by Ar et al. (3) were used. Rats were anesthetized by intraperitoneal injection of 60 mg/kg ketamine hydrochloride (Ketalar; Panpharma) and 6.5 mg/kg xylazine hydrochloride (Rompun 2%; Bayer). Anesthesia was maintained by an infusion pump (SE200; VIAL Medical, La Forteresse) with a mixture of ketamine hydrochloride (10 mg/ml) and xylazine hydrochloride (0.4 mg/ml) in Ringer lactate at a constant rate of 2 ml/kg h⁻¹. A temperature-regulated electric cushion kept the body temperature at 36.2°C (SD 0.32) measured with an intrarectal thermistor thermometer with temperature control feedback (Homeothermic Blanket Control Unit, Phymed, Paris, France).

Rats maintained under general anesthesia were microscopically examined for absence of perforation of the TM and for otitis media to exclude the possibility of any TM and ME pathology. The external auditory canal (EC) was cleaned with polyvidone (Betadine 10%; Merignac), and the TM was punctured. A transparent glass capillary (inner diameter 700 μm; outer diameter 2 mm) was bent 4 cm from the proximal end to make a 135° angle and connected hermetically with cyanocrilacle glue (Superglue3; Henkel, Boulogne-Billancourt, France) to the EC.

Rats were placed ventral side down, covered with a blanket, with heads exposed to allow spontaneous ventilation under general anesthesia and eyes covered with moist gauze to prevent drying. The glass capillary was then placed horizontally on a ruler with millimeter resolution (Fig. 1). Horizontality was checked using a level. A 1-mm displacement of the droplet movement in the glass capillary corresponded to a 0.385-μl ΔV. Readings were recorded at 5-min intervals and were accurate to 0.25 mm; that is, the accuracy of measurement reached ~0.1 μl. Hence, the cumulative changes in ME gas volume could be determined and plotted graphically against time.

**Disturbance of the steady-state gas composition of the ME and subsequent changes in ME gas volume.** ΔV were compared after a 5-ml syringe filled with pure N₂ (N₂ group; n = 10) or room air (air group; n = 10) was fitted to the far end of the horizontal capillary for flushing the ME. The gas was slowly introduced into the ME at a rate of ~100 μl/s for 20 s. The superfusate gas administered presumably left through the ET, which is known to open under hydrostatic pressure (5, 21–23). After the system was flushed, the syringe was unplugged to relieve any remaining pressure differences. A droplet of colored water, containing antifoam detergent to reduce friction and surface tension, was quickly introduced into the glass capillary internal lumen ~5 cm away from the EC to seal the system (30). Hence, any ΔV over time could be followed, and the rate of ΔV defined by the slope of the curve ΔV/Δt was determined. The symbol ΔV/Δt was defined as the slope of the curve for ME gas ΔV (at 37°C and 763 mmHg, which is the average barometric pressure in Paris) per unit time (Δt). As previously reported, this system can be used to measure transmucosal gas exchange values in the ME (3, 30).

**Bulla volume measurements.** At the end of the experiments, the glass capillary and perfusion tube were removed. A dissecting microscope was used to read the measurement of the volume of the ME (V) to the level of the punctured TM. This was done by filling the ME with saline containing Coomassie Brilliant Blue R-250 (5%; Sigma) and antifoam detergent using a microburette. Care was taken to eliminate any gas bubbles in the ME cavity. Assuming that the bulla of the rat can be considered as a sphere with an effective area A and a volume V, A relates to V as follows: \( A = \sqrt{36 \cdot \pi \cdot V^3} \).

**Thickness measurements.** ME bullae were harvested immediately after the measurements. Specimens were fixed in 10% phosphate-buffered formalin, decalcified with 10% EDTA buffer, embedded in paraffin wax, cut in 5-μm-thick sections, and stained with hematoxylin-eosin. Sagittal sections were taken in the center of the bulla. The thickness of the mucosa (L) was measured under a Leica DMLB microscope with a Leica DC 200 digital camera and analyzed by an image analysis system (Leica Microsystems Imaging Solutions, Cambridge, UK). The distance between the apical surface of the mucosa and the bony side was measured and defined as L. Five sections from each ear and at least five fields of each section were analyzed. Images were stored as digitized images. The L of the mucosa was measured from the digitized images displayed on the computer screen.

**Statistical analysis.** Statview (SAS Institute) was used to store and calculate data. Results were expressed as means (SD). Distributions around the means were tested for normality. When positive net gas ΔV were observed, an index was used to estimate volume differences between control and experimental groups, which was the product of the total positive ΔV (+ΔV) and the time it took to reach this value.

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**Fig. 1.** Schematic representation of the setup for measuring changes in middle ear (ME) volume at constant pressure. ET, eustachian tube.
For the rates of negative $\Delta V$ ($\sim \Delta V$), linear and exponential regressions and Pearson’s correlation coefficients of the function relating the ME $\Delta V$ to time were calculated. The nonparametric Mann-Whitney $U$-test was used to compare the slopes of change in ME volume with time, the bulla volume, and the thickness of the mucosa between the air and $N_2$ groups. Statistical significance was set at $P = 0.05$.

Mathematical Modeling

According to Fick’s first law of diffusion, the rate of gas diffusion in a steady state in a given direction through a barrier is directly proportional to the partial pressure difference of that gas between the two sides of the diffusion barrier, the gas solubility ($\alpha$), the diffusion coefficient ($D$) of the gas in the diffusion barrier, and the surface area available for diffusion ($A$) and is inversely proportional to the effective thickness of the barrier ($L$). Previously, it has been shown that, for a healthy ME at low-gas loss rates similar to those measured in the present study (see RESULTS below), most of the resistance to the gas loss rate in phase II is diffusive (3). Thus, for a constant mucosal blood flow rate, the following equation may be used to describe the steady-state gas volume loss with time from the ME:

$$-\frac{\Delta V_m}{\Delta t}F_{N_2} = \frac{A}{L} \alpha_{N_2}D_{N_2}(P_{meN_2} - P_{baN_2}) \tag{1}$$

where $-\Delta V_m/\Delta t$ is the rate of volume loss from the ME in phase II, and $F_{N_2}$ is the fraction of $N_2$ in the ME gas; thus $-\Delta V_m/\Delta t - F_{N_2}$ is the rate of $N_2$ loss from the ME. $P_{meN_2}$ and $P_{baN_2}$ are the partial pressures of $N_2$ in the ME gas and the arterial blood entering the ME circulation, respectively, and $(P_{meN_2} - P_{baN_2})$ is the partial pressure difference of $N_2$. The $\alpha_{N_2}$ and $D_{N_2}$ in this case are the solubility and diffusion coefficients, respectively, of $N_2$ in the mucosa (at body temperature). The term $(A/L\alpha_{N_2}D_{N_2})$ is the $N_2$ diffusive conductance of the ME mucosa. It represents the physical $(\alpha_{N_2}, D_{N_2})$ and morphological $(A, L)$ parameters involved in the rate of $N_2$ loss.

All of the parameters on the right side of Eq. 1 can be either measured or calculated. The values for $\alpha_{N_2}, D_{N_2}$, and $F_{N_2}$ were taken from Fink et al. (17). The value for $P_{meN_2}$ was calculated by use of a normal ME pressure of 760 Torr and $H_2O$ pressure of 47 Torr. Values for $P_{meO_2}$ and $P_{meCO_2}$ were assumed to be equal to $P_{O_2}$ and $P_{CO_2}$ values measured in subcutaneous gas pockets of Sprague-Dawley rats (2), which supposedly represent any kind of non- or poorly ventilated gas pocket, including the ME cavity (32).

Similarly, the value for $P_{baN_2}$ was calculated from arterial blood gas values of the same strain of rats given in the literature (38, 43).

The assumption in Eq. 1 that mucosal thickness $L$ may represent the gas diffusion barrier was discussed in depth in Ar et al. (3). To validate these assumptions and since all other parameters in Eq. 1 are known, we calculated the expected $-\Delta V_m/\Delta t$ using our measured $L$ and compared it with the experimentally obtained value. Similarly, from Eq. 1, we can estimate the effective diffusion barrier $L$ from the measured $-\Delta V_m/\Delta t$. The consistency between expected values using the mathematical model and experimental data was the hypothesis to test, which would suggest, if verified, a predominant role of $N_2$ in transmucosal gas exchange during steady-state conditions.

RESULTS

ME Gas Volume Variations with Time ($\pm \Delta V_m/\Delta t$)

In accordance with previous studies, three distinct phases (I to III) could be identified (29, 30). As depicted in Fig. 2, the mean $\Delta V$ with time showed an initial ME gas volume increase (phase I), followed by a linear decrease (phase II), which was gradually reduced with time (phase III).

Phase I. Phase I was less prominent in the air than in the $N_2$ group. The net volume increase above zero $\Delta V$ observed in phase I can be used to characterize this phase. Table 1 summarizes the differences in volume increase between the air and $N_2$ groups.

Phase II. Phase II showed a significant linear decrease in ME gas volume with time in all animals of both the air and $N_2$ groups. The $r^2$ values for the individual animals of the air group ranged from 0.945 to 0.997 ($n = 10$) [mean 0.981 (SD 0.018)] and those of the $N_2$ group from 0.950 to 0.999 ($n = 10$) [mean 0.983 (SD 0.016)].

The mean slope for the regression equations for the air group was $-0.128 \mu l/min$ (SD 0.023) ($n = 10$), and for the $N_2$ group it was $-0.105 \mu l/min$ (SD 0.032) ($n = 10$). The groups did not differ significantly in slope values ($P = 0.13$), and the overall mean slope was $-0.117 \mu l/min$ (SD 0.030) ($n = 20$).

Figure 2 shows the mean $\Delta V$ in relation to time. The slopes were calculated from the means of each measuring time point for the air and $N_2$ groups. Because of the nonnormal distribution of the individual slopes in the air group, the mean slope values for the mean time points were somewhat different: $-0.108$ and $-0.107 \mu l/min$ for the air and $N_2$ groups, respectively.

Phase III. At $\sim 35$ min after the initial ME washout, the rate of gas loss started to gradually decrease (Fig. 2, phase III). The mean decrease in gas loss with time could be described for the air group (Eq. 2) and the $N_2$ group (Eq. 3) as follows:

$$\Delta V_{\Delta t} = -4.42 + 16.63e^{-0.071\Delta t} \tag{2}$$

$$\Delta V_{\Delta t} = -2.41 + 10.31e^{-0.042\Delta t} \tag{3}$$

where $\Delta V_{\Delta t}$ is the total $\Delta V$ from time zero up to any given time, and $\Delta t$ is the time lapse from time zero.

The rates of $\Delta V$ between 35 and 60 min were not significantly different between the air and $N_2$ groups.

Bulla Volume Measurements

The overall average volume of the ME gas space was 42.0 $\mu l$ (SD 3.4) ($n = 20$), with no significant difference between the air and $N_2$ groups ($P = 0.21$).

Thickness Measurements

The mean thickness of the mucosa and submucosa up to the underlying bone was 22.8 $\mu m$ (SD 10.3) ($n = 20$), with no...
Significant difference between the air and N₂ groups (P = 0.60).

**Mathematical Modeling**

Consistency between expected value and experimental data was verified. The expected -ΔV/Δt was -0.119 μl/min adjusted to the experimental conditions of temperature, barometric pressure, and tissue shrinkage during fixation (20%). This value was not significantly different from the experimental value of -0.117 μl/min (SD 0.030) (P = 0.99). Similarly, the calculated value of L was 31.6 μm (SD 10.3) and did not differ significantly from the measured L value (P = 0.14).

**DISCUSSION**

The present study documents quantitatively transmucosal ME gas exchange in an anesthetized rat model and suggests that N₂ diffusion is predominantly responsible for the observed rate of gas loss.

Quantification of ME gas exchange from pressure measurements is difficult because it requires the knowledge of the ME volume and “dead” volumes involved. Our method quantitatively evaluates gas ΔV at a constant (ambient) pressure and bypasses possible errors introduced by tubing materials, length of tubing, and pressure difference measurements (28, 30). It is independent of both the ME cavity gas volume and the measuring system volume; it estimates quantitatively the amount of gas that leaves or enters the ME, and it avoids variations in ME volume due to TM displacements and ET openings.

We aimed to disturb the steady-state gas composition of the ME by flushing it with ambient air or N₂ and then observed how this steady-state situation is reestablished. During the experiments, in accordance with previous observations (29, 30), we found that the general course of ME ΔV could be divided into three phases (Fig. 2) discussed below.

**Phase I**

When the ME is flushed with air, its cavity has initially almost no CO₂ and is rich in O₂. As can be seen from the partial pressure differences presented in Table 2, O₂ is driven from the ME cavity to the surrounding tissues and blood, and, conversely, CO₂ moves into it from tissues and blood (34). The initial gradients of partial pressures under which gases are exchanged are between the arterial blood and the ME cavity. Compared with the other gases involved, N₂, which has low solubility in tissue and blood and initially no partial pressure gradient between arterial blood and ME gas, must have a low net gas exchange rate. The initial partial pressure of O₂ in the ME is higher than that of the arterial blood (~60 Torr difference, Table 2). However, the product of gas solubility times diffusivity for O₂ is 1.74 times that of N₂. Binding to hemoglobin should not occur at this stage, since the incoming arterial blood is almost completely hemoglobin saturated. Considering the above ratio and the initial partial pressure differences given in Table 2, the initial rate at which O₂ diffuses into the blood is calculated to be 4.3 times the rate at which N₂ diffuses into the ME. For CO₂, the solubility times diffusivity product is ~35 times higher than that of N₂ (7, 8, 11, 14, 15, 17). Taking into account the initial gradient, CO₂ is initially transported into the ME cavity at ~480 times the N₂ rate. Analysis of the contribution of all three gases to the total initial rate of ΔV shows that O₂ accounts for less than 1% and N₂ for a little more than 0.2%. The rest of the change is due to CO₂ diffusion into the ME.

With similar considerations, the respective roles of O₂ and N₂ when pure N₂ was initially flushed are minute, and most of the change is due to CO₂ diffusion. In our experiments, we could not measure the true initial gas exchange at time zero because of the delay in sealing the system.

However, Fig. 2 shows that, over the entire duration of phase I, the net amount of gas entering the ME was larger in the N₂ than in the air group (Table 1). This observation can be explained by the difference in initial partial pressures of O₂ and

<p>| Table 1. Parameters used to estimate the properties of phase I for air- and N₂-flushed middle ears |</p>
<table>
<thead>
<tr>
<th>Conditions</th>
<th>Maximal ΔV, μl</th>
<th>Peak Time, min</th>
<th>Volume (index)</th>
<th>n</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>0.166 (0.171)</td>
<td>5</td>
<td>0.82</td>
<td>10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N₂</td>
<td>2.405 (1.102)</td>
<td>10</td>
<td>24.05</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Ratio(N₂/air)</td>
<td>14.75</td>
<td>2</td>
<td>29.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Values are means (SD); n, no. of animals. ΔV, volume change. The index used to estimate volume increase differences was the product of the maximal increase in ΔV and the time it took to reach this value. *For maximal ΔV.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Table 2. Gas composition in blood and in each phase and for air- and N₂-flushed middle ears |

<table>
<thead>
<tr>
<th>Group</th>
<th>Gas Partial Pressure Of/in</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Arterial*</td>
</tr>
<tr>
<td>Air</td>
<td>O₂</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>CO₂</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>N₂ (+ Ar)</td>
<td>588</td>
</tr>
<tr>
<td></td>
<td>H₂O</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>760</td>
</tr>
<tr>
<td>N₂</td>
<td>O₂</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>CO₂</td>
<td>36</td>
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<tr>
<td></td>
<td>H₂O</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>760</td>
</tr>
</tbody>
</table>

ME Gas Partial Pressures in Different Phases and Their Differences From Blood

<table>
<thead>
<tr>
<th>Phase I</th>
<th>Phases II and III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>Initial difference</td>
</tr>
<tr>
<td>ME-a</td>
<td>ME-v</td>
</tr>
<tr>
<td>Air O₂</td>
<td>149</td>
</tr>
<tr>
<td>N₂ O₂</td>
<td>0</td>
</tr>
<tr>
<td>N₂ O₂</td>
<td>564</td>
</tr>
<tr>
<td>N₂ O₂</td>
<td>47</td>
</tr>
<tr>
<td>N₂ O₂</td>
<td>760</td>
</tr>
<tr>
<td>N₂ O₂</td>
<td>760</td>
</tr>
<tr>
<td>N₂ O₂</td>
<td>760</td>
</tr>
</tbody>
</table>

Gas partial pressures are expressed in Torr. ME, middle ear; a, arterial; v, venous. *Taken from Refs. 38 and 43. †Taken from gas pocket values (Refs. 4, 18, 20, 37).
N₂ (Table 2) in addition to the entry of CO₂. Taking these pressures into account and the physical properties of diffusion and solubility, O₂, in addition to CO₂, would enter the ME at 1.24 times the rate of N₂ leaving it. Hence the net increase in gas volume with time in the ME is expected to be higher and last longer with an N₂-flushed ME than with an air-flushed ME (Fig. 2, Table 1). The initial conditions change continuously with time as gases are exchanged, toward a reestablishment of the steady-state gas composition in phase II (17, 33, 40–42).

**Phase II**

As can be seen in Table 2, the ME-to-blood partial pressure differences for CO₂ and O₂ disappear at phase II, whereas for N₂, a steady-state pressure difference is established. As a result, the increase in ME gas volume diminishes with time (Fig. 2). The PmₑN₂ stays higher than that of the venous blood, because of the equilibration of CO₂ and O₂ with the blood on one hand and the total ME pressure maintained at atmospheric values on the other. The total pressure equilibration is due to either equilibration through the ET in an awake animal (24–26, 36, 41) or equilibration with the moving droplet in our model (see MATERIALS AND METHODS). It should be noted that, unlike the partial pressures of CO₂ and O₂, N₂ partial pressure is approximately the same in both venous and arterial blood, because the equilibration of N₂ with the blood occurs in the lungs and because N₂ is neither consumed nor produced in the body. The difference in maintained N₂ partial pressure between the ME and the blood perfusing it leads to a continuous loss of N₂ from the ME cavity into the blood. N₂ is the rate-limiting factor of ME gas loss because it diffuses slower than CO₂ and O₂, which equilibrate relatively quickly with the venous blood. This relatively slow rate of N₂ loss dictates the overall rate of gas loss from the ME and makes it essentially dependent, for a given mucosal blood flow, on the rate of N₂ diffusion between the ME and the blood.

The present observation agrees with that of previous studies based mainly on pressure measurements, showing that changes in ME pressure are driven by the difference in N₂ partial pressure between the ME and blood (1, 11, 13, 14, 33, 41, 42).

The r² values of the individual linear regression lines of ΔV (−ΔVₘ) with time (Δt) during phase II fit a linear model. A consistency between the expected values provided by the mathematical model and the experimental data for the calculation of −ΔVₘ/Δt and effective diffusion barrier L was found. Thus the assumption that the measured L represents the diffusion barrier between the ME gas and mucosal blood may be accepted. Similarly, the estimation of L from the measured −ΔVₘ/Δt was in accordance with experimental data. Hence the experimental results fit the mathematical model for phase II, which suggest a predominant role of N₂ diffusion as a limiting factor in the rate of gas loss we observed. Other experimental studies and mathematical models based on ME pressure changes attribute to the ME to blood partial pressure difference of N₂ the role of dominating the ME gas economy (3, 9, 10, 17, 35, 39).

Since blood flow is expected to take part in the clearance of gas from the ME, we estimated the effective blood flow rate of the ME mucosa in steady state conditions using Eq. 4:

\[
\frac{\Delta Q_m}{\Delta t} = \left( - \frac{\Delta V}{\Delta t} \cdot F_{N_2} \right) \cdot \left[ \alpha_{N_2} \cdot (P_{N_2} - P_{N_2}) \right]^{-1}
\]

where ΔQₘ/Δt is the rate of effective blood flow in the ME mucosa per unit of time and PₑN₂ is the partial pressure of N₂ in the venous blood. This value is about the same as PmₑN₂, since, in steady-state conditions, it is assumed that the blood leaving the ME circulation equilibrates with the gas composition of the ME (Table 2). The equation describes the increase in the amount of N₂ per unit time in the blood between entering and leaving ME circulation, taking into account its solubility and the increase in its partial pressure. From Eq. 4, the calculated effective blood flow in the ME is ΔQₘ/Δt = 310 μl/min. Since in awake animals and in steady state the rate of ME ventilation is the same as the rate of gas absorption, it was possible from the rates of measured gas loss and estimated blood flow to estimate a ratio of ME perfusion to ventilation ΔVₘ/ΔQₘ in an awake animal of ~2.650. These values are about an order of magnitude higher for blood flow and lower for ΔVₘ/ΔQₘ than the ones calculated by Ar et al. (3) on the basis of both gas diffusion and blood perfusion limitations. However, both estimates, which should be verified experimentally, show that, in steady-state conditions, the ME is a relatively poorly ventilated and highly perfused organ.

**Phase III**

The linearity of Phase II can be observed as long as all of the parameters of Eq. 1 are not changed (Fig. 2). At ~35 min after the initial ME washout, the rate of gas loss gradually decreases (Fig. 2, phase III). Thus it is assumed that one or more of the parameters in Eq. 1 changes gradually. The mechanisms involved are not yet known. One possible explanation may be related to the effects of prolonged anesthesia on the cardiopulmonary system and ME blood flow.

However, we speculate that the gradual decrease in gas loss is related to a gradual increase in effective mucosal thickness due to effusion and thickening of the mucous layer above the mucosa, which cannot be cleared with a closed ET. For this latter hypothesis, we obtained a calculated change in thickness value, using the exponential equations, which were fitted to the data of −ΔVₘ/Δt in phase III (Fig. 2; Eqs. 2 and 3). Then using Eq. 1, we obtained new thickness values of the mucosa + effusion for each time point. The calculated values of mucosa + effusion correspond to an increase in mucosa thickness of ~2.9 μm within the first hour of the experiment, which (from the calculated ME cavity surface area) is equivalent to a total amount of effusion of ~0.17 μl in the same hour.

**Limitations**

1) Since the model is sensitive to thickness variations, small variations may explain the variations in the ΔVₘ values around the means (Fig. 2). In addition, the model assumes that L corresponds to the diffusive barrier between the ME gas space and the mucosal circulating blood. This assumption was discussed above.

2) The data obtained may be different in awake animals.

3) Our model assumes that ME and blood partial pressures of the gases are similar to those measured in other studies, both directly and indirectly. However, because of technical limitations, we were not able to directly measure these partial pressures in the ME.
4. Our simplified model assumes constant blood flow and blood gas partial pressures in the ME.

5. The data used for diffusion and solubility coefficients were taken from the literature and may be somewhat different in the ME of the rat.

6. The fraction of N\textsubscript{2} in phase II in the ME is assumed to be the same as in subcutaneous gas pockets.

7. The calculated area must be considered only as an estimate of the actual area available for diffusion.

In conclusion, we have developed quantitative experimental and mathematical three-phase models for transmucosal gas exchange in the ME of the anesthetized rat. These models provide evidence for gas loss through the mucosa of the ME. Phase I demonstrated that the rate of establishment of the steady state in the ME depends on gas composition. However, during the steady-state gas loss phase (II), the gradient of partial pressure difference in N\textsubscript{2} between the ME and blood is responsible for the rate of \( \Delta V \) and is independent of the initial type of steady-state disturbance. The experimental setup and mathematical model may be useful in further research in the field of ME gas economy in pathological conditions, including the role of the perfusion and secretion of the mucosa.

ACKNOWLEDGMENTS

We are in debt to Ann Belinsky for reading and commenting on the manuscript, Margriet Huisman for help in image analysis, and Dr. Eric Lecain, who was very helpful during the experiments.

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