Measurement of \( \dot{V}O_2 \), \( \dot{V}CO_2 \), and evaporative water loss with a flow-through mask

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Withers, Philip C. Measurement of \( \dot{V}O_2 \), \( \dot{V}CO_2 \), and evaporative water loss with a flow-through mask. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 42(1): 120-123, 1977. Equations for the calculation of \( \dot{V}O_2 \), \( \dot{V}CO_2 \), and evaporative water loss were derived for use of a paramagnetic gas analyzer with a flow-through respirometry system and an open or closed mask. The magnitude of errors involved in the determination of \( \dot{V}O_2 \) with no \( CO_2 \) absorbent are small (\( \pm 3\% \)) but may be greater if no \( H_2O \) absorbent is used, unless an appropriate correction is made for evaporative water loss. Data collected for hummingbirds (Selasphorus sasin) and monitor lizards (Varanus gouldii) indicate the validity of the techniques described for the measurement of \( \dot{V}Q \) and evaporative water loss and demonstrate the use of paramagnetic gas analyzers in monitoring respiratory patterns.

flow-through respirometry, RQ

PARAMAGNETIC GAS ANALYZERS have traditionally been used to measure rates of oxygen consumption (\( \dot{V}O_2 \)) but considerable additional information can be obtained by slight modification of the respirometry system. The rate of carbon dioxide production (\( \dot{V}CO_2 \)), RQ, and evaporate water loss (EWL) can be determined rapidly and accurately by sequential removal of the \( CO_2 \) and \( H_2O \) absorbents. Rapid changes in \( \dot{V}O_2 \) and breathing patterns can be observed by minimizing the volume of the respirometry chamber and the downstream dead space.

Masks with flow-through respirometry systems have been used for the measurement of \( \dot{V}O_2 \) of active animals (9-11). Flow-through respirometry systems can be adapted for use with a mask by sealing the mask to the head of the animal (closed mask; Fig 1A). A second design for a mask involves a suction pump downstream of the mask which draws air past the face of the animal and into the mask (open mask; Fig 1B). The present study describes open-flow respirometry systems for use with open or closed masks and presents a rapid and accurate technique for the determination of \( \dot{V}O_2 \), \( \dot{V}CO_2 \) and evaporative water loss.

TERMINOLOGY

The symbols to be used follow those previously established (4, 5) and for convenience are assumed throughout the text to be converted to standard temperature (\( 0°C \)) and pressure (760 Torr) rather than ambient temperature and pressure (atpt). \( V \) represents a flow rate (STP) and \( F \) a fractional concentration. Then

\[
\begin{align*}
\dot{V}O_2 &= \text{rate of } O_2 \text{ consumption (STPD)} \\
\dot{V}CO_2 &= \text{rate of } CO_2 \text{ production (STPD)} \\
V_I &= \text{rate of airflow into the mask (STPD)} \\
V_E &= \text{rate of airflow out of the mask (STPD)} \\
V_{in} &= \text{rate of airflow into the mask (STP)} \\
V_{out} &= \text{rate of airflow out of the mask (STP)} \\
F_{\dot{V}O_2} &= \text{fractional concentration of } O_2 \text{ entering the mask} \\
F_{\dot{V}O_2} &= \text{fractional concentration of } O_2 \text{ leaving the mask}
\end{align*}
\]

The flow rate (STP) due to ambient water vapor and evaporative water loss of the animal are designated \( V_A \) and \( V_{EWL} \), respectively. If \( CO_2 \) has been removed from the air, the appropriate parameters are denoted with a prime notation, e.g., \( F'_{\dot{V}O_2} \), \( V'E \).

ERROR TOLERANCES

The calculation of \( \dot{V}O_2 \) using flow-through respirometry can involve large errors since \( F_{\dot{V}O_2} = F_{\dot{V}O_2} \). Normally, \( F_{\dot{V}O_2} = 0.20946 \) (8) and 0.2000 < \( F_{\dot{V}O_2} \) since lower \( F_{\dot{V}O_2} \) would subject the experimental animal to low levels of \( O_2 \). Note that the actual values of \( F_{\dot{V}O_2} \) (e.g., 0.2093, 0.20946) is unimportant because the span is set relative to \( F_{\dot{V}O_2} \) so that the absolute value of \( F_{\dot{V}O_2} - F_{\dot{V}O_2} \) is independent of \( F_{\dot{V}O_2} \).

Two types of error, termed proportional and nonproportional, may be involved in the calculation of \( \dot{V}O_2 \). A constant fractional error of 0.01 in \( V \) or \( F \) will result in an error of 0.01 in \( \dot{V}O_2 \). A nonproportional error in \( V \) or \( F \) (i.e., no error in inlet value but error in outlet, or vice versa) will result in a larger error in \( \dot{V}O_2 \). For example, if \( V_I = 100 \pm 1 \), \( V_E = 100 \), \( F_{\dot{V}O_2} = 0.20946 \), and \( F_{\dot{V}O_2} = 0.2000 \), then \( \dot{V}O_2 = 0.946 \pm 0.209 \) (fractional error = 0.22). This type of nonproportional error is encountered with flow-through respirometry since only one of \( V_I \) and \( V_E \), and \( F_{\dot{V}O_2} \) and \( F_{\dot{V}O_2} \), is measured at a given time.

EQUIVALENCE OF OPEN AND CLOSED MASK SYSTEMS

Despite the fact that open mask systems utilize a downstream suction pump whereas closed masks normally have an upstream positive-pressure pump, the derivations of equations for each mask system are identical. The rate of flow of air from an open mask is constant (as determined by the downstream pump) and RQ and EWL alter the inlet flow rate. The flow rate into a closed mask is constant, and RQ and EWL alter the outlet flow rate.
LOW-THROUGH RESPIROMETRY

\[ V_{EWL} = \frac{P_d \cdot \dot{V}_L}{760 - P_d} = \frac{P_d \cdot \dot{V}_O_2}{(760 - P_d) \cdot E} \]  

(2b)

If H\(_2\)O vapor is not removed from the air ahead of the paramagnetic gas analyzer, the fractional concentration of O\(_2\) will be altered by the added volume of water and by the magnetic susceptibility of water. The paramagnetic effect of H\(_2\)O relative to that of O\(_2\) can be calculated from the magnetic susceptibility of O\(_2\) (taken as +1.0) and the susceptibility of ~0.0038 for H\(_2\)O (1), and their fractional concentrations, Fe\(_{O_2}\) and Fe\(_{H2O}\). Figure 2 depicts the relationship between Fe, the fractional concentration of O\(_2\) read from the analyzer, and Fe\(_{H2O}\), the actual fractional concentration of O\(_2\), for different water vapor pressures. The error involved is nonproportional since V\(_{EWL}\) alters Fe\(_{O_2}\) and not Fe\(_{H2O}\), but this error is insignificant compared to the precision of the paramagnetic gas analyzer if the water vapor pressure is less than 20 Torr, corresponding to about \( \beta = 2 \text{ mg H}_2\text{O/ml O}_2\) consumed.

The effect of CO\(_2\) on the analyzer can be calculated from its relative susceptibility of ~0.0063 (1) and its fractional concentration. Figure 2 shows the relationship between Fe and Fe\(_{H2O}\) for different \( \alpha \) and RQ values. The nonproportional error due to CO\(_2\) is negligible under normal operating conditions of \( \alpha < 0.01 \).

The error involved with the calculation of V\(_O_2\) due to ambient water vapor and the evaporate water loss of the animal is an overestimation equal to (VA + V\(_{EWL}\)) / V\(_{out}\). The error in V\(_O_2\) if the air leaving the mask were saturated at 37°C (water vapor pressure = 47 Torr) would be about 0.062, or 6.2%.

H\(_2\)O ABSORBENT, NO CO\(_2\) ABSORBENT

Water vapor must be absorbed before both the flowmeter and the paramagnetic gas analyzer for the following analyses to apply. V\(_O_2\) can be determined independently of VA and V\(_{EWL}\) from Eq. 1d since VA = V\(_{EWL}\) = 0 under these conditions.

H\(_2\)O ABSORBENT, NO CO\(_2\) ABSORBENT

\[ P \cdot V = n \cdot R \cdot T \]
\[ \therefore \frac{n}{V} (\text{mol/ml}) = \frac{P}{R} \cdot \frac{1}{T} \]
\[ \therefore \frac{n}{V} (\text{ml H}_2\text{O/ml air}) = 0.359 \cdot \frac{P}{T} \]  

(2a)

where P = water vapor pressure (atm) and T = ambient temperature (°C). Hence, VA = 0.359 P / T.

EWL can be determined directly by freezing and collecting H\(_2\)O from the outlet air or from the weight gain of the H\(_2\)O absorbent, or calculated by measuring the water vapor pressure of the inlet and outlet air. If the ratio of ml O\(_2\) consumed/ml air leaving the mask = \( \alpha \) and the EWL = \( \beta \) mg H\(_2\)O/ml O\(_2\) consumed, then V\(_{EWL}\) = 1.24 \( \alpha \beta \dot{V}_{out} \) (ml H\(_2\)O/min). Alternatively, and if only pulmonary water loss is collected into the outlet air, V\(_{EWL}\) can be calculated from the O\(_2\) extraction of the animal (E) and the water vapor deficit (stpd) between inspired and expired air (Pd). The rate of airflow through the lungs (stpd) = \( \dot{V}_L = \dot{V}_{O_2} / E \). Then, ml H\(_2\)O/ml air breathed = Pd/(760-Pd), and
\[
\dot{V}_O_2 = \frac{\dot{V}_E \cdot (F_{I_O_2} - F_{E_O_2})}{1 - (1 - RQ) \cdot F_{I_O_2}} \quad (3a)
\]

If \(V_{CO_2}\) is independently determined, Eq. 3a can be rearranged as
\[
\dot{V}_O_2 = \frac{\dot{V}_E \cdot (F_{I_O_2} - F_{E_O_2}) - \dot{V}_{CO_2} \cdot F_{I_O_2}}{1 - F_{I_O_2}} \quad (3b)
\]

The fractional error in \(\dot{V}_O_2\) is \(\pm 3\%\) if RQ is assumed to be 0.85 but actually is 1.0 or 0.7.

**H\(_2\)O and CO\(_2\) Absorbers**

If both H\(_2\)O and CO\(_2\) absorbents are used before the flowmeter and analyzer, the \(\dot{V}_O_2\) is calculated from Eq. 1d by substituting \(V_A = \dot{V}_{EWL} = RQ = 0\)
\[
\dot{V}_O_2 = \frac{\dot{V}'E \cdot (F_{I_O_2} - F'_{E_O_2})}{1 - F_{I_O_2}} \quad (4a)
\]

Equation 4a is that of Depocas and Hart (4) and Hill (5) for an open flow respirometry system with a downstream flowmeter.

If the CO\(_2\) absorbent is placed downstream of the flowmeter, the measured flow rate is \(\dot{V}_E = V_l - (1 - RQ) \cdot \dot{V}_O_2\), whereas the flow rate through the analyzer is \(\dot{V}'E = V_l - \dot{V}_O_2\). Substitution of \(\dot{V}'E = \dot{V}_E - RQ \cdot \dot{V}_O_2\) into Eq. 4a yields
\[
\dot{V}_O_2 = \frac{\dot{V}_E \cdot (F_{I_O_2} - F'_{E_O_2})}{1 - F_{I_O_2} + RQ \cdot (F_{I_O_2} - F'_{E_O_2})} \quad (4b)
\]

Equation 4b is nearly independent of RQ as \(RQ \cdot (F_{I_O_2} - F'_{E_O_2}) \ll 1 - F_{I_O_2}\).

**Calculation of RQ and EWL**

The RQ can be determined by alternately inserting and removing a CO\(_2\) absorbent in the system. A similar technic has been used to determine the CO\(_2\) content of air samples (G. Maclean, personal communication). If the CO\(_2\) absorbent is placed before the flowmeter, equating Eqs. 3a and 4a gives
\[
\text{RQ} = \frac{(1 - F_{I_O_2}) \cdot \dot{V}_E \cdot (F_{I_O_2} - F'_{E_O_2})}{\dot{V}_E \cdot (F_{I_O_2} - F'_{E_O_2})} \quad (5a)
\]

Substitution of \(\dot{V}_E = \dot{V}_E - RQ \cdot \dot{V}_O_2\) yields a complex quadratic equation which can be solved for RQ.

If the CO\(_2\) absorbent is placed past the flowmeter but ahead of the analyzer, then equating Eqs. 3a and 4b gives
\[
\text{RQ} = \frac{(1 - F_{I_O_2}) \cdot (F'_{E_O_2} - F_{E_O_2})}{F_{E_O_2} \cdot (F_{I_O_2} - F'_{E_O_2})} \quad (5b)
\]

Figure 3 shows the relationship between RQ, \(F'_{E_O_2} - F_{E_O_2}\), and \(F_{I_O_2}\) for Eq. 5b. \(F'_{E_O_2} - F_{E_O_2}\) is sufficiently great over the normal physiological range of RQ to allow its accurate determination.

After \(\dot{V}_O_2\) and RQ are determined, EWL of the animal into the mask can be measured by removing the H\(_2\)O absorbent and equating the appropriate equations.

Values of \(\dot{V}_O_2\), RQ, and total EWL were determined for Allen’s hummingbird (Selasphorus sasin) in a respiratory chamber by application of the preceding technics (Fig. 4). The respiratory patterns of euthermic hummingbirds were impossible to discern because the respiratory frequency at 15°C is 200–300 breaths/min (unpublished data). However, pneumatic respiratory patterns of torpid hummingbirds were readily observed with a sensitive respirometry system (Fig. 4). High sensitivity was achieved by minimizing the chamber volume (35 ml) and dead space and by using small volumes of CO\(_2\) and H\(_2\)O absorbents. The lag time of the system was 40 s with a 99% response time of about 20 s. The \(\dot{V}_O_2\), RQ, and EWL data for hummingbirds compare favorably with expected values (Table 1).

The \(\dot{V}_O_2\) of large monitor lizards (Varanus goulditi) was measured with an open mask, and the respiratory patterns were readily discerned (Fig. 4B). Planimetry of individual breaths gave a mean value for \(\dot{V}_O_2\) of five

![Fig. 3. Relationship between RQ and concentration of O\(_2\) (F\(_{E_O_2}\)) and change in concentration (Δ = F\(_{E_O_2}\) - F\(_{E_O_2}\)) when CO\(_2\) absorbent is removed from respirometry system.](image)

![Fig. 4. A: typical changes in O\(_2\) concentration of outlet air for S. sasin with removal of CO\(_2\) absorbent (heavy upward arrow), H\(_2\)O absorbent (light upward arrow), and replacement of CO\(_2\) and H\(_2\)O absorbents (light downward arrow). B: respiratory patterns of torpid S. sasin and resting monitor lizards (V. goulditi).](image)
jizards which was identical to previous values (see Table 1).

<table>
<thead>
<tr>
<th>Table 1. Metabolism, RQ, and EWL of hummingbirds in metabolic chambers, and metabolism of monitor lizards with open masks</th>
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<tbody>
<tr>
<td><strong>Hummingbirds (S. sasin)</strong></td>
</tr>
<tr>
<td>$V_{O2}$, ml O$_2$ g$^{-1}$ h$^{-1}$</td>
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<tr>
<td>RQ (not fasted)</td>
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<tr>
<td>RQ (fasted 36 h)</td>
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<tr>
<td>EWL, mg H$_2$O g$^{-1}$ h$^{-1}$</td>
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<tr>
<td><strong>Monitor lizards (V. gouldii)</strong></td>
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<tr>
<td>$V_{O2}$, ml O$_2$ g$^{-1}$ h$^{-1}$</td>
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<tr>
<td>Ambient temperature = 15°C. Hummingbirds: body weight = 3.8 g; body temperature = 37-40°C. Monitor lizards: body weight = 200-1,300 g; body temperature = 15°C.</td>
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It is apparent that mathematical analyses which are required to calculate $V_{O2}$ for a system with an open or closed mask are those for standard respirometry techniques (4, 5). If H$_2$O is not absorbed ahead of the flowmeter and analyzer, then $V_{O2}$ may be overestimated by as much as 6% depending on the operating conditions. However, the EWL can readily be determined by direct measurement (gravimetric or freezing), by measuring the water vapor pressure, or by removing the H$_2$O absorbent. The error in $V_{O2}$ if CO$_2$ is not absorbed ahead of the flowmeter and gas analyzer is low (± 3%).

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REFERENCES