Apneic Oxygen Uptake in the Torpid Pocket Mouse *Perognathus parvus*

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ABSTRACT

The apneas of many torpid mammals can persist longer than estimated O_2 stores allow. This suggests that some O_2 is acquired during these apneas by either cutaneous uptake or by a nonventilatory flux down an open airway (tracheal flux). Previous experiments confirmed apneic O_2 uptake in the bat *Eptesicus fuscus* with the conclusion that the uptake most likely occurred by tracheal flux. However, the bat's large cutaneous wing area remained a potential source of cutaneous O_2 uptake, leaving uncertainty regarding the mechanism of O_2 uptake, particularly in regard to some evidence suggesting that small mammals might be obligated to maintain a closed glottis during apnea. This study sought experimental confirmation of passive O_2 uptake in the pocket mouse *Perognathus parvus,* torpid at a body temperature of 10°C, body mass 16.0 ± 0.6 g ($N = 9$). Ventilation bouts lasted 1.49 ± 0.06 min, whereas apneas lasted 4.51 ± 0.14 min, despite estimated O_2 stores able to support apneas of only 1.0 min. The maximum predicted cutaneous O_2 uptake was 0.67 μmol O_2/h, whereas the theoretically calculated tracheal flux was 20.2 μmol O_2/h. This theoretical rate of tracheal flux compared favorably to the measured plateau apneic O_2 uptake rate of 16.7 μmol O_2/h. However, the diffusional component of tracheal flux was 3.6 times greater than predicted, indicating an important contribution from cardiogenic mixing. Overall, apneic O_2 uptake provided 10.2% of the mouse's total O_2 uptake. We conclude that passive tracheal flux is the most likely mechanism by which this animal acquires O_2 during apnea.

Introduction

Many heterothermic mammals breathe intermittently during torpor. This pattern of breathing often consists of a bout of rhythmic tidal breathing lasting one or more minutes separated by periods of apnea that can last up to several hours. As first suggested by Kayser in 1940 (A. Malan, personal communication) and later by Clausen and Ersland (1968), some mechanism for acquiring O_2 during apnea must exist because these apneas can continue beyond the capacity of estimated O_2 stores. Malan (1982) demonstrated that a hibernating hedgehog could theoretically sustain its 1-h apneas by acquiring O_2 through a passive tracheal flux combining diffusion and bulk convection. A more recent investigation applied Malan's analysis to the 15-g big brown bat, *Eptesicus fuscus,* to account for direct laboratory measurements of apneic O_2 uptake from this bat (Szewczak and Jackson 1992*a*). Estimated O_2 stores for this bat suggest that apneas of only 17 min could be supported at a body temperature (T_b) of 10°C, despite observed apneas as long as 147 min at this T_b.

With the bat, however, some uncertainty remains whether cutaneous exchange across the wing membranes could account for the observed O_2 uptake during apnea, instead of the proposed tracheal flux. Herreid et al. (1968) attempted to measure cutaneous gas exchange across the wing membranes of euthermic *E. fuscus.* Although they detected some CO_2 exchange, they failed to detect significant O_2 uptake through the wing. However, the rate of apneic O_2 uptake measured by Szewczak and Jackson (1992*a*) in torpid *E. fuscus* may have been beyond their threshold for detection. In addition, some evidence suggests that small mammals may be obligated to maintain a closed glottis during apnea (Crossfill and Widdicombe 1961; Koo et al. 1976). This, of course, would obviate the potential for any tracheal flux.

To address these issues, we undertook the present study to determine whether apneic O_2 uptake could be confirmed in another small, torpid mammal. Specifically, we desired to investigate apneic O_2 uptake in a torpid mammal lacking the confounding influence of the large cutaneous area available to the bat. Similar in body mass to the big brown bat, the Great Basin pocket mouse, *Perognathus parvus,* readily enters heterothermic torpor with an intermittent pattern of breathing and thus provided a suitable subject for this investigation. As a further objective, we analyzed the most plausible route by which the mouse acquired measured apneic O_2. We supported this analysis with measurements of the pocket mouse's airway morphometry.

Material and Methods

Experimental Animals

We captured nine *Perognathus parvus* specimens using Sherman live traps set in Deep Springs Valley, Inyo County, Califor-

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nia. Their body mass was 16.0 ± 0.6 g. The mice were maintained in an animal holding room on a 10L : 14D light cycle. They were housed in separate 23 × 27 × 19 cm stainless steel cages lined with local soil (necessary to keep fur from matting) and provided with lengths of PVC tubing for shelter. Temperature in the room ranged from 10° to 15°C, which encouraged the mice to utilize torpor. Diets consisted of mixed seeds supplemented with lettuce or apple slices for moisture.

Measurement of Instantaneous Oxygen Uptake

A small volume, flow-through metabolic chamber permitted simultaneous measurements of continuous O_2 uptake and ventilation. The basic design of this chamber followed that used to measure apneic O_2 uptake in *Eptesicus fuscus* (Szewczak and Jackson 1992*a*). The mouse chamber was constructed from PVC tubing reinforced to a wall thickness of 1.5 cm to ensure rigidity. Identical tubing was used in the cages to accustom the mice to entering torpor within this "artificial burrow" before the experiments. The chamber was closed with 1.2-cm black acrylic caps fastened with toggle clamps and sealed with rubber gaskets. Ports were fitted in the center of each end cap for tubing connections. The internal dimensions of the chamber measured 3.8 cm diameter by 7.3 cm long, which provided approximately 50 mL of dead space in the chamber when occupied by a mouse.

Two measurements experimentally determined instantaneous O_2 consumption: (1) the difference in O_2 fraction between dry, CO_2-free incurrent and excurrent chamber flow, and (2) the time-volume of dry, CO_2-free gas flowing from the chamber (Depocas and Hart 1957; Hill 1972). Using dry, CO_2-scrubbed gas provided a determination of $\dot{V}O_2$ independent of the respiratory quotient (see appendix in Frappell et al. [1992]). Incurrent and excurrent flow were desiccated with indicating Drierite while indicating soda lime was used to remove CO_2. A pressurized air tank ($FO_2 = 0.2144$) provided an invariant source of incurrent gas to eliminate minor compositional fluctuations inherent in room air. This source supplied both the chamber circuit and a parallel reference circuit. During experiments a multistage regulator on the air tank provided steady flow rates through each chamber of approximately 120 mL/min. Flow rate through the chamber was measured on the excurrent flow to provide a reliable reference against ambient room pressure. This option was unavailable for the incurrent gas flow because of the positive pressure flow through the chamber (chamber to room pressure differential was ca. 12 cm H_2O).

A differential O_2 analyzer (Applied Electrochemistry, model S-A3II; model N-37M sensor) determined the change in O_2 fraction by comparing the excurrent metabolic chamber flow to that from the parallel reference chamber. To maximize accuracy, we followed the manufacturer's recommendation of flowing 10 mL/min of gas from the air tank through the sensor cabinet to provide an invariant reference. In addition to the reference circuit, a second parallel circuit bypassed the metabolic chamber for calibrating the experimental-to-reference circuit difference while the mouse was in the chamber. We matched the flow rates and resistances in all three circuits to deliver gas to the O_2 sensors identically, irrespective of which circuit was open. Overflow connections enabled O_2 determinations to be made at room pressure. Incurrent and excurrent flow of each circuit passed through CO_2 and water-removing cartridges. To optimize responsiveness, we minimized the volumes of all excurrent gas circuitry.

The signal output from the O_2 analyzer was acquired with a computer for real time monitoring and later analysis (Apple Quadra 700 equipped with National Instruments Lab-NB A/D board and LabVIEW software). After low-pass filtration to eliminate noise spikes, the signal was amplified to exploit full resolution of the 12-bit analog-to-digital conversion, then sampled at 40 Hz. Ten point averages of this signal were stored to yield data files with a time resolution of 0.25 s. An electronic flowmeter (McMilan; range: 0–185 mL/min) similarly interfaced with the computer to calculate O_2 consumption from the analyzer data. To confirm reliable readings the electronic flowmeter was checked against a glass float flowmeter (Cole-Parmer Instrument, ±2% accuracy).

At the start of an experiment, a pocket mouse was removed from its cage, weighed, and then placed into the chamber. The chamber setup was then immersed in a water bath controlled at 9.6°C, the temperature previously determined with rectal temperature measurements to achieve a T_b of 10.0°C during torpor. Entry into torpor at the target temperature was indicated by intermittent breathing and a heart rate less than 60/min, which was determined from observation of cardiogenic pulsations on the pressure trace during apneas (Fig. 1). Only mice exhibiting these characteristics proved to have rectal temperatures of 10.0° ± 0.3°C on removal from the chamber. Most mice entered torpor within several hours. If they did not readily enter torpor after this time they were removed from the chamber. Torpid mice were monitored for several more hours before collecting data to ensure a steady-state metabolic and ventilatory response. The duration of data records ranged from 30 to 90 min, with multiple records collected while the mouse remained torpid. If a mouse aroused from torpor, the experiment was ended and the mouse was removed from the chamber.

Oxygen uptake data acquisition began and ended with 5-min segments in which the gas flowing to the metabolic chamber was redirected through the bypass circuit to compare it with the gas flowing through the reference circuit. (During this procedure a separate supply provided room air to the mouse.) This procedure established a zero baseline that was used to correct for any instrument signal drift, if present. Drift corrections were only made if they appeared linear over the course of the record and if the baseline drift from the beginning to

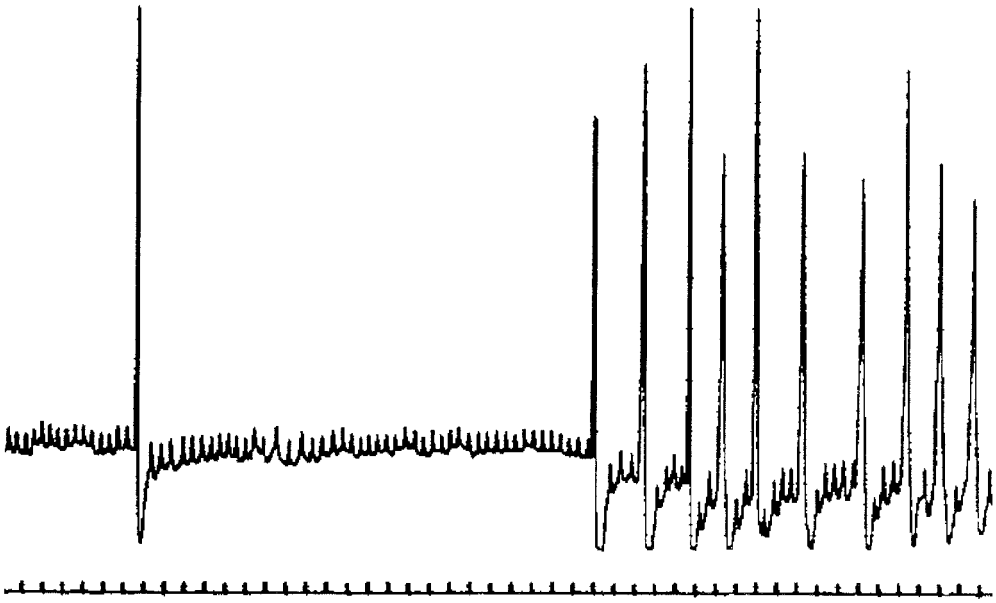

Figure 1. Detail of plethysmographic pressure trace. Ventilations are revealed by large spikes, with inspiration upward. Note the cardiogenic pulsations visible between breaths. The tick count below the trace displays seconds.

end did not exceed one half the magnitude of apneic uptake. Switching the bypass flow back through the metabolic chamber introduced an artificial spike in the O_2 consumption record as room air, lower in O_2 concentration than the tank gas, was flushed from the chamber (Fig. 2). A similar artifact occurred at the end of the file on switching back to the bypass for calibration as a bolus of room air resident in the bypass circuit passed through the system.

The reliability of this experimental protocol was tested using a granite pebble as a faux mouse. The pebble was selected to displace a similar volume to that of a mouse to maintain equivalent flow dynamics to that of a mouse-occupied chamber. The same protocol of flows, temperatures, CO_2 and water-removing cartridges, and valve settings were maintained identical to those used with the mouse experiments.

Measurement of Ventilation Activity

The metabolic chamber doubled as a plethysmograph permitting barometric detection of ventilation movements (Jacky 1978). Ports on the side of the metabolic chamber and the reference chamber connected tubing to either side of a differential pressure transducer (Validyne, model DP103-18). This arrangement isolated the metabolic chamber transducer from ambient pressure noise. Incurrent and excurrent air through both chambers passed through flow resistors. These flow resistors were constructed by fitting pieces of PE 10 tubing (Clay Adams, no. 7401) in 3 mm i.d. polyvinyl tubing (Tygon, #3603; Cameron 1986). The lengths of PE tubing (range: 1–3 cm) in each resistor were adjusted to balance the total resistance in each circuit and between the chambers. These flow resistors served as low-pass filters to retain transient ventilatory-induced pressure changes for detection, yet allowed continuous gas flow through the chambers. They also smoothed the flow through the chambers, providing a low noise pressure signal. The pressure transducer signal was demodulated (Validyne, model CD-15) and sent to a chart recorder (Gould, model 2400S) for amplification and low-pass filtering. The processed ventilation signal was also sent to the computer, which used a software routine to detect ventilation peaks and then stored them concurrent with the metabolic rate data. Only the ventilatory activity was monitored; no effort was made to determine ventilatory volumes.

Calculation of Apneic Oxygen Uptake

Although we endeavored to minimize dead space volume in the experimental system, it could not be eliminated entirely. We therefore applied the instantaneous transformation algorithm of Bartholomew et al. (1981) to achieve data records displaying instantaneous O_2 uptake. This calculation requires knowledge of the system's effective washout volume. We used the initial artifact spikes from the faux mouse trials to determine the effective chamber washout volume to be 44.6 ± 0.9 mL. The instantaneous transformation resolved the ventilation peaks more sharply, but revealed no significant difference on the plateau rates of apneic O_2 uptake when compared to the untransformed data, $P = 0.58$.

For each data record, we referenced O_2 uptake against a regression line calculated from the initial and final zero seg-

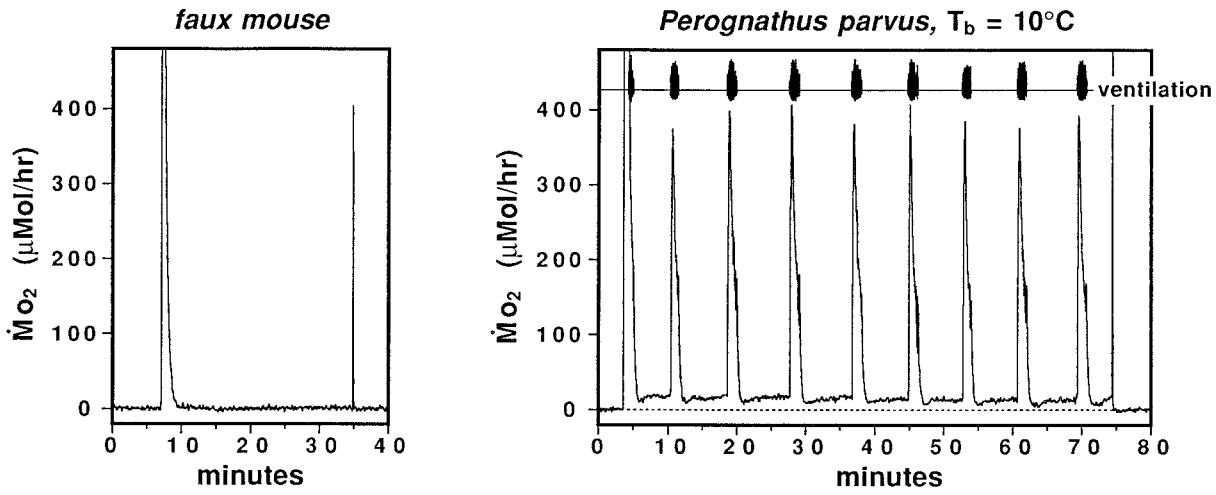

Figure 2. Sample data records of instantaneous O_2 uptake from a faux mouse and real mouse displayed at identical scales. The same procedures were used in each trial. The first and last spikes of the O_2 uptake trace result from the introduction of a bolus of room air during the calibration procedure (see "Material and Methods"). Note that the faux mouse initial artifact spike quickly returned to baseline, demonstrating the fast washout response of the metabolic chamber arrangement. The real mouse record shows concurrent ventilatory activity and displays sharp spikes in O_2 uptake from ventilation and nonzero O_2 uptake during apnea. Minor fluctuations in the apneic uptake record reflect fluctuations in the O_2 analyzer operating near the limit of its sensitivity.

ments of the record. Total O_2 consumption was determined by integrating these records over a consecutive span of paired ventilation and apneic intervals. Apneic O_2 uptake was determined by separately integrating the apneic intervals over this span (Fig. 3). The fraction of O_2 uptake occurring during apnea was calculated as the apneic uptake divided by the total O_2 consumption. The plateau apneic O_2 uptake rate was measured as the final, steady value reached during an apnea (Fig. 3). Plateau apneic O_2 uptake rate was only calculated for apneas that persisted long enough to display a clearly defined plateau.

Measurement of Airway Morphology

The University of California White Mountain Research Station donated three frozen specimens of *P. parvus* previously captured during small mammal surveys conducted approximately 5 km from our collection site. An additional three specimens from the metabolic study group were sacrificed to provide a sample size of six. We revealed the airways through dissection and, holding each specimen in a typical torpor posture, measured the length from the airway opening at the nose to the bronchial bifurcation with the aid of a stereo dissecting microscope and calipers (0.05 mm resolution). We next removed the lungs and revealed the primary bronchi. We then measured the length from the tracheal bifurcation to the point at which the primary bronchus became indistinct, as we considered this far enough into the lung for gas exchange to occur. Keeping the tissue moist with normal saline, we then sliced thin cross sections from the midsections of the trachea and primary bronchi. We measured the distance across the lumen of each section by placing them on an etched microscope slide with 0.01 mm gradations. Total cross-sectional area of the lumen was calculated assuming circular geometry at these diameters.

Results

Oxygen Uptake

We determined the O_2 uptake from 151 apneas and their paired ventilation bouts from 29 records ($N = 9$ animals) of duration 12.2–66.6 min ($\bar{X} = 31.0$ min). Ventilation bouts averaged 1.49 ± 0.06 min (range: 0.1–4.1 min), and typically consisted of about 30–60 breaths. Apneas lasted 4.51 ± 0.14 min (range: 0.9–9.1 min). Thus, on average, these pocket mice remained apneic 75.2% of the time when torpid at $T_b = 10°C$. The total O_2 consumption at $T_b = 10°C$ (combined ventilatory and apneic uptake) was 94.6 ± 7.6 μmol/h. For the 29 records the average rate of O_2 uptake during apnea was 11.7 μmol/h, and the fraction of total O_2 uptake during apnea was 0.102 ± 0.007. The measured apneic O_2 uptake plateau rate was 16.7 ± 0.7 μmol/h.

Records of instantaneous O_2 uptake and ventilatory activity displayed sharp positive spikes in O_2 uptake corresponding with ventilatory bouts. (The response time of the O_2 analyzer lagged the ventilation signal by about 28 s.) The rate of O_2 uptake trailed off as the bout progressed, then steeply dropped to near zero as the bout ended (Fig. 2). All data records exhibited above-zero O_2 uptake during the apneic intervals. Apneic O_2 uptake rose from a low level immediately following a ventilatory bout to a steady plateau level that persisted until the next

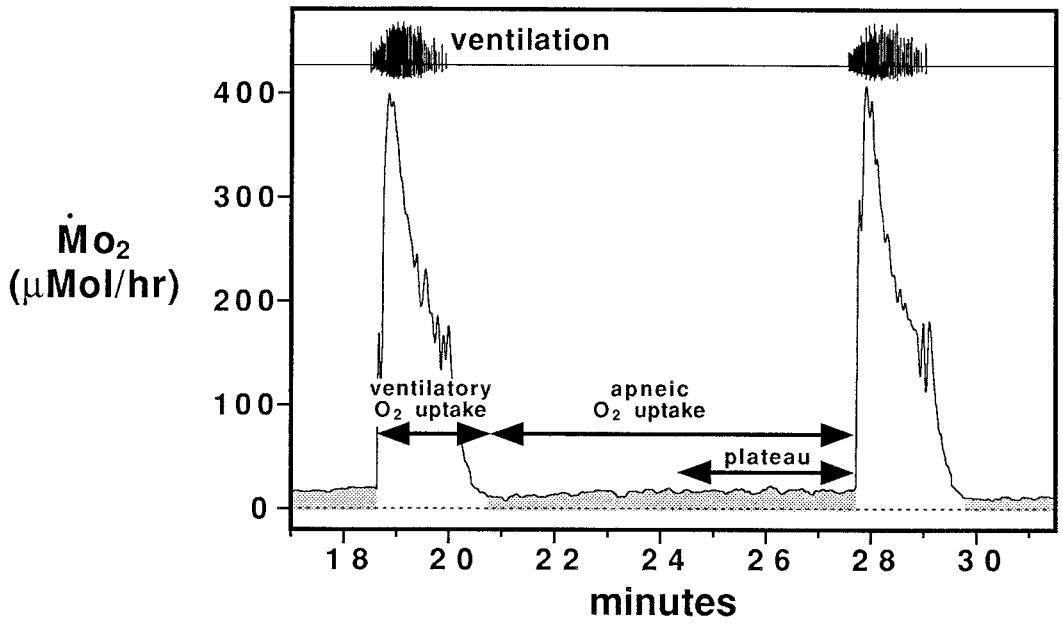

Figure 3. Detail from same experimental record of Figure 2 illustrating terminology used in the text. Ventilatory activity is shown on the top trace with concurrent O_2 uptake below. The dashed baseline represents zero O_2 consumption. Ventilatory O_2 uptake was integrated over the unshaded portion, while apneic O_2 uptake was integrated over the shaded portion. The plateau rate for apneic O_2 uptake was determined over the steady-state range indicated.

ventilation. This pattern of O_2 uptake reflects that observed in the torpid bat *Eptesicus fuscus* (Szewczak and Jackson 1992*a*).

Fifteen faux mouse trials yielded a mean O_2 uptake rate of 0.879 ± 0.319 μmol/h (range: −0.843 to 2.54). This value was 5.2% of the measured plateau rate of apneic O_2 uptake in the mouse, which differed significantly from the mouse plateau rate of apneic O_2 uptake with a *P*-value <0.0001 (nonparametric *t*-test). The artifact spike following the initial switch from the bypass flow quickly returned to baseline, demonstrating the fast washout response of the metabolic chamber arrangement (Fig. 2).

Airway Morphology

The six specimens of *Perognathus parvus* used for the airway morphometry measurements weighed 16.0 ± 0.8 g. The primary bronchi measured 5.1 ± 0.3 mm in length, with a diameter of 0.44 ± 0.02 mm for a cross-sectional area of 0.15 mm^2. The airway from the nose to the bronchial bifurcation was 30.2 ± 0.6 mm in length. The tracheal diameter was 0.96 ± 0.04 mm for a cross-sectional area of 0.72 mm^2.

Discussion

To interpret the mechanism of apneic O_2 uptake in the pocket mouse, we shall calculate estimates of cutaneous and passive tracheal O_2 flux and compare them with the directly measured rates of this investigation. We will further compare these with apneic O_2 uptake at the same T_b of 10°C in the only other species for which data are available, *Eptesicus fuscus,* the big brown bat (Szewczak and Jackson 1992*a*).

Available Oxygen Stores during Apnea in Perognathus parvus

Blood Stores. Malan (1982) performed a calculation of O_2 stores available during apnea for a hibernating hedgehog at a body temperature of 10°C. In that calculation, he used the allometric relationship of Dejours (1975) to calculate blood volume and assumed 25% arterial blood with an O_2 carrying capacity of 6.83 mmol/L. Venous blood was assumed to fall to 70% saturation during apnea, considering the blood's increased O_2 affinity at the lower body temperature, and an end-apneic arterial blood partial pressure of 1.4 kPa. Applying that calculation to the 16-g body mass of a pocket mouse, with adjustments of the scaling equations, yields an estimated blood store of 0.49 μmol O_2.

Lung Stores. Malan used the allometric relationship of Dejours (1975) to calculate total lung capacity of the hibernating hedgehog and then assumed functional residual capacity to be 20% of total lung capacity. Applying that calculation to the 16-g body mass of a pocket mouse yields an estimated lung store of 1.08 μmol O_2.

Tissue myoglobin stores are unavailable to reenter circulation (Dejours 1981) and are considered inconsequential as an O_2 store (Malan 1982). The combined total of estimated the blood and lung stores is 1.57 μmol O_2. Without an auxiliary influx of O_2, these stores could support an apnea of just 1.0

min at the pocket mouse's mean metabolic rate of 94.6 μmol O_2/h.

Theoretical Rate of Cutaneous Gas Exchange in Perognathus parvus

Herreid et al. (1968) measured cutaneous gas exchange from the outstretched wings of two bat species at different ambient temperatures. Although these bats were euthermic, the wing temperatures approach ambient and provide some indication of cutaneous gas exchange across mammalian skin at reduced temperatures. Their study did not measure a statistically significant O_2 uptake; however, based on the measured rate of CO_2 output and O_2's known lower cutaneous rate of about 0.04 that of CO_2 (Herreid et al. 1968), an estimate of potential O_2 cutaneous uptake can be calculated. At 15°C, the lowest temperature from which they obtained data, the measured rate of cutaneous CO_2 output was 633 μmol/($m^2 \times h$), implying a cutaneous O_2 uptake rate of 25.3 μmol/($m^2 \times h$). According to Meeh's equation (Herreid et al. 1968), the body surface area for a 16.0 g mouse would be 0.00641 m^2, which would yield an estimated cutaneous CO_2 output of 4.06 μmol/h and O_2 uptake of 0.17 μmol/h. However, this rate is based on the naked wings of a bat; the actual values in the mouse should be lower owing to its colder temperature (10°C) and furred body. An estimate based on the largest measured value of CO_2 output at 37.5°C (2,520 μmol/($m^2 \times h$)) still provides only 0.67 μmol O_2/h. Therefore, it thus seems unlikely that cutaneous O_2 uptake could account for the measured apneic rate of 16.7 μmol O_2/h.

Theoretical Rate of Tracheal Apneic O_2 Uptake in Perognathus parvus

The model we used to estimate for tracheal apneic flux was proposed by Malan (1982). It has two components: diffusional and convective. We derive an estimate for each separately, then combine them with a simple summation as per Malan. The interaction of these two components likely creates a more complex interaction than this summation; however, all the components used in this model of tracheal flux are physiologically reasonable. We present the estimate to explore the potential for tracheal apneic O_2 uptake as a comparison to the potential cutaneous O_2 uptake.

Diffusional O_2 Influx. To estimate O_2 diffusive potential, we presumed a partial pressure gradient from ambient to alveolar gas and applied this to a trachea model derived from our measurements of airway morphometry. For our estimate of alveolar gas, we presumed a mean of ventilatory and end-apneic arterial O_2 pressures. Such data are unavailable for the pocket mouse, but for the purpose of this estimate we used the mean ventilatory to end-apneic value of 8.9 kPa determined from *E. fuscus* at T_b = 10°C (Szewczak and Jackson 1992*b*). The upper end of the gradient was the O_2 available to the mouse in the chamber, which at the elevation of the site of the experiments (1,600 m), and the fraction of the dry tank gas (0.214) was 18.0 kPa. Thus, the partial pressure difference, ΔP_{O_2}, was 9.1 kPa. For the diffusion geometry, we presumed an equivalent cylinder reaching to the middle of the mouse's lungs. The primary bronchi function as parallel conductors that can be represented as a single equivalent conductor 5.1 mm in length with a lumen double the area of a single bronchus, that is, 0.30 mm^2. This equivalent conductor is, in turn, in series with the trachea. These series conductors can be represented as a single equivalent conductor, which if maintaining the cross-sectional tracheal lumen of 0.72 mm^2 would have a length of 42.4 mm. The diffusional flux, Q, is expressed by

$$\dot{Q} = \frac{\Delta P_{O_2} \times \text{area}}{\text{distance}} \times D, \quad (1)$$

where D is the diffusivity of O_2 (29.8 (μmol)/(kPa × mm × h) at 10°C; Schmidt-Nielsen 1979; Weast 1979). These values provide a diffusional influx estimate of 4.60 μmol O_2/h. However, the actual diffusive drive may be somewhat higher than this because of the convective influx (see below) that would bring a higher O_2 concentration within a shorter distance.

Diffusional CO_2 Efflux. The diffusional efflux of CO_2 is less effective than the diffusional O_2 influx due to its smaller concentration gradient and lower diffusivity (22.9 μmol/(kPa × mm × h) at 10°C; Weast 1979). We also presumed a diffusional limitation and used a mean ventilatory to apneic arterial concentration of CO_2 as the basis for an estimate of CO_2 efflux. Again, data for apneic arterial concentration of CO_2 are unavailable for the pocket mouse, but using the available data from *E. fuscus* at T_b = 10°C (Szewczak and Jackson 1992*b*) provides a concentration gradient of 3.2 kPa for CO_2. Taken with the mouse airway morphometry data and equation (1), this yields an efflux estimate of 1.2 μmol CO_2/h. The effective CO_2 efflux is probably less because it must go against a convective inflow (see below).

Bulk Convection of O_2 Down the Airway. Bulk convection provides a second continuous process to deliver O_2 down the airway. This occurs as the volume of O_2 removed from the lungs is incompletely replaced by an equivalent volume of CO_2. Less CO_2 fills the lungs as a consequence of the respiratory quotient and storage of CO_2 in the tissues. An assumed respiratory quotient of 0.78 and a total O_2 consumption of 94.6 μmol O_2/h implies a net CO_2 production of 73.8 μmol CO_2/h. Subtracting the potential CO_2 tracheal efflux of 1.2 μmol CO_2/h and the estimated cutaneous efflux of 4.06 μmol CO_2/h leaves 68.5 μmol CO_2/h to accumulate in the mouse. The

lungs would equilibrate with the arterial CO_2 concentration, but because they cannot clear all of the CO_2 production, an equilibrium is reached in which the lungs can accept no further CO_2. The retained CO_2 would remain dissolved in the blood and tissues and consequently would not contribute to gaseous volume in the lungs. Assuming that the bulk of the 68.5 μmol CO_2/h remains in solution would imply an equivalent gaseous volume of 1.90 mL/h that does not fill the lung (at body temperature and pressure). Absorption of the 4.60 μmol O_2/h diffusional influx provides an additional volume deficit of 0.13 mL/h at body temperature and pressure. Together, these effects result in a lung volume deficit of 2.03 mL/h. By virtue of pulmonary mechanics, the lungs tend to maintain a constant volume, which they achieve by drawing an influx of ambient air to replace this volume deficit. In the mouse experimental setup, 21.4% of this influx was O_2, thus delivering an additional 15.6 μmol O_2/h.

The Tracheal Flux Model in Operation. The combined estimates of diffusion (4.60 μmol O_2/h) and bulk convection (15.6 μmol O_2/h) would deliver 20.2 μmol O_2/h to the apneic mouse. In the transition from ventilation to apnea, absorption of O_2 from the lung (and cutaneous CO_2 efflux) would continue unabated, and this would drive some bulk convection. Similarly, because even during ventilation the alveolar O_2 concentration is less than ambient, a partial pressure gradient already exists at the onset of apnea to drive the diffusive flux of O_2 down the airway. Thus, O_2 uptake should fall to a minimal, but nonzero, rate as apnea begins. Furthermore, immediately following ventilation, the ventilatory dead space is filled with ambient air and the diffusional distance from ambient to alveolar air is minimal. As apnea continues, the arterial O_2 concentration would fall as the mouse consumes its O_2 stores. This would enhance the diffusional gradient and thereby increase the rate of diffusive flux. However, unless the rate of apneic O_2 uptake can match the somatic demand requirements, the flux would only increase until achieving an equilibrium between demand and the combined diffusive/bulk potential. The observed time course of O_2 uptake during apnea (Figs. 2 and 3) was consistent with such a process: apneic O_2 uptake never fell to zero, and an apparent equilibrium rate was reached (the "plateau rate" of our measurements).

However, presuming that this plateau rate falls short of total somatic O_2 requirement, then arterial O_2 concentration would decline until the resumption of breathing. Given the small amount of O_2 that reach the alveoli by this apneic process, we would not expect any limitation in the blood's ability to transport it, that is, a diffusion-limited process. (Any increase in blood O_2 affinity that may occur from the cold body temperature would only further ensure its transport.) Eventually, blood gas levels of either O_2 or CO_2 become intolerable and trigger the next ventilation bout.

An Alternate Explanation?

Instead of the passive process assumed above, could O_2 uptake occur during apnea by an active process of very shallow breathing? Several lines of evidence run contrary to this possible explanation. First, this would be an unprecedented ventilatory maneuver that would contradict accepted explanations for intermittent breathing, namely, that the instantaneous frequency within an intermittent bout exhibits a naturally efficient rate for the animal (Otis et al. 1950; Crossfill and Widdicombe 1961). Thus, a slow inspiration would be energetically disadvantageous and contrary to the energy-sparing function of torpor.

Second, the O_2 uptake between the ventilation bouts is always positive; thus, only a single slow inspiration could possibly occur. The plethysmographic pressure traces always showed breaths initiating with inspiration (upward), followed by expiration (downward) (Fig. 1). A single inspiration between intermittent ventilation bouts would necessitate an expiration at the start of the next bout, and this was never observed. Furthermore, with the 30–60 breaths per ventilation bout, a single "apneic" breath could at most provide 1/30 the O_2 uptake of a ventilation bout, or about 3% (perhaps a bit more with a larger tidal volume or increased extraction from the prolonged retention time). However, it seems unlikely that a single breath could provide the 10.2% of total O_2 uptake measured to occur during apnea.

Finally, given that the plethysmographic pressure traces were sensitive enough to detect the mouse's heartbeat (Fig. 1), they would most likely have also detected any such unusual ventilatory maneuvers.

General Discussion

The Malan model predicted a continuous influx of 20.2 μmol O_2/h compared to the measured plateau rate of 16.7 μmol O_2/h. Despite this apparent agreement, the experimental results did not entirely support the model on which the prediction was based. Our system could not directly measure O_2 uptake resulting from bulk convection at the predicted level of 15.6 μmol O_2/h. Bulk convective influx of ambient chamber air by the pocket mouse would manifest itself as a subtle reduction in airflow out of the chamber without reducing the excurrent O_2 fraction. Although we measured excurrent flow, the estimated bulk flow rate of 2.03 mL/h was below the ~6 mL/h noise level of our flowmeter. If apneic O_2 uptake operated as predicted by the Malan model, we should have only measured the diffusional component of 4.60 μmol O_2/h. The predicted diffusional rate of 4.60 μmol O_2/h was at twice the noise level of our O_2 analyzer and therefore measurable. Such accuracy was unnecessary, though, as our measured apneic O_2 uptake rate of 16.7 μmol O_2/h was 3.6 times this predicted diffusional rate, suggesting the need for a modification to the tracheal flux model.

The apneic O_2 uptake predicted from the Malan model depended on an assortment of presumed values; changes in any of which could affect its value. In addition to those variables described in the tracheal flux, less tractable variables such as upper airway resistance could inhibit both diffusion and bulk convection, whereas cardiogenic mixing may enhance tracheal flux by improving diffusion in the airways (West and Hugh-Jones 1961; Drechsler and Ultman 1984; Szewczak and Jackson 1992*a*). Fukuchi et al. (1976) determined that cardiogenic mixing increased the effective diffusivity of gases 5.6-fold over that of the expected molecular diffusivity. In support of this effect, we observed cardiogenic pulsations on our respiratory traces that approached 12% of the tidal volume (Fig. 1). Allometric scaling predicts the tidal volume in a 16-g pocket mouse to be 0.104 mL (Stahl 1967), and 12% of this would imply a pulsation volume of 0.013 mL (near the allometric prediction of cardiac stroke volume of 0.010 mL (Stahl 1967)). Based on our airway morphometry measurements (see "Results"), the combined volume of the bronchial and tracheal airways was 0.023 mL. Thus, these pulsations would displace approximately half the volume of the major airways with each heartbeat. It is a reasonable expectation that such agitation could sufficiently facilitate tracheal diffusion to account for our measured rate. Although the Malan model accounts for the factors that drive tracheal flux, the contribution of cardiogenic mixing should be considered to thoroughly interpret the process of apneic O_2 uptake.

Of course an open glottis is essential for tracheal flux to occur. However, some uncertainty exists whether small mammals maintain an open glottis during apnea. Vinegar et al. (1979) found that mice actively elevate their functional residual capacity above that of their static relaxation volume. Other studies indicate that static relaxation volume in mice and hamsters approaches the volume at which the airways will close (Crossfill and Widdicombe 1961; Koo et al. 1976). This evidence suggests that perhaps small mammals must defend lung volume during apnea by closing their glottis to prevent atelectasis. The results of this study, however, suggest that the glottis remains open during apnea in the pocket mouse. The cardiogenic pulsations recorded on the plethysmographic pressure trace (Fig. 1) also support an open glottis during apnea (Malan 1973, 1982). Furthermore, the presence of an open glottis would leave the glottal muscles atonal during apnea, a state consistent with the energy-sparing goal of torpor.

The tracheal flux hypothesis can also provide some insights into hibernation energetics and observed differences of intermittent breathing patterns among small torpid mammals. Compared to the similar-sized bat, *E. fuscus,* the pocket mouse had a higher total metabolic rate when torpid at $T_b = 10°C$, 94.6 versus 35.2 μmol O_2/h; or 5.9 versus 2.3 μmol O_2/(g × h). The higher metabolic CO_2 production of the pocket mouse would result in a greater pulmonary volume deficit to drive the hypothetical bulk convection, 15.6 versus 4.3 μmol O_2/h for the pocket mouse and bat, respectively. However, the resultant greater CO_2 production likely accounts for the shorter apneas of the pocket mouse compared to the bat (4.5 vs. 56.7 min) because it would more quickly bring the mouse to a ventilation-stimulating state of acidosis (particularly given the ineffectiveness of apneic CO_2 removal in the pocket mouse). In addition, the differences in airway morphometry gave the mouse a considerably less calculated rate of O_2 diffusion than the bat, 4.60 versus 16.6 μmol O_2/h. Furthermore, despite similar body weights, the heart of the bat is approximately 1.5 times as large as that of the pocket mouse (Jurgens et al. 1981), and this may enhance the effect of cardiogenic mixing discussed above. Together, these differences suggest why the mouse's measured rate of apneic O_2 uptake provided just 10% of its total O_2 requirement compared to 54% for the bat.

The bat has a shorter airway and a larger tracheal lumen than the pocket mouse. These differences can be attributed to the bat's adaptation to support the intense aerobic demands of flight. However, by enhancing the bat's ability for apneic O_2 uptake, this adaptation also serves it well during the comparatively minuscule O_2 demands of torpor. As an insectivorous hibernating bat, *E. fuscus* must endure the winter season by relying entirely on stored lipid reserves, the quantity of which must conform to payload limitations. In contrast, the pocket mouse can access food caches throughout the winter. Thus, alleviating some of the metabolic cost of mechanical ventilation through apneic O_2 uptake may provide an arguably greater selective advantage for the bat than for the mouse.

This investigation supports the hypothesis that O_2 uptake observed during apnea in small torpid mammals occurs by passive flux down an open airway. The actual process probably involves some combination of these effects. However, despite these uncertainties, all of the components used to estimate the tracheal flux are physiologically reasonable, and we present the estimate to demonstrate the physical plausibility of passive tracheal flux as a mechanism by which O_2 uptake occurs during apnea. Tracheal flux can account for the experimentally measured rate of apneic O_2 uptake, whereas the estimated cutaneous uptake falls short by two orders of magnitude. Unlike the bat, the pocket mouse lacks a large cutaneous surface area that might contribute as a mechanism for acquiring O_2. Differences between the pocket mouse and the bat in their measured rates of apneic O_2 uptake and apnea duration also suggest tracheal flux as the probable path. The direct observation of apneic O_2 uptake in these distinct species suggests that this process may be a shared phenomenon among small heterothermic mammals.

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