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APNEIC OXYGEN UPTAKE IN THE TORPID BAT, *EPTESICUS FUSCUS*

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Summary

Like many mammalian heterotherms, the big brown bat, *Eptesicus fuscus*, breathes intermittently during torpor. By exploiting this bat's preference to roost in crevices, we could separately measure O_2 uptake during ventilatory bouts and apneic periods using a flow-through metabolic chamber with a small dead space volume and short time constant. Oxygen uptake was measured during apneas ranging from 10 to 150min duration at body temperatures of 20, 10 and 5°C. The fraction of total O_2 uptake acquired during apnea was 0.26±0.03 (9), 0.54±0.10 (5) and 0.35±0.04 (3) for body temperatures of 20, 10 and 5 °C, respectively. Cardiogenic pulsations during apnea visible on plethysmographic pressure traces and theoretical calculations of airway and cutaneous diffusion potentials support the notion that apneic O_2 uptake occurs down an open airway by both diffusion and bulk convection.

Introduction

Many hibernating mammals alternate periods of breathing with apneas of variable duration. During studies of acid–base state and ventilation in the torpid bat, *Eptesicus fuscus*, it became apparent that significant O_2 uptake was occurring during these apneas. A previous study demonstrated cutaneous exchange of CO_2 in euthermic *Eptesicus fuscus* (Herreid *et al.* 1968), but did not reveal significant cutaneous exchange of O_2. Theoretical calculations support the notion that apnea is prolonged in torpid hedgehogs by passive diffusion of O_2 into the lungs, a process termed apneic oxygenation (Clausen and Ersland, 1968; Malan, 1982). This study presents direct evidence for O_2 uptake during apnea in *Eptesicus fuscus* and how it is influenced by changing body temperature (T_b).

Materials and methods

Animals

Big brown bats, *Eptesicus fuscus*, were captured locally in accordance with a scientific

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collector's permit issued by the Rhode Island Department of Environmental Management. Bats were kept in a specially prepared colony room maintained at 25°C. Outgoing ventilation from this colony room flowed through anti-viral filters. The bats were housed in stainless-steel cages in groups according to cage size and fed live mealworms raised on a diet of flour, chicken feed, potato slices and monkey chow. They had free access to vitamin-supplemented water (Poly-Vi-Sol or equivalent) at all times. Individual bats were identified by numbered bands on their forearms. Body mass upon capture ranged from 14 to 16g, but increased to over 20g in some individuals during captivity.

Experimental procedure

Bats were placed in the experimental chamber at ambient temperatures selected to reach target T_b values of 5, 10, 20 or 30°C while simultaneously monitoring T_b, ventilation, instantaneous O_2 uptake and the electrocardiogram (ECG). At least 2h of steady-state physiological conditions were observed before acquiring data.

Physiological chamber

A specially made chamber permitted simultaneous monitoring of T_b, ventilation, instantaneous O_2 uptake and ECG without disturbing the bat. Details of the chamber and analytical procedures are described elsewhere (Szewczak and Jackson, 1992*a*,*b*). In brief, the chamber exploits this bat's penchant for roosting in crevices. Although the chamber's interior is only slightly larger than the bat's, they voluntarily crawled into it without a complete arousal from torpor after only a few training sessions. The small dead space within the chamber facilitated the detection of respiratory movements, while providing a short time constant for monitoring gas exchange with a sensitivity sufficient to reveal O_2 uptake from single breaths. To ensure that the system reliably measured apneic O_2 uptake, it was tested using a *faux* bat in the chamber. This procedure yielded zero O_2 uptake following the identical calibration protocol used with the experimental bats. A computerized data-acquisition system collected oxygen uptake and respiratory movement data using programs that we had developed (IBM XT with Data Translation DT2801 A/D board, ASYST data acquisition and data management software: Keithly-ASYST Software Technologies, Inc., version 3.0).

Calculation of oxygen uptake

During experiments, gas flow through the chamber was from tanks of pressurized air. This eliminated any potential errors from variations in gas composition. A bypass circuit, however, enabled room air to ventilate the chamber during T_b equilibration and analyzer calibration of the experimental gas flow (Szewczak and Jackson, 1992*b*). Total O_2 uptake was calculated by integrating the instantaneous rate of O_2 uptake record with respect to time. As far as possible, total O_2 uptake was calculated in phase with ventilatory cycles (the onset of a ventilatory bout to the end of an apneic interval). Steady-state sections from apneic periods of the instantaneous O_2 uptake recording were separately integrated with respect to time to determine rates of apneic O_2 uptake. The fraction of time that was apneic was calculated from the ventilation recordings and then applied to the rates of

apneic O_2 uptake to determine the total apneic O_2 uptake. We assumed somatic metabolism to be independent of ventilatory state because of the uniform heart rate from ventilation to apnea.

Results

Below T_b=30°C, a typical ventilatory cycle consisted of a 1- to 9-min bout of rather evenly spaced breaths, followed by an apneic interval. Occasional sporadic breaths punctuated the apneic interval, most commonly at T_b=20°C. (Apneic O_2 uptake was calculated only from apneic intervals free from these sporadic breaths.) Apneas averaged longer at T_b=10°C (56.7±15.3min) than at T_b=20°C (6.1±0.4min; maximum: 13.7min) or T_b=5°C (6.5±0.8min; maximum: 40.9min). Nevertheless, the fraction of time apneic was similar for these temperatures (Table 1). The longest recorded apnea was 147min at T_b=10°C. This bat was apneic for 95% of a complete ventilatory cycle with an apneic O_2 uptake rate of 24.9 μmol O_2 h^{-1}.

Heart rate was essentially constant from ventilation to apnea, particularly if compared to heart rates of typical diving mammals, which may reduce heart rate by 80% during apnea (Elsner, 1965). Cardiogenic pulses synchronous with ECG signals were visible in the plethysmograph pressure records (Fig. 1). These pulses were most pronounced following a ventilatory bout, then steadily decreased during apnea, often becoming lost in the noise. Heart rates were 8.6±0.6, 13.5±0.4 and 35.4±3.8beatsmin^{-1} at T_b=5, 10 and 20°C, respectively.

Instantaneous O_2 uptake records (Fig. 2) consisted of spikes due to ventilatory bouts, separated by periods during apnea with O_2 uptake remaining above zero. Simultaneous plethysmography confirmed the apneas indicated in these records. Apneic O_2 uptake was often lower immediately following a ventilatory bout, then increased to a steady level. Total $\dot{M}_{O_2}$ fits well with allometric data from other hibernators (Geiser, 1988) only if the O_2 uptake calculation includes the apneic contribution between ventilatory spikes. Apneic intervals were too brief to measure apneic O_2 uptake confidently at T_b=30°C.

Table 1. *Total and apneic rates of oxygen uptake in the torpid bat,* Eptesicus fuscus, *at* T_b*=5, 10 and 20°C*

T_b (°C)	Total O_2 uptake (μmol h^{-1})	Rate of apneic O_2 uptake (μmol h^{-1})	Fraction of time apneic	Total apneic O_2 uptake (μmol h^{-1})	Fraction of total O_2 uptake during apnea	*n* (*N*)
5	24.0±5.5	8.93±1.15 [24]	0.88±0.01	7.9±0.9	0.35±0.04	3 (1)
10	35.2±2.2	21.3±3.1 [24]	0.88±0.03	18.9±2.9	0.54±0.10	5 (3)
20	110.0±7.0	33.9±1.9 [47]	0.80±0.03	27.2±1.9	0.26±0.03	9 (6)

Total apneic oxygen uptake is calculated from the rate of apneic oxygen uptake and the apneic time fraction (see Materials and methods).

Data are presented as mean±S.E.; *n* represents the number of experiments, *N* the number of different animals for each T_b; numbers in brackets represent the number of apneic intervals used to determine the rate of apneic oxygen uptake.

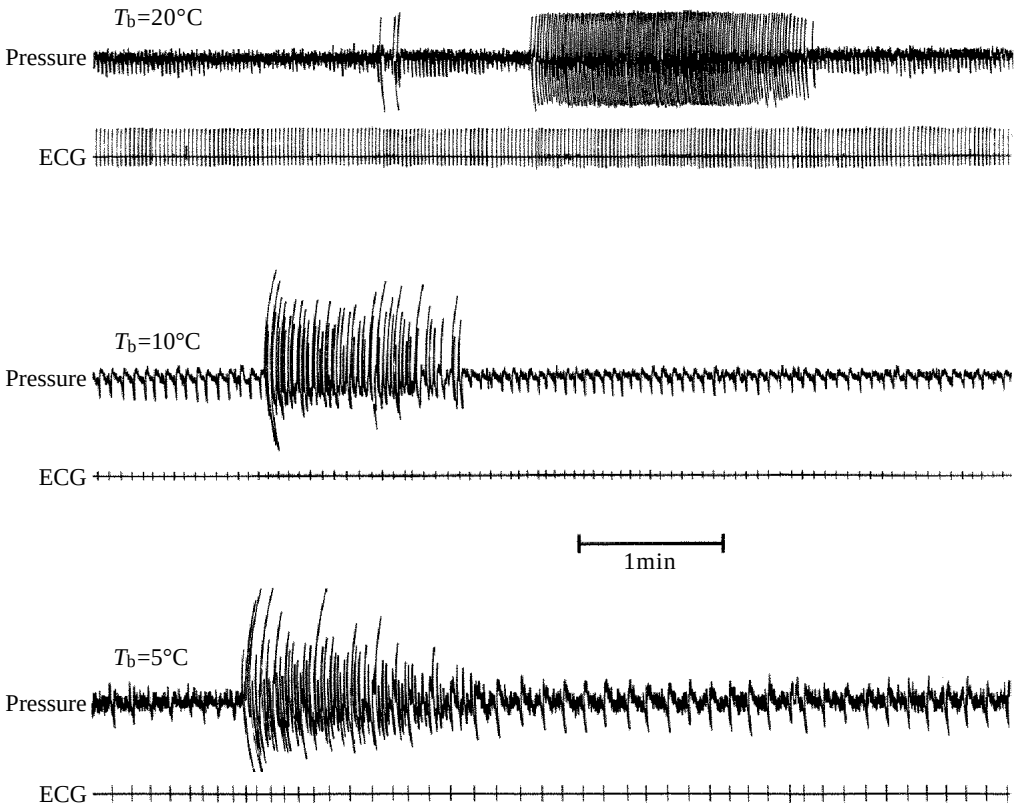

Fig. 1. Simultaneous plethysmograph pressure and ECG recordings from torpid *Eptesicus fuscus* at T_b=20, 10 and 5˚C. Ventilatory movements are revealed by the large pen excursions. Note the cardiogenic pulsations synchronous with the ECG signals. All records are on the same time scale; however, the pressure traces are not to the same scale because adjustments in amplifier gain were necessary at different temperatures.

The rate of apneic O_2 uptake increased as T_b was raised from 5 to 20˚C, but did not maintain a constant fraction of total O_2 uptake (Fig. 3). The maximum fraction of total O_2 uptake from apnea was at T_b=10˚C.

Discussion

Mode of apneic oxygen uptake

Oxygen uptake during apnea may occur either by cutaneous exchange of gas or *via* the airways into the lung. Several lines of evidence indicate that airway exchange is the more likely process in *Eptesicus fuscus.* A study of cutaneous gas exchange in euthermic *Eptesicus fuscus* measured gas exchange from the head and body separately (Herreid *et al.* 1968). The cutaneous output of CO_2 ranged from 18.8 to 104 μmolCO_2 h^{-1} for ambient temperatures of 18 and 37.5˚C, respectively, with cutaneous O_2 uptake reported to be insignificant. In that study, the wings of the bat were held open, increasing the

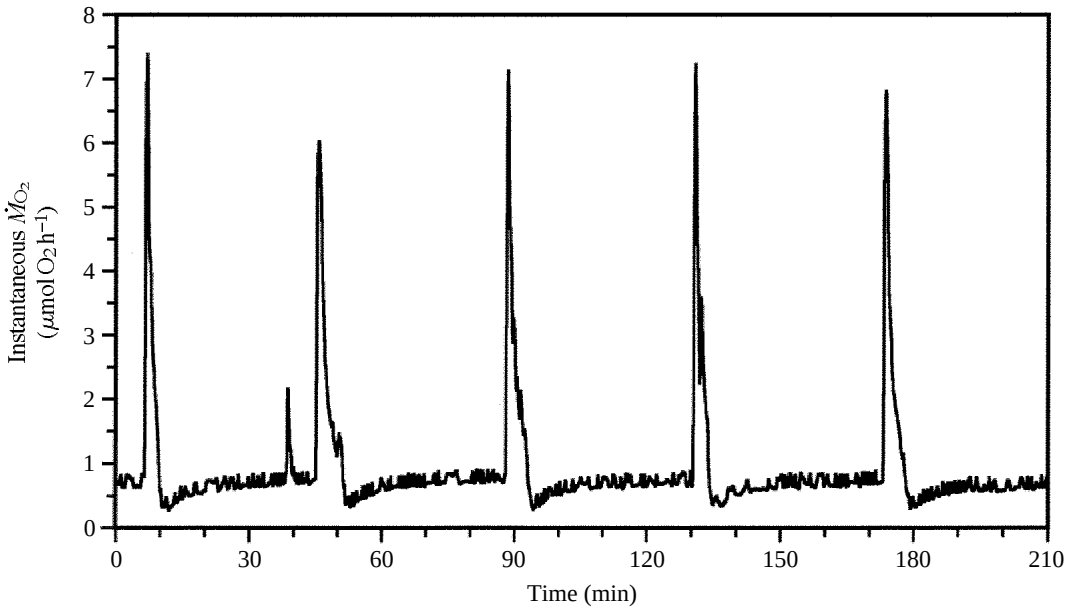

Fig. 2. Continuous oxygen uptake recording from torpid *Eptesicus fuscus* at T_b=10˚C. Intermittent ventilation bouts are revealed by the spikes. The small spike at 38min is from a pair of sporadic breaths. Apneic periods were confirmed by simultaneous plethysmography. Minor fluctuations are instrument artifacts.

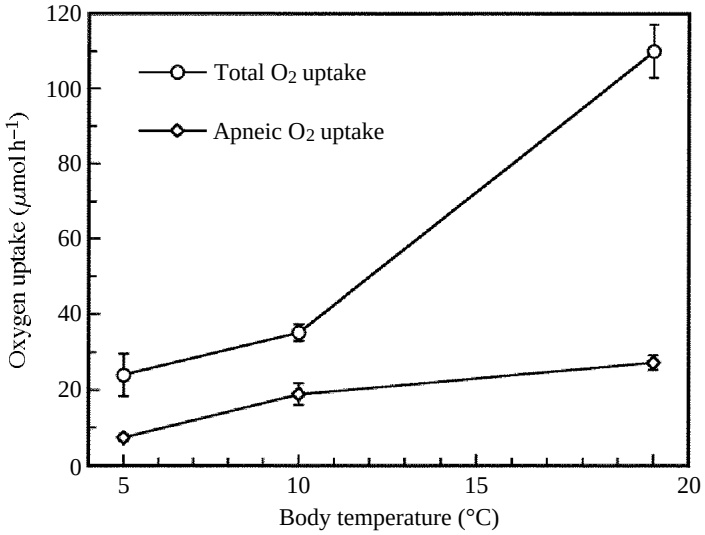

Fig. 3. Comparative mean rates of total to apneic oxygen uptake in torpid *Eptesicus fuscus* as a function of body temperature from 5 to 20˚C. Note that the greatest contribution of apneic O_2 uptake to the total O_2 uptake was at T_b=10˚C. Error bars represent standard error.

available cutaneous surface area by approximately five times over the folded wing posture of the torpid bats in the present study. Because the cutaneous conductance of O_2 is 20 times less than that of CO_2 (Piiper *et al.* 1976), the potential for cutaneous O_2 uptake of the bats in the present study would be two orders of magnitude less than the cutaneous CO_2 exchange of the bats in the prior study. Therefore, the expected cutaneous O_2 uptake would be at most 0.2 μmol O_2 h^{-1} for bats at T_b=20°C and below, considerably less than the 8.9–33.9 μmol O_2 h^{-1} range of apneic O_2 uptake measured in the present study. It is thus unlikely that cutaneous O_2 uptake could account for more than a minor portion of the apneic O_2 uptake measured in this study.

For effective gas exchange to occur by diffusion down the airway, the glottis must remain open during apnea. The cardiogenic pulsations recorded on the plethysmographic pressure trace (Fig. 1) strongly support an open glottis during apnea (Malan, 1973, 1982). There is no abrupt change in the quality of the pulsations in the transition from breathing to apnea that would indicate glottal closing. Based upon an analysis of breathing pattern and oxygen uptake, it was similarly concluded that the glottis remained open during apnea in torpid pipistrelle bats (Hays *et al.* 1991). Nevertheless, an investigation of non-ventilatory O_2 uptake during apnea in the little brown bat, *Myotis lucifugus*, concluded that its glottis was closed during apnea (Thomas *et al.* 1990). This conclusion was based upon measured rates of non-ventilatory O_2 uptake derived from numerical transformation of the instantaneous O_2 uptake data. The transformation procedure may possibly have introduced uncertainty, particularly at the low levels of O_2 uptake of 6.5 g *Myotis lucifugus* at T_b=5°C. Nonetheless, it is possible that there may be interspecies differences and that the glottis of *Myotis lucifugus* does remain closed during apnea, but the direct measurements of apneic O_2 uptake and observation of cardiogenic pulses of the present study suggest otherwise. To explore this issue further, the following sections quantitatively examine O_2 management during apnea in *Eptesicus fuscus*.

Available oxygen stores during apnea

During apnea, the bat's heart rate remains essentially constant. This continued circulation enables the tissues to withdraw O_2 stored in the blood, which reduces its P_{O_2} and enables it to remove O_2 stored in the lungs. The extent of these O_2 stores will be calculated and then used to determine the duration of apnea that they can support. Comparing this result with the observed durations of apnea should suggest whether it is necessary to invoke a mechanism for replenishing O_2 during apnea. The following calculations assume a fully O_2-loaded bat at the onset of a 1h apnea at T_b=10°C. The bat is presumed to be consuming O_2 at the experimentally measured metabolic rate of 35.2 μmol O_2 h^{-1}. All gas volumes are corrected for 10°C; the results from these calculations are compiled in Table 2.

Blood stores

The blood volume of bats is greater than that of other mammals; it has been determined to be about 13ml100 g^{-1} bodymass (M) in the bat *Myotis lucifugus* (Kallen, 1960). Because blood volume scales proportional to $M^{1.02}$ (Stahl, 1967), it may be estimated to be 1.9ml for a 15 g *Eptesicus fuscus*. Assuming 25% of this to be arterial blood with an

Table 2. *Estimated sources of oxygen in the torpid bat,* Eptesicus fuscus, *for 1h of apnea at* T_b*=10°C*

Source	O_2 (μmol)	Percentage of total O_2 requirement for 1 h
Blood stores	5.4	15.3
Lung stores	1.9	5.4
Cutaneous O_2 absorption	0.2	0.6
O_2 diffusion	16.6	47.2
Initial O_2 convection	0.4	1.1
Continuous O_2 convection	4.3	12.2
Total	28.8	81.8

The experimentally measured metabolic rate at T_b=10°C was 35.2 μmol O_2 h^{-1}.

O_2-carrying capacity of 6.83mmol l^{-1} (Malan, 1982), arterial blood should hold 3.2 μmol O_2. Assuming a decrease in arterial saturation to 50% provides 4.9 μmol O_2 in venous blood for a total blood store of 8.1 μmol O_2 (an estimate, since the respiratory properties of this bat's blood have not been determined). However, bats are known to reduce haematocrit during torpor by splanchic sequestering (Kallen, 1960; Martin and Stehn, 1977), so the total blood store of O_2 is probably unavailable. Thus, for this calculation, the estimated blood store will be reduced by one-third, yielding 5.4 μmol O_2.

Myoglobin stores

Because of its high oxygen affinity, oxygen bound to myoglobin is not available to re-enter the circulation (Dejours, 1981), and thus is only useful to the tissue in which it resides, which is primarily muscle. Since apneic bats are inactive, myoglobin O_2 stores are considered to be inconsequential.

Lung stores

Allometric lung data, corrected for bats, suggest a functional residual capacity of 0.322ml for *Eptesicus fuscus* (Brody, 1945; Maina and King, 1984). The end ventilatory Pa_{O_2} was 16kPa, and the end apneic Pa_{O_2} was 1.8kPa (Szewczak and Jackson, 1992*a*). Assuming the alveolar P_{O_2} to be similar, the concentration change may be estimated from:

$$\Delta C_{O_2} = \frac{\Delta P_{O_2}}{\boldsymbol{R}T}, \quad (1)$$

in which $\boldsymbol{R}$ is the gas constant (0.0624 l kPa $^{-1}$ mmol $^{-1}$ K^{-1}) and T is absolute temperature (Dejours, 1981). These P_{O_2}values indicate a lung O_2 concentration change of 6.03mmol l^{-1}, for a quantity of 1.9 μmol O_2 (0.044ml) stored at the onset of apnea.

Initial blood and lung O_2 stores can account for only 21% of the metabolic O_2 requirement during a 1 h apnea and are thus incapable of supporting apneas much longer than 12min. If the presumption of splanchic sequestering were removed, and all blood was available to the bat, then initial O_2 stores would be capable of supporting an apnea of

17min, which is still less than the 56.7min mean duration of apnea at T_b=10°C. There must therefore be a process for replenishing O_2 during apnea, which is consistent with the apneic O_2 uptake measured by the present study.

Available sources of oxygen replenishment during apnea

Oxygen can enter the blood either through the skin or *via* the respiratory airways and membranes. The theoretical calculation of airway diffusion potential follows from Malan (1982). The conditions stated above also apply to the following calculations.

Cutaneous oxygen absorption

Based upon the study by Herreid *et al.* (1968) the cutaneous O_2 uptake would be at most 0.2 μmol O_2 h^{-1}, which is probably a conservative overestimate for T_b=10°C. This is equivalent to only 0.6% of the bat's total O_2 requirement, and capable of extending apnea by perhaps 1min following exhaustion of initial O_2 stores.

Diffusion of oxygen into the lung

The geometry of the airway can be estimated from reported values in *Myotis lucifugus* (Thomas *et al.* 1990) and scaled to fit *Eptesicus fuscus* using length and diameter scaled to $M^{0.33}$ (Leith, 1982). This gives a tracheal length of 1.72cm and a cross-sectional area of 0.0095cm^2. Sample measurements of available *Eptesicus fuscus* specimens confirm these estimates (O. Mathieu-Costello, unpublished data). The airway length from the nares to the lung bifurcation was 1.6cm, with a mid-trachea lumen of 0.10cm×0.12cm providing a cross-sectional area of 0.00942cm^2. The airway length from the bifurcation down the primary bronchi was 0.50cm with a cross-sectional area of 0.0101cm^2. The diffusional path can then be modeled as a cylinder of 2.1cm length with a cross-sectional area of 0.00958cm^2. The net diffusion rate ($\dot{Q}$) is expressed by:

$$\dot{Q} = \frac{\Delta C_{O_2} \times \text{area}}{\text{distance}} \times D, \qquad (2)$$

where D is the diffusivity of O_2 at 10°C (0.187cm^2 s^{-1}) (Schmidt-Nielsen, 1979; Weast, 1979). The concentration gradient may be estimated by subtracting the mean ventilatory and apneic Pa_{O_2} value (8.9kPa) (Szewczak and Jackson, 1992*a*) from the ambient atmospheric P_{O_2} (21.2kPa, assuming normal pressure and dry air in the chamber). This gives an O_2 concentration gradient of 12.3kPa, or 5.41 μmol O_2 cm^{-3} in consistent units. Applying equation 2 to all these figures yields a net O_2 diffusion of 16.6 μmol O_2 h^{-1} (0.385ml O_2 h^{-1}), which is 47.2% of the bat's total O_2 requirement at T_b=10°C.

Bulk convection of oxygen into the lung

As a consequence of the respiratory quotient and the high CO_2 capacitance of blood and tissues (Dejours, 1981), CO_2 incompletely replaces the volume of O_2 absorbed from the lungs. Instead of contracting, the mechanical forces acting on the lung maintain it at a constant volume, and thus it draws an influx of ambient air (Malan, 1982). The diffusive efflux of CO_2 is limited by flowing counter to this influx and by its smaller concentration gradient and lower diffusivity compared to O_2 (0.144cm^2 s^{-1} at 10°C) (Weast, 1979).

The mean ventilatory to apneic $P_{a_{CO_2}}$ was 3.2kPa (Szewczak and Jackson, 1992*a*). From this value, the net diffusive efflux of CO_2 is estimated to be $3.98\,\mu mol CO_2\,h^{-1}$ ($0.092ml\,h^{-1}$). For an assumed respiratory quotient of 0.78, the total $\dot{M}_{CO_2}$would be $27.5\,\mu mol\,h^{-1}$ ($0.635ml\,h^{-1}$). This leaves $23.5\,\mu mol\,h^{-1}$ ($0.543ml\,h^{-1}$) of CO_2 to accumulate in the blood and tissues, with some cutaneous release, but it is unlikely that all of it fills the lungs. Metabolic absorption of the diffusive influx of $0.385ml\,O_2\,h^{-1}$, along with the diffusive efflux of $0.092ml CO_2\,h^{-1}$, combine to yield a continuous convective influx of $0.477ml\,h^{-1}$ of air, providing an additional $4.3\,\mu mol\,O_2\,h^{-1}$. Absorption of the initial lung store of $0.044ml\,O_2$ provides a one-time bulk convection of 0.044ml of air, furnishing $0.4\,\mu mol\,O_2$. Thus, the total bulk influx of O_2 during the first hour of apnea would be $4.7\,\mu mol$. This process would contribute 13.3% of the bat's total O_2 requirement.

The processes of diffusion and bulk convection combine to provide $21.3\,\mu mol\,O_2$ during this theoretical first hour of apnea. This provides 60.5% of the bat's O_2 requirement. Because it is based upon diffusion, the rate of this process should be dependent upon concentration gradient. This notion is consistent with the time course of instantaneous O_2 uptake recordings (Fig. 2). Following a ventilatory bout, O_2 stores are at capacity. This minimizes the concentration gradient, and hence O_2 influx. Consuming the initial O_2 stores improves the gradient and enhances the rate of O_2 uptake. This continues until an equilibrium is achieved between O_2 consumption and diffusion capacity, which then stabilizes the rate. The calculated equilibrium apneic O_2 uptake rate of $20.9\,\mu mol\,O_2\,h^{-1}$ (diffusive plus convective flux, following exhaustion of initial stores) also compares favorably with the measured rate of $18.9\pm2.9\,\mu mol\,O_2\,h^{-1}$ for bats at T_b=10°C. We thus conclude that passive O_2 influx down the airway is the most likely mechanism by which these bats acquire O_2 during apnea.

Possible role of cardiogenic mixing and the influence of temperature on apneic oxygen uptake

The idealized airway cylinder used in the above calculation did not consider potential resistances such as those from oral or nasal airways. Thus, the actual O_2 influx might be expected to be less than the calculated value. However, some experimentally measured rates of apneic O_2 uptake were actually higher (e.g. $24.9\,\mu mol\,O_2\,h^{-1}$ measured *vs* $20.9\,\mu mol\,O_2\,h^{-1}$ calculated for T_b=10°C). Because the above calculation assumed passive diffusion in still air, the difference may result from mechanical agitation of gases within the airways. It has been previously suggested that the mechanical action from the heart, i.e. cardiogenic mixing, may facilitate diffusion potential (West and Hugh-Jones, 1961; Fukuchi *et al*. 1976). Indeed, the cardiogenic pulses recorded from *Eptesicus fuscus* are suggestively prominent relative to the ventilation movements (Fig. 1).

Cardiogenic mixing may also influence the observed temperature-dependency of apneic O_2 uptake. The Q_{10} for diffusion of O_2 in air is about 1.2 (Dejours, 1981), whereas the Q_{10} of the rate of apneic O_2 uptake from T_b=10 to 20°C was 2.4, and from T_b=5 to 10°C was 4.5. The departure of these values from a Q_{10} of 1.2 indicates a dependency upon some other factor in addition to temperature. (Any temperature effects on haemoglobin affinity for O_2 would favorably influence the O_2 concentration gradient and

reduce the Q_{10}, rather than increase it.) The effect of cardiogenic mixing may be accentuated at low heart rates, in which it first breaks up the stratification of gases that inhibits diffusion (Fukuchi *et al.* 1976). At T_b=5˚C, the average interval between heartbeats is 7s, a plausible interval for stratification to occur in small airways. Thus, the large Q_{10} of 4.5 from T_b=5 to 10˚C could be attributed to the differential effects of cardiogenic mixing at these temperatures.

The observation of cardiogenic pulsations and a theoretical calculation of O_2 diffusion support the conclusion that apneic O_2 uptake occurs down an open airway. This process is apparently enhanced by the mechanical action of the heart, which could properly be considered to be an important respiratory organ for this animal.

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