Respiration Physiology, 88 (1992) 205–215

RESP 01886

Acid-base state and intermittent breathing in the torpid bat, *Eptesicus fuscus*

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(Accepted 6 January 1992)

Abstract. The effects of intermittent breathing on acid-base state and blood gases were characterized in the torpid bat, *Eptesicus fuscus*, during steady-state torpor between body temperatures (Tb) of 5 and 37 °C. Arterial blood samples were taken from indwelling catheters without disturbing the torpid state. Arterial pH (pHa) of samples taken without knowledge of ventilatory state rose by 0.15 units from 37 to 5 °C with a ΔpHa/ΔTb slope over this range of −0.0055 U/°C. However, at and below Tb = 20 °C, *Eptesicus fuscus* breathes intermittently with typical apneic periods of 40–150 min and 4–12 min at 10 and 20 °C, respectively. Samples taken at the end of a ventilatory bout and near the end of an apneic period at Tb = 20 °C revealed cyclic changes in pH (from 7.49 ± 0.02 to 7.34 ± 0.01), P_{O_2} (from 96.6 ± 3.4 to 30.8 ± 3.9 Torr), and P_{CO_2} (28.2 ± 1.4 to 45.9 ± 1.5 Torr). Between 10 and 37 °C, end-ventilatory pHa varied inversely with temperature with a ΔpHa/ΔT slope of −0.011 U/°C. Because intermittent breathing is common to many animals during hibernation, these results demonstrate the importance of coordinating blood sampling with ventilatory state for a reliable interpretation of acid-base regulation under these conditions.

Acid-base balance, hibernation, intermittent breathing (bat); Blood gases, hibernation, intermittent breathing (bat); Hibernation, torpor (bat); Mammal, bat (*Eptesicus fuscus*); Pattern of breathing, intermittent (bat)

Acid-base regulation in heterothermia is of particular interest because of the pH sensitivity of proteins and because changing temperature affects pH (Rosenthal, 1948; Reeves, 1969). Ectothermic vertebrates exhibit a general trend of increasing arterial pH (pHa) with decreasing body temperature (Tb) (Howell *et al.*, 1970; Cameron, 1984). This has been considered a strategy for maintaining a constant charge state for proteins, thereby preserving functional competency and thus, homeostasis (Reeves, 1972; Jackson, 1982; Cameron, 1989).

In contrast, the pattern of acid-base regulation exhibited by heterothermic mammals is one of a nearly constant pHa with decreasing Tb (Malan, 1982; Heisler, 1986). On first appearance this may seem homeostatic, but when the various physicochemical factors involved are considered, this strategy is revealed to be a progressive acidosis with

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decreasing Tb. This regulatory pattern may reflect the different requirements of heterothermic mammals from that of ectothermic vertebrates (Kayser, 1964; Malan, 1988). Ectotherms must maintain competent function throughout a range of Tbs, whereas mammals undergo heterothermia only when inactive. For mammals, heterothermy is engaged as an energy conservation strategy as in hibernative torpor. In this regard, the maintenance of complete functional competency may actually be undesirable; instead, a state in which metabolism is reduced by perturbation may be the goal (Malan, 1986, 1988).

This study investigated the acid-base state of the heterothermic mammal *Eptesicus fuscus*, the big brown bat. This bat is very temperature labile in that it exhibits both seasonal hibernation and daily heterothermic torpor throughout the year. Previous studies have not adequately addressed acid-base regulation in members of the Order Chiroptera. A further objective of this study was to characterize the effects of intermittent breathing upon acid-base state.

Methods

Animals. Big brown bats, *Eptesicus fuscus*, were captured locally with permission from 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 daily 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 weight ranged from 14–25 g.

Physiologic chamber. The physiologic chamber that was used permitted undisturbed sampling of arterial blood while simultaneously monitoring Tb, ventilation, metabolic rate, and ECG. The chamber was constructed of 12.5 mm acrylic painted black to render it opaque. The internal dimensions of the chamber (1.8 × 4.7 × 9.8 cm) are not much larger than the bat, but because these bats naturally roost in crevices they are at ease in this snug confinement. Most bats would voluntarily crawl into the chamber without a complete arousal from torpor after only two or three training sessions.

The bat assumed its natural inverted posture in the chamber by clinging to four copper screen patches that were attached to a panel of perforated fiberglass circuit board. These screen patches were individually wired and when coated with electrode gel served as passive ECG leads. A copper-constantan temperature probe protruded from the center of the panel to make firm contact with the bat's abdomen. The panel slid out of the top of the chamber for the removal of torpid bats. Luer Lok ports on the chamber provided for gas flow through the chamber and connection to a pressure transducer. The lid of the chamber was secured by two adjustable toggle fasteners and had a flexible gasket that provided an air-tight seal for the exteriorization of wires and catheter. Facilitated

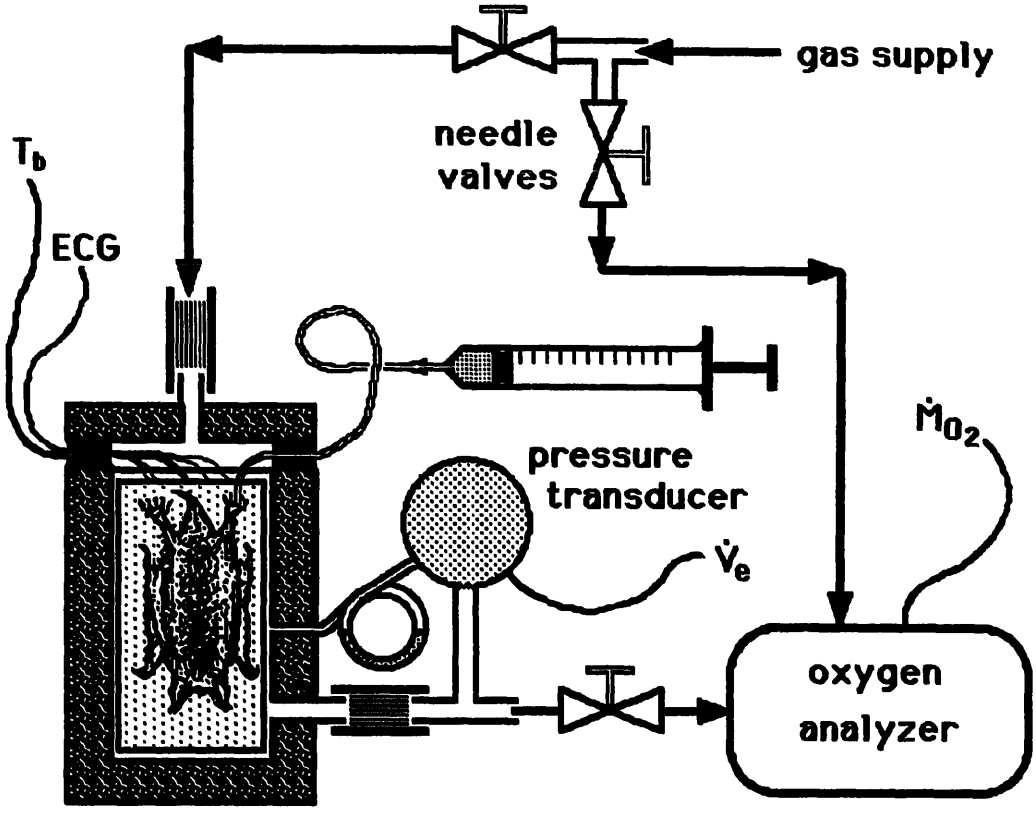

Fig. 1. Schematic diagram of chamber used for acid-base determinations. Body temperature, ventilation, metabolic rate, and ECG were simultaneously monitored to indicate physiological state during blood sampling.

by a lead ballast, the entire chamber was immersed into a temperature controlled water bath. Gas flowing into the chamber first circulated through coils in the water bath for temperature equilibration. A schematic representation of the chamber set-up is shown in Fig. 1.

Body temperature. A skin contact temperature probe was used because rectal probes agitated the bats and prevented entry into torpor. Preliminary work in this lab and others (Audet and Fenton, 1988; Hirshfield and O'Farrel, 1976) has demonstrated that skin contact readings were consistent with rectal temperature determinations. Skin contact temperatures were typically 0.5 °C or less above ambient temperature for steady-state torpid bats. A higher value above ambient indicated thermogenesis or transitory passive cooling from a higher temperature, *i.e.* entry into torpor. This strategy was also consistent with the other physiologic parameters monitored (ECG, metabolic rate, and ventilation). Body temperatures chosen for this study were 5, 10, 20, 30 and 37 °C. Bats at Tb = 37 °C were considered to be in torpor if they passively followed the ambient temperature, *i.e.* they exhibited no active thermoregulation. These bats were brought up to Tb = 37 °C from a lower Tb by adjusting the ambient temperature of the chamber.

Surgical procedure for blood collection. Bats were lightly anesthetized with ketamine hydrochloride (Vetalar®, Parke Davis) and xylazine hydrochloride (Rompun®, Haver, Bayvet Division, Miles Laboratories). The dosage used was 22 mg/g body weight of each compound. This was administered with a 28-gauge needle syringe into the pectoralis muscle. This light dose was used because heterothermic bats are extremely sensitive to most anesthetic agents. The bats often required inhalation of methoxyflurane as a supplemental anesthetic. Once soundly under the influence of these agents, the bats usually remained so for up to 1.5 h, which was sufficient time to complete the catheterization procedure. If a bat displayed any sign of arousal, additional methoxyflurane was administered to return the bat to a level of anesthesia unresponsive to stimuli.

Catheters were specially prepared from PE 10 tubing. The insertion end of the tubing was carefully drawn down over a flame to a size having a lumen of 100–150 μm and cut to a 30° angle. Small beads were formed along the tubing to secure it to the artery and tissue (Robinson *et al.*, 1969). The distal end of the catheter was enlarged by holding it above a flame to facilitate insertion onto a blunt 28-gauge needle tube (Heatley and Weeks, 1964). The completed catheter, which measured 10–12 cm in length, was sterilized in zephrine hydrochloride and coated with an antithrombogenic (North American Science Associates, Cleveland, OH).

Surgery was carried out using sterile technique. Bats were secured to a platform ventral side up, and catheters were implanted in the posterior tibial artery. This artery is superficial and easily accessed with minimal trauma. Furthermore, placing the catheter in this site facilitated exteriorization from the chamber. Following catheterization, 0.5–1 ml of normal saline (dependent upon Tb) was injected subcutaneously between the scapulae. This prevented dehydration which otherwise resulted from breathing the dry air ventilating the chamber.

Following experiments, catheters were aseptically removed under anesthesia, and the bats recovered well with no indication of impaired function distal to the traumatized artery. Bats used for a second blood sampling experiment were allowed to recover for at least 8 weeks following their previous donation.

Physiologic state during sampling. During experiments, ECG and ventilation movements were monitored on a polygraph; Tb and metabolic rate were monitored by computer. Following post-surgical arousal, bats were placed in the chamber and typically re-entered torpor within 1 h, reaching a steady-state Tb in 1.5–2 h. Sampling began only after several hours of steady physiological conditions. Values designated as 'ventilatory' were taken toward the end of a bout of rhythmic breathing, and values designated 'apneic' were taken toward the end of an apneic period in anticipation of the next ventilatory bout (Fig. 2).

Blood collection and analysis. Arterial blood samples were collected anaerobically using 1-ml insulin syringes (Becton-Dickinson) with blunted needle tubes. The needles on these syringes are attached directly to the syringe barrel yielding a very small dead space

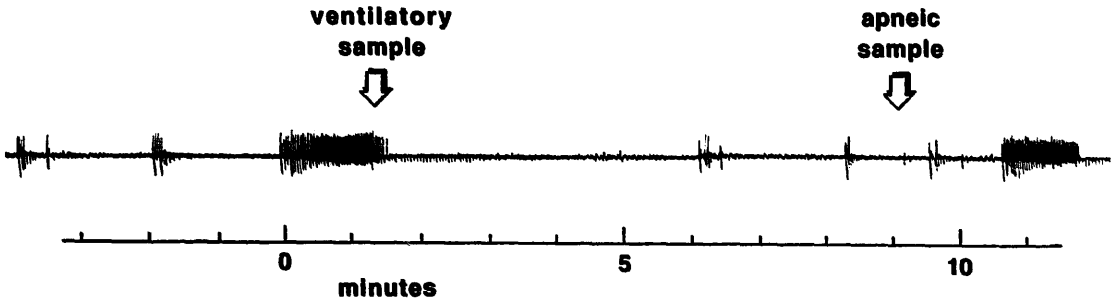

Fig. 2. Representative respiratory trace from a bat at Tb = 20 °C. Locations of ventilatory and apneic blood sampling are shown at their respective positions within a respiratory cycle.

(approx. 6 μl), which was filled with sodium heparin (1000 U/ml). Because of the comparatively large fluid resistance of the small catheter, the syringes could be interchanged directly on the catheter fitting without significant loss of blood. This obviated the need for more complicated and volume-consuming fittings. A syringe of normal saline was used to maintain catheter patency by gentle, hourly flushings. When sampling, arterial blood was drawn into this syringe before switching to the sampling syringe to obtain pure arterial blood. Neither flushing of the catheter nor sampling evoked arousal from torpor or respiratory movements during apnea.

As many as three individual samples of 120–150 μl were obtained from a bat during each experiment. The total blood volume in *Eptesicus fuscus* is estimated to be 1.9 ml based on data from a similar species, *Myotis lucifugus* (Kallen, 1960). The blood volume of bats is larger than comparably-sized terrestrial mammals, presumably to enable the aerobic demands of flight. Additionally, bats are known to sequester blood in their spleen during torpor when it is unnecessary to circulate such a large volume (Martin and Stehn, 1977), and there is probably splanchic compensation to sampling. Thus, we believe the volume of blood removed during torpor sampling was insufficient to compromise physiological function, and none of the physiological monitoring indicated otherwise.

Samples were immediately injected into a Radiometer BMS 3 MK2 for determination of pHa, Pa_{CO_2}, and Pa_{O_2}. The BMS was equilibrated to Tb and calibrated using Radiometer buffers and gases mixed by Wösthoff precision gas mixing pumps. Bicarbonate concentrations were calculated using the Henderson-Hasselbalch equation. Equilibrium constants were selected in reference to Tb and pHa (Reeves, 1976). The values for CO_2 solubility were taken from Severinghaus *et al.* (1956). Hematocrit was determined using specially prepared microcapillary tubes. Lactate concentrations were measured using a commercially available assay kit modified for small blood volumes (Sigma, St. Louis, MO).

Results

Data collected without knowledge of ventilatory state. Table 1 presents pHa, Pa_{CO_2}, $[HCO_3^-]$, [lactate], and hematocrit. These data were derived from arterial blood samples collected blindly; *i.e.* from a chamber arrangement in which it was not possible to determine the ventilatory status of the animal. *In vivo* pHa rose by 0.15 units from 37 to 5 °C with a ΔpHa/ΔTb slope over this range of −0.0054 U/°C; $r^2 = 0.85$, based upon Tb means. A linear regression of the raw data set yields a ΔpHa/ΔTb = −0.0060, and $r^2 = 0.49$; with the low correlation coefficient resulting primarily from the 20 °C data. *In vivo* Pa_{CO_2} decreased from 33.0 at Tb = 37 °C to 19.8 Torr at Tb = 5 °C. Lactate concentrations decreased slightly with decreasing Tb, but were never high enough to have a significant effect upon pHa, assuming normal blood buffering. Variations in hematocrit are consistent with bat hematocrit lability during torpor found by others (Kallen, 1960; Martin and Stehn, 1977).

Figure 3 displays the acid-base data on an *in vivo* pHa *vs* Tb plot. The large pHa variability at Tb = 20 °C motivated the investigation of how intermittent breathing affects acid-base state. Those results are presented next.

Data collected with knowledge of ventilatory state. Table 2 presents pHa, Pa_{CO_2}, $[HCO_3^-]$, and Pa_{O_2}, correlated with ventilatory state. For Tb = 20 °C the minimum apneic *in vivo* Pa_{O_2} recorded was 17 Torr, and the maximum ventilatory Pa_{O_2} was 104 Torr. At Tb = 20 °C the largest pHa oscillation during a respiratory cycle was 0.244 pH unit.

Figure 4 displays the acid-base data on an *in vivo* pHa *vs* Tb plot. Blind values for Tb = 30 and 37 °C are included as ventilatory samples since it was determined that

TABLE I

Acid-base data collected without knowledge of ventilatory state

Tb (°C)	37	30	20	10	5
pHa	7.30 ± 0.02	7.36 ± 0.01	7.44 ± 0.08	7.49 ± 0.01	7.45 ± 0.01
Pa_{CO_2} (Torr)	33.0 ± 3.0	23.2 ± 1.1	26.1 ± 5.3	21.6 ± 1.3	19.8 ± 0.5
$[HCO_3^-]$ (mEq/L)	16.2 ± 0.9	13.9 ± 0.3	20.8 ± 1.4	26.3 ± 1.5	24.6 ± 0.3
[lactate] (mM)	1.22 (6)	1.52 (1)	0.88 (2)	0.82 (6)	0.70 (2)
Hct (%)	42.7 ± 6.2	38.8 ± 3.4	40.6 ± 4.2	45.0 ± 1.9	40.0 ± 2.4
n	7	4	4	6	2
N	6	4	4	5	2

Values are means ± SE (*n*). *N* represents number of different animals, *n* represents total number of samples.

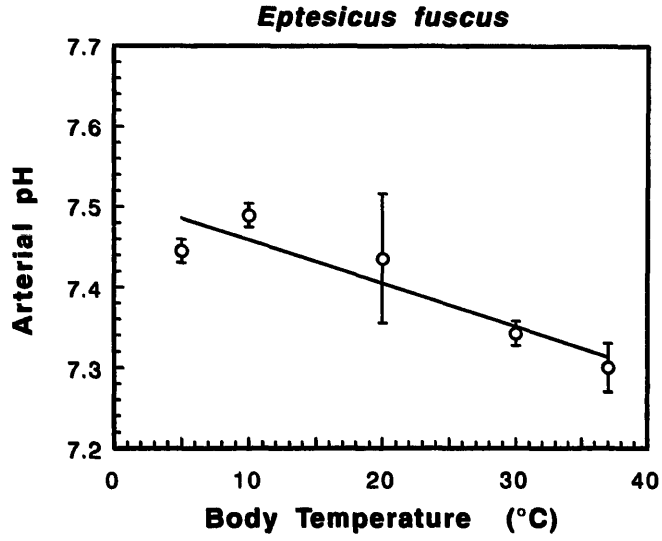

Fig. 3. *In vivo* arterial blood pH of *Eptesicus fuscus* as a function of body temperature. Data collected without knowledge of ventilatory state. The ΔpHa/ΔTb slope from 37 to 5 °C is −0.0054 U/°C; $r^2 = 0.85$, based upon Tb means. A linear regression of the raw data set yields a ΔpHa/ΔTb = −0.0060, $r^2 = 0.49$; with the low correlation coefficient resulting primarily from the wide variation in 20 °C data. Errors bars represent standard error.

Eptesicus fuscus does not exhibit significant apneas at these body temperatures. The regression line was calculated using mean ventilatory values and the one Tb = 10 °C ventilatory value; for this regression ΔpHa/ΔTb = −0.011 and $r^2 = 0.98$. A linear regression of the raw data set yields a ΔpHa/ΔTb = −0.011 and $r^2 = 0.74$. A regression based upon the apneic values at Tb = 10 and 20 °C and the Tb = 30 and 37 °C values yields a ΔpHa/ΔTb = −0.0057 and $r^2 = 0.81$.

TABLE 2

Acid-base data collected with known ventilatory state

Tb (°C)	20 ventilatory	20 apneic	10 ventilatory	10 apneic
pHa	7.49 ± 0.02	7.34 ± 0.01	7.57	7.47
Pa_{CO_2} (Torr)	21.9 ± 1.0	34.8 ± 1.1	19.4	28.2
[HCO_3^-] (mEq/L)	21.8 ± 0.9	23.4 ± 0.8	29.2	32.8
Pa_{O_2} (Torr)	96.6 ± 3.4	30.8 ± 3.9	120	13.5
n	15	9	1	1
N	12	6	1	1

Values are means ± SE. *N* represents number of different animals, *n* represents total number of samples.

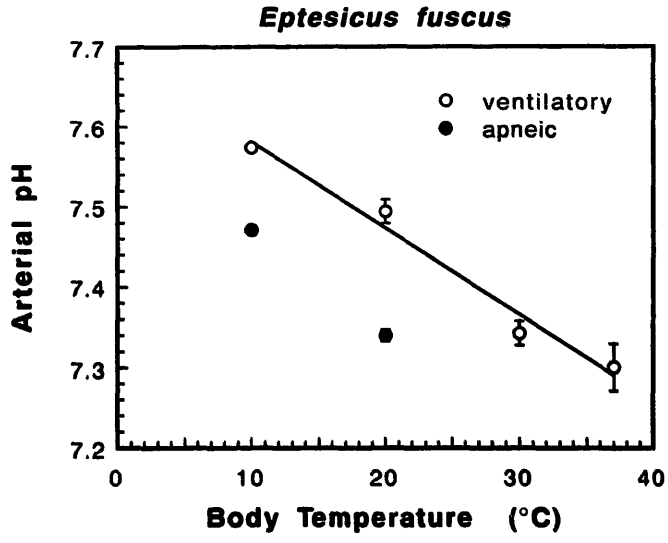

Fig. 4. *In vivo* arterial pH of *Eptesicus fuscus* as a function of body temperature. Data collected and correlated with regard to ventilatory state. Because significant apneic periods do not occur at Tb = 30 °C or 37 °C, these points are considered to be ventilatory. Regression line is drawn using only ventilatory data; $\Delta pHa/\Delta Tb = -0.011$, $r^2 = 0.98$, based on means. Error bars represent standard error.

Discussion

Among mammalian heterotherms the average $\Delta pHa/\Delta Tb = -0.0036$ U/°C with a range from 0.005 to −0.008 (Heisler, 1986). The $\Delta pHa/\Delta Tb$ of −0.0054 for *Eptesicus fuscus*, based upon acid-base data acquired without knowledge of ventilatory state, would appear to be consistent with other mammalian heterotherms. But when the acid-base data is correlated with ventilatory state, a different strategy is revealed. During torpor at and below Tb = 20 °C, *Eptesicus fuscus* breathes intermittently, with consequential oscillations in pHa. Is it appropriate to describe the temperature-dependency of this bat's acid-base regulation in terms of some average pHa, or should the data be interpreted in reference to ventilatory state?

Ventilatory control during intermittent breathing may be simply modeled by considering two thresholds: an 'on threshold', in which the status of pH, Pa_{O_2}, or Pa_{CO_2} become intolerable, and ventilation is initiated; and an 'off threshold', in which the combined status of these parameters are satisfactory, and ventilation ceases. Since breathing is the active effector for short-term acid-base control, it is reasonable to conclude that the acid-base state when ventilation ceases is the preferred state for the animal; *i.e.* the set point of the acid-base controller. It is then sensible to interpret the temperature-dependency of acid-base state at the end-ventilatory state.

When the end-ventilatory data for *Eptesicus fuscus* is considered in this way, the $\Delta pHa/\Delta Tb$ is −0.011 U/°C. This is equivalent to the average value reported for all heterothermic species (Heisler, 1986), which are believed to follow this relationship to maintain protein charge state and thus, defend enzyme function.

Interpreting the set point in this way is theoretically satisfying, because it avoids the

need to invoke adjustments of set point with heterothermy. From the view point of the controller, it may be 'temperature-blind' and merely acting to maintain the sensor proteins at an equivalent state, with the $\Delta pHa/\Delta Tb$ of -0.011 being a passive manifestation of changing physicochemistry. Cameron (1989) has suggested such a regulatory strategy be termed 'Z-stat', to reflect the goal of maintaining net protein charge. This is an evolution of Reeves' (1972) alphastat theory and the constant relative alkalinity theory of Howell *et al.* (1970).

During apnea the acid-base state becomes progressively acidotic. This acidosis may be explained by an increased tolerance to acid-base state deterioration during intermittent breathing, rather than a change in set point of the active controller. The end-ventilatory set point remains at a constant state, but with intermittent breathing a greater tolerance may be permitted before resuming ventilation (the 'on threshold'). This may be an essential adaptation in facilitating intermittent breathing in smaller mammals. Intermittent breathing is considered important in minimizing the work of breathing during low metabolic states (Milsom, 1988). Thus, an adaptive tolerance for acid-base oscillations may have a role in reducing the energetics of torpor, and enhancing hibernation survival.

Of course, the integrated effect of these acid-base oscillations will be some mean of the end-ventilatory and end-apneic states, and the intracellular oscillations are likely to be less pronounced due to intracellular buffering. An alternative explanation is that the acid-base controller is acting to maintain this mean value. However, this requires a more complicated scenario in which the pH set point must be adjusted with temperature, and overshot during ventilation.

In some instances the low $\Delta pHa/\Delta Tb$ slope reported for heterothermic mammals may actually be artifact. In the awake heterothermic ground squirrel, *Spermophilus tereticaudus*, sampling without knowledge of ventilatory state revealed an *in vivo* $\Delta pHa/\Delta Tb = -0.012$ between $Tb = 30–40\,^{\circ}C$ (Bickler, 1984), similar to the value of -0.011 we observed in ventilating *Eptesicus fuscus* between 10–37 °C. However, below 30 °C the squirrels exhibited the progressive acidosis typically reported for heterothermic mammals. These results are similar to our data collected without knowledge of ventilatory state. Since other members of the genus *Spermophilus* breathe intermittently during torpor (Milsom, 1988), it is likely that *Spermophilus tereticaudus* does as well. It is intriguing to speculate what would be the acid-base state of *Spermophilus tereticaudus* when correlated with ventilatory state.

At $Tb = 20\,^{\circ}C$ *Eptesicus fuscus* typically ventilated for 0.5–2 min followed by 4–12 min of apnea. This yields about a 14% probability of obtaining a blood sample during a ventilatory bout if the ventilatory state is unknown. At $Tb = 10\,^{\circ}C$, *Eptesicus fuscus* typically ventilated for 2–8 min separated by 40–150 min of apnea. At this Tb, the probability of randomly obtaining a blood sample during a ventilatory bout is less than 10%. With multiple sampling the contribution of any ventilatory samples acquired might be subdued by statistical smoothing, yielding a false impression of the acid-base regulation of this intermittent breather. However, the exact extent of this error cannot be determined without a complete profile of the acid-base variation with time.

An earlier study (Malan *et al.*, 1973) of intermittently-breathing hibernating marmots (2–3 kg) in which acid-base sampling was correlated with ventilatory state, observed maximum pHa oscillations of 0.042 pH unit at Tb = 11 °C. Similarly, an investigation of acid-base oscillations with the respiratory cycle of hibernating hedgehogs, *Erinaceus europaeus* (640 g) reported pHa oscillations of 0.04 pH unit at Tb = 4.5 °C (Tähti and Soivio, 1975). The greater pHa oscillations in 15 g *Eptesicus fuscus* may be the result of a scaling effect. Because of higher weight specific $\dot{M}_{O_2}$ in smaller mammals the acid-base state may deteriorate faster during apnea than in larger mammals. Larger hibernators may have greater acid-base inertia to carry them through apneic bouts with less variation in acid-base state, while other small hibernators may exhibit acid-base fluctuations similar to those observed in *Eptesicus fuscus*.

The authors have been unable to find any other studies using indwelling catheters to assess the acid-base state of any Chiropteran species. Methods requiring handling the animals should be considered unreliable, especially during torpor, because disturbances elicit arousal in which profound metabolic and respiratory changes occur swiftly. In a study of the bat *Myotis lucifugus* in which blood was sampled by cardiac puncture, a mean pHa of 7.53 ± 0.037 was found between 6–37 °C (Reeves and Wimsatt, 1966). However, the pHa varied in the range of Tb = 12–28 °C similar to that shown in Fig. 3 of this study, which are herein demonstrated to result from pHa oscillations during a respiratory cycle.

This study demonstrates that sampling should be correlated with ventilatory state for a thorough interpretation of acid-base regulation in intermittent breathers. Additionally, this study presents evidence that a nearly constant pH during heterothermy is not a general phenomenon in mammals, and in some instances may be artifact. The only other studies in which acid-base determinations were correlated with ventilatory state involved animals larger than *Eptesicus fuscus*, leading to a speculation that smaller animals may have an adaptive tolerance for large acid-base oscillations to facilitate intermittent breathing. Whether the magnitude of acid-base oscillations observed in *Eptesicus fuscus* are specific to this bat, Chiroptera, or smaller mammals must await further investigation.

Acknowledgements. We thank Dr. André Malan for his many helpful comments regarding this investigation. This work was supported by Grant DCB8802045 from the National Science Foundation (DCJ).

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