HEAT DISSIPATION, GAS EXCHANGE AND ACID–BASE STATUS IN THE LAND SNAIL *OREOHELIX* DURING SHORT-TERM ESTIVATION

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Summary

Within 4 days following entry into estivation, heat dissipation and oxygen consumption by the land snail *Oreohelix* spp. decreased by 83% compared to standard non-estivating rates. During both non-estivating and estivating conditions, the quantity of heat dissipated per mole of O_2 consumed was indicative of a completely aerobic metabolism. This calorimetric–respirometric (C/R) ratio was $-461\pm12\,\text{kJ}\,\text{mol}^{-1}\,O_2$ (S.E.M., $N=5$) under standard non-estivating conditions and $-464\pm26\,\text{kJ}\,\text{mol}^{-1}\,O_2$ ($N=4$) during estivation. Respiratory exchange ratios reflected a primary dependence upon carbohydrate as a metabolic substrate during both states. Carbon dioxide retention occurred during the first 36 h of estivation, resulting in an increase in hemolymph P_{CO_2} and a decrease in pH. The respiratory acidosis during short-term estivation was not compensated by elevation of hemolymph $[HCO_3^-]$ above levels predicted from the *in vitro* non-bicarbonate buffer value of hemolymph. A brief period of rapid CO_2 release, which caused hemolymph P_{CO_2} and pH to return to pre-estivation values, preceded the increase in O_2 consumption during arousal. Exposure of non-estivating snails to $4.67\,\text{kPa}$ P_{CO_2} ($1\,\text{kPa}=7.5\,\text{mmHg}$) caused a rapid and fully reversible 50% suppression of respiration rate. The temporal nature of CO_2 retention and release during entry into and arousal from estivation, and the suppression of O_2 consumption by artificial hypercapnia, support the hypothesis that elevated P_{CO_2} or the resultant acidosis may contribute to metabolic suppression during estivation by land snails.

Introduction

Many terrestrial pulmonate snails respond to desiccating environmental conditions by entering estivation, a behavior which minimizes evaporative water loss (Machin, 1975). The period of estivation, which may last up to several years, commences as the snail withdraws into its shell, secretes one or more epiphragma, and enters a metabolically quiescent condition. Rates of respiration may be

reduced by more than 80 % within days (Horne, 1973), and during extended estivation oxygen consumption may approach undetectable levels (Schmidt-Nielsen *et al.* 1971). While physiological alterations that occur during estivation have received renewed interest over the past few years (see Barnhart, 1989), major questions remain unresolved regarding the metabolism of estivating snails. We have undertaken the present study in an attempt to address some of these questions and to extend our understanding of metabolic rate suppression during estivation by land snails.

First, all previous studies of the metabolism of estivating gastropods have employed respirometry, an approach that assumes completely aerobic metabolism. The fact that land snails can survive anoxic conditions has long been appreciated (von Brand, 1946), although the extent to which anaerobic pathways of energy production are involved during estivation is unclear. While estivating snails do not typically experience environmental anoxia, ventilation is discontinuous (Barnhart and McMahon, 1987), and prolonged periods of apnea can result in pulmonary oxygen tensions as low as 0.35 kPa (Barnhart, 1986b). At such low tensions, anaerobic metabolism may be recruited to supplement aerobic energy production and, as a consequence, respiration rate would underestimate metabolic rate. Thus, we have utilized simultaneous calorimetry and respirometry to measure energy flow from snails before and during a short period of estivation. By comparing heat dissipation with oxygen consumption, we address the question of the possible involvement of anaerobic metabolism under these conditions.

Second, carbon dioxide retention during estivation has been inversely correlated with oxygen consumption (Barnhart and McMahon, 1987), leading to the suggestion that hypercapnia or the related acidosis may be causally associated with metabolic rate reduction during estivation (Barnhart, 1989). This hypothesis predicts that alterations in the acid–base status of the snail should be temporally related to changes in metabolic rate during entry into and arousal from estivation. We tested this hypothesis by monitoring oxygen consumption, carbon dioxide release and extracellular acid–base status of snails during these transitions. Additionally, recent work describing hypercapnia and acidosis in estivating snails has been performed with a single species, *Otala lactea* (family Helicidae). We have used *Oreohelix* spp. (family Oreohelicidae), native to semiarid western North America (Bequaert and Miller, 1973), to investigate whether the relationship between hypercapnia/acidosis and suppression of respiration is a common feature of estivation in land snails.

Materials and methods
Animals

Specimens of *Oreohelix* spp. were collected near Glenwood Springs, Colorado, in September 1988 and 1989. In this study, we did not distinguish between the two species of *Oreohelix* that occur at this collection site. [Previous electrophoretic and morphological studies (Rees, 1988; B. B. Rees and S. C. Hand, unpublished

observations) have shown that *O. strigosa* and *O. subrudis* constitute approximately 95 % and 5 %, respectively, of the snails collected here.] Snails were maintained in the laboratory at ambient temperature (20–25°C) and relative humidity (20–60 %). Water, lettuce, carrots and chalkboard chalk (a source of calcium) were provided for approximately 1 week each month, and snails were allowed to estivate between feeding intervals (Barnhart and McMahon, 1987). The whole mass (soft tissue plus shell) was about 0.3 g for snails used in calorimetry and about 1 g for all others. The dry tissue mass was 21.4±0.5 % (S.E.M., $N=13$) of the soft tissue mass for snails used in calorimetry and respirometry experiments.

Two to four days prior to experimentation, snails were given food and water to ensure active metabolism. Typically snails were removed from food and transferred to the experimental temperature (25°C) 4–6 h before measurements commenced. Metabolic state of the snails during experiments was controlled by altering relative humidity (Herreid, 1977). Experiments were begun with non-estivating snails held under conditions of high relative humidity (RH≥80 %), and estivation was induced by transferring snails to dry conditions (RH≤20 %).

Calorimetry

Measurements of heat dissipation of non-estivating and estivating snails were made with an LKB 2277 thermal activity monitor (Gnaiger, 1983b), and gas exchange was measured simultaneously (see below). Snails were individually housed in a 3.5 ml stainless-steel perfusion chamber, and the tubing for incurrent and excurrent gases was stainless steel or Viton. Heat dissipation in microwatts was time-corrected by the calibration unit of the calorimeter; typical time constants were $t_1=420$ s and $t_2=14\,000$ s. Data were sampled every 60 s and stored on an IBM XT personal computer, as well as on a strip chart recorder. All experiments were performed at 25°C.

Heat dissipation of non-estivating snails was measured while the chamber was perfused with air of 100 % relative humidity at 20 ml h^{-1}. Gas perfusion rate was controlled by a syringe pump. To ensure complete saturation, inflowing air was humidified at 26°C, and a piece of synthetic sponge (5 mm×5 mm×8 mm) and 0.3 ml deionized water were placed in the chamber. In the absence of an animal, heat dissipation was generally within 1 μW of the baseline achieved under dry conditions, indicating that no significant net evaporation or condensation occurred. Calorimetric measurements with non-estivating snails extended for 6–8 h. Following this interval, snails usually had their feet extended and no snail had formed an epiphragm.

Prior to measurements during estivation, data collection and gas perfusion were temporarily interrupted while the measuring chamber and snail were removed from the calorimeter and thoroughly dried. The chamber and snail were replaced into the calorimeter and perfused with dry air at an elevated flow rate (5–10 ml min^{-1}) for 12–24 h. The flow rate was reduced to 3 ml h^{-1} and measurements of heat dissipation and gas exchange were re-initiated. Measurements extended for 3–4 days during estivation. At the end of this period, we noted that

all snails had formed epiphragma and, when provided with moisture, all individuals became active. Immediately after each experiment, heat dissipation of the empty chamber was measured to correct for the presence of fecal material and/or baseline drift. This correction was typically less than $2\,\mu W$.

During estivation, it was necessary to determine accurately the amount of evaporative water lost from each snail in order to correct the measured heat dissipation. In each experiment, mass loss of the chamber plus snail was determined to the nearest 0.01 mg over an interval of 30–40 h. Because snails lose water and dry mass proportionately during estivation and maintain a constant 80 % tissue water (B. B. Rees and S. C. Hand, unpublished results), water loss was estimated as 0.8 times the measured mass loss. The amount of water loss was relatively consistent between experiments ($1.065\pm0.081\,\mu l\,H_2O$ per 40 h interval; $N=4$). Water loss was apportioned across the estivation interval based on the ventilatory/activity pattern of the snail, which was estimated by integrating the cyclic fluctuations in the calorimetric signal (taken as a relative indicator of ventilation/activity during estivation; see Fig. 1B). Standard conversion factors for the latent heat of vaporization at 25°C were used to calculate the endothermic signal generated by evaporative water loss.

Respirometry

Oxygen consumption ($\dot{n}_{O_2}$) and carbon dioxide release were monitored concurrently with measurements of heat dissipation. Excurrent gas was collected in glass syringes inverted under mineral oil. In a typical experiment, the gas collection intervals were 1 h during measurements with non-estivating snails and 8 h during estivation. When sufficient volume had been collected ($\geqslant16\,ml$), the gas was quantitatively removed and injected into an airstream drawn at $25\,ml\,min^{-1}$ through desiccant (Drierite), an Anarad CO_2 analyzer, an Applied Electrochemistry A-3A O_2 analyzer, and an R-1 flow controller. Signals from the analyzers were recorded on a strip chart recorder and read to 0.001 %. Changes in O_2 and CO_2 caused by the snails were usually less than 1 %. Diffusion of O_2 from ambient air into the collected gas was checked and found to be negligible. In the case of CO_2, a slow rate of diffusive loss into the mineral oil was measured, and the CO_2 values were corrected accordingly. The fractional content of water vapor and CO_2 in excurrent air were taken into account in the calculations of rates of gas exchange (Withers, 1977).

One objective of this study was to describe the temporal nature of metabolic suppression during entry into estivation and metabolic reactivation upon arousal. Since large heat signals are generated by evaporation and condensation of water, we could not use calorimetry to measure energy flow from snails accurately during transitions where relative humidity was changing. Thus, separate experiments were performed in which rates of gas exchange were monitored across these transitions. Individual snails were placed in glass flow-through chambers (approx. 13 ml volume) in a water bath maintained at 25 ± 1°C. Snails were in continuous darkness during measurement. Rates of gas exchange of non-estivating snails were

measured over 2 h during perfusion with air of 100 % relative humidity. Estivation was induced, without interrupting measurements, by perfusion of the chamber with dry air, and gas exchange was measured for the next 3 days. Arousal was effected by resuming perfusion with 100 % relative humidity.

To test the effect of elevated CO_2 on metabolism, we measured the $\dot{n}_{O_2}$ of snails exposed to 5.75 % CO_2 in air (P_{CO_2}=4.53–4.73 kPa, depending upon barometric pressure). $\dot{n}_{O_2}$ was measured over 1-h intervals in ambient air, during a 6-h hypercapnic exposure and following return to ambient air. The relative humidity of inflowing gas was maintained at 100 % throughout the experiment and the temperature was 25±1°C. A control experiment was performed with the same individuals to monitor $\dot{n}_{O_2}$ over a 9-h exposure to ambient air.

Hemolymph acid–base measurements

Hemolymph pH and P_{CO_2} were measured with a Radiometer Blood Micro System Mk II (BMS) at 25±0.5°C. The pH electrode of the BMS was calibrated with Radiometer precision pH buffers, and the CO_2 electrode was calibrated with CO_2 standards obtained from the London Company. Measurement of P_{CO_2} can be compromised by capacitance of the Radiometer P_{CO_2} electrode (Boutilier *et al.* 1978), and a correction is necessary when measuring small sample volumes (Barnhart, 1986*b*). To determine this correction for *Oreohelix* spp. hemolymph was tonometered with gases of known P_{CO_2}, and P_{CO_2} of 100 μl samples was measured. The true sample P_{CO_2} (P_{true}) was related to the initial reading of the electrode (P_{init}) and the measured P_{CO_2} (P_{meas}) by the following equation:

$$P_{true} = P_{meas} + F(P_{meas} - P_{init}).$$

The value of the correction factor, F, was found to be 0.34±0.11 (S.E.M., N=5). Accuracy of the corrected values was approximately ±0.27 kPa.

Non-estivating snails were kept in a humidified, darkened, temperature-controlled cabinet (25±1°C). Hemolymph (approx. 150 μl) from snails was sampled anaerobically from the perivisceral sinus (Barnhart, 1986*a*), and pH and P_{CO_2} were determined. After the initial sampling interval, the remaining snails were placed over desiccant, and dry air was perfused into the temperature cabinet to induce estivation. Hemolymph was sampled and measured as above at regular intervals during the next 3 days. Following estivation, one group of snails was moistened with water to promote arousal, and hemolymph was sampled 2 h later.

One group of non-estivating snails was exposed to 4.67 kPa CO_2 in humidified plastic bags for 2–6 h at room temperature (23–25°C). Hemolymph pH and P_{CO_2} were determined as above.

Hemolymph [HCO_3^-] was calculated with the Henderson–Hasselbach equation for each sample. The value of 6.189 was taken as the pK of carbonic acid in snail hemolymph at 25°C and physiological ionic strength (Barnhart, 1986*a*), and the solubility of CO_2 was assumed to be 0.33 mmol l^{-1} kPa^{-1} (Harned and Davis, 1943).

The non-bicarbonate buffer value was determined in hemolymph pooled from eight non-estivating snails. Samples of hemolymph (approx. $100\,\mu l$) were tonometered with humidified mixtures of CO_2 in N_2 provided by Wösthoff gas-mixing pumps. After 30–60 min of tonometry, pH was measured with the Radiometer BMS and total carbon dioxide (C_{CO_2}) was measured as described by Barnhart (1986a). Protein concentration in the pooled hemolymph was determined by the Lowry assay using bovine serum albumin as the standard (Peterson, 1977).

Statistics

Values are presented as means±the standard error of the mean (s.e.m.). Where appropriate, means were compared using the t-test. The slope of the in vitro non-bicarbonate buffer line was compared to the slope of the line drawn through experimental points with the test for equality of slopes in the analysis of covariance program of SPSS-X, version 3. In all cases, a probability of ≤0.05 was considered statistically significant.

Results

Measurements of metabolic variables

During perfusion with air of 100 % relative humidity, the metabolism of *Oreohelix* spp. was characterized by periods of reduced and stable heat dissipation, as well as periods of high and fluctuating heat dissipation (Fig. 1A). The periods of low heat dissipation probably reflected periods of minimal locomotor activity, while the periods of elevated heat dissipation were attributed to activity by the snail. For the purposes of data presentation, we will refer to these two conditions as standard and active metabolism, respectively (see Discussion). Rates of heat dissipation and gas exchange typically varied by a factor of 2–3 between the two states. Values for the quantity of heat dissipated per unit of oxygen consumed (C/R ratio) were $-461\pm12\,\text{kJ}\,\text{mol}^{-1}\,O_2$ ($N=5$) during standard metabolism and $-487\pm8\,\text{kJ}\,\text{mol}^{-1}\,O_2$ ($N=5$) during active metabolism. These values are close to theoretical oxycaloric values for aerobic metabolism (-445, -451 and $-478\,\text{kJ}\,\text{mol}^{-1}\,O_2$ for catabolism of lipid, protein and carbohydrate, respectively; Gnaiger, 1983a), and fall in the range of experimentally determined C/R ratios for aerobic organisms (Gnaiger and Staudigl, 1987). Respiratory exchange ratios (R) for non-estivating snails were consistent with a primarily carbohydrate-based metabolism during both standard and active conditions (Table 1).

When snails were transferred to dry conditions, heat dissipation and $\dot{n}_{O_2}$ dropped dramatically. Within 4 days, both measures of energy flow had declined to approximately 17 % of the standard non-estivating rate (Fig. 1B, Table 1). The C/R ratio for each individual was computed from the heat dissipated and oxygen consumed between consecutive measurements of mass (e.g. 57–89 h in Fig. 1B). These values were consistent with a completely aerobic metabolism during estivation ($-464\pm26\,\text{kJ}\,\text{mol}^{-1}\,O_2$; Table 1). The respiratory exchange ratio of
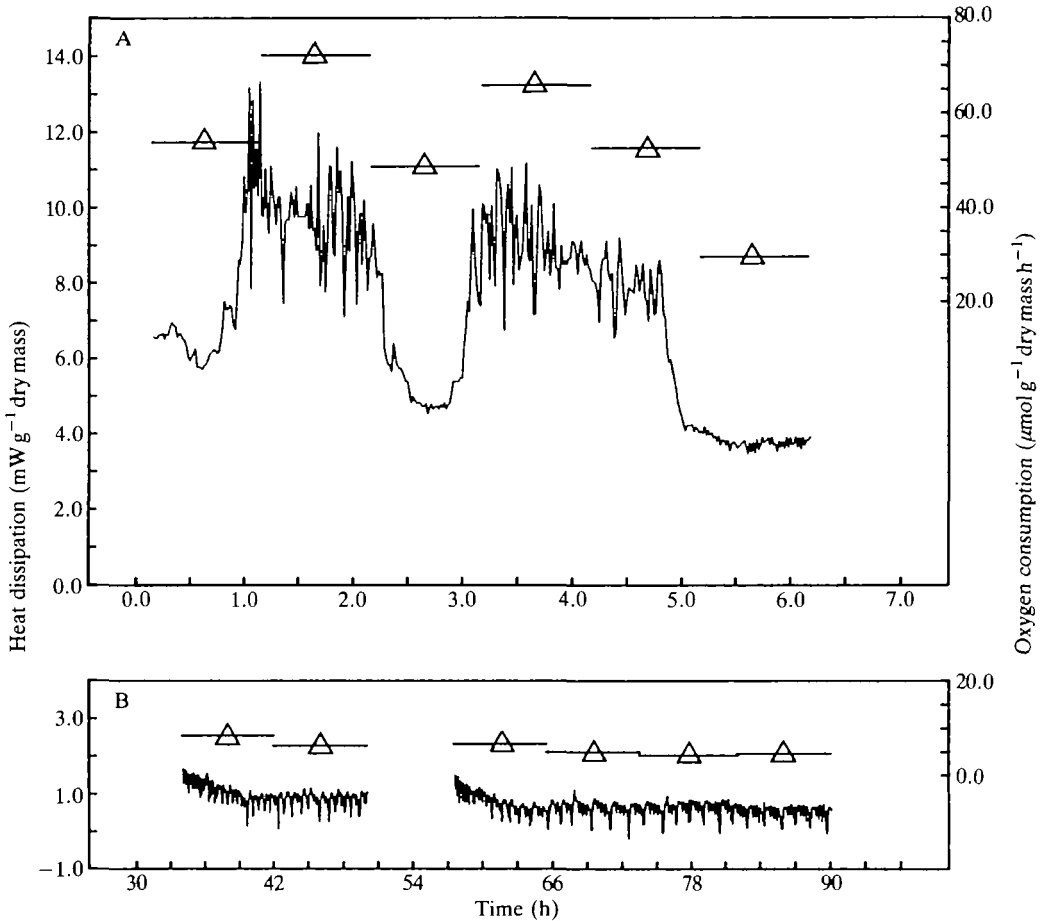
Fig. 1. Calorimetric and respirometric data collected from an individual *Oreohelix* before and during estivation. (A) Continuous measurement of heat dissipation and oxygen consumption (△) during a 6-h control period prior to induction of estivation. (B) Continuous measurement of heat dissipation and oxygen consumption (△) during a period of estivation of approximately 2.5 days. Data collection was interrupted between 50 and 57 h during which time the snail and measuring chamber were removed and weighed. The trace of heat dissipation has been corrected for evaporative water loss. In both A and B, the length of the horizontal bars indicates the time over which gas was collected for measurements of gas exchange. Artifactual heat transients of 2–5 min caused by gas sampling have been deleted from the record. The abscissa is time measured from the beginning of the experiment. Heat dissipation and gas exchange were not measured between 7 and 30 h, while the snail and measuring chamber were perfused with dry air at 5–10 ml min^{-1}.

0.953 (Table 1) suggested a primary dependence upon carbohydrate catabolism early in estivation.

Based upon measurements of $\dot{n}_{O_2}$, metabolism in *Oreohelix* spp. was rapidly arrested during entry into estivation, and this suppression was quickly reversed

Table 1. *Heat dissipation, oxygen consumption* ($\dot{n}_{O_2}$), *calorimetric–respirometric ratios (C/R) and respiratory exchange ratios (R) for non-estivating and estivating* Oreohelix *spp.*

	Heat dissipation (J g^{-1} dry mass h^{-1})	$\dot{n}_{O_2}$ (μmol g^{-1} dry mass h^{-1})	C/R (kJ mol^{-1} O$_2$)	R (mol CO$_2$ mol^{-1} O$_2$)
Standard (N=5)	16.5±1.3	35.8±3.0	−461±12	0.992±0.021
Active (N=5)	34.3±1.3*	70.4±2.5*	−487±8	0.932±0.014*
Estivating (N=4)	2.9±0.3*	6.2±0.5*	−464±26	0.953±0.032

Values for standard and active states are from the 1-h intervals during the control period (perfusion with 100 % RH) characterized by the lowest and highest heat dissipation, respectively.

Values for estivating snails were determined from 28–32 h of continuous data collection, beginning no earlier than 2 days after induction of estivation.

Asterisks indicate significant differences when compared to the value during standard metabolism (*t*-test, $P < 0.05$).

Values are means±S.E.M.

upon arousal (Fig. 2A). The non-estivating $\dot{n}_{O_2}$ ranged from 50 to 100 μmol O$_2$ g^{-1} dry mass h^{-1}. Upon reduction of ambient relative humidity, $\dot{n}_{O_2}$ decreased to approximately 25 μmol O$_2$ g^{-1} dry mass h^{-1} within 12 h, and continued to decline over the next 36 h. Between 48 and 72 h from the beginning of the bout of estivation, $\dot{n}_{O_2}$ fluctuated around 10 μmol O$_2$ g^{-1} dry mass h^{-1}. When snails were returned to an environment of high relative humidity, $\dot{n}_{O_2}$ rose over 2 h and peaked at about 45 μmol O$_2$ g^{-1} dry mass h^{-1}. Values during arousal were more variable than at other times, probably reflecting variation in the timing and extent of arousal among individuals (Herreid, 1977).

Measurements of carbon dioxide retention

Respiratory exchange ratios (Fig. 2A) indicated that CO$_2$ retention occurred during the early part of estivation and that CO$_2$ release occurred during arousal from estivation. Over the first 24 h of estivation, the average value for R ranged from 0.63 to 0.70. During the first hour of returning to perfusion with air of 100 % relative humidity, R increased to 1.47±0.10 (N=8) and was as high as 1.9 in one individual. The period of CO$_2$ release was brief, and R was 0.94–1.00 following initial arousal.

The rate of change in whole-body CO$_2$ content (ΔC_{CO_2}; Fig. 2B) can be calculated as the product of $\dot{n}_{O_2}$ and the difference between the true respiratory quotient (RQ) and the measured respiratory exchange ratio (Barnhart and McMahon, 1987):

$$\Delta C_{CO_2} = \dot{n}_{O_2}(RQ - R).$$

The value of 0.953 (Table 1) was assumed for RQ because it was determined over an extended period of estivation and presumably reflected a steady state between
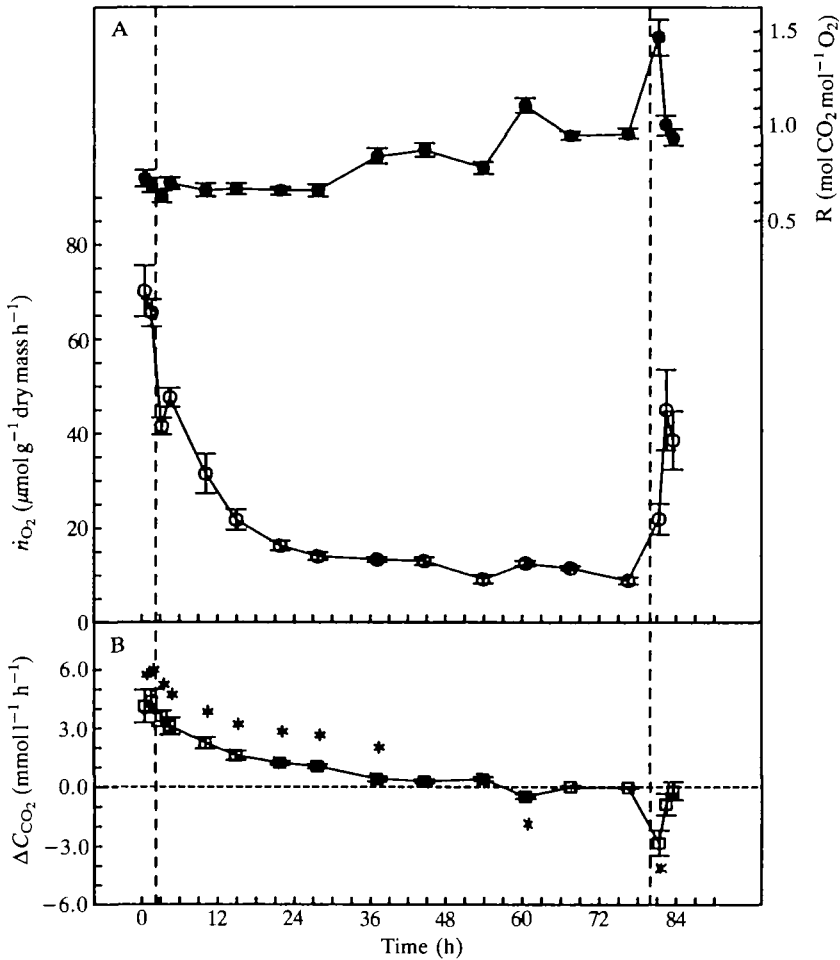
Fig. 2. Measurements of gas exchange by eight individuals during a cycle of activity, estivation and arousal. Vertical dashed lines indicate induction of estivation with dry air (at 2 h) and arousal from estivation with air of 100 % relative humidity (at 80 h). (A) Oxygen consumption (○) and respiratory exchange ratios (●). (B) ΔC_{CO_2} (□) corrected for tissue water content of each snail. Asterisks indicated values significantly different from $0\,\mathrm{mmol\,l^{-1}\,h^{-1}}$ (95 % confidence intervals do not overlap $0\,\mathrm{mmol\,l^{-1}\,h^{-1}}$). Means±s.e.m are presented.

CO_2 production at the tissue and its release from the organism. Over the first part of the estivation experiment, ΔC_{CO_2} was approximately $4\,\mathrm{mmol\,l^{-1}\,h^{-1}}$, gradually decreasing to values indistinguishable from $0\,\mathrm{mmol\,l^{-1}\,h^{-1}}$ after 36 h. Upon perfusion with air of 100 % relative humidity the value for ΔC_{CO_2} became negative, indicating CO_2 release. Interestingly, during arousal, the greatest rate of CO_2 release preceded the peak value of $\dot{n}_{O_2}$ by approximately 1 h. After the initial release of CO_2, ΔC_{CO_2} was again not different from $0\,\mathrm{mmol\,l^{-1}\,h^{-1}}$.

Carbon dioxide retention by snails during estivation resulted in inverse changes
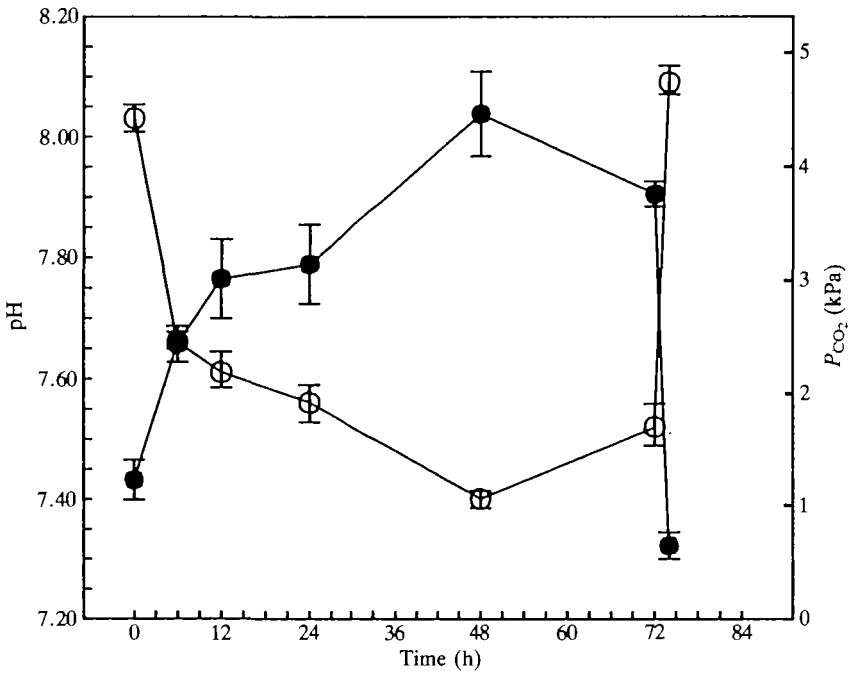
Fig. 3. Hemolymph P_{CO_2} (●) and pH (○) measured in *Oreohelix* spp. before, during and after arousal from estivation. The 0 h values were determined with non-estivating individuals and 74 h values were determined 2 h after arousal. Means±S.E.M are presented, and the number of snails was seven or eight at each time.

in hemolymph P_{CO_2} and pH (Fig. 3). Hemolymph P_{CO_2} increased from 1.24±0.17 kPa ($N=8$) in active snails to 3.15±0.35 kPa ($N=8$) at 24 h estivation, and to 4.47±0.35 kPa ($N=7$) by 48 h. A concomitant drop in hemolymph pH occurred from 8.03±0.02 ($N=8$) to 7.40±0.01 ($N=8$) by 48 h. Between 48 and 72 h, hemolymph P_{CO_2} and pH were relatively stable. Arousal caused a rapid decline in hemolymph P_{CO_2} and an increase in pH.

The data from Fig. 3 were plotted as a Davenport diagram (Davenport, 1974) in order to assess whether the acidosis was respiratory or metabolic in nature, and whether compensation had occurred (Fig. 4). Values measured during estivation were distributed above the *in vitro* buffer line, although the slope of the line describing the experimental points was not significantly different from that of the buffer line ($P=0.934$).

The effect of imposed hypercapnia

Exposure of non-estivating snails to 4.67 kPa ambient CO_2 resulted in an elevation of hemolymph P_{CO_2} to 4.0±0.21 kPa ($N=4$) and a concomitant decrease in pH to 7.59±0.02 ($N=6$). The changes occurred within 2 h and were stable through 6 h of hypercapnic exposure. An increase in hemolymph [HCO_3^-]
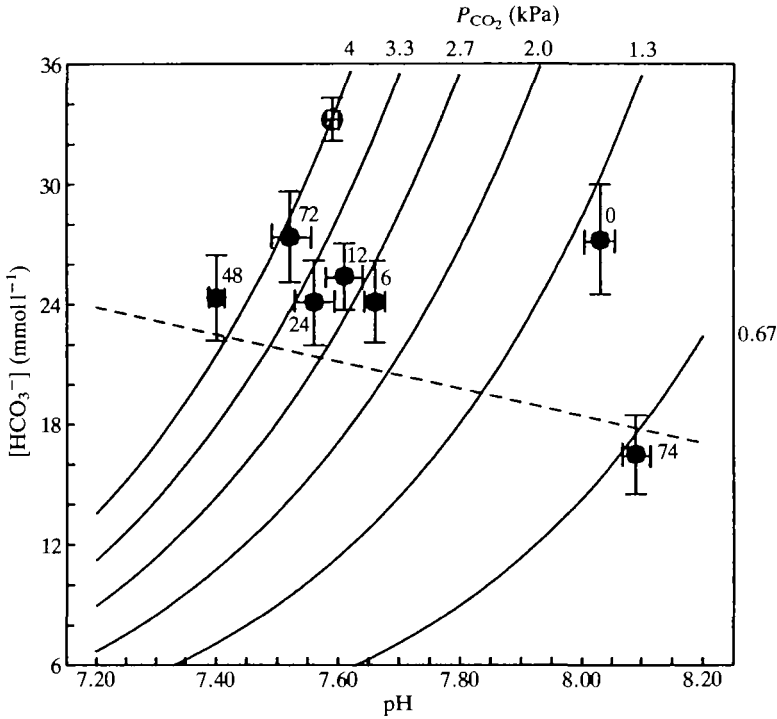
Fig. 4. Data from Fig. 3 plotted as a Davenport diagram. The time of sampling (h) is denoted above and to the right of the symbols. The open circle represents values obtained from non-estivating snails made hypercapnic by exposure to 4.67 kPa CO_2 ($N=4$). In a separate experiment, the slope and elevation of the buffer line were determined *in vitro* on hemolymph pooled from eight non-estivating individuals (dashed line). The slope of the buffer line is $-7.0 \, \text{mmol} \, l^{-1} \, HCO_3^- \, \text{pH unit}^{-1}$ and the protein content of the sample was $10 \, \text{mg} \, \text{ml}^{-1}$. Means$\pm$s.e.m are presented.

suggested that the respiratory acidosis during artificial hypercapnia was partially compensated (open circle, Fig. 4).

The effect of hypercapnia on $\dot{n}_{O_2}$ of non-estivating snails is shown in Fig. 5. Exposure to 4.67 kPa CO_2 caused $\dot{n}_{O_2}$ to fall to from control values to approximately $27 \, \mu\text{mol} \, g^{-1} \, \text{dry mass} \, h^{-1}$. The drop in respiration rate was fast and fully reversible. Compared to the $\dot{n}_{O_2}$ of the same individuals exposed to ambient CO_2 (approx. 0.03 kPa), the reduction in respiration was approximately 50 %.

Discussion

Metabolism of non-estivating snails

Oreohelix spp. are characterized by respiration rates during non-estivating conditions that are typical of other terrestrial pulmonate gastropods (see Table 2). Continuous measurements of metabolic heat production and gas exchange revealed two rather discrete states during the non-estivating period, and we have
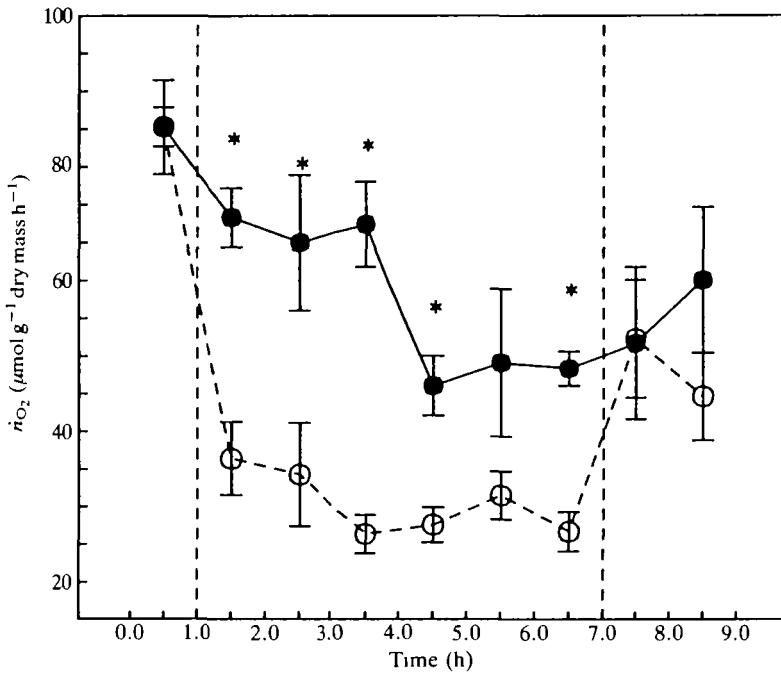
Fig. 5. The effect of imposed hypercapnia on respiration rate of non-estivating *Oreohelix* spp. ($N=4$). Snails were exposed to ambient air (●) or 4.67 kPa CO_2 in air (○). The vertical dashed lines bracket the period of elevated P_{CO_2}. The relative humidity of the perfusion gas was 100 % throughout. Asterisks indicate a significant reduction of $\dot{n}_{O_2}$ by hypercapnia compared to control value at that time (*t*-test). Means±S.E.M are presented.

referred to these states as standard and active metabolism. Rates of energy flow from snails varied by a factor of 2–3 between these two states, similar to the increment between average and peak oxygen consumption of non-estivating *Otala lactea* (Herreid, 1977; Table 2). In our experiments, it is possible that true maximal activity was not achieved by the animals due to the length of time since feeding and restricted movement in the measuring chamber. Hence the ratio of the active to standard rates of metabolism observed in this study may underestimate the animal's scope for activity (cf. Bayne and Newell, 1983). Of more interest in the context of this study is that the amount of heat dissipated per unit of oxygen consumed (C/R ratio) did not deviate from the theoretical value for fully aerobic metabolism in either state. Thus, we conclude that anaerobic pathways are not involved in standard or active metabolism. In terms of metabolic fuel for non-estivating metabolism, the respiratory exchange ratios indicate a primary dependence upon carbohydrate during both standard and active metabolic states.

Metabolism during estivation

Using the technique of simultaneous calorimetry and respirometry, we found

that the quantity of heat dissipated per mole of oxygen consumed during estivation did not differ from the theoretical values for aerobic metabolism. We conclude, therefore, that during early estivation anaerobic processes do not contribute to the energy metabolism in this organism. This is in accord with the finding that products of anaerobic metabolism do not accumulate in the land snail *Otala lactea* over 3 days of estivation (Churchill and Storey, 1989). After 3 weeks of estivation, however, lactate has been measured in *Otala lactea* (Churchill and Storey, 1989), and alanine and succinate accumulate in *Achatina achatina* (Umezurike and Iheanacho, 1983). Combined calorimetry and respirometry would be useful in substantiating and quantifying the contribution of anaerobic metabolism to the total energy metabolism during extended estivation.

Another interesting feature of the reduction in heat dissipation and gas exchange during entry into estivation is the rapidity with which it occurs. In the two snails for which the time course of metabolic suppression has been studied (*Bulimulus dealbatus* and *Oreohelix* spp.), the most rapid decline in respiration occurs during the first few days of estivation. Within 4 days of entry into estivation, respiration rate is reduced sixfold in both snails and, in *Bulimulus dealbatus*, respiration rate does not decrease further over 6 months (Horne, 1973). In the context of metabolic rate reduction, it is of interest to note that Churchill and Storey (1989) documented a positive metabolic cross-over at the phosphofructokinase and pyruvate kinase reactions during a 3-day bout of estivation in *Otala lactea*. These data were taken to indicate an activation of glycolysis during short-term estivation. This conclusion is difficult to reconcile with the rapid suppression of metabolic rate observed in other studies.

A correlate of reduced rates of respiration is a decrease in evaporative water loss (Barnhart, 1986*b*). Thus, the capacity to reduce respiration may represent an adaptation to survival in desiccating environments (Machin, 1975). Representative examples of oxygen consumption by active and estivating pulmonate gastropods are presented in Table 2. The slug *Limax flavus*, which occurs in mesic habitats and has a limited tolerance of desiccating conditions, is characterized by the highest respiration rate during estivation. At the other extreme is *Sphincterocila boissieri*, a snail found in the deserts of the Near East, which is characterized by the lowest rate of respiration during estivation. The estivating rates of oxygen consumption are intermediate in *Bulimulus dealbatus*, *Oreohelix* and *Otala lactea*, all of which occur in semiarid environments (Bequaert and Miller, 1973). Hence, the extent of reduction in respiration appears to be related to environmental water availability, such that lower rates of respiration, and thus lower rates of water loss, occur in individuals inhabiting more xeric environments.

Carbon dioxide retention and extracellular acidosis

Carbon dioxide retention and extracellular acidosis occur during entry into estivation with a timing comparable to that of the reduction in respiration rate. CO_2 retention occurred over the first 36 h of estivation, causing substantial changes in hemolymph P_{CO_2} and pH, coincident with a rapid decrease in

Table 2. *Respiration rates ($\mu mol\, O_2\, g^{-1}\, h^{-1}$) by terrestrial pulmonate gastropods during non-estivating and estivating conditions, standardized to wet and dry masses*

Species (study temperature, °C)	Non-estivating		Estivating		Reference
	Wet mass	Dry mass	Wet mass	Dry mass	
Limax flavus (30)	10.3	60	7.1	42	Horne, 1979
Bulimulus dealbatus (22)	5.4	31	1.1	6.6	Horne, 1973
Oreohelix spp. (25)	8.2–16.2	36–70	1.4	6.2	Present study
Otala lactea (20)	3.9–6.3*	20–32	1.1*	5.6	Herried, 1977
Otala lactea (23–25)	3.8	26	0.61	4.2	Barnhart, 1986a; Barnhart and McMahon, 1987
Sphincterochila boissieri (25)	NA†	NA†	0.17‡	0.9	Schmidt-Nielsen et al. 1971

Wet-mass-specific rates of oxygen consumption were adjusted to dry-mass-specific rates with factors reported in original studies.

* The value of 0.57 was used to convert whole mass (including shell) to wet tissue mass (Herreid, 1977).

† Values for oxygen consumption of non-estivating snails were not available.

‡ Whole snail oxygen consumption rates were divided by 1.8 g soft tissue per snail (Schmidt-Nielsen *et al.* 1971).

respiration rate. Between 48 and 72 h, hemolymph P_{CO_2} and pH were relatively stable, as was oxygen consumption. During arousal, a period of CO_2 release occurred, resulting in restoration of normal hemolymph P_{CO_2} and pH prior to the increase in oxygen consumption. In an extensive study of the pattern of carbon dioxide release from *Otala lactea* during estivation, Barnhart and McMahon (1987) found a correlation between periods of CO_2 release and periods of elevated oxygen consumption. During a 3-day bout of estivation, respiration rate in *Oreohelix* spp. was similarly elevated during a brief period of CO_2 release (approx. 60 h, Fig. 2). This temporal relationship between respiration rate and acid–base status suggests that alterations of P_{CO_2} and/or pH may contribute to the suppression of metabolic rate during estivation.

Furthermore, when non-estivating *Oreohelix* spp. were exposed to elevated ambient P_{CO_2}, oxygen consumption was markedly reduced. The reduction in respiration, however, was only 50 %, compared to 83 % during estivation. This discrepancy may be explained, in part, by the differing degree of compensation that occurred during artificially imposed hypercapnia relative to estivation (Fig. 4). Although imposed hypercapnia caused hemolymph P_{CO_2} to increase to levels approximating those measured during estivation, hemolymph pH dropped only to 7.59 compared to 7.40. The relatively higher pH and [HCO_3^-] during artificial hypercapnia compared to estivation may have acted to lessen the drop in respiration, similar to the findings of Walsh *et al.* (1988) for trout hepatocytes. In

addition to acid–base variables, it is likely that other factors, that were not operative during artificial hypercapnia, contribute to metabolic rate suppression during estivation. We speculate that metabolic suppression during estivation may be the result of multiple determinants, possibly including neurohormonal agents.

Oreohelix spp. displayed an acid–base condition and suppression of metabolism similar to those documented for *Otala lactea*. The comparison between snails, however, reveals interesting differences. Extracellular acidification in *Oreohelix* spp. during the first 3 days of estivation was greater than that observed in *Otala lactea* (Barnhart, 1986a). In addition, respiration rate of *Oreohelix* spp. was more affected by ambient hypercapnia: oxygen consumption decreased by 50 % at ambient P_{CO_2} of approximately 4.67 kPa, whereas a 50 % reduction was not observed in *Otala lactea* until P_{CO_2} had been increased to 8.66 kPa (Barnhart and McMahon, 1988). Both observations may be a result of differing responses to respiratory acidosis. *Otala lactea* displayed marked compensation (Barnhart, 1986a), whereas compensation by *Oreohelix* spp. was slight or non-existent. If hypercapnia is indeed involved in the suppression of metabolism, a species difference in the extent of compensation may modulate how fast metabolic rate is reduced during entry into estivation. While speculative, lack of compensation could reflect an adaptation to more xeric environments, in which bouts of estivation are more frequent or more profound.

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