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DISCONTINUOUS CARBON DIOXIDE RELEASE AND METABOLIC DEPRESSION IN DORMANT LAND SNAILS

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SUMMARY

The respiration of dormant land snails (*Otala lactea* Müller) is characterized by periodic retention and release of CO_2. Rates of oxygen uptake ($\dot{V}_{O_2}$) and CO_2 release ($\dot{V}_{CO_2}$) of individuals were recorded continuously for up to 21 days. $\dot{V}_{O_2}$ was usually low ($5{\cdot}6\,\mu l\,g^{-1}$ tissue h^{-1}) but increased up to five-fold at intervals between 20 and 50 h. Snails hypoventilated and retained CO_2 when $\dot{V}_{O_2}$ was low, while periods of elevated $\dot{V}_{O_2}$ commenced with hyperventilation and net CO_2 release. The ratio $\dot{V}_{CO_2}/\dot{V}_{O_2}$ varied between about 0·2 and 4·8 during these cycles. Calculated whole-body CO_2 content fluctuated over a range of about 4·3 mmol l^{-1} H_2O, and was inversely correlated with $\dot{V}_{O_2}$.

Cycles of CO_2 retention and release might be the result and/or the cause of changes in metabolic rate during dormancy. Ventilation is sensitive primarily to O_2, and O_2 transport appears to be diffusion-limited. A simple model based on these characteristics predicts hypoventilation and consequent CO_2 retention when $\dot{V}_{O_2}$ is reduced. Also, the close correlation of $\dot{V}_{O_2}$ and whole-body CO_2 content in snails suggests that CO_2 or acid–base balance might influence metabolic rate during dormancy. The relationship between discontinuous CO_2 release and respiratory water loss in insects and snails is discussed.

INTRODUCTION

Pulmonate land snails respond to dehydrating conditions by entering a state of quiescence (dormancy) in which the rates of oxygen consumption and evaporative water loss are greatly reduced. The ability to survive for months or years in this inactive condition permits snails to live in seasonally arid habitats in spite of their need for moisture during activity and feeding (reviewed by Nopp, 1974; Machin, 1975).

Ventilation of the snail lung occurs by diffusion across a closable pore, the pneumostome (Krogh, 1941). P_{CO_2} in the lung and haemolymph of *Otala lactea* increases and P_{O_2} decreases during dormancy, indicating that specific ventilation (i.e. ventilation relative to metabolic rate) is reduced (Barnhart, 1986*b*). External gas exchange becomes discontinuous as the pneumostome, which is open nearly

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continuously in active snails, opens only intermittently during dormancy (Nopp, 1971). In addition, $\dot{V}_{O_2}$ of dormant helicid snails shows large fluctuations in apparently quiescent and undisturbed individuals (Schmidt-Nielsen, Taylor & Shkolnik, 1971; Kratochvil, 1976). These bursts of O_2 consumption are sustained for hours or days and are distinct from short-term changes in O_2 uptake that result from intermittent breathing.

The retention of CO_2 in dormant snails strongly depresses extracellular pH (Burton, 1976; Barnhart, 1986*a*). One goal of the present study was to investigate the apparent correlation between CO_2 retention and reduction of metabolic rate during dormancy in *Otala*. Previous results indicated that fluctuations of $\dot{V}_{O_2}$ in dormant *Otala* might be correlated with a cycle of CO_2 retention and release (Barnhart, 1986*b*). In the present study, flow-through respirometry was used to investigate the temporal patterns of O_2 uptake and CO_2 release and to estimate the changes which occur in whole-body CO_2 content during dormancy.

A second objective of this study was to compare the pattern of gas exchange of dormant snails with that of insects. Certain large insects exhibit a discontinuous pattern of CO_2 release during rest or diapause, to which has been attributed a role in reduction of respiratory water loss (Miller, 1974, 1981). Although they are quite different morphologically, snails and insects encounter similar problems in resisting desiccation and starvation during prolonged dormancy. Comparison of the patterns of gas exchange in insects and snails is therefore of interest.

MATERIALS AND METHODS

Subjects

Snails were collected from an introduced population at Playa del Rey in Los Angeles County, California. The snails were transported to the University of Calgary and maintained in the laboratory on a diet of cabbage, carrots and Purina rat chow. Blackboard chalk was provided as a calcium source. Water and food were provided for approximately 1 week of each month. On this regimen, juvenile snails matured rapidly and only 5 of 160 adult specimens had died after 1 year of captivity. At least 2 weeks prior to respirometry, dormancy was induced by withholding food and water. Only adult snails having a fully formed shell were used for measurements. Whole mass (including shell) ranged between 7 and 10 g (Table 1).

Respirometry

An open mask flow-through system was used for respirometry. A transparent plastic mask was sealed over the aperture of the shell with dental wax, leaving a space between the mask and the mantle collar of the snail. Air was drawn through the mask at 10 ml min^{-1}, and then successively through desiccant (Drierite), CO_2 analyser, O_2 analyser and pump. Stainless-steel tubing was used throughout, except for the glass desiccant tube (3 ml) and a 6-cm length of narrow-bore Tygon tubing connecting the mask and desiccant tube.

The instruments used were an Applied Electrochemistry S-3A O_2 analyser, CD-3A CO_2 analyser and R-2 flow controller. The gas analysers were calibrated with air and with $O_2/CO_2/N_2$ standards analysed with a Scholander device (Scholander, 1947). Outputs from the gas analysers were sampled at a frequency of about 50 Hz, digitized by a 12-bit a./d. converter, averaged over 5-s intervals and displayed and recorded automatically by a microcomputer. Air flow rate ($10\,ml\,min^{-1}$) was measured downstream of the desiccant tube by means of a bubble flowmeter and was constant to within ±2 % during the measurement periods.

Rates of O_2 uptake ($\dot{V}_{O_2}$) and CO_2 release ($\dot{V}_{CO_2}$) were calculated using the following equations (see Withers, 1977, equation 3b and derivation):

$$\dot{V}_{O_2} = [\dot{V}_E \times (Fi_{O_2} - Fe_{O_2}) - (\dot{V}_{CO_2} \times Fi_{O_2})]/(1 - Fi_{O_2}) \quad (1)$$

$$\dot{V}_{CO_2} = \dot{V}_E \times (Fe_{CO_2} - Fi_{CO_2}), \quad (2)$$

where $\dot{V}_E$ is the flow rate ($l\,min^{-1}$), and Fi and Fe are the fractional concentrations of a gas entering and leaving the mask, respectively. Equation 1 corrects for changes in O_2 input to the mask due to difference between $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ (Withers, 1977). Equation 2 is simplified because Fi_{CO_2} is nearly zero, making the correction unnecessary.

The accuracy of the system was checked by injecting known volumes of a calibration gas (5 % CO_2, 95 % N_2) into the gas flow. This method mimics CO_2 release by adding CO_2 and mimics O_2 uptake by displacing a volume of air (20·95 % O_2) equal to the total injection volume. $\dot{V}_{CO_2}$ was calculated using equation 2 and $\dot{V}_{O_2}$ was calculated using the following:

$$\dot{V}_{O_2} = \dot{V}_E \times (Fi_{O_2} - Fe_{O_2}). \quad (3)$$

The resulting peaks were integrated for comparison with the injection volumes. Agreement was within ±2 % when measuring volumes similar to individual breaths (10–20 μl O_2 or CO_2). Time lags in the system were determined and subtracted to coordinate the O_2 and CO_2 records during data analysis.

Gas exchange was recorded in constant conditions of humidity, temperature and illumination. The snail with mask was placed on a lump of dental wax and covered by an inverted beaker, which was flushed continuously with air. Humidity in the beaker was controlled at 32 ± 2 % by bubbling the air through a saturated solution of $MgCl_2$ (Winston & Bates, 1960). Temperature was monitored with a thermocouple and remained at 24 ± 1 °C.

Changes in F_{O_2} and F_{CO_2} of the airstream due to respiration were usually less than 0·3 vol%. Over several hours, calibration of the S-3A may drift by up to 0·1 vol% at an F_{O_2} of 20·95 vol%, so frequent checking of Fi_{O_2} (baseline) was important for accuracy. The composition of air drawn through the mask when the pneumostome was closed did not differ measurably from ambient. Therefore, Fe_{O_2} and Fe_{CO_2} during each 'breath' (opening of the pneumostome) were compared with adjacent records of F_{O_2} and F_{CO_2} between breaths (pneumostome closed) in calculating gas exchange.

After baseline subtraction and synchronization, the $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ records were treated in two ways. First, in order to represent the rate of metabolic oxygen consumption more closely, a smoothed record of gas exchange was derived by integrating the data over successive, non-overlapping intervals which were of at least 30 min duration and included at least three breaths. Second, instantaneous rates (i.e. corrected for system washout characteristics) were calculated over the course of individual breaths in some records, in order to examine the dynamic aspects of diffusion gas exchange. Equations used were those of Bartholomew, Vleck & Vleck (1981). These calculations were also used to estimate the percentage of time that the pneumostome was open.

Whole-body CO_2 content

The overall metabolic respiratory quotient (RQ) estimated from long periods of respirometry (7–21 days) was 0·96 (Table 1). However, over shorter measurement intervals the ratio of CO_2 release to O_2 uptake varied between 0·22 and 4·8. These deviations of the respiratory exchange ratio (R) from the (presumed constant) metabolic RQ are assumed to indicate change of the whole-body CO_2 content (ΔC_{CO_2}), which was calculated for each measurement interval from O_2 uptake (M_{O_2}, mmol), R and RQ of each individual according to the following equation:

$$\Delta C_{CO_2} = M_{O_2} \times (RQ - R). \quad (4)$$

Lung volume

Following respirometry, the lung gas volume of each snail was measured by the method of Jones (1961). Briefly, the animal was placed in a closed, water-filled chamber connected with a manometer and a micrometer burette. The volume of the chamber was reduced by a known amount with the burette and the resulting change in pressure read from the manometer. The apparatus was calibrated with measured volumes of air. Precision was about $\pm 5\,\mu l$.

RESULTS

Oxygen uptake

$\dot{V}_{O_2}$ was often elevated for a day or two following placement of a dormant snail in the respirometry system. After this period of acclimation, long-term records of $\dot{V}_{O_2}$ show a pattern of periodic elevation above a relatively stable minimum (Fig. 1). The regularity and frequency of the fluctuations varied considerably within and between individuals, but the minimum or 'basal' $\dot{V}_{O_2}$ varied little (Table 1). Two individuals secreted calcareous epiphragms during respirometry (see below). In both cases, the periodic bursts of oxygen consumption appeared to be less frequent following this event (Fig. 1).

CO_2 release

Simultaneous records of $\dot{V}_{CO_2}$, $\dot{V}_{O_2}$ and other respiratory parameters in a representative individual are shown in detail in Fig. 2. When $\dot{V}_{O_2}$ was high R remaine

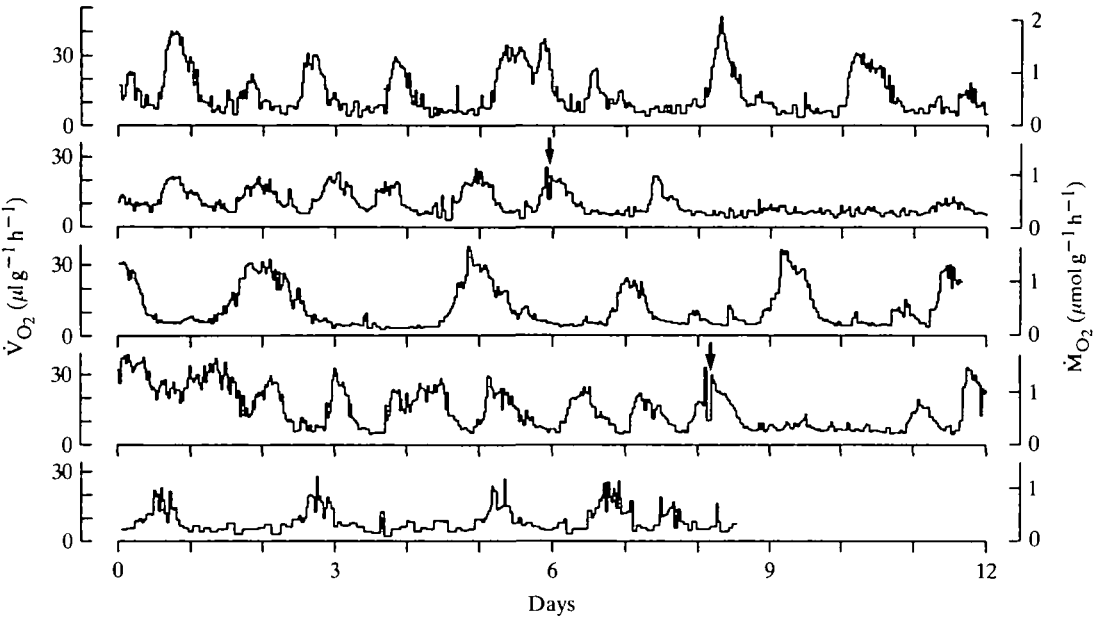

Fig. 1. Long-term records of oxygen consumption of five dormant individuals (23–25 °C, 32 % relative humidity, constant light). Each plot begins at midnight. Records were smoothed by calculating $\dot{V}_{O_2}$ over intervals which were at least 30 min and included at least three breaths (openings of the pneumostome). Arrows indicate events of epiphragm secretion.

Table 1. *Respiratory parameters of dormant* Otala lactea

Mass including shell (g)	8·56 ± 1·28 (6·99–9·95)
Wet tissue mass (g)	6·58 ± 1·17 (4·82–7·64)
Tissue water (% mass)	0·853 ± 0·015 (0·824–0·872)
Lung volume (μl ATP)	283 ± 109 (181–475)
Basal $\dot{V}_{O_2}$ (μl STPD g^{-1} wet tissue h^{-1})	5·6 ± 0·57 (5·0–6·5)
Burst $\dot{V}_{O_2}$ (μl STPD g^{-1} wet tissue h^{-1})	27·0 ± 5·39 (19·8–32·7)
Mean $\dot{V}_{O_2}$ (μl STPD g^{-1} wet tissue h^{-1})	13·7 ± 1·94 (11·2–16·3)
Respiratory quotient	0·964 ± 0·026 (0·913–0·990)
Minimum respiratory exchange ratio	0·22 ± 0·055 (0·16–0·31)
Maximum respiratory exchange ratio	4·77 ± 0·901 (3·19–5·26)
Range of C_{CO_2} (mmol l^{-1} H_2O)	4·3 ± 0·41 (3·5–4·8)

The data are derived from recordings of 7–21 days duration from each of seven dormant individuals.

Basal $\dot{V}_{O_2}$ is the lowest average $\dot{V}_{O_2}$ exhibited over 12 h by each individual.

Burst $\dot{V}_{O_2}$ is the average peak $\dot{V}_{O_2}$ of three or more bursts in each individual.

Range of C_{CO_2} is the average change during three or more major fluctuations of C_{CO_2} in each snail.

Values are mean ± S.E.M. (range).

Temperature was 23–25 °C, relative humidity 32 %.

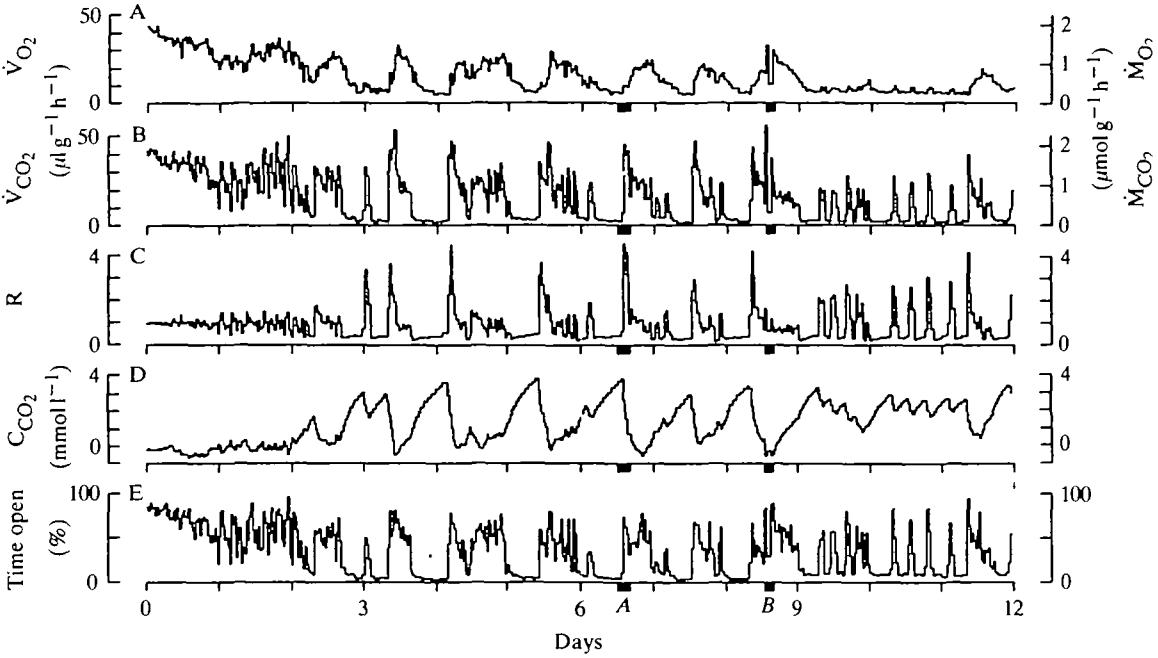

Fig. 2. Respiration of a dormant individual over 12 days. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ (A,B) were calculated for intervals of at least 30 min including at least three breaths. R (C) is the respiratory exchange ratio for each interval ($\dot{V}_{CO_2}/\dot{V}_{O_2}$). Total R (= RQ) for the entire measurement period was 0·98. Whole-body CO_2 content (D) was estimated at the end of each measurement interval as the cumulative difference between metabolic CO_2 production and CO_2 release. The C_{CO_2} scale is an interval scale without true zero (see Materials and Methods). *A*, bar indicates the 4-h interval shown in detail in Fig. 3. *B*, bar indicates a 3-h interval during which an epiphragm was secreted, shown in detail in Fig. 7.

relatively close to RQ and calculated changes of whole-body CO_2 content were minor (Fig. 2C,D). As $\dot{V}_{O_2}$ declined, R dropped below RQ and C_{CO_2} increased. Subsequent periods of elevated $\dot{V}_{O_2}$ always commenced with elevation of R and a large burst of net CO_2 release. During the longest periods of reduced $\dot{V}_{O_2}$, the increase of C_{CO_2} was limited by small bursts of CO_2 release, which were accompanied by only a slight change of $\dot{V}_{O_2}$ (e.g. last quarter of record in Fig. 2).

Breathing pattern

During periods of reduced $\dot{V}_{O_2}$ and CO_2 accumulation the pneumostome was open only about 3–5 % of the time (Fig. 2E). During these periods, breaths were brief (30–60 s) and breath frequency was low (2–3 h^{-1}) (Fig. 3). In contrast, during periods of elevated $\dot{V}_{O_2}$ and CO_2 release, breaths were more frequent and of longer duration (Fig. 3) and the pneumostome was open about 70 % of the time (Fig. 2E).

Periods of increased ventilation and net CO_2 release began with a series of prolonged breaths (Fig. 3). Comparison of instantaneous $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ during sustained opening of the pneumostome (Fig. 3) shows that $\dot{V}_{O_2}$ is initially highe

than $\dot{V}_{CO_2}$, but declines much more rapidly than $\dot{V}_{CO_2}$. CO_2 release continues at a high rate due to the high capacitance of the body fluids for CO_2. Thus, brief breaths are associated with net CO_2 accumulation, and prolonged breaths with net CO_2 release.

$\dot{V}_{O_2}$ and C_{CO_2}

$\dot{V}_{O_2}$ was inversely and linearly related to the logarithm of C_{CO_2} as these parameters varied over time (Fig. 4). The correlation was highly significant ($P < 0{\cdot}001$) in each of seven individuals tested. However, further analysis is difficult because the slope of y on log x depends on the absolute values of x. Absolute values of x (i.e. of C_{CO_2}) in this case are unknown, because the method used for calculating C_{CO_2} measures only change and not absolute values (see Materials and Methods). $\dot{V}_{O_2}$ varied about five-fold as C_{CO_2} ranged over about 4·3 mmol l^{-1} (Table 1).

Effect of changing external gas tensions

The effects of elevated external CO_2 (2%) and O_2 (42%) were tested in three individuals during periods of basal $\dot{V}_{O_2}$ and CO_2 accumulation. Results were qualitatively similar in each individual. During exposure to 2% CO_2, $\dot{V}_{CO_2}$ was reduced by about half compared to $\dot{V}_{CO_2}$ in air (Fig. 5). This change apparently

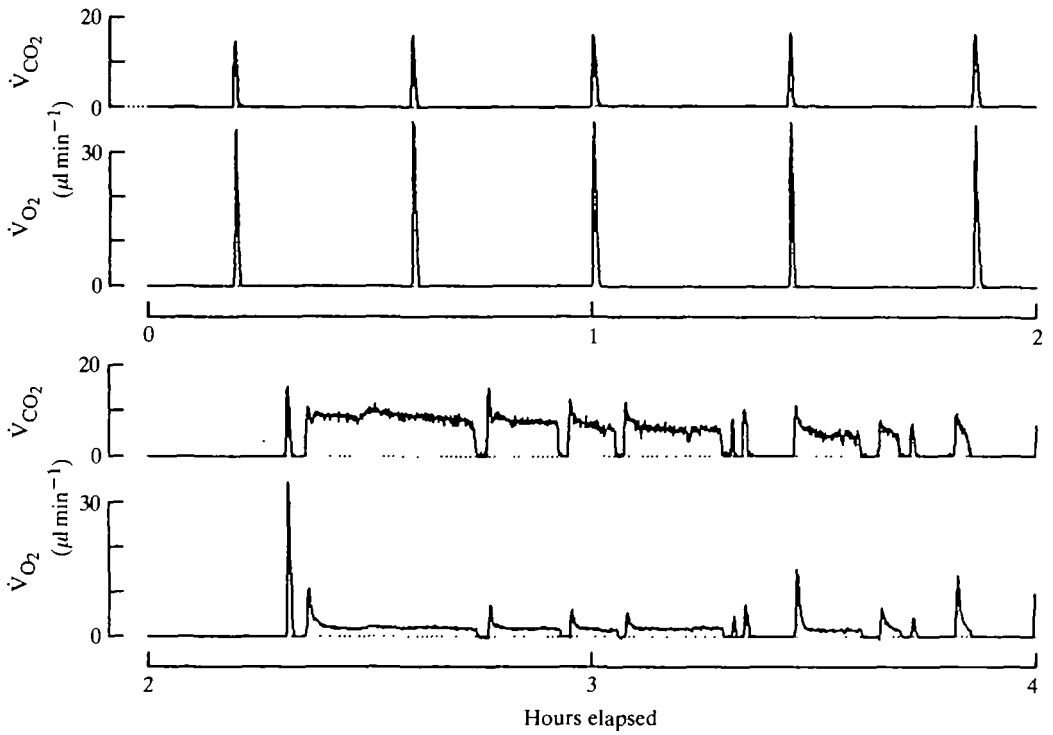

Fig. 3. Instantaneous rates of CO_2 release and O_2 uptake showing individual breaths, before and during a burst of CO_2 release (detail of record shown in Fig. 2,*A*). No measurable gas exchange occurred between breaths when the pneumostome was closed.

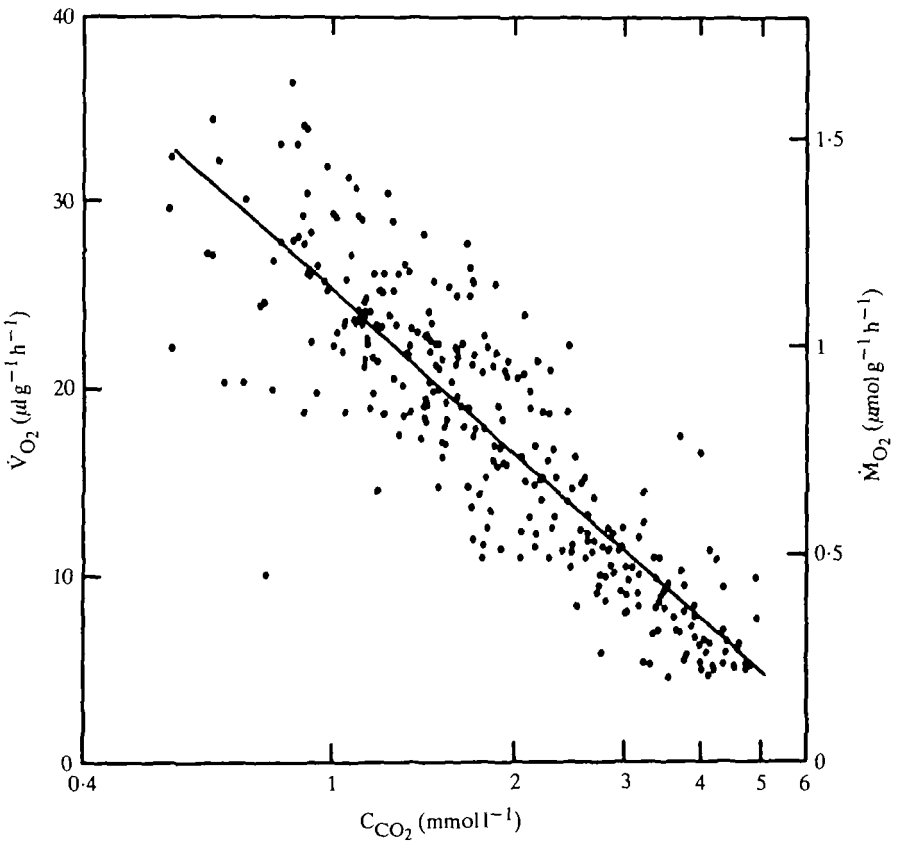

Fig. 4. Correlation between rate of oxygen consumption and whole-body CO_2 content as these parameters vary over time in a dormant individual (data from Fig. 2). Note log-transformed x-axis. Whole-body CO_2 content was estimated at the end of each measurement interval as the cumulative difference between metabolic CO_2 production and CO_2 release (see Materials and Methods). The C_{CO_2} scale is an interval scale without true zero, and indicates magnitude of change but not absolute values of C_{CO_2}.

results from the reduced gradient for diffusion of CO_2 out of the lung. $\dot{V}_{O_2}$, breath frequency and breath duration were not affected, indicating that 2% CO_2 had no immediate (2 h) effect on the pattern of ventilation (i.e. pneumostome opening).

When snails were exposed to 42% O_2, $\dot{V}_{O_2}$ during the interval including the first breath increased approximately three-fold, which may be attributed to the increased gradient for O_2 uptake (Fig. 6). Breath frequency then dropped by about half but O_2 uptake per breath remained elevated, so $\dot{V}_{O_2}$ returned to the previous level. Breath duration was essentially unchanged. $\dot{V}_{CO_2}$ decreased by about half owing to reduced breath frequency. On return to air, O_2 uptake during the first breath dropped by about two-thirds, then returned to the previous level within a few breaths. $\dot{V}_{CO_2}$ was unchanged during the first breath, then rose gradually. Breath frequency increased two- to three-fold on return to air.

Similar tests during periods of elevated $\dot{V}_{O_2}$ were inconclusive because of the high variability of respiration over time. However, in contrast to the results during lo

$\dot{V}_{O_2}$, in 2 % CO_2 there was a large (about five-fold) increase in breath duration when $\dot{V}_{O_2}$ was elevated.

Epiphragm secretion

Two instances of epiphragm secretion occurred during respirometry. Both events occurred during periods of elevated $\dot{V}_{O_2}$ (Fig. 1) and in both instances gas exchange was apparently blocked for 1·5–2 h following secretion as the epiphragm dried

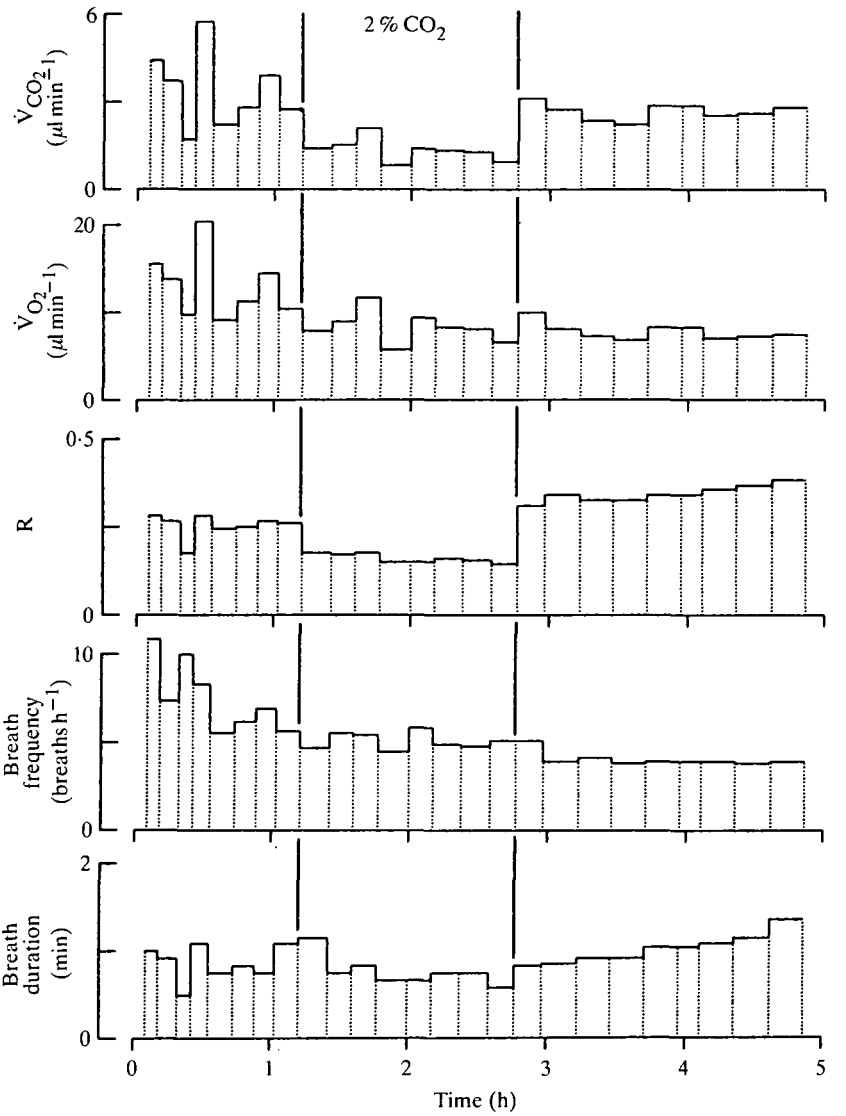

Fig. 5. Effect of 2 % CO_2 on gas exchange. Each histogram interval represents the period from the end of a breath up to and including the next breath. The subject had just entered a period of low $\dot{V}_{O_2}$ and CO_2 accumulation. The chamber was flushed with 2 % CO_2, 21 % O_2, 77 % N_2 (32 % relative humidity) over the interval indicated.

(Fig. 7, see Discussion). The pattern of instantaneous gas exchange was otherwise apparently similar whether epiphragms were present or absent.

DISCUSSION

Periodic CO_2 release

Periodic CO_2 release in dormant *Otala* was previously inferred from measurements of lung gas tensions and from closed-chamber respirometry (Barnhart, 1986*b*). In a broad sense, periodic or intermittent CO_2 release evidently occurs in a

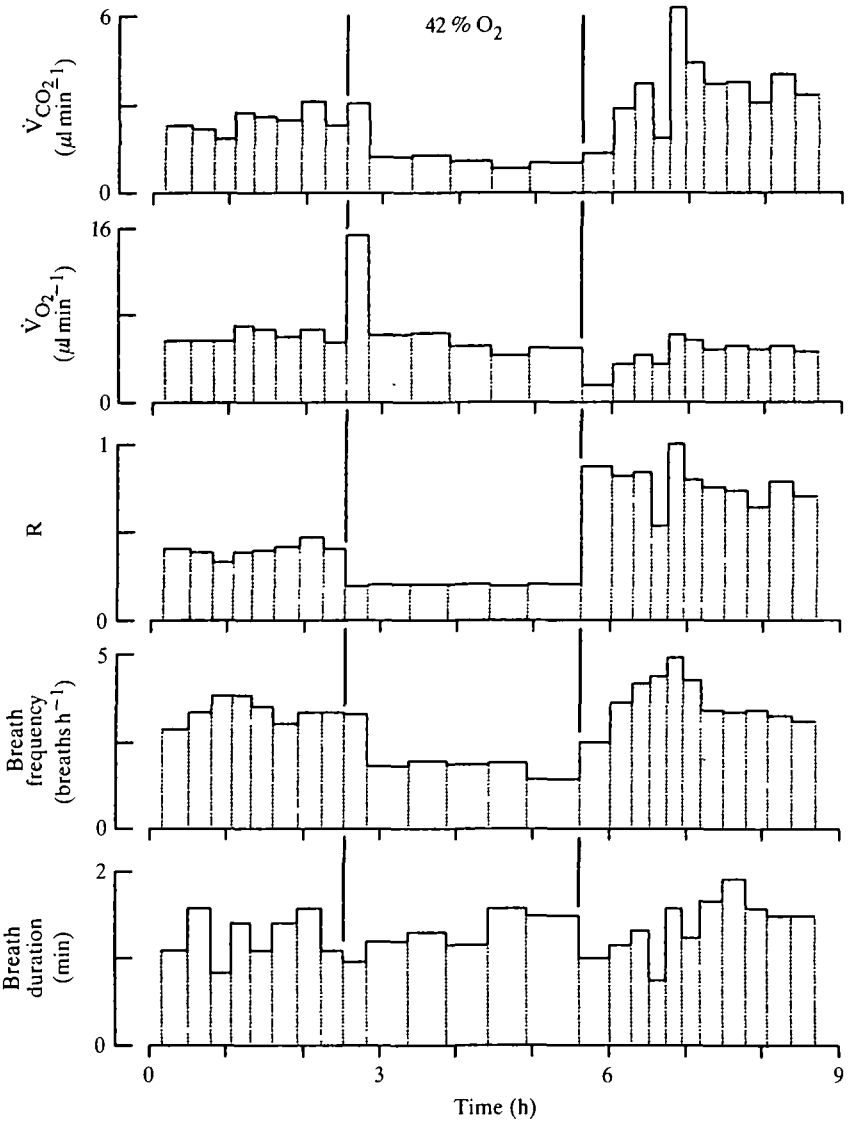

Fig. 6. Effect of 42 % O_2 on gas exchange. Conditions were as in Fig. 5. The chamber was flushed with 42 % O_2, 58 % N_2.

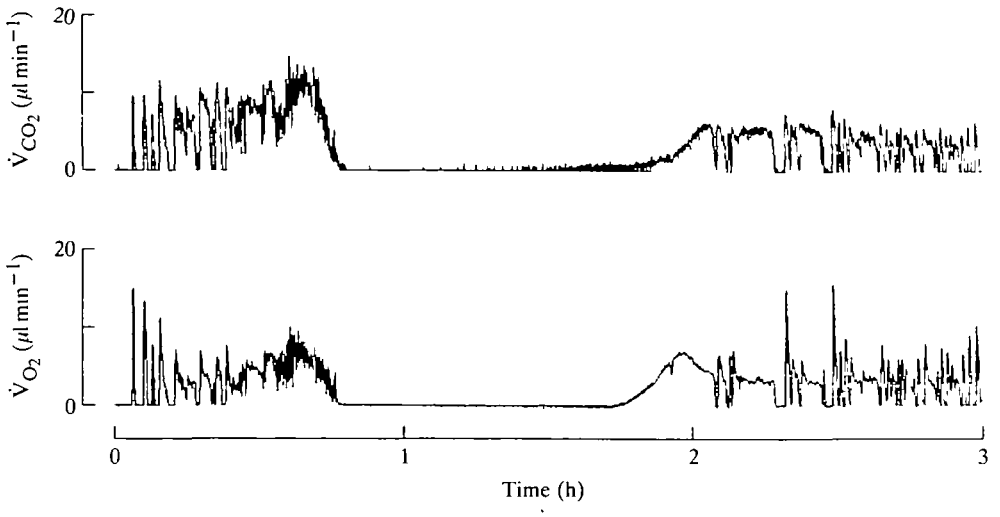

Fig. 7. Instantaneous rates of gas exchange during the secretion of an epiphragm (detail of Fig. 2,*B*). Gas exchange appears to be blocked until the epiphragm dries (see Discussion).

wide variety of animals including hibernating mammals (Bickler, 1984), reptiles (Ackerman & White, 1979; Glass & Johansen, 1979; Hicks & Riedesel, 1983) and in resting or dormant insects (see reviews by Miller, 1974; Edney, 1978) as well as in snails. Accumulation and release of CO_2 result from changes in the level of ventilation relative to the rate of aerobic metabolism ('specific ventilation'), which alters partial pressures and concentrations of O_2 and CO_2 in the body fluids. Equal changes of partial pressure involve a much larger amount of CO_2 than O_2 because capacitance of body fluids is much higher for CO_2 (Dejours, 1981). Therefore, a change in specific ventilation may only slightly affect body O_2 content but cause a prolonged period of net CO_2 accumulation or release from the body fluids.

Periodic CO_2 release is most thoroughly described in diapause pupae of large saturniid moths, especially *Hyalophora cecropia* (see reviews by Miller, 1974; Edney, 1978). These pupae have a constant $\dot{V}_{O_2}$ but release CO_2 in discrete bursts, often hours apart. The spiracles open slightly and close at high frequency ('flutter') during CO_2 accumulation but open widely and continuously during CO_2 release (Schneiderman, 1960). This pattern apparently results from differential sensitivity of ventilation to O_2 and CO_2. Low P_{O_2} at the abdominal ganglia causes spiracle flutter (Burkett & Schneiderman, 1974) which keeps tracheal O_2 at about 3·5 % (Levy & Schneiderman, 1966*a*). In contrast, CO_2 acts directly at the spiracles and triggers prolonged opening when tracheal CO_2 exceeds about 6 % (Levy & Schneiderman, 1966*a*; see Miller, 1981, for discussion of CO_2 and ventilation in insects).

Gas exchange of pupae and snails appears similar in several respects. First, periodic CO_2 release in each is associated with dormancy and reduced $\dot{V}_{O_2}$. CO_2 release is relatively continuous when $\dot{V}_{O_2}$ is high (Schneiderman & Williams, 1953, 1955; see Fig. 2). Second, ventilation of both pupae and snails evidently responds primarily to O_2 and not CO_2 during periods of CO_2 accumulation. Neither spiracle

movement nor tracheal P_{O_2} of pupae changes appreciably as CO_2 accumulates and tracheal P_{CO_2} increases (Schneiderman, 1960; Levy & Schneiderman, 1966*a*). Likewise, the pneumostome activity of *Otala* apparently remains constant as CO_2 accumulates during periods of low $\dot{V}_{O_2}$ (Fig. 2E). Effects of experimental hypercapnia in *Otala* also suggest that ventilation is insensitive to CO_2 during periods of basal $\dot{V}_{O_2}$. 2% CO_2 impeded CO_2 diffusion out of the lung but had no obvious effect on O_2 uptake, breath frequency or duration (Fig. 5). In contrast, hyperoxia caused an immediate reduction of breath frequency and, consequently, of the rate of CO_2 release (Fig. 6).

In both pupae and snails, hypoventilation accompanies reduced $\dot{V}_{O_2}$. Thus, interburst tracheal P_{O_2} in pupae decreases as $\dot{V}_{O_2}$ decreases (Levy & Schneiderman, 1966*a*). In *Otala*, P_{O_2} values in the lung gas and the haemolymph are greatly reduced when $\dot{V}_{O_2}$ is low (Barnhart, 1986*b*). Interestingly, tarantulas also exhibit lower arterial P_{O_2} at rest (28 Torr) than following activity (74 Torr) when $\dot{V}_{O_2}$ is presumably higher (Angersbach, 1978). It appears that a change in specific ventilation may be a common mechanism for matching of O_2 transport to metabolic demand in terrestrial invertebrates. The rate of internal O_2 transport in these animals may be diffusion-limited, so that P_{O_2} in the lung or tracheal gas affects the rate of O_2 transport. In *Otala*, O_2 transfer from lung to arterial haemolymph is strongly diffusion-limited (Barnhart, 1986*b*).

Based on the above observations, periodic CO_2 release in snails and pupae might be explained as follows. O_2 and CO_2 may both influence ventilation, to some degree independently. Fall of internal P_{O_2} to a threshold level stimulates a pattern of ventilation (flutter in pupae, brief breaths in *Otala*) which maintains P_{O_2} at or near the threshold level. When $\dot{V}_{O_2}$ is low, the lung or tracheal P_{O_2} necessary to supply respiration is reduced and hypoventilation ensues. Consequently, P_{CO_2} slowly increases towards a new equilibrium level as CO_2 accumulates. If P_{CO_2} exceeds a critical level, prolonged opening of the spiracles or pneumostome is induced and may reduce P_{CO_2} well below the triggering level. A periodic pattern of CO_2 release ensues. When $\dot{V}_{O_2}$ is high, high P_{O_2} is required in the lung or tracheae and the specific ventilation is therefore elevated. The equilibrium P_{CO_2} at this high specific ventilation will be low, and if it lies near or below the P_{CO_2} threshold, CO_2 release will be continuous.

An interesting question is whether the P_{CO_2} sensitivity of ventilation changes with metabolic state. P_{CO_2} varies between about 25 and 100 Torr in dormant *Otala*, but is apparently controlled rather precisely at a much lower level in active snails. Moreover, specific ventilation of active snails changes with temperature, so P_{CO_2} and haemolymph pH change with temperature in the 'alphastat' pattern (Barnhart, 1986*a*,*b*).

Fluctuations of $\dot{V}_{O_2}$ during dormancy

Major episodes of CO_2 release in *Otala* were usually followed by prolonged increase of $\dot{V}_{O_2}$ (Fig. 2). In contrast, saturniid pupae maintain constant $\dot{V}_{O_2}$ (Schneiderman & Williams, 1953, 1955; Buck & Keister, 1955). Certain other insect

pupae show infradian cycles of $\dot{V}_{O_2}$ during diapause, but CO_2 release has not been investigated in those species (Denlinger, Willis & Fraenkel, 1972; Beck, 1980). Semiperiodic bursts of O_2 uptake appear to be characteristic of dormant helicid snails (see Schmidt-Nielsen *et al.* 1971; Kratochvil, 1976). Dormant snails are quite sensitive to disturbance and $\dot{V}_{O_2}$ may increase in response to a variety of factors including vibration, light, temperature and humidity (Machin, 1975; Herreid & Rokitka, 1976). In specimens kept in constant conditions, simply passing a shadow across the shell affects respiration (M. C. Barnhart, personal observations) so that it is very difficult to rule out external influences. However, Schmidt-Nielsen *et al.* (1971) believed that bursts are an inherent feature of snail metabolism and not necessarily a response to external stimuli. Possibly bursts of oxygen uptake represent occasional attempts to arouse from dormancy, perhaps necessary for a full assessment of external conditions. The reduction of $\dot{V}_{O_2}$ burst frequency following epiphragm secretion (Fig. 1) might indicate that frequent bursts are not a physiological necessity.

The mean and range of $\dot{V}_{O_2}$ in dormant *Otala* (Table 1) are similar to those in diapausing cecropia pupae, i.e. $16{\cdot}3\,\mu l\,g^{-1}\,h^{-1}$, $6{\cdot}8$–$38\,\mu l\,g^{-1}\,h^{-1}$ at 25°C (Schneiderman & Williams, 1953). The 'basal' $\dot{V}_{O_2}$ of dormant *Otala* is about 7% that of active snails at similar temperature ($85\,\mu l\,g^{-1}\,h^{-1}$; Barnhart, 1986*a*). Peak $\dot{V}_{O_2}$ of active *Otala* may be as high as $140\,\mu l\,g^{-1}\,h^{-1}$ (calculated from Herreid, 1977), so the factorial aerobic scope is about 24. Given the often low frequency of breaths, gas exchange should be used only cautiously as a measure of metabolic rate in dormant pulmonates. Care must be taken that the respirometry measurement period includes several breaths. Closed-chamber respirometry over 5-h periods gave results similar to those reported here (mean $\dot{V}_{O_2} = 13{\cdot}1\,\mu l\,g^{-1}\,h^{-1}$; Barnhart, 1986*b*).

Effect of epiphragms on gas exchange

During epiphragm formation a complex mucous layer is secreted on the mantle collar, then separated from it by expulsion of air from the lung. The mucus dries to form a membrane across the aperture of the shell and may attach the shell to the substrate. Epiphragms of *Otala* include a porous area which permits the diffusion of gases (Barnhart, 1983). However, the perforations form only as the epiphragm dries, and the freshly secreted mucus evidently prevents gas exchange (Fig. 7). The epiphragm has no obvious effect on the pattern of gas exchange after drying, as was predicted on the basis of its high permeability (Barnhart, 1983).

$\dot{V}_{O_2}$ versus *whole-body* CO_2 *content*

A negative correlation between $\dot{V}_{O_2}$ and whole-body CO_2 content (Fig. 4) might arise by at least two mechanisms. First, reduced metabolic rate may induce hypoventilation and thereby cause CO_2 accumulation (see above). Second, accumulation of respiratory CO_2 might depress cellular metabolism, by affecting intracellular pH. Possibly both mechanisms operate simultaneously. Acid–base changes are associated with metabolic transitions in a variety of cells and organisms (Busa & Nuccitelli, 1984; Bickler, 1984). The idea that dormancy in snails might involve 'CO_2 narcosis'

is not new (Picher, 1971, and references therein) but little direct evidence is available. $\dot{V}_{O_2}$ of non-dormant *Otala* is depressed by about half on exposure to 10% CO_2, coincident with depression of intracellular pH (M. C. Barnhart, unpublished).

Suction ventilation

In *H. cecropia* pupae, the volume deficit that results from low R causes negative pressure in the tracheal system. Opening of the spiracles during flutter is sufficiently small and brief that the pressure gradient is maintained and causes air to flow into the spiracles (suction or passive ventilation) which may further enhance retention of CO_2 and water vapour (Levy & Schneiderman, 1966*b*; Kestler, 1985). The degree to which CO_2 retention is enhanced by suction ventilation is not clear. Assuming diffusion only, the lowest R predicted in cecropia pupae is about 0·3 (calculated from tracheal partial pressures in table 2 of Levy & Schneiderman, 1966*a*). Measured R of cecropia pupae during CO_2 accumulation is 0·17 and ranges from 0·01 to 0·56 (RQ = 0·78) (Schneiderman & Williams, 1955).

Reduction of lung volume probably occurs in dormant *Otala* between breaths (Barnhart, 1986*b*). Compared to insect spiracles, however, the pneumostome opens very widely (about 1–3 mm^2; M. C. Barnhart, personal observations) and breath duration is seldom less than 30 s. Any pressure gradient that develops during apnoea must be abolished almost immediately after the pneumostome opens. The lowest R observed in the present study was about 0·22, which is similar to the lowest predicted R based on measurements of P_{O_2} and P_{CO_2} in the lung of dormant *Otala* and assuming gas exchange by diffusion alone (Barnhart, 1986*b*). Thus, it appears that suction ventilation may be negligible in snails.

Respiratory water loss

Dormancy probably first developed in littoral marine ancestors of land snails (see Little, 1983) and primitively appears to be a strategy for avoiding desiccation. Respiration is significant in this regard. In *Otala*, the lung is a major potential site of water loss; the total rate of water loss in dormancy would be at least 54% higher without hypoventilation, and 460% higher if the aerobic metabolic rate were not also reduced (Barnhart, 1986*b*).

It is often stated that suction ventilation reduces water loss in insects by retarding efflux of water vapour from the spiracles while enhancing O_2 uptake, and that this effect provides an adaptive advantage for periodic CO_2 release (Buck, 1958; Miller, 1974, 1981; Edney, 1978). Such an interpretation is probably misleading, however. It should be emphasized that, considering the entire respiratory cycle, the need for CO_2 release, not O_2 uptake, appears to limit the extent to which ventilation and consequent water loss can be reduced. The maximum partial pressure gradient for CO_2 loss is only about one-third that for O_2 uptake in cecropia pupae (Levy & Schneiderman, 1966*a*). Apparently, therefore, the minimum conductance necessary for CO_2 release (i.e. to keep P_{CO_2} tolerably low) is roughly three times greater than that needed for O_2 uptake (assuming RQ = 1). Suction ventilation does not enhance CO_2 release relative to water loss; water vapour and CO_2 presumably encounte

similar convective and diffusive conductance across the spiracles. The ratio of water vapour loss to CO_2 release must depend directly on their respective partial pressure gradients. Assuming a constant pressure gradient for water loss, the ratio will be low when the P_{CO_2} gradient is large. In this respect, there is no inherent advantage to suction ventilation or periodic CO_2 release. In fact, a constant minimum level of ventilation that maintained the highest tolerable internal P_{CO_2} would theoretically result in less water loss than the periodic pattern, in which specific ventilation fluctuates (see Kestler, 1985, p. 183).

There appear to be only two basic mechanisms for reducing respiratory water loss. The rate of aerobic metabolism may be reduced, and/or the specific ventilation may be reduced, with consequent decrease of internal P_{O_2} and elevation of P_{CO_2}. Intermittent CO_2 release in insects and snails appears to be a consequence of these changes and of characteristics of ventilatory control.

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