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DEPRESSION OF AEROBIC METABOLISM AND INTRACELLULAR pH BY HYPERCAPNIA IN LAND SNAILS, *OTALA LACTEA*

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Summary

The pulmonate land snail *Otala lactea* undergoes simultaneous hypercapnia, hypoxia, extracellular acidosis and metabolic depression during dormancy. We tested the effects of ambient hypercapnia and hypoxia on oxygen consumption ($\dot{V}_{O_2}$) and on extracellular and intracellular pH of active (i.e. non-dormant) individuals. Active snails reduced $\dot{V}_{O_2}$ by 50 % within 1 h when exposed to 65 mmHg (1 mmHg = 133·3 Pa) ambient P_{CO_2}, and by 63 % in 98 mmHg. These levels of CO_2 are within the range that occurs naturally in the lung and blood during dormancy. $\dot{V}_{O_2}$ of hypercapnic snails remained below that of controls for the duration of exposure (up to 9 h) and returned to control levels within 1 h when CO_2 was removed. Both pHe and whole-body pHi (measured using [^{14}C]DMO) fell with increasing haemolymph P_{CO_2} by approximately $0{\cdot}7\log P_{CO_2}$. Critical ($\dot{V}_{O_2}$-limiting) ambient P_{O_2} of active snails was 90 mmHg in the absence of CO_2 and dropped to 50 mmHg when $\dot{V}_{O_2}$ was reduced 45 % by exposure to CO_2. Estimated critical P_{O_2} at the lower $\dot{V}_{O_2}$ typical of dormancy is well below the typical lung P_{O_2} of dormant *Otala*, suggesting that P_{O_2} in the lung does not normally limit oxygen consumption during dormancy. These results support the hypothesis that hypercapnia or resulting respiratory acidosis depresses metabolic rate during dormancy, and argue against a limitation of $\dot{V}_{O_2}$ by hypoxia.

Introduction

Studies of the physiology and biochemistry of metabolic arrest suggest that hypoxia and changes of intracellular pH are possible factors governing reversible transitions between activity and dormancy in many animals (Hochachka, 1988; Busa & Nuccitelli, 1984; Hochachka & Guppy, 1987). Pulmonate land snails provide an interesting opportunity to investigate both these mechanisms. Dormancy in *Otala lactea* (Helicidae) is characterized by reduced aerobic metabolism

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and by simultaneous hypoxia, hypercapnia and respiratory acidosis brought about by hypoventilation of the lung. Mean $\dot{V}_{O_2}$ declines to about 15 % of that of resting, non-dormant individuals (Barnhart & McMahon, 1987). P_{O_2} in the lung often falls below 10 mmHg, compared with 137 mmHg in active snails, whereas P_{CO_2} reaches 100 mmHg in dormancy, compared with 12 mmHg in active snails. Mean haemolymph pH falls 0·4 units as a result of hypercapnia and up to 0·5 units further during prolonged dormancy as $[HCO_3^-]$ decreases (Barnhart, 1986*a*,*b*).

Cycles of CO_2 accumulation and release coincide with fluctuations of $\dot{V}_{O_2}$ in dormant *Otala*, and $\dot{V}_{O_2}$ is inversely related to whole-body CO_2 content (Barnhart & McMahon, 1987). Both the broad correlation between hypercapnia and dormancy and the short-term correlation between body CO_2 content and $\dot{V}_{O_2}$ suggest that CO_2 accumulation and/or resultant acidosis might be factors in the control of metabolic rate during dormancy. However, low P_{O_2} values in the lung and haemolymph suggest that $\dot{V}_{O_2}$ might be oxygen-limited during dormancy. To test these possibilities, the present study examines the effects of acute experimental hypercapnia and hypoxia on the oxygen consumption of active *Otala*. The effect of hypercapnia on whole-body intracellular pH (pHi) was also measured because intracellular acidification is a possible mechanism by which CO_2 could affect metabolism.

Materials and methods

Subjects

Adult *Otala* were collected at Playa del Rey in Los Angeles County, California, and maintained in the laboratory as described previously (Barnhart & McMahon, 1987). The individuals used had a mean whole mass of 8·44 ± 0·84 g (S.D.).

Respirometry

O_2 uptake and CO_2 release of individuals were measured over successive 1-h intervals by closed-chamber respirometry, using 60 ml syringes as chambers (Barnhart, 1986*b*). For measurements in the absence of CO_2, an open vial holding a folded wick and 1 ml of 5 % KOH was placed in the chamber to absorb respiratory CO_2. For measurements in the presence of CO_2 the KOH was replaced with distilled water. Temperature was 20–23 °C.

After a brief period (2–7 days) of dormancy, snails were placed on wet paper towelling for 2 h to induce arousal and permit hydration, and then placed in the respirometry chambers for 1 h of acclimation. To measure gas exchange, the chambers were flushed with mixtures of air and CO_2 or N_2 prepared by Wösthoff gas-mixing pumps, closed for 1 h, and then placed in an infusion pump to expel the gas successively through a dessicant tube, an Applied Electrochemistry S3A electrochemical O_2 analyser and a CD3A infrared CO_2 analyser. Oxygen and CO_2 fractions were measured to the nearest 0·01 % volume. The analysers were calibrated using nitrogen, dry CO_2-free air and a 5 % CO_2 standard analysed with

a Scholander device. Change in oxygen fraction in the respirometers was typically 1–2 % of volume. Gas exchange was calculated according to Vleck (1987).

Effects of P_{CO_2} and P_{O_2} on $\dot{V}_{O_2}$

The effect of ambient P_{CO_2} on $\dot{V}_{O_2}$ was examined in four experiments which ranged in duration from 5 to 14 h. In each experiment, 24 individuals were distributed randomly among a control (zero CO_2) and several CO_2 treatment groups. $\dot{V}_{O_2}$ of all individuals was first measured in the absence of CO_2. The treatment groups were subsequently exposed to ambient P_{CO_2} between 13 and 98 mmHg.

The relationship between P_{O_2} and $\dot{V}_{O_2}$ was measured in two experiments, one in the absence of CO_2 and the other at 66 mmHg P_{CO_2}. $\dot{V}_{O_2}$ of each individual was determined initially in air and afterwards at one of six levels of P_{O_2} between 14 and 160 mmHg.

Effects of hypercapnia on pH

The effects of hypercapnia on pH of venous haemolymph (pHe) and of intracellular fluids (pHi) were measured in individuals which had previously been catheterized to permit marker injection and serial sampling of haemolymph. pHi was estimated with the labelled weak acid distribution technique (Waddell & Butler, 1959), using 5,5-dimethyl[^{14}C]oxazolidine-2,4-dione (DMO) as the pH indicator and polyethylene glycol[1,2^{3}H] (PEG) as the extracellular fluid volume marker (isotopes from New England Nuclear). Catheters consisted of 21 gauge hypodermic infusion needles with attached tubing (Deseret Company). The needle was lightly coated with cyanocrylate glue just behind the tip, then inserted through a hole in the shell into the visceral sinus. If no leakage occurred around the insertion, the needle was sealed to the shell and supported with epoxy paste. A 1 cm length of tubing was left attached to the needle, and was reversibly closed by a crimp secured by a band of larger tubing. Animals were placed on moist paper towelling and used within 1–2 days of preparation.

The catheterized snails were placed in plastic bags for exposure to gas mixtures. The bags were flushed continuously with humidified mixtures of CO_2 and air to maintain 0, 46 or 98 mmHg P_{CO_2}. After waiting 3 h to permit equilibration with ambient CO_2, each snail was injected with 0·15 μCi of DMO and 0·20 μCi of PEG in 30 μl of 200 mmol l^{-1} NaCl, followed by 100 μl of snail haemolymph to flush the catheter. Haemolymph samples (about 60 μl each) were collected at hourly intervals thereafter for 6 h. The catheter dead-space volume was discarded. The next 40 μl was transferred anaerobically to a Radiometer capillary pH electrode for determination of pHe. Subsamples (10 μl) were analysed for isotope activities (see below).

Haemolymph P_{CO_2} was estimated from pH using the *in vitro* relationship between pH and P_{CO_2} (Astrup method). Haemolymph from each individual was equilibrated at 46 and 98 mmHg P_{CO_2} in a microtonometer and pH was measured. The results were plotted, assuming a linear relationship between pH and logP_{CO_2}

(Barnhart, 1986*a*). *In vivo* pH was compared with these plots to estimate *in vivo* P_{CO_2}.

Isotope activities in haemolymph (disints min^{-1} ml^{-1}) were measured using a dual-channel liquid scintillation counter with external standard (Tracor Mark III). Haemolymph was digested in NCS tissue solubilizer and counted in OCS (Amersham), with correction for counting efficiencies in the calculation of activity. Extracellular volume was calculated from PEG dilution, extrapolating the exponential decline of haemolymph PEG activity to the time of injection. Extracellular water content (ECW, g) was calculated from extracellular volume assuming haemolymph water content to be 1 g ml^{-1}. Total body water (TBW, g) was measured as the difference between wet and dry body mass, and intracellular water (ICW, g) as the difference between TBW and ECW. pHi was calculated for the total intracellular space using the equation:

$$\mathrm{pHi} = \mathrm{pK_{DMO}} + \log\frac{[\mathrm{DMO}]_i}{[\mathrm{DMO}]_e}(1 + 10^{\mathrm{pHe}-\mathrm{pK_{DMO}}}) - 1 \qquad (1)$$

(Waddell & Butler, 1959), assuming $pK_{DMO} = 6{\cdot}27$ (Boron & Roos, 1976). $[DMO]_i$ was calculated as follows:

$$[\mathrm{DMO}]_i(\text{disints min}^{-1}\text{ g}^{-1}) = \frac{\mathrm{DMO_{inj}}(\text{disints min}^{-1}) - \{[\mathrm{DMO}]_e(\text{disints min}^{-1}\text{ g}^{-1}) \times \mathrm{ECW(g)}\}}{\mathrm{ICW(g)}}, \qquad (2)$$

where DMO_{inj}(disints min^{-1}) is the total DMO injected.

Results

Effect of CO_2 on $\dot{V}_{O_2}$

$\dot{V}_{O_2}$ decreased with time in snails respiring in the absence of CO_2 (see Fig. 2). Typically, $\dot{V}_{O_2}$ declined gradually over 6–8 h to about 70 % of the initial level, then remained stable for the remainder of the measurement periods (up to 14 h).

$\dot{V}_{O_2}$ was rapidly depressed by ambient hypercapnia, and the reduction of $\dot{V}_{O_2}$ was greater at higher P_{CO_2}. The relationship between $\dot{V}_{O_2}$ and P_{CO_2} appears to be nonlinear, with maximum slope at roughly 40–60 mmHg P_{CO_2} (Fig. 1). The reduction of $\dot{V}_{O_2}$ relative to the control group was maximal within the first hour of exposure (Fig. 2), although equilibration with altered ambient P_{CO_2} requires more than 2 h (Fig. 3, see below). When CO_2 was removed, $\dot{V}_{O_2}$ increased within 1 h to a level similar to or somewhat higher than that of the control group (Fig. 2).

The behaviour of each animal was noted at hourly intervals. All individuals remained at least partly extended from the shell during respirometry. Crawling was infrequent in all groups (2 % of observations). Over time, an increasing proportion of the snails partly retracted into the shell; this occurred sooner in low CO_2 than in high CO_2. In the experiment illustrated in Fig. 2, half or more of the snails in 0 mmHg P_{CO_2} were partly retracted after 2 h, whereas this occurred only after 7 and 11 h in 46 and 98 mmHg P_{CO_2}, respectively.

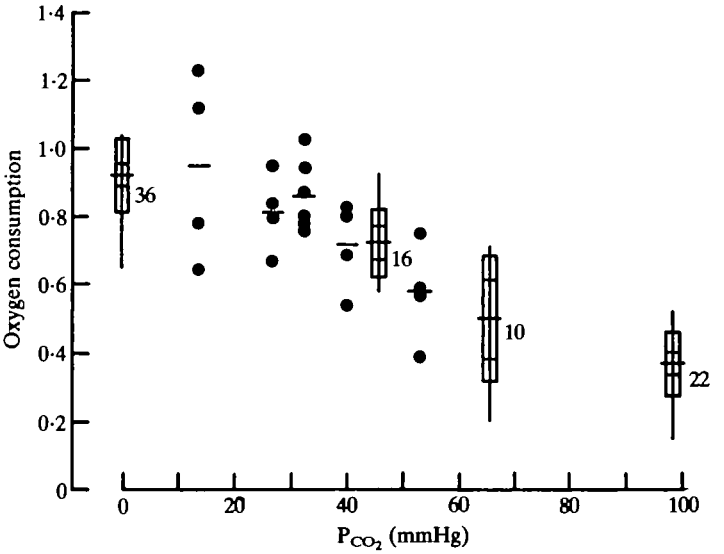

Fig. 1. Effect of carbon dioxide on oxygen consumption of active *Otala lactea*. Ordinate shows the rate of oxygen consumption during hypercapnia as a fraction of that in the absence of CO_2. Oxygen consumption of each individual was measured over two consecutive 1-h periods: first without CO_2, then with CO_2 present. Horizontal bars, boxes and vertical bars indicate, respectively, mean, 95 % confidence interval of mean, standard deviation and range. Numbers beside boxes indicate sample size. Points show individual values where sample size was less than 10.

Equilibration with ambient P_{CO_2}

The time course of change of internal P_{CO_2} during ambient hypercapnia can be inferred from the respiratory exchange ratio, R (Fig. 3). R was low or negative immediately following an increase of ambient P_{CO_2}, which may be attributed to the accumulation of CO_2 in the body fluids as P_{CO_2} increased. R increased rapidly and became positive within 2 h, which shows that P_{CO_2} of the body fluids rose rapidly and exceeded ambient P_{CO_2} within this period. R eventually rose beyond the control level (Fig. 3). The overshoot was greater at higher P_{CO_2}, and may have been due to an increase of haemolymph [HCO_3^-] during hypercapnia (Burton, 1976; Barnhart, 1986*a*). Addition of HCO_3^- to the body fluids shifts the CO_2–carbonic acid–bicarbonate equilibrium and should therefore increase CO_2 loss and elevate R.

Effects of hypercapnia on pHe and pHi

Mixing of the isotopes was judged to be complete within 3 h of injection; after this period the mean $[DMO]_e$ was stable, although PEG levels declined exponentially, as indicated by the linearity of plots of the logarithm of activity (disints min^{-1}) *vs* time from 3 to 6 h after injection. These plots were extrapolated to time zero to estimate extracellular fluid volumes for each individual. Mean extracellular water as a percentage of total body water was 51·1 ± 6·74 % (S.D., $N = 15$).

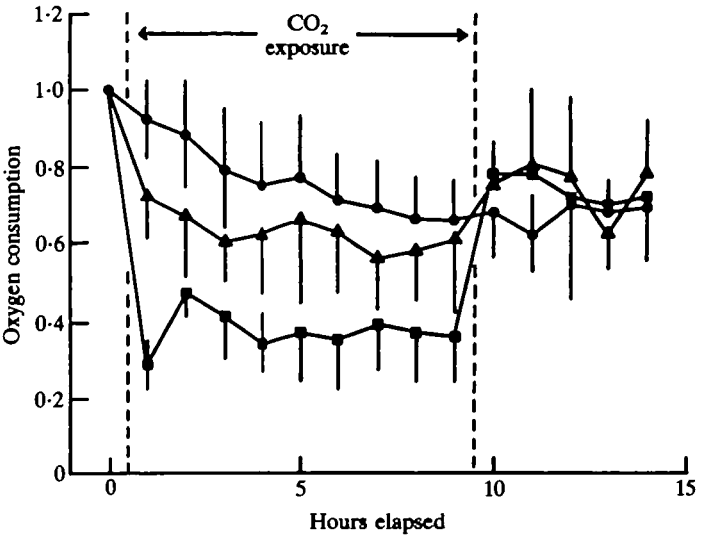

Fig. 2. Time course of change in rate of oxygen consumption in the presence and absence of CO_2. Points and bars indicate means and 95 % confidence limits of means, $N = 8$. $\dot{V}_{O_2}$ of each individual was expressed as a fraction of an initial control measurement in the absence of CO_2. Dotted lines indicate the period of CO_2 exposure. $P_{CO_2} = 0$ mmHg (●); $P_{CO_2} = 46$ mmHg (▲); $P_{CO_2} = 98$ mmHg (■).

Between 3 and 6 h after injection the mean values of $[DMO]_e$ and pHe in the three groups of snails did not change significantly. Therefore, the $[DMO]_e$ and pHe results were averaged over this period for each individual and used to calculate a single estimate of pHi (from equations 1, 2) and P_{CO_2} (from the Astrup line) for each (Fig. 4). Both pHe and pHi declined similarly with increase of haemolymph P_{CO_2}. Linear regressions of pHe and pHi on the logarithm of haemolymph P_{CO_2} yielded the following equations:

$$pHi = 8{\cdot}446 - 0{\cdot}667 \log P_{CO_2} \ (r^2 = 0{\cdot}81), \quad (3)$$

$$pHe = 8{\cdot}631 - 0{\cdot}700 \log P_{CO_2} \ (r^2 = 0{\cdot}93). \quad (4)$$

The regression coefficients for pHe and pHi did not differ significantly. Mean pHi was lower than pHe by 0·12 units; this difference was significant (analysis of covariance, critical $P = 0{\cdot}05$).

Effect of hypoxia on $\dot{V}_{O_2}$

$\dot{V}_{O_2}$ was independent of ambient P_{O_2} above about 90 mmHg in the absence of CO_2, and independent above about 50 mmHg when $\dot{V}_{O_2}$ was lowered 45 % by the presence of CO_2 (Fig. 5). Below these levels, $\dot{V}_{O_2}$ varied with P_{O_2}. The relationship between ambient P_{O_2} and P_{O_2}-limited $\dot{V}_{O_2}$ is approximately described by the following regression equation (see Fig. 5):

$$\dot{V}_{O_2}(\mu l\, g^{-1}\, h^{-1}) = 1{\cdot}05 P_{O_2}(\text{mmHg}). \quad (5)$$

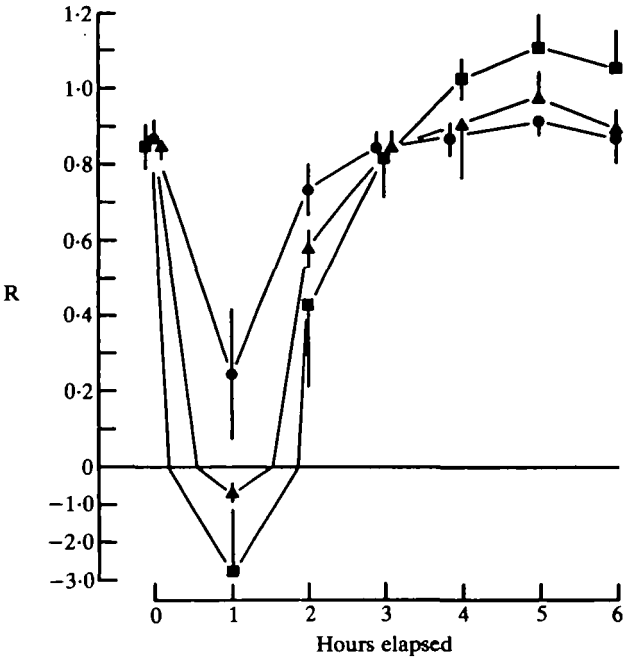

Fig. 3. Time course of change in the respiratory exchange ratio ($R = \dot{V}_{CO_2}/\dot{V}_{O_2}$) during CO_2 exposure. Points and bars indicate means and 95 % confidence limits of means ($N = 6$). Note that the vertical scale differs above and below zero. $P_{CO_2} = 33$ mmHg (●); $P_{CO_2} = 66$ mmHg (▲); $P_{CO_2} = 98$ mmHg (■).

The y-intercept did not differ significantly from zero (5·1 mmHg; $P = 0{\cdot}2$); consequently, the regression was forced through zero. This equation was used to predict the limit of $\dot{V}_{O_2}$ as a function of P_{O_2} (see Discussion).

Discussion

Effect of hypercapnia on $\dot{V}_{O_2}$

Previous work has shown that CO_2 retention is correlated with lowered $\dot{V}_{O_2}$ in dormant *Otala* (Barnhart & McMahon, 1987). The present results show that the oxygen consumption of active *Otala* is rapidly and reversibly depressed by imposed hypercapnia. Both results appear to be consistent with the hypothesis that hypercapnia resulting from hypoventilation reduces metabolic rate during dormancy. However, $\dot{V}_{O_2}$ of hypercapnic active snails was not depressed to the same degree as during dormancy. The highest ambient P_{CO_2} tested lowered mean $\dot{V}_{O_2}$ by only 63 % (Fig. 1) compared with the 85 % reduction during dormancy (Barnhart & McMahon, 1987). In absolute terms, the mean $\dot{V}_{O_2}$ in 98 mmHg ambient P_{CO_2} (37 μl g^{-1} h^{-1}) was more than double the mean dormant $\dot{V}_{O_2}$ and six times the minimum dormant $\dot{V}_{O_2}$ observed previously (13·7 and 5·6 μl g^{-1} h^{-1}, respectively; Barnhart & McMahon, 1987). Thus, it is still uncertain whether the

effects of hypercapnia limit $\dot{V}_{O_2}$ during true dormancy. Moreover, hypercapnia did not hasten withdrawal into the shell or induce epiphragm formation, events which normally accompany entry into dormancy.

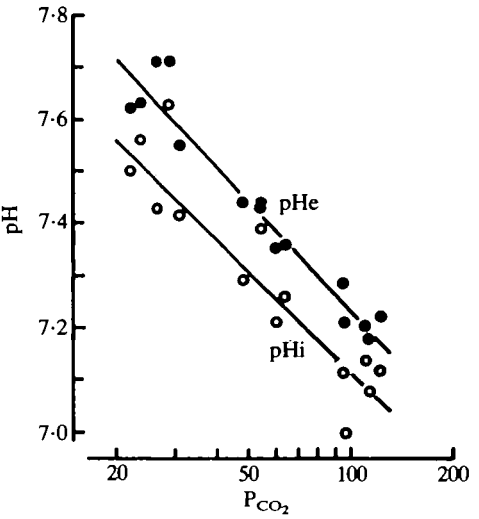

Fig. 4. Relationship between extracellular (i.e. haemolymph) pH (pHe, ●), whole-body intracellular pH (pHi, ○), and haemolymph P_{CO_2} in active *Otala lactea* after 6–9 h of ambient hypercapnia. pHe was measured directly, pHi was estimated using DMO, and P_{CO_2} was estimated from pHe using the Astrup method. Points represent individual animals; data for each individual are based on averages of 3–4 measurements of pHe and $[DMO]_e$ (see Results).

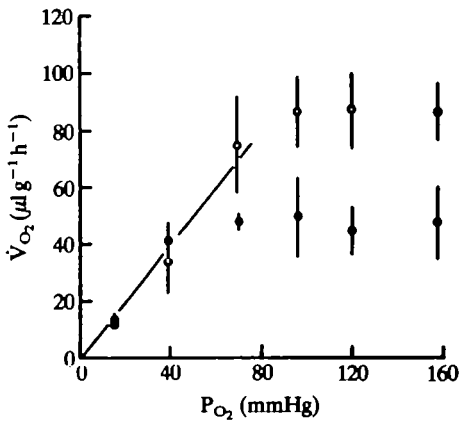

Fig. 5. Effect of P_{O_2} on $\dot{V}_{O_2}$. Symbols and bars indicate means and 95 % confidence limits of means ($N = 4$ for each). Open and closed circles are animals respiring in 0 and 66 mmHg P_{CO_2}, respectively. $\dot{V}_{O_2}$ is expressed per gram whole body mass (including shell). The line is the least-squares linear regression of P_{O_2}-dependent $\dot{V}_{O_2}$ on P_{O_2}. The regression was forced through zero (see Results).

Hypercapnia and O_2 delivery

A possible mechanism for depression of $\dot{V}_{O_2}$ by CO_2 in *Otala* is through interference with O_2 delivery to the tissues, either by depressing circulation (heart rate decreases in hypercapnic individuals) or by the reversed Bohr effect of haemocyanin (Barnhart, 1986*b*) which might reduce P_{O_2} at the tissues. Either effect might conceivably cause oxygen limitation of $\dot{V}_{O_2}$ during hypercapnia. However, if hypercapnia inhibited oxygen transport, oxygen-limited $\dot{V}_{O_2}$ should be lower at similar P_{O_2} during hypercapnia due to reduced conductance for oxygen (Herreid, 1980). This is not the case (Fig. 5), and the results therefore argue that hypercapnia does not interfere with oxygen transport.

Hypercapnia and acid–base balance

Change of intracellular pH is one of several possible mechanisms by which hypercapnia could initiate metabolic effects (Walsh *et al.* 1988). The present results show that intracellular acidosis must be considered as a potential mechanism for the observed effect of CO_2 on $\dot{V}_{O_2}$ in *Otala*. The whole-body pHi measurement leaves open the question of the exact values of pHi in different tissues. These values were not determined in the present study because of uncertainty regarding the fate of the extracellular volume marker (PEG) as it left the haemolymph. The similar extracellular and intracellular acidosis during hypercapnia is curious because of the presumably higher buffering capacity of the intracellular space (Burton, 1976). Similar results were, however, obtained in the bivalve *Mytilus* (Lindinger *et al.* 1984).

Although intracellular pH has not been measured in dormant *Otala*, Rees & Hand (1987) report that pHe and whole-body pHi decline by similar increments during dormancy in the land pulmonate *Oreohelix*. It appears that pHi may not only fall but also fluctuate over a substantial range during dormancy due to changes in P_{CO_2}. CO_2 release of dormant *Otala* is periodic (Barnhart & McMahon, 1987) and haemolymph P_{CO_2} ranges between 25 and 100 mmHg (Barnhart, 1986*b*). Comparison of this range with Fig. 4 suggests that pHi fluctuates over about 0·4 units due to changes of P_{CO_2} during dormancy. These changes of pH may occur rapidly, particularly during CO_2 release. Measurement of pHi during dormancy using DMO thus presents special difficulties. It should be noted in this regard that dormant pulmonates are sensitive to disturbances associated with handling (Machin, 1975; Herreid & Rokitka, 1976; Herreid, 1977). The manipulations required by the present application of the DMO method for measuring pHi induce hyperventilation, release of CO_2 and increase of $\dot{V}_{O_2}$ in dormant *Otala* (M. C. Barnhart, unpublished results).

Effects of acidosis on metabolism

Although the biochemical mechanisms that reduce the rate of energy utilization during dormancy and other hypometabolic states are not yet understood, considerable evidence indicates that glycolysis is suppressed (Hochachka &

Guppy, 1987). Intracellular acidosis appears to suppress glycolysis in hibernating mammals (see review by Malan, 1986) and in the brine shrimp, *Artemia*, during metabolic arrest (Busa & Nuccitelli, 1984; Carpenter & Hand, 1986; Hand & Carpenter, 1986). Among molluscs, acid pH reduces phosphofructokinase activity in the adductor muscle of *Mytilus* (Ebberink, 1982), and acidosis has been implicated in reduction of phosphofructokinase activity and glycolysis during anoxia in the whelk *Busycon* (Ellington, 1983). Pyruvate kinase from *Otala* shows decreased affinity for phosphoenolpyruvate and ADP, increased inhibition by alanine and ATP, and decreased activation by fructose 1,6-diphosphate *in vitro* when pH is lowered from 7·0 to 6·5 (J. Fields, personal communication).

Other mechanisms which have been proposed to suppress glycolysis during hypometabolism involve neither CO_2 nor change in pH. These include phosphorylation of regulatory enzymes, dissociation of enzyme complexes to produce less active soluble forms, and decreased levels of fructose 2,6-bisphosphate, an activator of phosphofructokinase (Storey, 1988). Recent studies show that fructose 2,6-bisphosphate levels fall by 86–93 % in *Otala* during 4 days of dormancy and subcellular binding of glycolytic enzymes decreases (K. Storey, personal communication). Thus, evidence suggests that both pH-dependent and pH-independent mechanisms may suppress glycolysis in *Otala* during dormancy.

Hypoxia and $\dot{V}_{O_2}$

P_{O_2} in the lung and haemolymph of dormant *Otala* is on average only about one-third that of active snails, and is often less than 10 mmHg owing to hypoventilation (Barnhart, 1986*b*). Might $\dot{V}_{O_2}$ be oxygen-limited during dormancy? One approach to this question is to compare $\dot{V}_{O_2}$ in dormancy with the limit of $\dot{V}_{O_2}$ predicted at the low levels of P_{O_2} typical of dormancy. As a first approximation, lung P_{O_2} can be substituted for ambient P_{O_2} in equation 5 [lung and ambient P_{O_2} do not differ greatly in active *Otala* (Barnhart, 1986*b*)]. The mean of the lowest 10 % of lung P_{O_2} values observed in dormant *Otala* was 4·5 mmHg (data of Barnhart, 1986*b*). The predicted limit of $\dot{V}_{O_2}$ at this P_{O_2} is about 5 $\mu l\,g^{-1}\,h^{-1}$ (from equation 5). This value is similar to the minimum sustained $\dot{V}_{O_2}$, observed during continuous respirometry ($5{\cdot}6 \pm 0{\cdot}57\,\mu mol\,g^{-1}\,h^{-1}$, S.E.M.; Barnhart & McMahon, 1987). Thus it appears that lung P_{O_2} sometimes approaches a critical level. However, the data do not indicate that P_{O_2} normally limits $\dot{V}_{O_2}$ during dormancy. Mean lung P_{O_2} during dormancy is 38 mmHg (Barnhart, 1986*a*), which corresponds to a $\dot{V}_{O_2}$ limit of 40 $\mu l\,g^{-1}\,h^{-1}$, much higher than the mean $\dot{V}_{O_2}$ during dormancy (14 $\mu l\,g^{-1}\,h^{-1}$; Barnhart & McMahon, 1987).

In conclusion, the effects of acute hypercapnia and hypoxia on oxygen consumption of active *Otala lactea* indicate that hypercapnia, but not hypoxia, explains much of the reduction of aerobic metabolism during dormancy. However, $\dot{V}_{O_2}$ of acutely hypercapnic active snails is not depressed to the lowest levels typical of dormancy, suggesting that other factors also contribute to metabolic depression.

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