# GAS EXCHANGE AND VENTILATORY RESPONSES TO HYPOXIA AND HYPERCAPNIA IN *AMPHISBAENA ALBA* (REPTILIA: AMPHISBAENIA)

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### SUMMARY

1. Total and cutaneous gas exchange and ventilatory responses to breathing hypoxic and hypercapnic gases were studied in *Amphisbaena alba* (Linnaeus), a burrowing squamate reptile.

2. This species shows a very low oxygen uptake rate ($\dot{V}_{O_2}$) compared with other squamates of the same size ($\dot{V}_{O_2} = 15\cdot4$, $36\cdot2$ and $49\cdot0\,\mathrm{ml\,kg^{-1}\,h^{-1}}$, at 20, 25 and 30°C, respectively). Cutaneous gas exchange represents a large fraction of the total uptake. Oxygen uptake was strongly affected by temperature [$Q_{10} = 5\cdot5$ (20–25°C); $1\cdot8$ (25–30°C); $3\cdot2$ (20–30°C)].

3. *A. alba* shows a biphasic ventilatory pattern under hypoxic and hypercapnic conditions. A single breathing cycle, consisting of expiration–inspiration, includes a ventilatory period (VP) followed by a non-ventilatory (breath hold) period (NVP) of variable duration. When breathing air at 25°C the NVP typically occupied about 2 min. The ventilatory period occupied only 0·075 parts of a complete breath-to-breath cycle. Breathing hypoxic gases caused a pronounced rise in ventilation volume ($\dot{V}_E$) from an increase in tidal volume ($V_T$) and frequency (f) at inspired $O_2$ concentrations below 7%. Breathing hypercapnic gas mixtures induced a minor change in $V_T$ at $CO_2$ concentrations below 3%, and $\dot{V}_E$ increased mostly because of increases in f. End tidal $O_2$ ($P_{ET_{O_2}}$) and $CO_2$ ($P_{ET_{CO_2}}$) tensions changed with increasing $\dot{V}_E$ while breathing hypoxic and hypercapnic gas.

4. The results are discussed in relation to the fossorial habits of *A. alba*, and are compared with data from other squamates.

### INTRODUCTION

Amphisbaenians are subterranean reptiles limited to tropical and subtropical regions. In spite of a high cutaneous water loss, they can be found even in semi-desert areas (Gans, 1968). They have been separated from lizards and snakes since the Palaeocene or perhaps the Cretaceous period (Gans, 1969; Romer, 1966). It

seems a reasonable assumption that subterranean life in amphisbaenians is phylo-genetically old, and that many morphological and physiological adaptations for subterranean life have been selected for. Although the anatomy and biochemistry of amphisbaenians is relatively well known, less is known about their physiology than that of any other reptiles (Johansen, Abe & Weber, 1980).

*Amphisbaena alba* has a wide geographical distribution throughout South America, occurring under different climatic conditions (Vanzolini, 1968). Living underground this species may face variable hypoxic and hypercapnic conditions, which may be intensified after heavy rain and in rain forest soils with high rates of biological decomposition. *A. alba* often digs its tunnels in hard compact soil. The limbless body is extremely muscular and contains more myoglobin than is known for other reptiles (Weber *et al.* 1980). Confined to these tunnels, which may reach depths of 1 m or more, *A. alba* may be exposed to an atmosphere both hypoxic and hypercapnic. The $O_2$ concentrations may be less than 10 % (Rawitcher, 1944). Fossorial mammals which burrow at similar depths have shown burrow $CO_2$ concentrations as high as 6 % (McNab, 1966).

In this study we investigated the gas exchange at different temperatures and the ventilatory responses to hypoxic and hypercapnic breathing in *A. alba*.

## MATERIALS AND METHODS

The amphisbaenians were collected in the State of Sao Paulo, Brazil, and air-freighted to Aarhus, Denmark. The 10 specimens used weighed from 130 to 210 g (mean mass 164 g) and were housed in individual cages at 25 °C for several weeks. Once every 10 days they were fed minced fish until satiated but food was withheld for a week prior to any experiments.

Oxygen uptake and carbon dioxide output were measured using an open-circuit method (Servomex differential paramagnetic $O_2$ analyser and Beckman LB-2 $CO_2$ analyser) in a temperature-controlled room at 20, 25 and 30 °C. The effluent gas from the respirometer was dried with silica gel before entering the gas analysers. After the amphisbaenians had been placed in the Plexiglas respirometer, gas exchange was measured for a minimum of 5 h and a maximum of 24 h, preceded by 3 h of adjustment to the experimental situation. Whenever measurements lasted longer than 12 h, only diurnal resting values were considered for comparison since con-tinuous monitoring of $O_2$ uptake for 24 h revealed a nocturnal pattern of activity.

To determine the lung and cutaneous gas exchange, the head of the amphisbaenian was isolated from the body by a rubber collar placed just behind the occipit and fixed with surgical tape (Fig. 1). The body of the animal was then placed in an air-tight plastic tube, with the head protruding into a small polyethylene chamber tightly connected to a plastic tube, but completely isolated from it by the rubber collar. Gas exchange in the head chamber was determined with an open-circuit method, measuring pumped air flow and $O_2$ and $CO_2$ contents using a Servomex analyser for $O_2$ and a Beckman LB-2 for $CO_2$. Analysed outputs were connected to a Beckman recorder provided with an integrating circuit. This arrangement permitted the
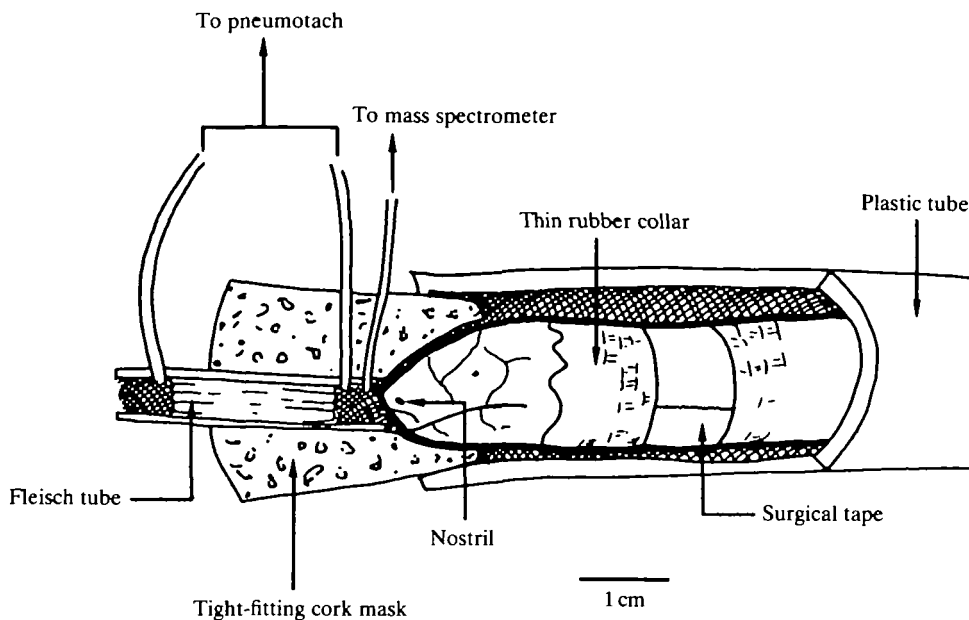
Fig. 1. Diagram of the mask and arrangement used to measure ventilatory parameters using a pneumotachographic method.

detection of very small gas tension differences. Cutaneous gas exchange was assessed by mass spectrometry (Medspect II, Searle) measuring the difference between the initial gas concentration in the plastic tube, followed by repeated measurements of tube gas composition at 15-min intervals. Prior to analysis of each 15-min sample, the gas in the posterior chamber was recirculated in the closed tube section, using a small air pump, to ensure complete mixing. After each reading the tube was flushed with room air and closed off again. The air volume in the tube was calculated after each experiment by water displacement, keeping the amphisbaenian's body inside the tube. Pieces of moist paper towel were kept inside the tube in order to maintain high humidity. All the values for gas exchange are expressed at STPD conditions.

Tidal volume, breathing frequency and total ventilatory volume were measured using a pneumotachographic method. A cork mask was tightly fixed to the head of the amphisbaenian and shielded from the rest of the body by a thin rubber collar and surgical tape, thus reducing the dead space to a minimum (Fig. 1). The cork and rubber collar arrangement were fitted into a thick-walled plastic tube containing and partly immobilizing the animal. The pneumotach transducer (Fleisch tube) was constructed as described elsewhere (Wood, Glass & Johansen, 1977), using a 1 ml syringe barrel and polyethylene tubing as air flow resistor. An opening close to the animal's nares was connected *via* plastic tubing to the mass spectrometer for continuous sampling and analysis of expired gas (Fig. 1). The tube containing the animal was then placed in a plastic bag which was kept inflated with normal air or different gas mixtures for testing ventilatory responses to selected gases. When the mask was in place, the amphisbaenians were allowed to rest for 3–4 h to become

accustomed to the experimental conditions. Whenever the amphisbaenians breathed hypoxic or hypercapnic gas mixtures, the bag was flushed with normal air afterwards until the normoxic breathing pattern was re-established. The ventilatory parameters measured included variations in the end tidal $O_2$ and $CO_2$ ($PET_{O_2}$ and $PET_{CO_2}$), tidal volume and breathing frequency, weight-specific ventilation ($\dot{V}E$), and the $P_{O_2}$ difference between inspired and expired air, which allows estimation of oxygen extractions from the ventilated air. All ventilatory parameters were measured at 25 °C in resting conditions. Surrounding movements and audible disturbances were restricted to a minimum. Tests for statistical significance were performed using a $t$-test for the difference between means. Whenever more than two groups were compared, one-way analysis of variance (ANOVA) with range test was used if significant intergroup variation was found. Differences were considered to be significant at the 0·05 level.

Tracheal volume of dead amphisbaenians was measured as described by Gratz (1978).

## RESULTS

### Oxygen uptake and carbon dioxide output

The oxygen uptake ($\dot{V}_{O_2}$) and carbon dioxide output ($\dot{V}_{CO_2}$) at different temperatures are shown in Fig. 2, and Table 1 summarizes the values of $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and respiratory quotient (RQ). The gas exchange values for A. alba showed a high temperature sensitivity shown by high $Q_{10}$ values for $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$. The $Q_{10}$ values between 20 and 25 °C were high: 5·5 for $\dot{V}_{O_2}$ and 3·9 for $\dot{V}_{CO_2}$. Between 25 and 30 °C the respective values were 1·8 and 2·5, and for the entire temperature interval studied (20–30 °C) $Q_{10}$ values were 3·2 and 2·5, respectively. Table 1 also shows a decrease in the RQ value with increase in temperature.

Simultaneous recordings of lung and cutaneous gas exchange demonstrated that most of the $O_2$ is taken up via the lungs, which are also the main route for $CO_2$ elimination (Table 2). Note that nearly 20 % of the total $\dot{V}_{O_2}$ and more than 43 % of the $CO_2$ exchange occur across the skin. The values for gas exchange of the lung and skin expressed in Table 2 may have been influenced by the mask technique needed to compartmentalize the pulmonary and cutaneous gas exchange surfaces. However, this contingency will in no way invalidate the finding of a very large fraction of cutaneous gas exchange in A. alba.

### Ventilation pattern and responses to breathing various gas mixtures

The ventilatory pattern in A. alba is biphasic with expiration followed by inspiration interspaced by a breath-hold period of variable duration, the non-ventilatory period (NVP) (see Fig. 4). When breathing sea level atmospheric air, the ventilatory period (VP) relative to the sum of the ventilatory period and non-ventilatory periods VP/(VP+NVP) was very short: 0·075 at 25 °C. No variation in this pattern, except changes in breath-hold duration and tidal volume, was found during hypoxic or

hypercapnic breathing. The ventilatory variables when breathing atmospheric air (21 % $O_2$) are shown in Tables 3 and 4.

Analysis of variance (ANOVA) showed that the ventilatory variables did not change between 21 % and 10 % oxygen. At 7 % $O_2$ (Table 3; Fig. 3) ventilatory
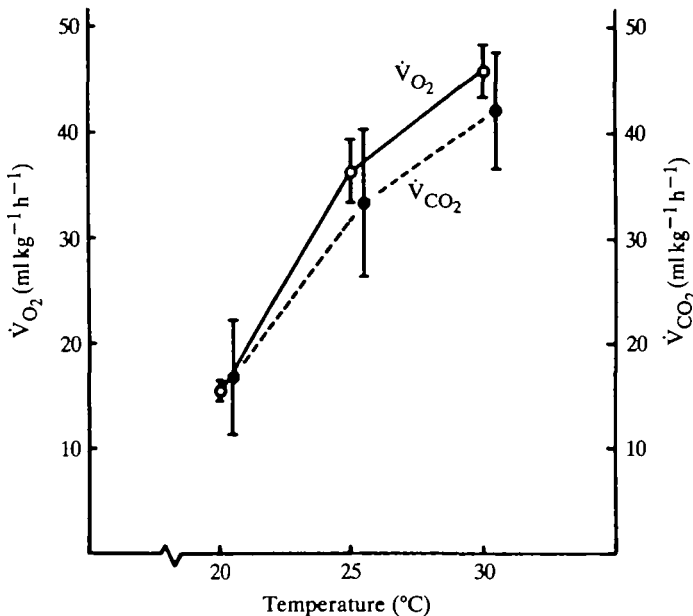
Fig. 2. Gas exchange of *Amphisbaena alba* at different temperatures. Open circles and solid lines, $\dot{V}_{O_2}$; solid circles and dashed lines, $\dot{V}_{CO_2}$. Mean ± s.e.m. $N = 4$ (20°), $N = 11$ (25°), $N = 9$ (30°).

Table 1. $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ *and RQ for* Amphisbaena alba *at different temperatures*

| Temperature (°C) | $N$ | Mass (g) | $\dot{V}_{O_2}$ (ml kg$^{-1}$ h$^{-1}$) | $\dot{V}_{CO_2}$ (ml kg$^{-1}$ h$^{-1}$) | RQ |
|---|---|---|---|---|---|
| 20 | 4 | 170·3 ± 6·5 | 15·4 ± 0·4 | 19·9 ± 5·5 | 1·094 ± 0·035 |
| 25 | 11 | 165·0 ± 11·7 | 36·2 ± 3·0 | 33·3 ± 7·2 | 0·905 ± 0·089 |
| 30 | 9 | 162·4 ± 11·5 | 49·0 ± 2·5 | 42·2 ± 5·5 | 0·877 ± 0·056 |

Values are mean ± s.d.

Table 2. *Lung and skin gas exchange in* Amphisbaena alba *at 25°C*

| | Lung | Skin | Total |
|---|---|---|---|
| $\dot{V}_{O_2}$ (ml kg$^{-1}$ h$^{-1}$) | 29·2 ± 4·2 | 6·5 ± 0·9 | 35·8 ± 5·1 |
| $\dot{V}_{CO_2}$ (ml kg$^{-1}$ h$^{-1}$) | 12·6 ± 1·6 | 9·2 ± 0·8 | 21·5 ± 2·8 |
| RQ | 0·433 ± 0·007 | 1·42 ± 0·08 | 0·927 ± 0·043 |
| % of total $\dot{V}_{O_2}$ | 81·8 ± 0·05 | 18·2 ± 0·01 | |
| % of total $\dot{V}_{CO_2}$ | 56·9 ± 1·80 | 43·1 ± 1·80 | |

Values are mean ± s.e., $N = 3$.

A. S. ABE AND K. JOHANSEN

Table 3. *Ventilatory responses of Amphisbaena alba to hypoxia at 25°C*

| % $O_2$ (mmHg) in inspired gas | 21 (158) | 10 (81) | 7 (52) | 6 (45) | 5 (39) | 3 (23) |
|---|---|---|---|---|---|---|
| End tidal $O_2$ (mmHg) | 102·2 ± 6·3 | 37·1 ± 2·5 | 29·2 ± 1·9 | 25·1 ± 1·6 | 23·2 ± 1·6 | 11·6 ± 1·4 |
| End tidal $CO_2$ (mmHg) | 21·5 ± 1·7 | 20·2 ± 1·2 | 15·2 ± 1·1 | 16·6 ± 0·8 | 14·2 ± 1·0 | 15·6 ± 0·9 |
| $V_T$ [ml(BTPS) $kg^{-1}$] | 20·6 ± 0·3 | 22·3 ± 3·1 | 28·1 ± 1·5 | 31·2 ± 0·9 | 31·4 ± 2·8 | 33·7 ± 2·2 |
| f ($h^{-1}$) | 31·4 ± 4·8 | 32·4 ± 6·0 | 57·6 ± 12·6 | 48·0 ± 10·2 | 77·4 ± 9·0 | 147·0 ± 25·8 |
| $\dot{V}_E$ [ml(BTPS) $kg^{-1}$ $h^{-1}$] | 642 ± 42·6 | 690 ± 36·6 | 1620 ± 264 | 1500 ± 96 | 2478 ± 294 | 4956 ± 330 |
| % $O_2$ extraction | 34·7 ± 3·5 | 54·2 ± 4·7 | 43·8 ± 5·0 | 44·2 ± 4·4 | 40·5 ± 3·6 | 50·4 ± 7·8 |
| $\dot{V}_{O_2}$ [ml(STPD) $kg^{-1}$ $h^{-1}$] | 35·6 ± 4·0 | 28·4 ± 2·5 | 37·7 ± 4·3 | 30·2 ± 3·0 | 38·1 ± 3·4 | 56·9 ± 8·8 |
| $\dot{V}_E/\dot{V}_{O_2}$ | 18·0 ± 1·9 | 24·3 ± 3·5 | 43·0 ± 4·0 | 49·7 ± 2·2 | 65·0 ± 4·5 | 87·1 ± 6·3 |

Values are mean ± S.E.M., N = 4.
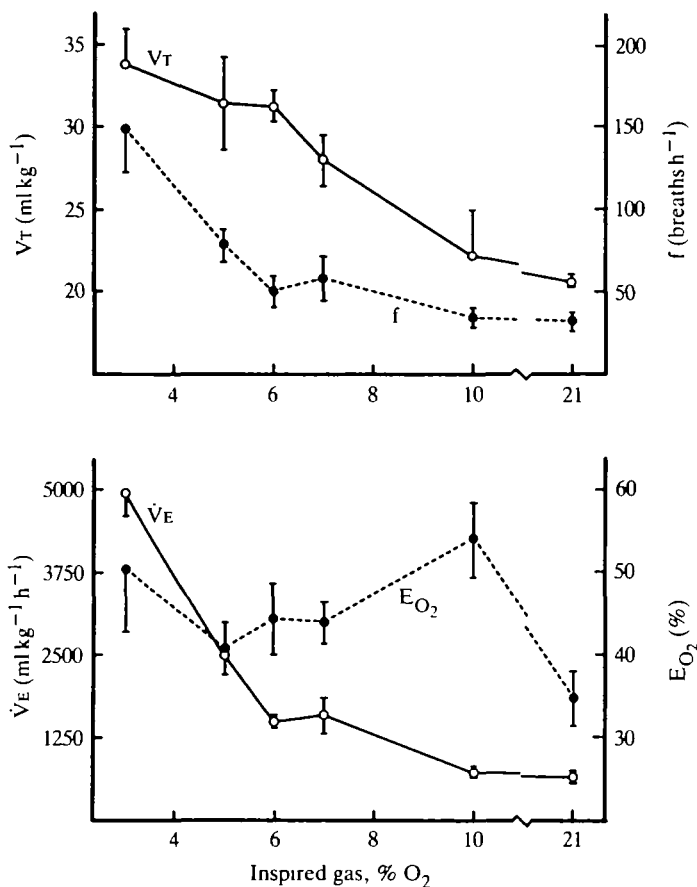
Fig. 3. Variations of tidal volume (VT), respiratory rate (f), ventilation volume (V̇E), and $O_2$ extraction $E_{O_2}$ at different inspired $O_2$ concentrations. Mean $\pm$ s.e.m., $N = 4$.

volume increased because of significant increases in tidal volume and frequency. Below 6 % $O_2$ however, V̇E increased only because of a rise in frequency. Ventilatory responses to hypercapnia were rather different from the hypoxic responses in spite of a rather similar increase in lung ventilation. In hypercapnic conditions (Figs 4, 5; Table 4) V̇E did not change significantly at $CO_2$ concentrations below 3 % $CO_2$. At 3 % $CO_2$, f was significantly increased (Table 4), but tidal volume (VT) remained unchanged at inspired $CO_2$ tensions as high as 4 %, although ventilation had increased more than four-fold due to an increase in breathing frequency (f) (Fig. 4). The difference in f between 4 % and 5 % $CO_2$ was not significant, but f values were significantly higher than at 3 % and lower than at 6 % $CO_2$. VT, however, remained unchanged and increased significantly only when 5 % and 6 % $CO_2$ were inspired.

Increased ventilation values in response to hypercapnia were correlated with a marked decrease in the inspired − expired $P_{O_2}$ differences ($\Delta P_{O_2}$) and thus % $O_2$ extraction compared to hypoxic breathing (Tables 1, 3, 4). Fig. 5 shows the ventilatory responses to increasing inspired $CO_2$ tension. It was not possible to obtain

data at concentrations higher than 6 % $CO_2$ because of struggling movements by the animals.

Tracheal volume for *A. alba* was $2 \cdot 96 \pm 0 \cdot 05 \, \mathrm{ml \, kg^{-1}}$.

## DISCUSSION

The oxygen uptake rates for *A. alba* at 20, 25 and 30 °C are considerably lower than values reported for lizards and snakes in the same weight range. The value at 20 °C was also lower than for some turtles of similar weight. In the fossorial caecilian *Bolengerula taitanus*, oxygen uptake values were in the range reported for other amphibians of similar size (Wood, Weber, Maloiy & Johansen, 1975). These data may suggest that reduced oxygen uptake in a caecilian may not be a specific adaptation for fossorial life. Kamel & Gatten (1983), comparing $O_2$ uptake in three species of fossorial reptiles with data from similar sized, non-fossorial reptiles, found that resting metabolic rate was 34–67 % lower in the fossorial species. Our data on *A. alba* also support the suggestion that a reduced $O_2$ uptake may be an adaptation for fossorial life.

*A. alba* is probably one of the largest amphisbaenids (Gans, 1975). The species must be periodically active because of the considerable mechanical activity involved in digging, often in hard-packed soil. The oxygen availability in the tunnels and the low rate of oxygen diffusion through the soil may set limitations for the maximum $\dot{V}_{O_2}$ possible. As pointed out by Hayward (1966), the oxygen concentration in some rodent burrows and galleries may be quite low (McNab, 1966). *A. alba* is reported to come to the soil surface during heavy rain (Beebe, 1945) which is likely to flood its tunnels. During active burrowing at greater depths, *A. alba* may experience
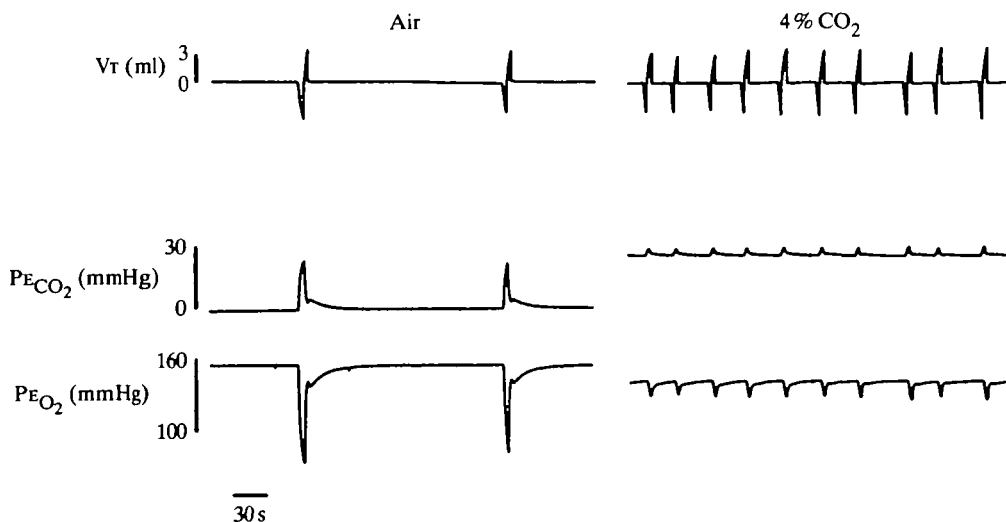


Fig. 4. Tidal volume ($V_T$) and end-tidal gas compositions ($P_{E_{O_2}}$ and $P_{E_{CO_2}}$) during normal air and 4 % $CO_2$ breathing. Note unchanged tidal volume and increases in frequency at 4 % $CO_2$.
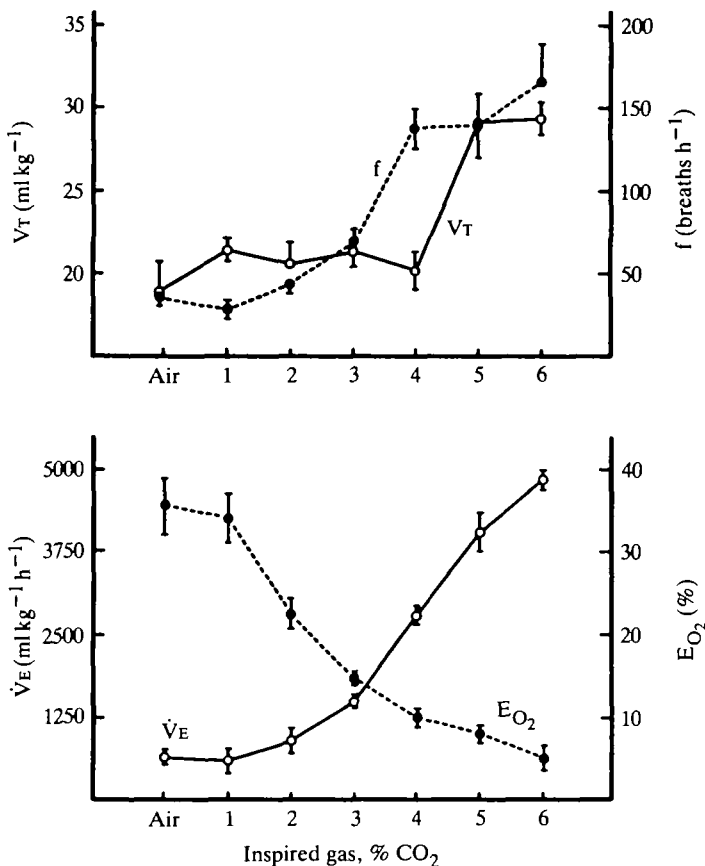
Fig. 5. Ventilatory parameters during hypercapnic breathing in *Amphisbaena alba*. Mean ± s.e., $N = 4$. $V_T$, tidal volume; f, respiratory rate; $\dot{V}_E$, ventilation volume; $E_{O_2}$, oxygen extraction.

low ambient oxygen concentrations. Its exceptionally high blood oxygen affinity (Johansen *et al.* 1980) and high muscle myoglobin content (Weber, Johansen & Abe, 1980) may help to ensure an adequate oxygen supply during activity in a hypoxic atmosphere.

It is proposed that *A. alba*, because of its fossorial habits, may have its most temperature-independent $O_2$ uptake at lower ambient temperatures than is typical for reptiles. Accordingly, its preferred temperature should be much lower than is typical for tropical reptiles. Hicks & Wood (1985) have demonstrated in the iguana a relationship between ambient $O_2$ availability and the preferred body temperature, which declined as inspired $O_2$ tension was reduced. Wood (1984) hypothesized, on the basis of a model analysis relating blood $O_2$ affinity to central cardiovascular shunting in ectotherms, how tissue $O_2$ uptake could be limited. He predicted that ectotherm vertebrates may thermoregulate behaviourally to a lower preferred body temperature when exposed to ambient hypoxia. *A. alba* may be one of very few reptiles naturally exposed to an $O_2$ availability considerably less than in atmospheric

Table 4. *Ventilatory responses of Amphisbaena alba to hypercapnia at 25°C*

| | Air | Inspired $CO_2$ concentration | | | | | |
| | | 1% | 2% | 3% | 4% | 5% | 6% |
|---|---|---|---|---|---|---|---|
| End tidal $O_2$ (mmHg) | 102·0 ± 7·5 | 104·0 ± 4·8 | 121·3 ± 1·9 | 132·5 ± 0·1 | 136·4 ± 2·2 | 136·7 ± 1·6 | 139·7 ± 2·0 |
| End tidal $CO_2$ (mmHg) | 23·0 ± 1·2 | 30·7 ± 0·7 | 29·1 ± 1·0 | 30·6 ± 1·2 | 32·9 ± 0·5 | 38·3 ± 0·1 | 44·3 ± 0·6 |
| $V_T$ [ml(BTPS) kg$^{-1}$] | 18·9 ± 2·0 | 21·5 ± 0·8 | 20·6 ± 1·3 | 21·5 ± 1·0 | 20·2 ± 1·2 | 29·3 ± 1·8 | 29·4 ± 1·0 |
| f (h$^{-1}$) | 34·2 ± 4·2 | 27·6 ± 6·0 | 44·4 ± 6·0 | 70·2 ± 7·2 | 138·0 ± 12·6 | 141·0 ± 19·2 | 165·0 ± 24·0 |
| $\dot{V}_E$ [ml(BTPS) kg$^{-1}$ h$^{-1}$] | 630 ± 72 | 582 ± 168 | 900 ± 173 | 1494 ± 90 | 2790 ± 96 | 4044 ± 318 | 4854 ± 126 |
| % $O_2$ extraction | 35·4 ± 3·6 | 33·8 ± 3·2 | 22·2 ± 1·9 | 14·6 ± 0·5 | 10·2 ± 1·3 | 8·2 ± 0·9 | 5·9 ± 1·5 |
| $\dot{V}_{O_2}$ [ml(STPD) kg$^{-1}$ h$^{-1}$] | 35·6 ± 2·5 | 31·2 ± 3·0 | 31·4 ± 2·5 | 34·1 ± 2·0 | 43·9 ± 4·6 | 50·1 ± 4·4 | 42·9 ± 7·7 |
| $\dot{V}_E/\dot{V}_{O_2}$ | 17·7 ± 1·2 | 18·7 ± 3·3 | 28·7 ± 3·5 | 43·8 ± 2·4 | 63·6 ± 2·9 | 80·7 ± 6·3 | 113·1 ± 10·9 |

Values are mean ± S.E.M., $N = 4$.

air. From field measurements (A. S. Abe, unpublished) a preferred body tempera-
ture for *A. alba* of about 25 °C is predicted.

Whether the inherently low $\dot{V}_{O_2}$ in *A. alba* at 30 °C is an adaptation for a burrowing
life or a characteristic of amphisbaenians must remain an open question.

*A. alba* has a surprisingly large cutaneous component of total gas exchange when
compared with other terrestrial squamates. Even aquatic snakes such as *Acrochordus*
and *Natrix* show a lower cutaneous gas exchange fraction than *A. alba* (Standaert &
Johansen, 1974; Gratz, 1978). Cutaneous $\dot{V}_{CO_2}$ greatly exceeded cutaneous $\dot{V}_{O_2}$, and
consequently pulmonary RQ values were low; this was confirmed from the end tidal
gas compositions analysed during normal breathing using the mass spectrometer.
A high fraction of cutaneous gas exchange, especially for $CO_2$, is also indicated by
the high cutaneous water loss, which reflects the humid environment in which
*Amphisbaena* lives (Krakauer, Gans & Paganelli, 1968). With respect to the overall
respiratory quotient determined for the long-term gas exchange studies, there was a
clear tendency for decreased RQ values with increasing temperature. The lowest
values were found between 25 and 30°C. Jackson, Palmer & Meadow (1974) have
suggested that $CO_2$ retention with increasing temperature in the turtle *Pseudemys
scripta* is related to pH regulation. The RQ values relative to the temperatures used
in the *A. alba* study were not different from those previously reported for other
reptiles (Bennett & Dawson, 1976).

Metabolic rate, expressed as oxygen consumption, was surprisingly temperature-
sensitive between 20 and 25 °C, and to a much lesser extent between 25 and 30 °C.
Such a significant thermal dependence can be related to the acclimation temperature,
since the amphisbaenians were kept at 25 °C for many weeks prior to use, and gas
exchange was measured in acute conditions at 20 and 30 °C. There was a clear trend
for $Q_{10}$ to decline with increasing temperature, a finding reported earlier for many
lizards and snakes (Bennett & Dawson, 1976). Such temperature dependence in
*Amphisbaena* might be related to the narrow range of temperature in which this
species is active.

Some burrowing lizards may follow thermal gradients within the soil (Brattstrom,
1965). Field observations showed that the amphisbaenian *Agamodon anguliceps*
moves vertically in the soil as the temperature rises (Gans, 1968). The soil has a quite
stable temperature at a given depth which, of course, varies according to its com-
position (Monteith, 1975). It is possible that amphisbaenians are able to maintain a
rather stable body temperature by vertical migration during diurnal and seasonal
activity.

Reptiles in general have a biphasic ventilatory pattern in undisturbed conditions
(Wood & Lenfant, 1976; Gans & Clark, 1978; Glass & Wood, 1983). Ventilation in
*A. alba* follows this general pattern (expiration–inspiration–pause) during normal
air breathing and in hypoxic and hypercapnic conditions. Tidal volume was high
and nearer to the range reported for snakes than for lizards (Dmi'el, 1972; Glass
& Johansen, 1976; Gratz, 1978; Stinner, 1982; Wood *et al.* 1977). Breathing
frequency at a given temperature, however, was low, even compared to values for
turtles. Among squamates, only *Acrochordus* (Glass & Johansen, 1976) has a lower

breathing frequency than *A. alba*. This low breathing frequency results in the low weight-specific ventilation and a ventilatory requirement of 17·6 (BTPS/STPD) for *A. alba* at 17°C. According to the Fick principle, the low ventilatory requirement must correlate with a high oxygen extraction such as has been demonstrated for *A. alba*. In fact, *A. alba* shows a higher $O_2$ extraction than all species of lizards and snakes studied, except *Acrochordus javanicus*. *A. alba* and the aquatic snake *A. javanicus* share many features, such as low values for oxygen uptake and low ventilatory requirements, in addition to very high blood oxygen affinities and relatively high blood $O_2$ capacities (Standaert & Johansen, 1974; Glass & Johansen, 1976; Johansen *et al.* 1980).

Turtles are very tolerant of low oxygen tensions, and the influence of low $P_{O_2}$ has been reported to be less important than hypercapnia in the control of breathing in these reptiles (Lenfant, Johansen, Petersen & Schmidt-Nielsen, 1970; Glass & Wood, 1983). Other reptiles seem to be less tolerant of hypoxia, and inspiration of oxygen mixtures between 15% and 10% $O_2$ produces a rapid ventilation increase in lizards and snakes (Randall, Stullken & Hiestand, 1944). When the lizards *Lacerta* and *Tarentola* were subjected to hypoxia, ventilation increased, by augmentation of tidal volume, and breathing frequency decreased (Nielsen, 1962). The aquatic snake *Acrochordus* showed a marked ventilation increase when inspired oxygen concentration was less than 10%. VT was not affected until 5% $O_2$ was inspired, while f increased also at higher $O_2$ concentrations (Glass & Johansen, 1976). The water snake *Natrix rhombifera* responds to hypoxia by increasing tidal volume and reducing ventilatory frequency, resulting in little change in ventilation (Gratz, 1979). Considering the weight-specific ventilation volume at 5% $O_2$ inspired gas, the values for *A. alba* were more than double those for *A. javanicus*, suggesting more efficient gas exchange in the aquatic snake. Most importantly, when discussing control of breathing in ectotherms, the animals' preferred temperature as well as the experimental temperatures must be considered (Glass & Wood, 1983).

An intact specimen of *A. alba*, resting completely buried for nearly 4 h in air-equilibrated soil, showed a $Pa_{O_2}$ of 49 mmHg (1 mmHg = 133·3 Pa), which corresponds to an arterial $O_2$ saturation of 93%. The high $O_2$ saturation of the blood at such low arterial $P_{O_2}$ correlates with an extraordinarily high $O_2$ affinity of the blood in *A. alba* compared to that of other squamate reptiles; this characteristic should be of adaptive value to a fossorial animal (Johansen *et al.* 1980). In addition, a low preferred temperature in *A. alba* will shift the $O_2$ dissociation curve of the blood to the left. As pointed out by Wood (1984), another consequence of a lower preferred body temperature in ectotherms may be a lowering of the arterial $O_2$ tension of the hypoxic ventilatory threshold.

High $CO_2$ concentration has a tendency to depress breathing frequency in lizards and snakes (Nielsen, 1961; Templeton & Dawson, 1963; Glass & Johansen, 1976; Gratz, 1979). For *A. alba*, however, f increased continuously above 1% $CO_2$ in inspired gas, with no reduction at higher $CO_2$ concentrations. However, VT remained unchanged at concentrations lower than 5% $CO_2$ and increased slightly at higher inspired $CO_2$ concentrations. This response pattern seems to be intermediate

between the increase in VT reported for *Lacerta* (Nielsen, 1962) and *N. rhombifera* (Gratz, 1979), and the pattern in *A. javanicus*, in which VT did not change significantly even at high $CO_2$ concentrations (Glass & Johansen, 1976).

Perhaps the most striking result obtained in this study of *A. alba* is the conspicuous reduction in $O_2$ extraction from the lung with increasing $CO_2$ content in the inspired air. The extraction declines from 35·4% when breathing atmospheric air to 5·9% when breathing 6% $CO_2$ (Table 4). Correlated with this decrease is a nearly eight-fold increase in ventilation.

We see two possible mechanisms behind this change in the $P_{O_2}$ gradient between inspired and expired gas (or % $O_2$ extraction). First, the increase in ambient $P_{CO_2}$ will change the diffusional conductance for $CO_2$ across the skin. At 6% $CO_2$ ambient, there will be hardly any gradient in $CO_2$ across the skin. This will cause retention of $CO_2$ and the high Bohr shift of the blood will lead to a very low affinity for $O_2$ which may compromise $O_2$ uptake from the lung gas. Second, the $CO_2$ retention will probably cause an increasing right-to-left shunt of the blood and a mismatch of ventilation or lung $O_2$ availability, with perfusion of the lung leading to decreased $O_2$ extraction.

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