VENTILATION AND GAS EXCHANGE IN LIZARDS DURING TREADMILL EXERCISE

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Summary

The extent to which lizards ventilate their lungs during locomotion is controversial. Direct measurements of airflow across the nostrils suggest a progressive reduction in tidal volume and minute ventilation with increased running speed, while other studies have demonstrated that arterial P_{O_2} remains constant during exercise. To resolve these conflicting findings, we measured minute ventilation and gas exchange rate in five specimens of *Varanus exanthematicus* and five specimens of *Iguana iguana* during treadmill locomotion at speeds between 0.14 and 1.11 m s^{-1} at 35 °C. These speeds are much lower than maximal running speeds, but are greater than the maximal aerobic speed. In both species, the ventilatory pattern during locomotion was highly irregular, indicating an interference between locomotion and lung ventilation. In *Varanus exanthematicus*, treadmill locomotion elicited a six- to eightfold increase in minute ventilation from a pre-exercise level of 102 ml kg^{-1} min^{-1}, whereas the rate of oxygen uptake increased approximately threefold (from 3.9 to 12.6 ml kg^{-1} min^{-1}). After exercise, both minute ventilation and gas exchange rate decreased immediately. Because minute ventilation increased more than did oxygen consumption, an increase in lung P_{O_2} during exercise is predicted and, thus, *Varanus exanthematicus* appears effectively to ventilate its lungs to match the increased metabolic rate during locomotion at moderate speed. In *Iguana iguana*, both minute ventilation and gas exchange rate increased above resting values during locomotion at 0.28 m s^{-1}, but both decreased with further increases in locomotor speed. Furthermore, following exercise, both minute ventilation and oxygen uptake rate increased significantly. *Iguana iguana*, therefore, appears to be unable to match the increased oxygen demand with adequate ventilation at moderate and higher speeds.

Key words: reptile, lizards, *Varanus exanthematicus*, *Iguana iguana*, exercise, locomotion, ventilation, breathing pattern, gas exchange, cardiovascular.

Introduction

In most vertebrates, ventilation increases during physical activity to meet the increased metabolic demands of the working muscles. In the lizards *Varanus exanthematicus* and *Iguana iguana*, measurements of arterial blood gases and pulmonary gas exchange rate indicate that ventilation adequately maintains arterial blood gas composition during treadmill exercise at speeds greater than their maximal aerobic speed (Mitchell *et al.* 1981*a*,*b*; Bennett, 1994). In a subsequent study, Carrier (1987*a*) measured inspired airflow across the nostrils in four species of lizards (*Iguana iguana*, *Ctenosaura similis*, *Varanus exanthematicus* and *Varanus salvator*) during rest, activity and recovery. At speeds greater than walking speed, all four species displayed a progressive decrease in tidal volume, resulting in a decrease in total ventilation in spite of an increased breathing frequency. On the basis of these findings and the musculoskeletal anatomy of lizards, Carrier (1987*a*,*b*) suggested that lizards are mechanically constrained

from breathing during rapid locomotion because the hypaxial muscles contribute to both ventilatory and locomotor movements. This interpretation was subsequently supported by electromyographic recordings from the hypaxial muscles during walking and ventilation in *Iguana iguana* (Carrier, 1989, 1990). The disparity between the studies by Mitchell *et al.* (1981*a*,*b*) and Carrier (1987*a*,*b*) is not easily explained since ineffective pulmonary ventilation during locomotion must result in predictable changes in the composition of arterial blood gases. However, because lizards often employ intermittent locomotion, even on treadmills, it is possible that blood gas composition is maintained by ventilating the lungs during brief pauses between locomotor activity. The possible inability of lizards to ventilate their lungs effectively during exercise is in sharp contrast to the pattern observed in birds and mammals and has important implications concerning the evolution of sustained locomotion in tetrapods (e.g. Carrier,

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1987*b*; Bramble and Jenkins, 1989; Bennett, 1994; Ruben, 1995).

Given the conflicting results of these studies, we re-examined ventilatory responses to exercise in *Varanus exanthematicus* and *Iguana iguana*. Ventilation and gas exchange were measured directly, both at rest and at speeds that were in excess of maximum aerobic capacity.

Materials and methods
Experimental animals

Five savannah monitors (*Varanus exanthematicus* Bosc) with body masses ranging from 260 to 660 g were purchased from a licensed supplier in Florida, USA, and transported by air to Irvine, CA, USA. In addition, five green iguanas (*Iguana iguana* L.) weighing 330–1900 g were obtained from local pet stores. All lizards were housed in large terraria with free access to water and a thermal gradient and were maintained on a light:dark cycle of 12 h:12 h.

Measurement of ventilation and gas exchange rate

In all experiments, the ventilatory airflows across the nostrils were measured using pneumotachography. To provide a gas-tight connection between the nostrils and the pneumotachograph, thin-walled Tygon tubing was inserted into each nostril of unanaesthetised animals and then merged and glued to the head using epoxy. In the smallest iguanas, the nostrils were too small for this arrangement, and a small plastic mask was glued over the nostrils instead. The pneumotachograph (8421, series 0-5 LPM, H. Rudolph, Inc., MO, USA), connected to a differential pressure transducer (Validyne MP 45-1-871), was attached to the tubing leaving the nostrils or mask. The pneumotachograph was calibrated by inserting a syringe where the tubing or mask was connected during the experiments and manually simulating breaths. All calibration procedures produced very tight correlations between injected gas volumes and the integrated flow signal ($r^2 \geqslant 0.98$) and were reproducible before and after the experiments. To provide an independent verification of our pneumotachograph recordings, we also collected the exhaled gases in a Mylar bag during many of the trials on the varanids. The volume of the exhaled gas in the Mylar bag was subsequently determined by withdrawing the gas into an airtight glass syringe. To collect exhaled gases, a miniature T-shaped two-way non-rebreathing valve (series 2384, H. Rudolph, Inc., MO, USA) was inserted between the tubes leaving the nostrils and the pneumotachograph (which, in turn, was connected to the Mylar bag). The inhalation and exhalation ports of this valve each contain a diaphragm creating a unidirectional airflow between inhaled air and the Mylar bag; thus, only exhaled airflow could be measured in this experimental design. There was excellent agreement between the direct determination of the exhaled gas and that determined by pneumotachography: a linear regression yielded $V_m = 0.999 V_p$ ($r^2 = 0.98$; $N = 52$), where V_m is the volume of exhaled gas in the Mylar bag and V_p is the volume of exhaled gas calculated from pneumotachography.

Gas exchange rate was measured differently in the two species of lizards. In preliminary experiments, we verified that the two experimental designs yielded similar values for O_2 uptake and CO_2 excretion rates. For the varanids, gas exchange rate was determined from minute ventilation (as measured by pneumotachography) and exhaled gas composition in the gas collected in the Mylar bags. Exhaled gas composition was analysed using Applied Electrochemistry O_2 and CO_2 analysers (S-3A and CD-3A, respectively) connected in series. From these measurements, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were calculated as $(F_{I_{O_2}} - F_{E_{O_2}})V$ and $(F_{E_{CO_2}} - F_{I_{CO_2}})V$, respectively, where V is the volume of exhaled gas, F_I designates the inspired gas fraction and F_E designates the expired gas fraction. All $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ measurements were corrected to STPD. For *Iguana iguana*, gas exchange rate was determined as described by Wang and Warburton (1995). Briefly, the tubing leaving the nostrils was connected to a T-piece attached at both ends to gas-tight Tygon tubing. One end fed into the gas analysers (described above), connected in series, whereas the other served as a reservoir. An Applied Electrochemistry flow pump, connected in series with the gas analysers, maintained a constant gas flow from the T-piece and the gas analysers. The pneumotachograph was inserted into the reservoir tubing and measured a decrease in the airflow rate during exhalation and an increase in the airflow rate during inhalation. In this system, the signal from the differential pressure transducer preceded that of the gas analysers by approximately 2 s. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were determined as the area below the baseline signal for each gas observed during a breath-hold; the relationship between this area and gas exchange rate was determined by simulating exhalations with known gas compositions and volumes.

Measurement of systemic blood flow and heart rate

After completing all measurements on the ventilatory responses to treadmill exercise, four varanids were instrumented using a blood-flow probe (2R, Transonic System, Inc., Ithaca, NY, USA) for determination of systemic blood flow and heart rates. Shortly before surgery, lizards were placed in a bucket with a cloth soaked in Halothane to induce light anaesthesia to allow intubation. After intubation, the lizards were artificially ventilated (8–15 breaths min^{-1} and a tidal volume of 10–20 ml kg^{-1}) using an SAR-830 CWE Inc. ventilator (Ardmore, PA, USA) and a gas mixture consisting of 30 % O_2, 3 % CO_2 (balance N_2) prepared by a gas-mixing flow meter (GF-3, Cameron Instruments, TX, USA). This gas mixture passed through a Halothane vaporizer (Dräger, Lubeck, Germany) initially set at 3–4 % and subsequently reduced to 0.5–1 % throughout the surgery. To implant the flow probe, a 2–3 cm incision was made on the ventral side caudal to the sternum. Because we felt it was imperative to minimize damage to the rectus abdominus muscle, the flow probe was inserted on the most easily accessible vessel. This differed among animals, and flow probes were, therefore, implanted around either the left aortic arch or a branch of the right aortic arch. After placement of the flow probe, the incision was closed with intermittent sutures and cyanacrylate tissue glue (Nexaband; S/C-TriPoint Medical, Raleigh, NC, USA). Artificial ventilation without Halothane was continued until the lizard regained

consciousness and started breathing on its own. It was then placed in a small container maintained at 30–35 °C and allowed to recover for at least 48 h.

Flow probes were connected to a dual-channel blood flow meter (T201, Transonic System, Inc.) for simultaneous measurement of both mean blood flow rate and instantaneous blood flow velocity, which was used for on-line determination of heart rate. Because we were not able to place flow probes around all the systemic outflow vessels, our measurements underestimate systemic blood flow rate and blood flow measurements are therefore expressed as relative values compared with rest.

Monitoring of the locomotor cycle

The locomotor cycle was monitored using a photocell apparatus that recorded lateral bending of the trunk. The apparatus consisted of a photocell and a light-emitting diode (LED) situated at opposite ends of a 10 cm piece of flexible tubing attached to the dorsal midline of the lizards using strips of Velcro. The photocell formed one arm of a Wheatstone bridge. As the lizard walked and ran on the treadmill, lateral bending of the trunk bent the tube and changed the amount of light from the LED illuminating the photocell, providing an analogue record of the locomotor cycle. In addition, all experiments were video-taped using a VHS video system (60 images s^{-1}), which enabled subsequent analysis of exercise performance.

Experimental protocols

In both species, ventilation and gas exchange rate were determined simultaneously (as described above) before, during and after exercise using a similar experimental protocol. At least 24 h prior to experimentation, lizards were equipped with masks or tubing inserted in the nostrils and allowed to acclimate to the experimental temperature (35 °C). A few hours before treadmill exercise, the lizard was placed on the treadmill and left undisturbed with the mask or tubing connected to the experimental apparatus. Immediately before running, pre-exercise ventilation and gas exchange rates were measured over a 10–20 min period. Although the lizards quickly relaxed when placed on the treadmill, these pre-exercise values do not represent true resting values. To minimize fatigue, each lizard was exercised on two consecutive days. For the varanids, the exercise regime commenced with a walking speed of 0.14 m s^{-1}, followed by 0.28, 0.42 and 0.56 m s^{-1} (in that order) on the first day, whereas the exercise regime consisted of 0.28 and 0.83 m s^{-1} on the second day. For the iguanas, the first day consisted of 0.28, 0.56 and 0.83 m s^{-1} (in that order), whereas the exercise regime consisted of 0.28 and 1.11 m s^{-1} on the second day. In both species, the maximum speed used represents the highest speed at which the lizards were able to match the belt speed for at least 2 min. At the lower locomotor speeds, the belt speed was kept constant for approximately 5 min. In each trial, the belt speed of the treadmill was slowly increased to the desired level and kept constant for at least 5 min or until the animal was no longer able to match the belt speed. In both species, gas exchange and minute ventilation were measured

during the last 2–3 min of each exercise trial. Following exercise, gas exchange rates and minute ventilation were measured for 3–4 min immediately following exercise (however, owing to the low ventilation following the lowest speeds in *Varanus exanthematicus*, it was necessary to sample for up to 7 min to collect sufficient gas for precise determination of gas exchange). This period immediately following exercise is referrred to as the recovery period. After each exercise trial, the lizard was allowed to recover further until the ventilatory pattern resembled that recorded during the pre-exercise period. In general, the period required for this criterion to be fulfilled increased with increasing running speed and lasted for between 15 and 60 min.

The iguanas were not able to perform sustained exercise. Therefore, to determine whether the pronounced increase in ventilation immediately following exercise (see Fig. 3A,B) resulted from a time-dependent change in the ventilatory response to exercise or to the cessation of locomotion proper, an additional experimental protocol was employed. In these experiments, iguanas were exercised repeatedly at 0.56, 0.83 or 1.11 m s^{-1} in exercise bouts lasting 30 s followed by a 2 min recovery period. This exercise regime was repeated six or seven times, or until the lizard displayed significant signs of fatigue and was no longer able to match the belt speed. It was assumed that this protocol resulted in an elevated metabolic rate during the period of recovery and allowed comparison of minute ventilation during exercise and immediately prior to and immediately preceding exercise.

Data collection, analysis and statistics

All signals from the differential pressure transducer, the blood flow meter and the photocell for measurement of lateral bending were collected on a computer at 50 Hz using Acknowledge data-acquisition system software (Biopac System, Goleta, CA, USA). All subsequent data analysis was performed using Acknowledge data-analysis software (version 3.0).

The effects of running speed on all parameters studied were assessed using a one-way analysis of variance (ANOVA) for repeated measures. Differences among means were distinguished using a Student–Newman–Keuls analysis. A fiducial limit for significance of $P<0.05$ was chosen, and all results are presented as mean ± 1 S.E.M.

Results

Critique of treadmill exercise

In this study, lizards were forced to walk and run on a treadmill, which is a very unnatural situation. In most lizards, the typical locomotor pattern consists of either slow walking or sprints of high speed and short duration. Nevertheless, the varanids performed consistently well at the lower speeds and were able to match the belt speed for longer than 10 min. In contrast, the iguanas performed poorly, and half of the animals purchased could not match the belt speed of the treadmill for any sustained period. Furthermore, these individuals exhibited behavioural traits such as defensive posturing and 'tail thrashing' and, consequently, we do not report the data

obtained on these animals. The data reported were obtained from five subjects of each species that performed consistently well. Within each species, the responses to exercise were consistent and, therefore, we feel that our data represent the cardiopulmonary response to increased metabolic rate during locomotion in these species.

Physiological variables were determined for lizards ambulating at speeds up to a maximum of $0.83\,\mathrm{m\,s^{-1}}$ for varanids and up to $1.11\,\mathrm{m\,s^{-1}}$ for iguanas. These speeds are well above the maximum aerobic speeds of both species ($0.33–0.54\,\mathrm{m\,s^{-1}}$ for *Varanus exanthematicus* and $0.14\,\mathrm{m\,s^{-1}}$ for *Iguana iguana*; Gleeson *et al.* 1980; see also Fig. 3), but are considerably lower than the maximum running speeds of both species ($2.0\,\mathrm{m\,s^{-1}}$ for *Varanus exanthematicus*, and $4.5\,\mathrm{m\,s^{-1}}$ for *Iguana iguana*; Carrier, 1987*a*). In the present study, $0.83\,\mathrm{m\,s^{-1}}$ and $1.11\,\mathrm{m\,s^{-1}}$, respectively, were the highest speeds that the lizards were able to maintain on the treadmill. The previous studies by Gleeson *et al.* (1980) and Mitchell *et al.* (1981*a,b*) reported physiological measurements only at speeds up to 0.28 and $0.54\,\mathrm{m\,s^{-1}}$ for *Iguana iguana* and *Varanus exanthematicus*, respectively.

Breathing pattern during and following locomotion

In all specimens of both species, the breathing pattern was altered during locomotion (Fig. 1). While the breathing pattern at rest in *Varanus exanthematicus* consisted of evenly spaced single breaths commencing with expiration followed by inhalation, the ventilatory airflows during locomotion were characterized by frequent and abrupt changes (Fig. 1A–C). The maximum airflow rate increased, whereas the inspiratory and expiratory times decreased relative to pre-exercise values. Following exercise, tidal volume immediately increased above pre-exercise values and the breathing pattern returned to distinct, evenly spaced breaths. In *Iguana iguana*, ventilation was typically continuous before exercise, which probably indicates that these animals were not in a resting state. At the onset of locomotion, the breathing pattern was characterized by a high-frequency ventilatory oscillation of low-volume inspiratory and expiratory flows, interrupted by larger breaths of high and erratic rates of airflow (Fig. 1D–F). The changes in tidal volume during and following exercise are presented in Fig. 2 for both species. In this analysis, only expiratory airflows that were separated by substantial inspiratory airflows are reported. Thus, many of the

Fig. 1. Sample recordings of ventilatory airflow rates in *Varanus exanthematicus* and *Iguana iguana* running on a treadmill at $0.83\,\mathrm{m\,s^{-1}}$. In each example, the upper trace is a recording of lateral bending of the trunk. A flat trace indicates that the lizard was stationary. Once the treadmill was turned on and the lizard began to move, each wave cycle on the trace represented one locomotor cycle. In each example, the lower trace is a pneumotachograph recording of ventilatory airflow rates at the nares. Expiratory flow occurred when the trace was above baseline. (A) A 30 s bout of running in *V. exanthematicus* illustrating ventilation before, during and after locomotion. (B,C) Enlarged segments of the running portion of trial A. (D) A 30 s bout of running in *I. iguana* illustrating ventilation before, during and after locomotion. (E,F) Enlarged segments of the running portion of trial D.

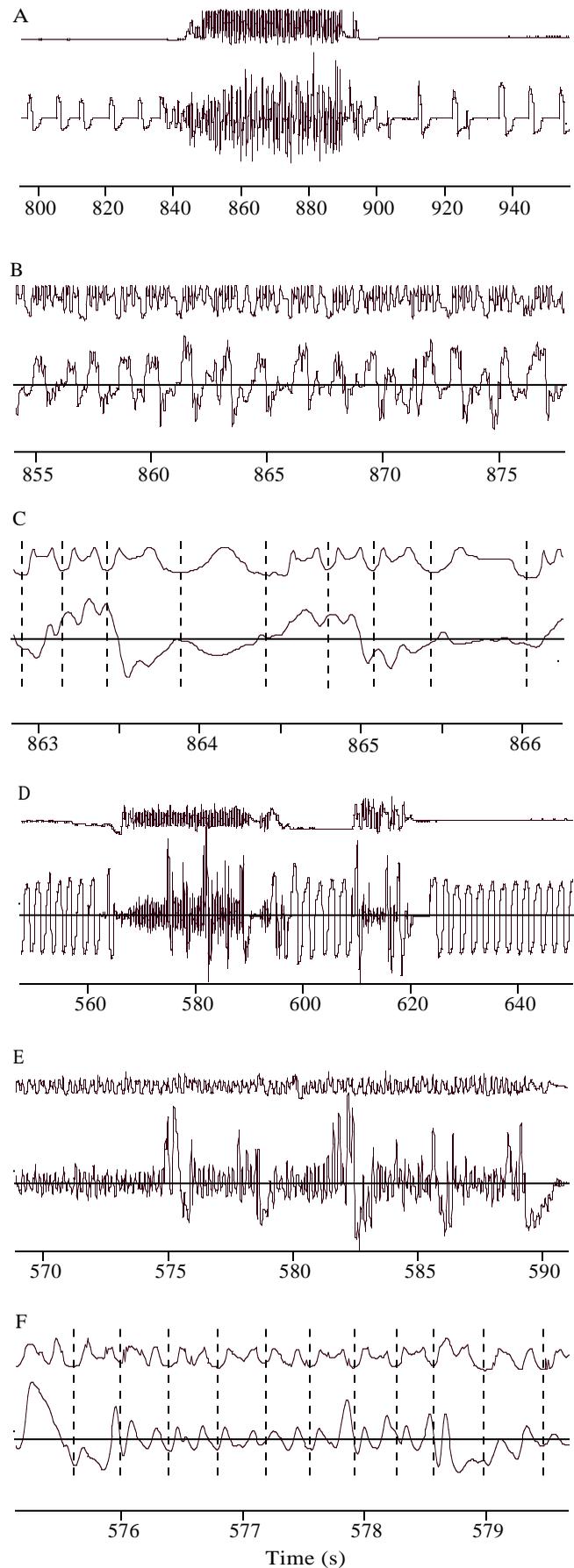*Varanus exanthematicus*   *Iguana iguana*

Fig. 2. Expiratory tidal volume during (●) and following (■) treadmill exercise at different locomotor speeds in *Varanus exanthematicus* (A) and *Iguana iguana* (B). Values are means ± 1 S.E.M. (*N*=5). Mean values that are significantly different from pre-exercise levels (▲) are marked with an asterisk. Mean values during recovery that are significantly different from those for exercise at the same speed are marked with a dagger.
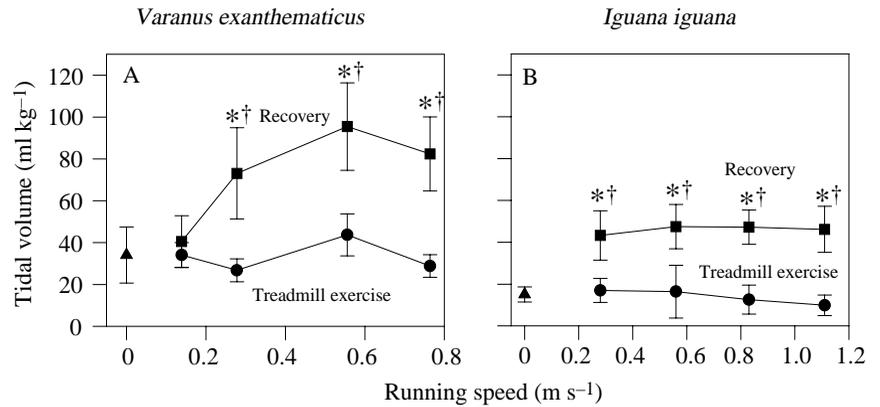
small breaths (which presumably do not contribute to gas exchange) are not included. In both species, tidal volume during exercise was not significantly different from that during rest, but increased two- to fourfold immediately following exercise.

Ventilation and gas exchange before, during and following locomotion

The data for minute ventilation and gas exchange rate in both species are presented in Fig. 3 and Tables 1 and 2. In *Varanus exanthematicus*, minute ventilation increased from a pre-exercise value of $102\pm23\,\mathrm{ml\,kg^{-1}\,min^{-1}}$ to a maximum of $771\pm91\,\mathrm{ml\,kg^{-1}\,min^{-1}}$ at $0.56\,\mathrm{m\,s^{-1}}$ (Fig. 3A). At speeds between 0.14 and $0.56\,\mathrm{m\,s^{-1}}$, minute ventilation increased significantly during the recovery period. The rate of oxygen uptake ($\dot{V}_{O_2}$) increased from a pre-exercise level of $3.9\pm0.7\,\mathrm{ml\,kg^{-1}\,min^{-1}}$ to a maximum of $12.6\pm2.2\,\mathrm{ml\,kg^{-1}\,min^{-1}}$ at $0.56\,\mathrm{m\,s^{-1}}$ and
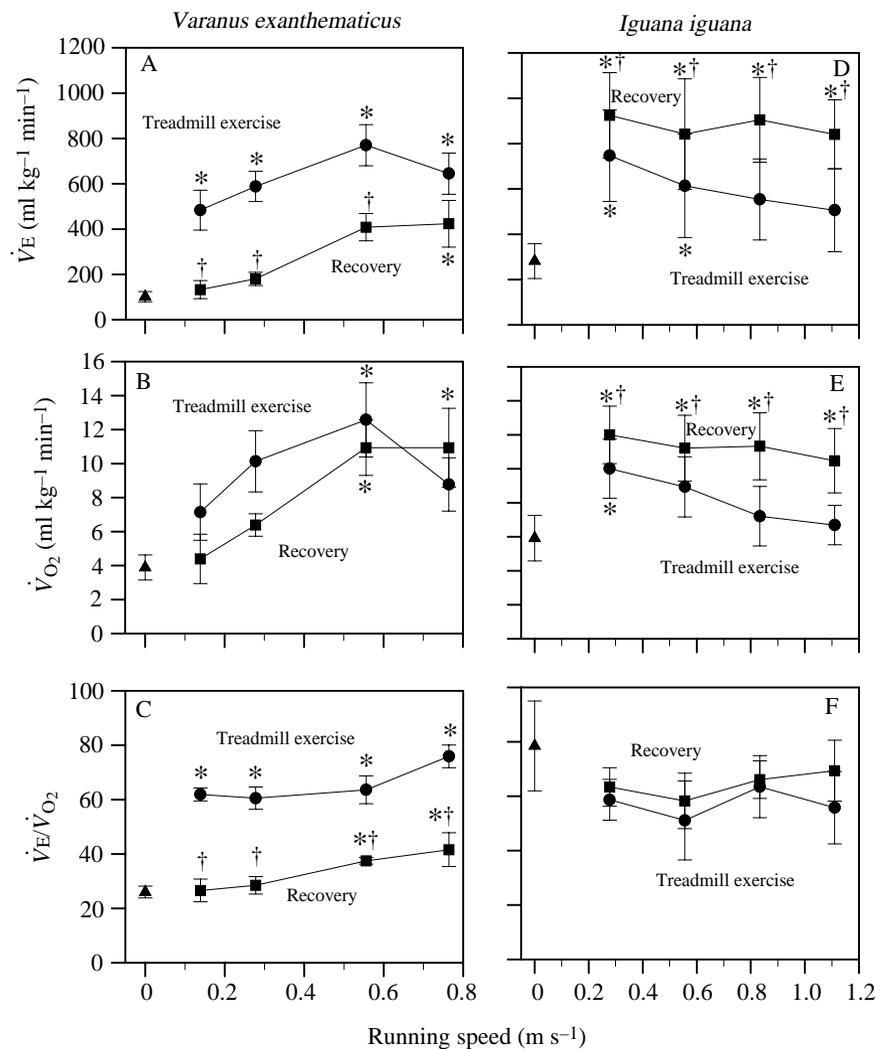

Fig. 3. The ventilatory and respiratory responses to exercise in *Varanus exanthematicus* and *Iguana iguana*. Minute ventilation ($\dot{V}_E$), oxygen uptake rate ($\dot{V}_{O_2}$) and air convection requirements ($\dot{V}_E/\dot{V}_{O_2}$) during treadmill locomotion (●) and during the immediate recovery period (■) in *Varanus exanthematicus* (A–C) and *Iguana iguana* (D–F). Values are means ± 1 S.E.M. (*N*=5). Mean values that are significantly different from pre-exercise levels (▲) are marked with an asterisk. Mean values during recovery that are significantly different from those for exercise at the same speed are marked with a dagger.

Table 1. *The respiratory gas exchange ratio during locomotion and immediately following exercise in* Varanus exanthematicus *and* Iguana iguana

| | *Varanus exanthematicus* | | | *Iguana iguana* | | |
| | | RER | | | | RER | |
Speed ($m\,s^{-1}$)	Locomotion	Recovery	Speed ($m\,s^{-1}$)	Locomotion	Recovery
Pre-exercise	0.77±0.07		Pre-exercise	0.70±0.08	
0.14	0.93±0.02	0.82±0.15	0.28	0.95±0.10	1.45±0.13*,†
0.28	0.85±0.04	0.78±0.08	0.56	0.78±0.08	1.27±0.08*,†
0.56	1.04±0.06*	1.23±0.05*	0.83	0.68±0.14	1.19±0.12*,†
0.76	1.01±0.11	1.12±0.06*	1.11	0.72±0.04	1.29±0.08*,†

*Significantly different from the pre-exercise value; †significantly different from the exercise value at the same speed.
The respiratory exchange ratio, RER, is calculated as $\dot{V}_{O_2}/\dot{V}_{CO_2}$.

decreased, although not significantly, with a further increase in speed (Fig. 3B). At lower speeds, $\dot{V}_{O_2}$ during recovery was lower than during exercise, but this difference disappeared at higher speeds (Fig. 3B). The rate of elimination of CO_2 ($\dot{V}_{CO_2}$) followed the same pattern as $\dot{V}_{O_2}$, although the changes in the recovery period were more pronounced (data not shown). The respiratory gas exchange ratio (RER; $\dot{V}_{CO_2}/\dot{V}_{O_2}$) increased from 0.77±0.07 to 1.04±0.06 with increased speed up to $0.56\,m\,s^{-1}$, reaching values as high as 1.23±0.05 following exercise (Table 1). At all speeds, minute ventilation increased relatively more than did $\dot{V}_{O_2}$ or $\dot{V}_{CO_2}$, resulting in increased air convection requirements ($\dot{V}_E/\dot{V}_{O_2}$ or $\dot{V}_E/\dot{V}_{CO_2}$; Fig. 3C; Table 2). During recovery, air convection requirement quickly decreased and $\dot{V}_E/\dot{V}_{O_2}$ was significantly higher than pre-exercise values only after the fastest running speeds. However, $\dot{V}_E/\dot{V}_{CO_2}$ during recovery did not differ significantly from the pre-exercise level of 35.2±3.1 (Table 2).

In *Iguana iguana*, minute ventilation increased significantly during exercise from a pre-exercise level of $282±77\,ml\,kg^{-1}\,min^{-1}$ to a maximum of $747±202\,ml\,kg^{-1}\,min^{-1}$ at $0.28\,m\,s^{-1}$ (Fig. 3D). Comparison of the minute ventilation at each locomotor speed (pre-exercise not included) using a one-way ANOVA for repeated measures revealed a statistically significant reduction in minute ventilation with increased running speed. In contrast to the varanids, minute ventilation increased significantly relative to pre-exercise levels immediately following locomotion and reached levels as high as $925±188\,ml\,kg^{-1}\,min^{-1}$. Similarly, $\dot{V}_{O_2}$ increased from a pre-exercise level of $5.92±1.34\,ml\,kg^{-1}\,min^{-1}$ to a maximum of $10.01±1.74\,ml\,kg^{-1}\,min^{-1}$ at $0.28\,m\,s^{-1}$ and was significantly increased relative to pre-exercise levels during recovery from all locomotor speeds, reaching a maximum value of $12.02±1.69\,ml\,kg^{-1}\,min^{-1}$ (Fig. 3E). Furthermore, at the two highest locomotor speeds, both minute ventilation and $\dot{V}_{O_2}$ were significantly lower ($506±183\,ml\,kg^{-1}\,min^{-1}$ and $6.69±1.16\,ml\,kg^{-1}\,min^{-1}$, respectively), than the levels measured at $0.28\,m\,s^{-1}$. There were no systematic changes in minute ventilation relative to $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ (Fig. 3D; Table 2), whereas RER increased significantly during the recovery period and reached maximum values as high as 1.45±0.13 from a pre-exercise value of 0.70±0.08 (Table 2).

The changes in heart rate and systemic blood flow rate during exercise in *Varanus exanthematicus* are presented in Table 3. Heart rate increased significantly from a pre-exercise level of $45.5±3.6\,beats\,min^{-1}$ to a maximum of $99.4±5.7\,beats\,min^{-1}$ at the highest speed, which was mirrored by a simultaneous significant increase in relative systemic blood flow rate.

Table 2. *Minute ventilation relative to ventilatory CO_2 production during locomotion and immediately following exercise in* Varanus exanthematicus *and* Iguana iguana

| | *Varanus exanthematicus* | | | *Iguana iguana* | | |
| | | $\dot{V}_E/\dot{V}_{CO_2}$ | | | | $\dot{V}_E/\dot{V}_{CO_2}$ | |
Speed ($m\,s^{-1}$)	Locomotion	Recovery	Speed ($m\,s^{-1}$)	Locomotion	Recovery
Pre-exercise	35.2±3.1		Pre-exercise	78.5±16.4	
0.14	61.9±2.4*	33.2±1.7†	0.28	78.1±12.1	55.0±8.1
0.28	60.5±4.1*	38.5±5.6†	0.56	76.7±13.5	54.8±6.2
0.56	63.6±5.1*	30.6±1.9†	0.83	134.7±31.1*	69.5±7.4
0.76	75.9±4.2*	37.1±5.3†	1.11	90.6±15.2	64.0±7.5

*Significantly different from the pre-exercise value; †significantly different from the exercise value at the same speed.
Minute ventilation, $\dot{V}_E$, and ventilatory CO_2 production ($\dot{V}_{CO_2}$) are measured in $ml\,kg^{-1}\,min^{-1}$.

Ventilation during and after repeated bouts of exercise

The ventilatory responses to exercising iguanas repeatedly in bouts of 30 s followed by 120 s of recovery are presented in Fig. 4. During this protocol, minute ventilation invariably decreased during exercise compared with the preceding recovery period, indicating that the increase in ventilation following exercise can be ascribed to the cessation of locomotion *per se*, rather than being the result of time-dependent changes in the ventilatory response to exercise.

Discussion

Previous studies on *Iguana iguana* and *Varanus*

exanthematicus demonstrated that arterial blood gas composition is maintained during treadmill exercise (Mitchell *et al.* 1981*a*,*b*), indicating that minute ventilation increases in proportion to metabolic rate. In contrast, a later study on the same species (Carrier, 1987*a*) showed a progressive decline in minute ventilation as speed increased above slow walking. Given this disparity, the primary purpose of the present study was to obtain direct measurements of minute ventilation and ventilatory patterns during locomotion in these lizards. Our data show that both *Varanus exanthematicus* and *Iguana iguana* are capable of ventilating their lungs during a locomotor stride (Fig. 1). However, in both species, the ventilatory pattern was influenced by locomotion, and in
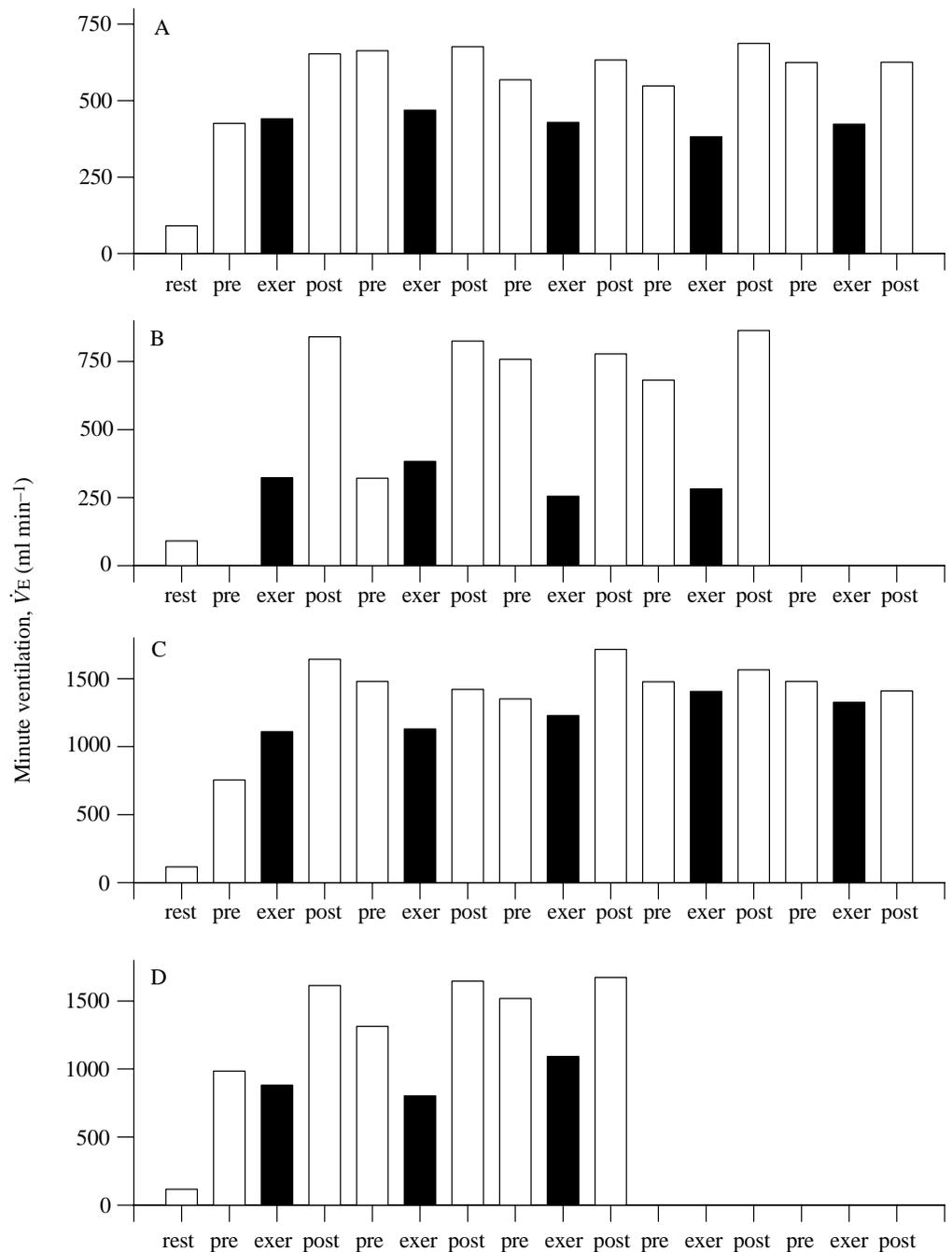
Fig. 4. Minute ventilation in two specimens of *Iguana iguana* during experiments in which the lizards repeatedly walked for 30 s and then rested for 2 min. In each histogram, 'rest' represents resting minute ventilation. The minute ventilation recorded during the 30 s period immediately prior to exercise (exer) is labelled 'pre' and the minute ventilation recorded immediately after the exercise is labelled 'post'. Trials are shown for a 0.33 kg lizard walking at 0.56 m s^{-1} (A) and 0.83 m s^{-1} (B), and for a 1.90 kg lizard walking at 0.56 m s^{-1} (C) and 1.11 m s^{-1} (D)

Iguana iguana there was a progressive decrease in minute ventilation with increased running speed (Fig. 3D).

Breathing patterns during exercise

The breathing pattern in both species was altered during exercise and was characterized by breaths of higher flow velocities and shorter duration than both before and after exercise (Fig. 1). This is in accordance with the findings of Carrier (1987*b*), but is in sharp contrast to earlier studies on ventilation during forced activity in restrained lizards and snakes, which report large increases in tidal volume and small changes in breathing frequency during activity (Wilson, 1971; Dm'iel, 1972; Bennett, 1973; Cragg, 1978). These differences are most probably explained by the differences in experimental design. In the earlier studies, the animals were physically restrained and activity was induced by pinching the legs or through electrical shocks, whereas the lizards in the present study ran freely on a treadmill. In humans, increases in tidal volume account for most of the exercise hyperpnoea that occurs at moderate levels of exercise, while increases in breathing frequency become more important during more severe or prolonged exercise (e.g. Hanson *et al.* 1982). In mammalian quadrupeds, the increased level of ventilation during exercise results primarily from an increase in breathing frequency, whereas changes in tidal volume are more variable; for example, in dogs, tidal volume decreases during exercise (Flandrois *et al.* 1971), whereas tidal volume increases slightly in ponies, goats and calves (Bisgard *et al.* 1978, 1982; Smith *et al.* 1983; Kuhlmann *et al.* 1985).

Immediately following cessation of locomotion, the breathing pattern in both species was characterized by large tidal volumes (Figs 1, 2). In *Varanus exanthematicus*, breathing frequency decreased following exercise, leading to a decrease in minute ventilation (Fig. 3). In contrast, ventilation following exercise in *Iguana iguana* was virtually continuous which, in concert with the increased tidal volume, resulted in a large increase in minute ventilation (Figs 1–3). Consistent with this observation, *Iguana iguana* exhibited an increased minute ventilation following locomotion in the experiments employing short bouts of repeated exercise (Fig. 4). These findings suggest that the observed changes in the breathing pattern are correlated with the cessation of exercise rather than resulting from time-dependent changes in the ventilatory response to exercise. Changes in breathing pattern following exercise have been reported only rarely for mammals. In dogs, tidal volume increases modestly following exercise, but returns to pre-exercise levels within minutes (Flandrois *et al.* 1971).

The effects of exercise on minute ventilation and gas exchange rates

The present study shows that minute ventilation and gas exchange rates increased during locomotion in both species, but that the response to exercise differed between *Varanus exanthematicus* and *Iguana iguana* (Fig. 3; Tables 1, 2). In *Varanus exanthematicus*, minute ventilation increased significantly above pre-exercise levels at all locomotor speeds and decreased immediately following exercise (Fig. 3A,B). A

similar pattern was seen for $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$. In *Iguana iguana*, minute ventilation, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ increased significantly above the pre-exercise levels at the slowest locomotor speeds, but decreased with further increases in running speed (Fig. 3D,E).

Previous studies on the ventilatory responses to exercise in these two species used different techniques from those employed here. Mitchell *et al.* (1981*b*; see also Mitchell *et al.* 1981*a*; Gleeson *et al.* 1980) measured arterial blood gas composition and gas exchange rates at treadmill speeds up to 0.28 and 0.56 m s^{-1} for *Iguana iguana* and *Varanus exanthematicus*, respectively. On the basis of arterial P_{CO_2} and pulmonary gas exchange rates, the effective minute ventilation of the lungs ($\dot{V}_{eff}$) and lung P_{O_2} were calculated using well-established equations for gas exchange in mammals (e.g. Otis, 1964). In these studies, arterial P_{CO_2} decreased during exercise in both species, suggesting that $\dot{V}_{eff}$ relative to $\dot{V}_{O_2}$ ($\dot{V}_{eff}/\dot{V}_{O_2}$) was increased.

The use of arterial P_{CO_2} to calculate $\dot{V}_{eff}$ relies on the assumption that arterial P_{CO_2} is identical to lung P_{CO_2} and that only ventilatory changes affect arterial P_{CO_2}. This, in turn, assumes no cardiac or pulmonary shunt and that the lung functions as a perfect gas exchanger (i.e. no ventilation–perfusion inhomogeneity and no diffusion limitation). However, factors that influence arterial P_{CO_2}, independently of minute ventilation, will alter this estimation. For example, if arterial P_{CO_2} is higher than lung P_{CO_2}, $\dot{V}_{eff}$ will be underestimated. Alternatively, improved lung function (e.g. less ventilation–perfusion inhomogeneity) or a reduction in the cardiac right-to-left shunt decrease arterial P_{CO_2} at constant minute ventilation relative to $\dot{V}_{CO_2}$. Therefore, if the cardiac shunt is reduced and/or lung function improved during exercise compared with rest, it is possible that the calculations of Mitchell *et al.* (1981*a,b*) will overestimate the relative increase in minute ventilation. Unfortunately, very little is known about changes in cardiac shunt and lung function during exercise in reptiles and, consequently, it is difficult to evaluate the magnitude of these possible errors. In *Varanus exanthematicus* walking at low speed (0.28 m s^{-1}), left atrial P_{O_2} increases during exercise, in spite of an increased ventilation–perfusion inhomogeneity (Hopkins *et al.* 1995), which supports the existence of an exercise hyperpnoea. Finally, using measurements of blood gas composition to calculate minute ventilation may not provide a good time resolution; because lizards often run intermittently (even on treadmills), it is conceivable that they maintain a constant blood gas composition by ventilating their lungs during brief pauses in locomotor activity (Carrier, 1987*a*). Presumably, this potential problem would be most pronounced at higher locomotor speeds.

Carrier (1987*a*) reported a substantial reduction in minute ventilation of both *Varanus exanthematicus* and *Iguana iguana* at running speeds that are equivalent to those of our study. It is possible that the discrepancy in the results from the present study is due to the different techniques employed. Carrier (1987*a*) assessed inspiratory airflow rates using the rate of heat loss from heated thermistors implanted over one nostril. This system was difficult to calibrate over a wide range of airflow rates and, because maximum airflow rates were predicted to occur

following locomotion, Carrier (1987*a*) calibrated the recording system using the airflows observed during recovery from exercise. However, the present study shows that the maximum flow rates occur during locomotion and that the difference in flow rate between exercise and recovery is more pronounced for *Varanus exanthematicus* than for *Iguana iguana* (Fig. 1). Consequently, an error in the calibration of the flow probe in Carrier's (1987*a*) study may have resulted in underestimated minute ventilation during exercise in *Varanus exanthematicus*.

In spite of differences in experimental design, there is reasonable agreement between our results and those from previous studies. For *Varanus exanthematicus*, Mitchell *et al.* (1981*b*) calculated a $\dot{V}_{eff}$ of approximately $60\,ml\,kg^{-1}\,min^{-1}$ at rest and a $\dot{V}_{eff}/\dot{V}_{O_2}$ of 18. During exercise, $\dot{V}_{eff}/\dot{V}_{O_2}$ increased to approximately 40, corresponding to a $\dot{V}_{eff}$ of approximately $800\,ml\,kg^{-1}\,min^{-1}$ (Fig. 1 in Mitchell *et al.* 1981*b*). Our determination of total minute ventilation (i.e. effective minute ventilation *and* dead-space minute ventilation) is comparable at rest ($102\,ml\,kg^{-1}\,min^{-1}$), while the maximum level during exercise is slightly lower ($771\,ml\,kg^{-1}\,min^{-1}$) than in this previous report. Carrier (1987*a*) reported a minute ventilation of $110\,ml\,kg^{-1}\,min^{-1}$ at rest and $304\,ml\,kg^{-1}\,min^{-1}$ during locomotion at $0.8\,m\,s^{-1}$ in *Varanus exanthematicus*, which is likely to be an underestimation for the reasons given above. Mitchell *et al.* (1981*b*) determined that $\dot{V}_{O_2}$ increased progressively with increasing locomotor speed from a resting value of $3.2\,ml\,O_2\,kg^{-1}\,min^{-1}$ to a maximum value of almost $20\,ml\,O_2\,kg^{-1}\,min^{-1}$ at $0.3\text{–}0.4\,m\,s^{-1}$ in *Varanus exanthematicus*. The maximum $\dot{V}_{O_2}$ in our study was lower ($12.6\,ml\,kg^{-1}\,min^{-1}$), whereas the pre-exercise level ($3.9\,ml\,kg^{-1}\,min^{-1}$) was slightly higher. For *Iguana iguana*, the pre-exercise $\dot{V}_{O_2}$ determined in the present study ($5.9\,ml\,kg^{-1}\,min^{-1}$) is almost twice as high as the resting value of $3.0\,ml\,kg^{-1}\,min^{-1}$ reported by Mitchell *et al.* (1981). This difference probably reflects the fact that the iguanas in the present study were not completely undisturbed and that our pre-exercise condition did not resemble true resting conditions. This explanation is also supported by the high pre-exercise minute ventilation and $\dot{V}_E/\dot{V}_{O_2}$ in the present study (Fig. 3F) compared with the previous studies. For *Iguana iguana*, Mitchell *et al.* (1981*b*) reported that $\dot{V}_{O_2}$ increased to $13.8\,ml\,kg^{-1}\,min^{-1}$ at $0.14\,m\,s^{-1}$ and calculated $\dot{V}_{eff}$ to be approximately $800\,ml\,kg^{-1}\,min^{-1}$ at this speed, while Carrier (1987*a*) reported a minute ventilation of approximately $900\,ml\,kg^{-1}\,min^{-1}$ at $0.1\text{–}0.2\,m\,s^{-1}$. These values are similar to the minute ventilation and gas exchange rates at $0.28\,m\,s^{-1}$ in the present study (Fig. 3D,E). As in our study (Fig. 3D), Carrier (1987*a*) also observed a progressive decrease in minute ventilation when locomotor speed was increased above $0.2\,m\,s^{-1}$; Mitchell *et al.* (1981*a,b*) did not study the physiological responses to speeds higher than $0.28\,m\,s^{-1}$.

In *Iguana iguana*, both experimental protocols show that minute ventilation increased significantly after exercise (Figs 3D, 4) and that this hyperpnoea during recovery from exercise was associated with a significant increase in $\dot{V}_{O_2}$ (Fig. 3E,F). In *Varanus exanthematicus*, minute ventilation decreased following exercise (Fig. 3A), and only after the

fastest running speeds did $\dot{V}_{O_2}$ remain high during recovery (Fig. 3B). Carrier (1987*a*) reported large increases in minute ventilation following locomotion in both species, but did not measure $\dot{V}_{O_2}$ or $\dot{V}_{CO_2}$. In the absence of blood and lung gas composition measurements, it is difficult to interpret increased $\dot{V}_{E.O_2}$ following exercise in *Iguana iguana*. In *Iguana iguana*, systemic blood flow appears to increase following exercise (Farmer *et al.* 1996), which may increase the convective transport of O_2 and thus $\dot{V}_{O_2}$, but this possibility must be verified experimentally. Furthermore, if effective ventilation ($V_T - V_{DS} \times f$, where V_T is tidal volume, V_{DS} is dead space volume and f is breathing frequency) is decreased during locomotion, lung and blood O_2 content would decline, and if these O_2 stores are replenished following locomotion, $\dot{V}_{O_2}$ (measured across the nostrils) would increase transiently during the recovery period. In this scenario, arterial P_{O_2} would be expected to decrease during exercise; this is not supported by direct measurements of blood gas composition (Mitchell *et al.* 1980*a,b*).

In summary, in *Varanus exanthematicus*, it appears that minute ventilation increases during exercise sufficiently to meet $\dot{V}_{O_2}$ during locomotion at speeds up to $0.8\,m\,s^{-1}$. This is indicated by the large increase in minute ventilation relative to $\dot{V}_{O_2}$ (Fig. 3A–C), also found by Mitchell *et al.* (1981*a,b*), and the decrease in $\dot{V}_{O_2}$ immediately following exercise. In *Iguana iguana*, however, the present study and that of Carrier (1987*a*) indicate that minute ventilation decreases progressively as locomotor speed is increased above $0.28\,m\,s^{-1}$ and that this decrease is associated with a decrease in $\dot{V}_{O_2}$. Furthermore, as tidal volume tended to decrease with increased locomotor speed (although the decrease was not statistically significant), it is possible that effective ventilation decreased at the highest locomotor speeds in spite of the increase in $\dot{V}_E/\dot{V}_{O_2}$ (Fig. 3D). This interpretation is supported by the increased $\dot{V}_{O_2}$ and minute ventilation following exercise, which may indicate a reduction in lung and blood O_2 content during exercise.

The observation that minute ventilation and $\dot{V}_{O_2}$ decrease in *Iguana iguana* as the speed of locomotion increases does not necessarily imply that these lizards are mechanically incapable of ventilating their lungs while running. It is possible, for example, that muscular exercise is associated with changes in the control of ventilation, such that exercise exerts an inhibitory action on the central motor output to the muscles responsible for ventilation. In this case, the proximal explanation for the reduction in ventilation would not be a mechanical limitation *per se*, but rather a result of the central integration of afferent input. It may, however, be argued that such changes in ventilatory control would only be of adaptive value (and presumably then selected for) if breathing during locomotion were energetically inefficient or if ventilatory efforts were to reduce locomotor performance (e.g. by affecting acceleration or maximum running speed).

The hypothesis of mechanical interference between locomotion and pulmonary ventilation

Given that lizards use their hypaxial muscles for both locomotion and ventilation (Carrier, 1989*a,b*, 1990), it is

plausible that the observed changes in breathing pattern result from interference between the locomotor and ventilatory functions. In the group of bony fishes from which tetrapods evolved, the hypaxial muscles did not contribute to lung ventilation (Liem, 1985), but were probably associated with lateral bending of the trunk and provided torsional stability during swimming. In salamanders, the obliquely oriented hypaxial muscles (the external oblique, internal oblique and transversalis muscles) are active during terrestrial walking in a manner that indicates that they stabilize the trunk against the long axis (Carrier, 1993). A similar pattern of activity during walking has been observed in the hypaxial muscles of *Iguana iguana* (Carrier, 1990). Recent observations suggest that the obliquely oriented hypaxial muscles of lizards are responsible for the lateral bending of the trunk during locomotion (Ritter, 1995, 1996). Finally, in trotting dogs, the locomotor action of the interosseous intercostal muscles has been found to predominate over their ventilatory action (Carrier, 1996). Therefore, it seems reasonable to suggest that locomotion may place limits on ventilatory function in lizards. The erratic nature of the breathing pattern observed during locomotion in both species in this study and the reduction in minute ventilation with increased running speed observed in *Iguana iguana* are consistent with mechanical interference between locomotor and ventilatory function as originally proposed by Carrier (1987a,b, 1989, 1990).

The findings for *Varanus exanthematicus* clearly conflict with the hypothesis of an ancestral conflict between ventilation and locomotion. Among lizards, varanids are thought to be a highly derived lineage, whereas iguanids are phylogenetically among the most basal of extant lizards (Estes *et al.* 1988). Given this phylogeny, the most parsimonious explanation is that the ability of *Varanus exanthematicus* to breathe effectively during locomotion is due to a modification of the ancestral condition to facilitate simultaneous running and breathing. In varanids, there is a pronounced anatomical distinction between the first few thoracic ribs and intercostal muscles and the ribs and intercostal muscle throughout the rest of the trunk (D. R. Carrier, unpublished observations). Visual observations indicate that the cranial ribs move during ventilation and that there may be a cranial-to-caudal division of labour in the intercostal musculoskeletal system of varanids that is not present in iguanids. Furthermore, the lizard *Uromastyx microlipes* can ventilate its lungs using a buccal pressure force pump (Al-Ghamdi *et al.* 1995), and it has recently been reported that *Varanus exanthematicus* can use contractions of the buccal cavity to inflate the lungs following exercise (Brainerd and Owerkowicz, 1996). Although the actual contribution of this mechanism during exercise remains to be determined, these gular movements may participate in lung ventilation.

Does ventilation limit $\dot{V}_{O_2}$ during exercise?

Carrier (1987b) suggested that limited minute ventilation, particularly at high running speeds, limits $\dot{V}_{O_2}$ and, therefore, renders lizards incapable of sustained locomotion. This

Table 3. *Heart rate and relative changes in systemic blood flow rate ($\dot{Q}_{sys}$) during locomotion in* Varanus exanthematicus

Speed ($m\,s^{-1}$)	Heart rate, f_H (beats min^{-1})	Relative change in $\dot{Q}_{sys}$
0	45.5±3.6	1.0±0.0
0.14	76.1±6.4*	2.0±0.4*
0.28	92.5±6.9*	2.5±0.7*
0.56	98.2±7.8*	2.7±0.6*
0.76	99.4±5.7*	2.8±0.6*

*Significantly different from the pre-exercise value.

argument assumes that minute ventilation limits oxygen transport during exercise and implies that an increased minute ventilation could sustain a higher $\dot{V}_{O_2}$. At a given systemic cardiac output and a fixed cardiac right-to-left shunt, minute ventilation limits systemic oxygen delivery ($\dot{Q}_{sys} \times [O_2]_a$, where $\dot{Q}_{sys}$ is the systemic blood flow and $[O_2]_a$ is the oxygen content of systemic arterial blood) if lung P_{O_2} falls to an extent where pulmonary venous blood is no longer saturated. The lung P_{O_2} at which the haemoglobin oxygen-saturation of pulmonary venous blood is compromised depends on both blood oxygen-affinity and the P_{O_2} difference between lung gas and blood leaving the lungs. This P_{O_2} difference is generally larger in reptiles than in mammals (1–3 kPa *versus* 0.5–13 kPa) and results from a combination of intrapulmonary shunts, diffusion limitation and ventilation–perfusion inhomogeneity (reviewed by Powell, 1994). In *Varanus exanthematicus*, minute ventilation increased proportionally more than did gas exchange rates during exercise, as demonstrated by the large changes in air convection requirement for both O_2 and CO_2 (Fig. 3C; Mitchell *et al.* 1981b). The resulting increase in lung P_{O_2} may help to overcome diffusion limitations or the inability to increase diffusion capacity during exercise (Mitchell *et al.* 1981b; Hopkins *et al.* 1995). Finally, in *Varanus exanthematicus* walking at low speed (0.28 m s^{-1}), Hopkins *et al.* (1995) reported an increase in left atrial P_{O_2} during exercise, in spite of an increased ventilation–perfusion inhomogeneity which, in combination with the increase in ventilation, does not support the suggestion that lung P_{O_2} limits gas exchange during exercise in this species.

A vigorous analysis of O_2 transport limitations during exercise must determine O_2 transfer rates between the inhaled gas, the lungs, pulmonary capillary blood, systemic blood O_2 transport and O_2 diffusion between systemic capillary blood and mitochondria. To complicate matters further, in reptiles, the time constant for attaining steady state during progressive exercise may be exceedingly long, and a transient reduction in transfer rate at one step in the O_2 transport cascade is therefore not necessarily indicative of an O_2 transport limitation. There are no studies that have measured all of the O_2 transfer components in a single species of reptile. Nevertheless, a previous study on *Iguana iguana* and *Varanus exanthematicus* concluded that sustained aerobic activity was limited not by minute ventilation, but rather by the circulatory

system (Gleeson *et al.* 1980; Bennett, 1994). This conclusion was based on the relative hyperventilation and the attainment of maximal cardiac output and arterial–venous oxygen differences at maximal aerobic speeds. The relative changes in systemic blood flow and heart rate shown in Table 3 for *Varanus exanthematicus* are consistent with this interpretation. In contrast, the reduced gas exchange observed in *Iguana iguana* at the higher locomotor speeds may be a consequence of an altered breathing pattern during locomotion. Additional studies are required to quantify the various transport steps before conclusions regarding physiological limitations can be seriously addressed.

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