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Oxygen transfer during aerobic exercise in a varanid lizard *Varanus mertensi* is limited by the circulation

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Summary

Oxygen transfer during sustained maximal exercise while locomoting on a treadmill at 0.33 m s^{-1} was examined in a varanid lizard *Varanus mertensi* at 35 °C. The rate of oxygen consumption ($\dot{V}_{O_2}$) increased with locomotion from 3.49±0.75 (mean ± S.D.) to 14.0±4.0 ml O_2 kg^{-1} min^{-1}. Ventilation ($\dot{V}_E$) increased, aided by increases in both tidal volume and frequency, in direct proportion to $\dot{V}_{O_2}$. The air convection requirement ($\dot{V}_E/\dot{V}_{O_2}$=27) was therefore maintained, together with arterial P_{aCO_2} and P_{aO_2}. The alveolar–arterial P_{O_2} difference (P_{AO_2}–P_{aO_2}) also remained unchanged during exercise from its value at rest, which was approximately 20 mmHg. Pulmonary diffusion for carbon monoxide (0.116±0.027 ml kg^{-1} min^{-1} $mmHg^{-1}$) was double the value previously reported in *V. exanthematicus* and remained unchanged with exercise. Furthermore, exercise was associated with an increase in the arterial–venous O_2 content difference (C_{aO_2}–C_{vO_2}), which was assisted by a marked Bohr shift in the hemoglobin saturation curve and further unloading of venous O_2. During exercise the increase in cardiac output ($\dot{Q}_{tot}$) did not match the increase in $\dot{V}_{O_2}$, such that the blood convection requirement ($\dot{Q}_{tot}/\dot{V}_{O_2}$) decreased from the pre-exercise value of approximately 35 to 16 during exercise. Together, the results suggest that ventilation and O_2 transfer across the lung are adequate to meet the aerobic needs of *V. mertensi* during exercise, but the decrease in the blood convection requirement in the presence of a large arterial–venous O_2 content difference suggests that a limit in the transport of O_2 is imposed by the circulation.

Key words: exercise, ventilation, oxygen consumption, oxygen transport, blood gas, breathing pattern, reptile, *Varanus mertensi*, lung diffusion, P_{50}, oxygen affinity.

Introduction

Metabolic rate, or more precisely, the rate of oxygen consumption ($\dot{V}_{O_2}$), is set by structural and functional parameters at each point in the oxygen pathway from lungs to the mitochondria in skeletal muscle. It is uniformly accepted that, in mammals, the 'limitation' to maximal $\dot{V}_{O_2}$ is distributed over all components of the pathway, though under certain conditions some steps may provide more resistance than others (Hoppeler and Weibel, 1998).

A number of studies have searched for the component(s) that limit oxygen transfer and hence aerobic metabolism in exercising varanid lizards, a group that is considered to have one of the highest aerobic scopes amongst reptiles (Gleeson et al., 1980; Mitchell et al., 1981a,b). Almost all of these studies have been restricted to one species, *Varanus exanthematicus*. It would appear that this species effectively ventilates its lungs to match the increase in metabolic rate achieved during sustained locomotion, at levels sufficient to achieve maximal rates of oxygen uptake (Hopkins et al., 1995; Wang et al., 1997). Indeed, ventilation has been shown to increase proportionally more than metabolic rate (Mitchell et al., 1981b; Wang et al., 1997), giving rise to an elevated alveolar partial pressure of oxygen (P_{AO_2}) (Mitchell et al., 1981b). This suggests that aerobic activity in varanids is not limited by ventilation, as originally suggested in the axial constraint hypothesis proposed by Carrier (1987b). In contrast to the elevated P_{AO_2}, arterial P_{O_2} (P_{aO_2}) remains somewhat constant (Mitchell et al., 1981b; Hopkins et al., 1995), implying that the observed alveolar–arterial P_{O_2} difference observed during exercise is potentially limited by pulmonary diffusion, the inability to increase diffusing capacity during exercise, ventilation/perfusion inequalities and/or cardiac shunting (Mitchell et al., 1981b; Hopkins et al., 1995).

It has been suggested that the greater factorial scope in *V. exanthematicus*, compared to other lizards such as *Iguana iguana*, is potentially due to a greater scope in both cardiac output and oxygen extraction (Gleeson et al., 1980; Bennett, 1994). The iguana, at moderate to high speeds, is also unable to match the increased metabolic rate with adequate ventilation; as speed increases ventilation decreases (Carrier, 1987a; Wang et al., 1997) and this decrease is accompanied with a decrease in $\dot{V}_{O_2}$ (Wang et al., 1997). Such a finding adds

support to the axial constraint hypothesis, at least for the iguana.

Despite the sustained interest in the determinants of aerobic scope in varanids, no study has yet measured all the O_2 transfer components in a single species. Further, the studies by Mitchell et al. (1981a,b) used arterial P_{CO_2} (Pa_{CO_2}) and pulmonary gas exchange rates to calculate ventilation and lung P_{O_2} from well-known alveolar gas equations. Such calculations assume that only ventilatory changes affect Pa_{CO_2}.

This study therefore analyses O_2 transfer during sustainable treadmill exercise in a semi-aquatic varanid lizard, *Varanus mertensi*, to broaden our understanding of O_2 transfer beyond one varanid species. The use of a treadmill as opposed to a water channel is justified as this species is just as home on land as it is in water, and the use of a treadmill permits comparison with the results of previous studies.

Materials and methods

Animals

Six water monitors *Varanus mertensi* Glauert 1951 (mass 1.39±0.39 kg, snout–vent length=40–45 cm) were obtained from the field (near Darwin, NT, Australia) and housed in temperature-controlled rooms (30 °C) with access to heat lamps. They were maintained on a diet of minced meat, insects and food supplement (Wombaroo, Australia), with water supplied *ad libitum*.

Protocol

Animals were initially acclimated to the experimental temperature (35 °C, body temperature, T_b, checked with an infrared thermometer) for at least 12 h before being run on a treadmill, set at speeds increasing from 0.2 to 0.42 m s^{-1}, and wearing a lightweight plastic mask that encompassed the head and enclosed the mouth and nostrils. A bias flow of air (2800 ml min^{-1} STPD, standard temperature and pressure, dry) was drawn through the mask, dried and analysed for the rate of oxygen consumption, $\dot{V}_{O_2}$, as outlined below. Prodding of the tail ensured that the animals kept pace with the treadmill. The speed at which the maximum level of $\dot{V}_{O_2}$ ($\dot{V}_{O_2max}$) was sustained for 2 min and showed no further increase with increasing speed was established as the speed to be used for subsequent experimental runs (0.33 m s^{-1}).

On a separate occasion, animals were fitted with Y-connector masks and ventilation and metabolism determined by the methods described below. The lizards were again run on a treadmill at 0.33 m s^{-1} until exhaustion. These measurements were used to ensure that the subsequent surgery and placement of arterial and venous catheters did not affect the performance of the lizards.

Following the placement of catheters (see below), the animals were again fitted with Y-connector masks and made to run on the treadmill (0.33 m s^{-1}). This time ventilation and metabolism, end tidal gases (see below) and various blood parameters (see below) were measured at specified time intervals: pre-exercise (i.e. the animal masked and resting quietly on the treadmill for at least 30 min), during exercise at 1 min and 5 min, exhaustion (i.e. the animal no longer able to maintain pace), and after 2 min recovery.

Ventilation and metabolism

Individual masks were constructed each time an animal was run by placing the main stem of a Y-connector in the mouth of the animal and sealing the nose and mouth with a quick-setting rubber (Impregum, ESPE). Plastic tubing was connected to the two arms of the Y-connector. At one end a pump drew air (2800 ml min^{-1} STPD) under negative pressure through the mask and a pneumotachometer connected at the other end. Flow was measured using a differential pressure transducer (PT5, Grass Instruments, ±5 cm H_2O), integrated and recorded digitally (50 Hz; DT2801-A, DataTranslations and ASYST, MacMillan Software) as volume. The bias flow from the pump was electronically offset to zero, thereby allowing the recording of tidal volume and breathing pattern. After passing through the pneumotachometer the gas was passed through a drying column (Drierite) and analysed for the fractional concentrations of O_2 (S3-AII dual channel O_2 analyser, Ametek) and CO_2 (CD-3 CO_2 analyser, Ametek), F_{O_2} and F_{CO_2}, respectively. The outputs from the analysers were recorded digitally (50 Hz) and used to calculate the overall rates of oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) by taking into account the respiratory exchange ratio (R), as outlined in Frappell et al. (1992). In brief,

$$\dot{V}_{O_2} = \text{flow}' \times (F'_{IO_2} - F'_{EO_2}) / (1 - F'_{IO_2}), \quad (1)$$

where the modifiers I and E represent incurrent and excurrent, respectively, and a prime ′ indicates dry CO_2-free gas, the CO_2 being mathematically scrubbed; $F'_{O_2}=F_{O_2}/(1-F_{CO_2})$. A similar equation can be written for $\dot{V}_{CO_2}$:

$$\dot{V}_{CO_2} = \text{flow}' \times (F'_{ECO_2} - F'_{ICO_2}) / (1 - F'_{ICO_2}), \quad (2)$$

where, in this case, prime ′ represents dry O_2-free gas. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ are expressed at STPD (0 °C, 760 mmHg, dry).

The breathing pattern was analysed in terms of tidal volume (V_T), expiratory, inspiratory, inspiratory pause and total times (T_E, T_I, T_P and T_{TOT}), frequency ($f=1/T_{TOT}\times60$) and minute ventilation ($\dot{V}_E=V_T\times f$) in a similar approach to that detailed in Frappell et al. (1992). On average, 25 consecutive breaths were analysed. Volumes are expressed at BTPS (T_b, barometric pressure, saturated).

End tidal gases

A 14-gauge needle was inserted from the centre of the two arms into the main stem of the Y-connector. This was connected directly to the other channel of the O_2 analyser and the CO_2 analyser with a short length of small-bore tubing (PE, i.d. 1.00 mm, o.d. 1.50 mm). Gas was subsampled through this circuit at a flow (τ=1.2 s) that permitted the determination of end tidal P_{O_2} and P_{CO_2} (P_{AO_2} and P_{ACO_2}, respectively) [i.e. alveolar; strictly speaking the surface of the lung wall is increased by a honeycomb-like (faviform) system of partitions, which bear a matrix of capillaries on both surfaces. The

individual chambers form the faveoli (Perry and Duncker, 1978)], determined as fractional concentration $F\times(P_B–P_{H_2O(35)})$, where P_B is barometric pressure and $P_{H_2O(35)}$ is partial pressure of water vapour at 35 °C. This gas then rejoined the main flow of air before it passed through the drying column.

For three animals the CO_2 analyser was placed in the subsampling circuit and used to determine end tidal P_{CO_2} (P_{ACO_2}); for the other three animals the CO_2 analyser was used to directly determine $\dot{V}_{CO_2}$.

Heart rate

Heart rate (f_H) was determined from an electrocardiogram (e.c.g.) measured with two small needle electrodes placed on the dorsal service and positioned diagonally across the heart. The signal was appropriately amplified (7P4F, Polygraph, Grass Instruments).

Catheterisation

Animals were anaesthetised with an i.v. dose of ketamine administered *via* the ventral coccygeal vein at a dose of 80–100 mg kg^{-1}. After induction of anaesthesia, a small mid-ventral incision (approximately 2 cm) was made in the neck. The common jugular vein was occlusively cannulated using a PE catheter (i.d. 0.58 mm, o.d. 0.96 mm). The tip of the catheter was directed past the brachiocephalic vein to rest in the anterior vena cava and was checked by measuring the length of the catheter on removal. The internal carotid artery was occlusively cannulated with PE catheter (i.d. 0.58 mm, o.d. 0.96 mm) and the tip advanced to rest as close to the heart as possible, presumably in the brachiocephalic artery or aorta. The catheters were sutured in place, looped loosely to avoid tension, led to the exterior through a small hole on the animal's dorsal surface just above the shoulders, filled with heparinized saline and sealed. The wound was closed with sutures. The surgical procedure lasted no more than 40 min and the animals were allowed to recover for at least 24 h prior to further testing.

Blood variables

Small blood samples (250–300 µl) were taken at designated times from both the artery (modifier, a) and the vein (mixed venous blood, modifier, $\bar{v}$) and stored anaerobically on ice. The partial pressures of blood gases P_{O_2}, P_{CO_2} and the pH were measured at 35 °C (BMS MK2, Radiometer). The electrodes were calibrated before and after each measurement. P_{O_2} and P_{CO_2} were measured over 3 min and regressed back to time zero; pH was measured on incremental volumes of blood until the variation between successive measurements was less than 0.005 units. The oxygen content, C_{O_2}, of each blood sample was determined on a 15 µl subsample of blood using a galvanic cell (Oxygen Content Analyser, OxyCon). Hematocrit, hemoglobin concentration [Hb] (Boehringer Mannheim kit no. 124729) and lactate concentration (Sigma kit no. 826) were also measured for each sample.

On another occasion a 2 µl blood sample was taken and oxygen equilibrium curves determined on a modified Hem-O-Scan (Aminco Instruments). The method followed is largely that presented by Holland et al. (1988), where gases of appropriate P_{O_2} are pulsed into the chamber and the percentage saturation determined. Curves were constructed from 10–15 points at 35 °C and at CO_2 tensions of 39 and 79 mmHg (1 mmHg=133.3 Pa); the gases mixed by a pump (Wosthoff). Each point was digitised and the P_{50} determined from the linear section of the Hill plot in the middle range of saturations. The Bohr factor was determined as ($\Delta\log P_{50}/\Delta$pH), pH being determined from blood tonometered at the P_{CO_2} values used in the Hem-O-Scan.

Resting lung volume and pulmonary diffusing capacity

Resting lung volume and pulmonary diffusing capacity were determined for six animals after quietly resting for 30 min and during exercise using helium dilution and carbon monoxide clearance techniques. The lizards were fitted with a mouthpiece as previously described. The mask was directly attached *via* a three-way stopcock to a closed circuit of known volume (V_{SYS}) that consisted of flexible tubing, helium and carbon monoxide analysers (CO and He Analysers, Morgan, UK), a collapsible reservoir and a pump that ensured a continual flow (approximately 1000 ml min^{-1}) through the circuit. The stopcock was initially open to enable the lizard to breathe room

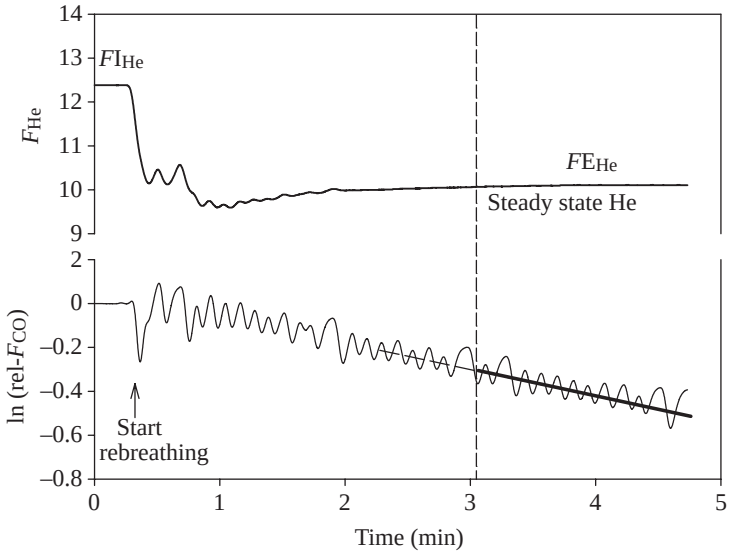

Fig. 1. A recording of gaseous washouts obtained during exercise in an animal rebreathing a mixture of He, CO and air. The initial fluctuations and sudden decrease in He represents breath-by-breath change in He as it is mixed with alveolar gas. Helium decreases to a stable concentration whereas CO continuously diffuses from alveolar gas to blood, causing a decrease of alveolar F_{CO}. The concentration of CO relative to He (rel-F_{CO}) changes exponentially with time such that a plot of ln(rel-F_{CO}) *versus* time yields a linear relationship, shown as the solid line fitted through the data after He has stabilized.

air. The circuit contained a test gas that comprised carbon monoxide (CO, 0.28 %), helium (He, 0.138 %) and air (the balance). During an end inspiratory pause, and with constant levels of He and CO in the circuit, the stopcock was turned so that the lungs were connected to the closed circuit. The breathing of the animal effected mixing of the test gas and alveolar gas. During this time the analysers continually monitored the levels of He and CO. The stopcock was opened to room air once the value of He reached a new steady state (Fig. 1).

As the solubility of He in blood is very low ($0.0095\,ml\,ml^{-1}\,atm^{-1}$) (Muysers and Smidt, 1969) the volume of the lung (V_L) and circuit combined ($V_{SYS}+V_L$) can then be determined from V_{SYS} and the known fractional concentrations of He initially and finally contained in the circuit ($F_{IHe} - F_{EHe}$) as previously described by Glass et al. (1981):

$$V_{SYS(BTPS)} + V_{L(BTPS)} = V_{SYS(BTPS)} \times (F_{IHe} / F_{EHe}), \quad (3)$$

where V_{SYS} was previously determined by injecting a known volume of air (V_{AIR}) into the circuit containing the test gas:

$$V_{SYS(BTPS)} = V_{AIR(BTPS)} \times [F_{EHe} / (F_{IHe} - F_{EHe})]. \quad (4)$$

The determination of carbon monoxide diffusing capacity of the lungs (D_{LCO}) was based largely on that described in Depledge (1985) and Crawford et al. (1976). During the rebreathing of the test gas the fraction of CO continually declined and D_{LCO} could be determined by the equation:

$$D_{LCO} = (V_{SYS(STPD)} + V_{L(STPD)}) / (P_B - P_{H2O(35)}) \times \ln \text{rel-}F_{CO}(t)/t, \quad (5)$$

where t is time and rel-$F_{CO}(t)$ is given by:

$$\text{rel-}F_{CO}(t) = F_{CO}(t) \times F_{He(max)} / [F_{CO(max)} \times F_{He}(t)]. \quad (6)$$

Right-to-left shunt fraction

The existence of a right-to-left (R–L) cardiopulmonary shunt was determined from measurements of arterial O_2 content Ca_{O_2}, venous O_2 content $C\bar{v}_{O_2}$ and P_{AO_2} during 100 % oxygen breathing for three lizards resting on a treadmill. The total amount of oxygen leaving the cardiopulmonary system is given by total cardiac output ($\dot{Q}_{tot}$)×Ca_{O_2}, which must equal the sum of the amounts of oxygen in the shunted blood, shunt blood flow ($\dot{Q}_{shunt}$)×$C\bar{v}_{O_2}$, and the pulmonary end-capillary blood, ($\dot{Q}_{tot}$–$\dot{Q}_{shunt}$)×Cc'_{O_2}, where Cc'_{O_2} is the O_2 content of end-capillary blood. This relationship can be rearranged to yield the R–L shunt fraction:

$$\dot{Q}_{shunt} / \dot{Q}_{tot} = (Cc'_{O_2} - Ca_{O_2}) / (Cc'_{O_2} - C\bar{v}_{O_2}). \quad (7)$$

During 100 % oxygen breathing, Hb is completely saturated and Cc'_{O_2} can be estimated as:

$$Cc'_{O_2} = (Hb \times 1.38) + P_{AO_2} \times \beta O_2, \quad (8)$$

where βO_2 is the capacitance of blood for oxygen ($=0.00135\,mmol\,l^{-1}\,mmHg^{-1}$).

Substitution of Cc'_{O_2} and Ca_{O_2} into the above equation yields:

$$\dot{Q}_{shunt} / \dot{Q}_{tot} = [(P_{AO_2} - Pa_{O_2}) \times \beta O_2] / [(P_{AO_2} - Pa_{O_2}) \times \beta O_2 + (Ca_{O_2} - C\bar{v}_{O_2}]. \quad (9)$$

In practice, when 100 % oxygen is breathed, P_{AO_2} can be calculated without knowledge of R as:

$$P_{AO_2} = P_B - P_{H2O(35)} - P_{ACO_2}. \quad (10)$$

This was confirmed in one lizard from direct measurement of P_{AO_2}, the observed value differed from the calculated value by 8 mmHg from about 700 mmHg.

Calculated variables

A number of variables were derived.

Total cardiac output ($\dot{Q}_{tot}$) was determined from rearrangement of the Fick equation to:

$$\dot{Q}_{tot} = \dot{V}_{O_2} / (Ca_{O_2} - C\bar{v}_{O_2}). \quad (11)$$

Alveolar ventilation ($\dot{V}_A$) was determined from the alveolar gas equation:

$$\dot{V}_A = \dot{V}_{CO_2} / Pa_{CO_2} \times \boldsymbol{R}T, \quad (12)$$

where $\boldsymbol{R}$ is the gas constant and T is absolute temperature (K) [$2.785\,L\,mmHg^{-1}\,(KL_{STPD})^{-1}$].

Data collection and statisitics

All data signals were fed into an A/D board (DT2801/A, Data Translations, MA, USA) collecting at a speed of 50 Hz using ASYST (Macmillen Software, Keithley Instruments, NY, USA) and stored on computer for later analysis. Data are presented as means ± 1 S.D. Differences between treatments and time intervals were analysed using repeated-measures ANOVA. *Post-hoc* modified two-tailed *t*-tests were used to assess differences between appropriate comparisons using the Bonferroni method. A significant difference was defined as $P<0.05$.

Results

Metabolic rate before, during and after locomotion

No difference existed in $\dot{V}_{O_2}$ between the pre- and post-surgical treatments ($P=0.254$, Fig. 2). The pre-exercise $\dot{V}_{O_2}$ ($3.49\pm0.75\,ml\,O_2\,kg^{-1}\,min^{-1}$, Table 1) was higher than that reported for standard metabolic rate in this species (Christian and Conley, 1994), though this is hardly surprising given that the lizards in the present study were wearing a mask and rested for only 20 min during the day before running. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ increased with locomotion, quickly reaching a plateau that was maintained until exhaustion, on average 11.8 min after commencing the run (Fig. 2). Averaged over the period of exercise, $\dot{V}_{O_2max}$ (Table 2) was similar to that previously reported ($15.4\pm2.4\,ml\,O_2\,kg^{-1}\,min^{-1}$; Christian and Conley, 1994). R increased from 0.71±0.03 at rest to 0.86±0.21 during exercise. In the 2 min following exercise $\dot{V}_{O_2}$ tended to decline while $\dot{V}_{CO_2}$ was still maintained at the

Table 1. *Measured respiratory values for* V. mertensi *at rest*

Metabolism		Ventilation		Oxygen transport	
$\dot{V}_{O_2}$ kg^{-1} (ml O_2 kg^{-1} min^{-1})	3.5±0.7	P_{AO_2} (mmHg)	121±9	Pa_{O_2} (mmHg)	100±3
$\dot{V}_{CO_2}$ kg^{-1} (ml CO_2 kg^{-1} min^{-1})	2.1±0.25	P_{ACO_2} (mmHg)	27±2	Pa_{CO_2} (mmHg)	29±4
R	0.71±0.04	$\dot{V}_E$ kg^{-1} (ml kg^{-1} min^{-1})	106±41	pHa	7.522±0.080
		$\dot{V}_A$ kg^{-1} (ml kg^{-1} min^{-1})	75±22	Ca_{O_2} (mmol l^{-1})	3.7±0.9
		V_T kg^{-1} (ml kg^{-1})	25.4±11.4	$P\bar{v}_{O_2}$ (mmHg)	61±7
		f (min^{-1})	4.1±1.2	$P\bar{v}_{CO_2}$ (mmHg)	35±8
		T_I (s)	2.3±0.7	$pH_{\bar{v}}$	7.496±0.096
		T_E (s)	1.7±0.4	$C\bar{v}_{O_2}$ (mmol l^{-1})	2.3±0.6
		T_P (s)	13.5±6.2	$\dot{Q}_{tot}$ kg^{-1} (ml kg^{-1} min^{-1})	127±57
		T_{TOT} (s)	17.4±7.3	$\dot{Q}_{shunt}/\dot{Q}_{tot}$ (%)	17.9±3.2
		$\dot{V}_E/\dot{V}_{O_2}$	26.8±11.0	f_H (min^{-1})	74±9
		$\dot{V}_E/\dot{V}_{CO_2}$	38.3±15.4	$\dot{Q}_{tot}/\dot{V}_{O_2}$	35±8.4
				[La]a (mmol l^{-1})	3.2±1.2
		V_R kg^{-1} (ml kg^{-1})	86±38	Hct	29.0±2.6
		D_{LCO} (ml kg^{-1} min^{-1} mmHg^{-1})	0.116±0.027	[Hb] (g dl^{-1})	7.9±2.2

Values are mean ± 1 S.D. For metabolism and blood variables N=6; for ventilation N=5, except for P_{ACO_2} where N=3.
Abbreviations as in main text.
T_b=35.0±0.5 °C, mass=1.39±0.39 kg.

level achieved during running; as a result R further increased (Table 2).

Ventilation and breathing pattern before, during and after locomotion

The breathing pattern at rest was typical for a lizard, i.e. evenly spaced single breaths, each interrupted by an end-inspiratory pause (Fig. 3). During sustained locomotion there was a substantial reduction in T_P (and a slight shortening of T_I and T_E, though this was not significant for T_I) (Fig. 3; Tables 1, 2). $\dot{V}_E$ increased (Table 2; Fig. 4), aided by increases in both V_T and f (Fig. 3B; Table 2), in direct proportion to $\dot{V}_{O_2}$ (Fig. 4). Therefore, the air convection requirement for oxygen, $\dot{V}_E/\dot{V}_{O_2}$, was maintained constant (Table 2; Fig. 4). A similar situation existed for $\dot{V}_E/\dot{V}_{CO_2}$ (Table 2). In the 2 min following exercise, V_T and f remained unchanged; as a result $\dot{V}_E$ was maintained at the exercise level and, as a consequence of the tendency for $\dot{V}_{O_2}$ to decline, the $\dot{V}_E/\dot{V}_{O_2}$ ratio tended to increase, though not significantly (Fig. 4). $\dot{V}_E/\dot{V}_{CO_2}$ was maintained at the same level as observed during exercise (Table 2).

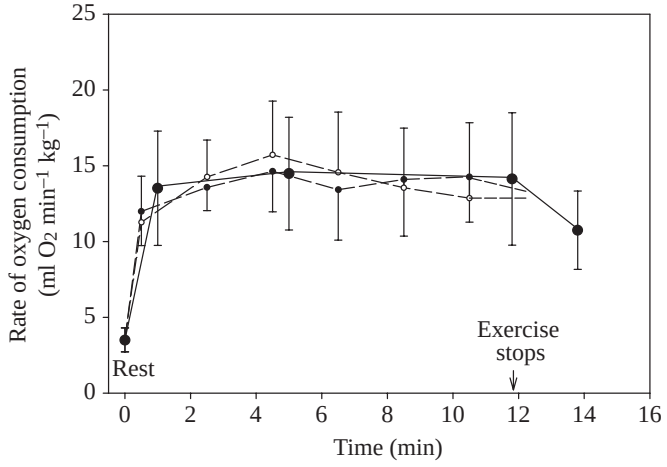

Fig. 2. Rate of oxygen consumption ($\dot{V}_{O_2}$) at rest and during maximum levels of sustained aerobic exercise on a treadmill for *V. mertensi*. The large solid symbols represent data collected during experimental runs, the small symbols connected by dashed lines represent data obtained pre- (small closed circles) and post-surgically (small open circles) with animals on the treadmill. Values are means ± 1 S.D. (N=6).

Oxygen transport before, during and after locomotion

The Hb affinity in *V. mertensi* (P_{50}=49.3±4.4 mmHg, 35 °C, P_{CO_2}=39 mmHg, pH approx. 7.5), together with the Bohr factor (–0.30±0.048), were similar to values previously published in varanid species; the P_{50} for *V. exanthematicus* is 47 mmHg at 35 °C, pH 7.5, Bohr factor –0.30 (Wood et al., 1977), and for *V. gouldii* P_{50} is 48 mmHg at 35 °C, pH 7.3 (Bennett, 1973a). Despite a small increase in P_{AO_2} and Pa_{O_2} (Tables 1 and 2), the alveolar–arterial P_{O_2} difference (P_{AO_2}–Pa_{O_2}) remained unchanged during exercise from its value at rest (approx. 20 mmHg) and decreased slightly during the first few minutes of recovery (Fig. 4). Arterial O_2 content, Ca_{O_2}, remained constant at all levels of $\dot{V}_{O_2}$, though due to the decrease in $P\bar{v}_{O_2}$ the venous O_2 content, $C\bar{v}_{O_2}$, dropped substantially (Tables 1 and 2). As expected, given no change in $\dot{V}_E/\dot{V}_{O_2}$, Pa_{CO_2} remained constant and no different from P_{ACO_2} at all levels of $\dot{V}_{O_2}$ (Table 1 and 2). Despite a tendency for blood pH to decrease with exercise and during recovery this was not significant. Lactate concentration [La] increased approx. 2.5-fold during and after exercise (Tables 1 and 2). Hct and [Hb] remained constant throughout exercise and in the recovery phase; the

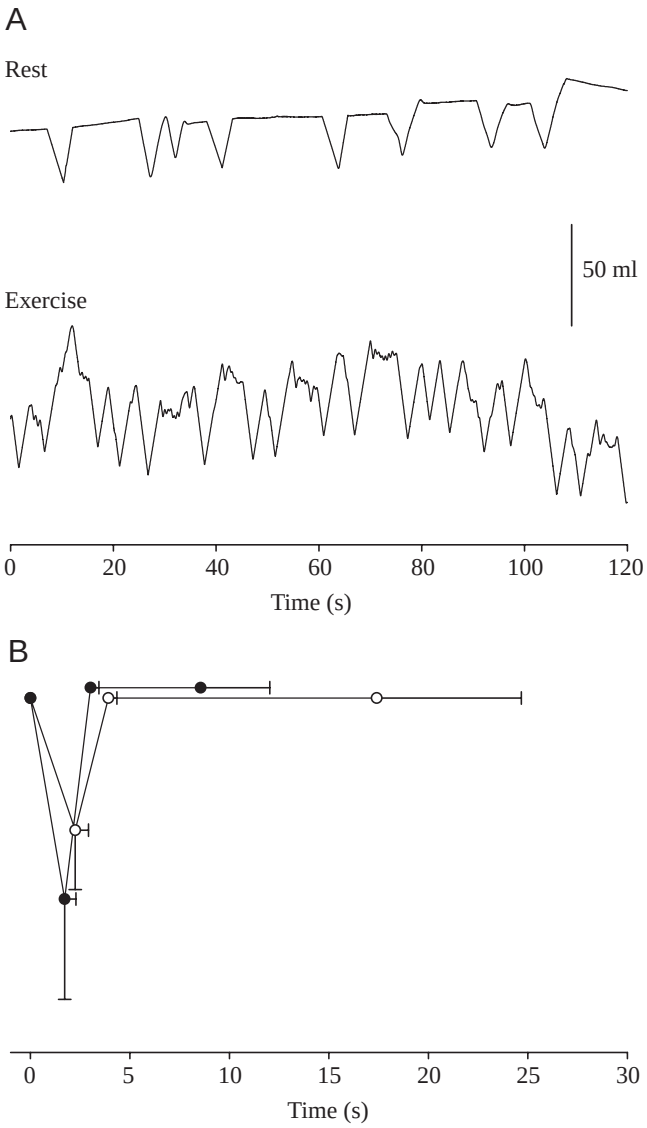

Fig. 3. (A) Typical breathing pattern obtained during rest (pre-exercise) and exercise in *V. mertensi*. (B) A spirogram for rest (open symbols) and exercise (closed symbols) that shows the changes in volume and timing that occur with exercise. Note that each breathing cycle starts with expiration (downward deflection), followed by an inspiration and a post-inspiratory pause. Values are mean ± 1 S.D. (N=5).

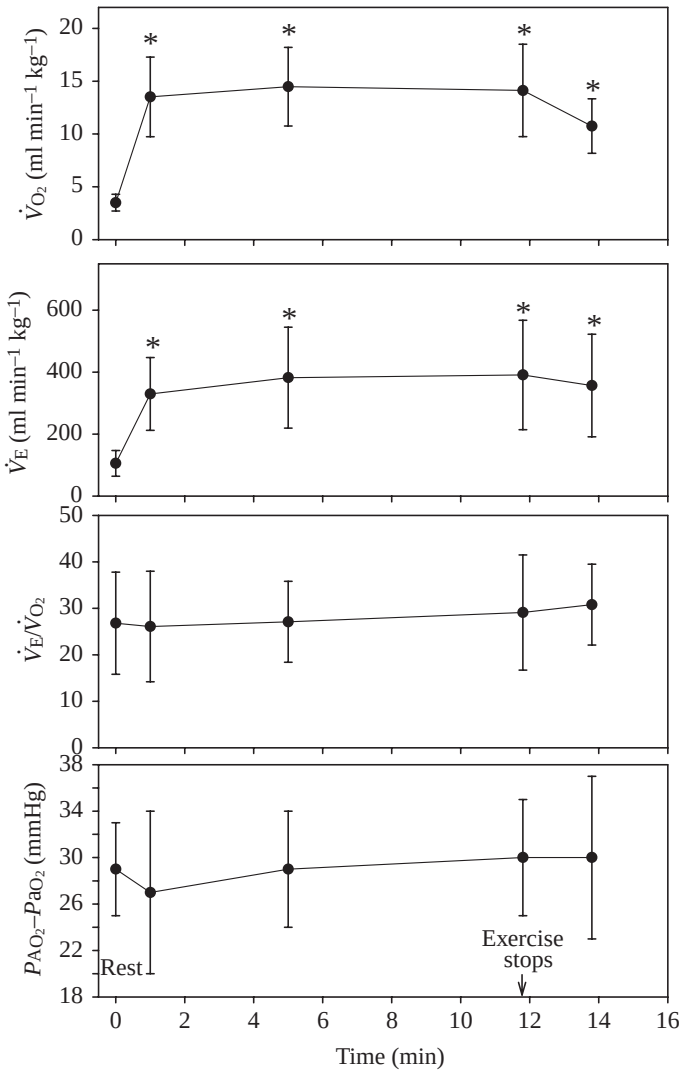

Fig. 4. Values obtained for $\dot{V}_{O_2}$, $\dot{V}_E$, $\dot{V}_E/\dot{V}_{O_2}$ and P_{AO_2}–Pa_{O_2} during rest, exercise and recovery in *V. mertensi*. Changes in $\dot{V}_{O_2}$ were closely matched by changes in $\dot{V}_E$ such that $\dot{V}_E/\dot{V}_{O_2}$ was maintained. P_{AO_2}–Pa_{O_2} was maintained at resting values during exercise and the first 2 min of recovery. Values are means ± 1 S.D. (N=5). *Significant difference from the value at rest.

value of Hct was identical to that reported for *V. gouldii* (Bennett, 1973a).

Systemic cardiac output and right-to-left shunt fraction

The associated increase in arterial–venous O_2 content difference (Ca_{O_2}–$C\bar{v}_{O_2}$) with exercise was not enough to offset any increases in cardiac output, hence $\dot{Q}_{tot}$ increased (Tables 1 and 2). The increase in $\dot{Q}_{tot}$ was achieved through a corresponding increase in f_H, and stroke volume remained unaltered (approximately 1.72 ml kg^{-1}). Both $\dot{Q}_{tot}$ and f_H remained high during the first 2 min of recovery. At rest *V. mertensi* possessed a considerable right-to-left shunt (Table 1), but unfortunately no information was obtained on the shunt during exercise.

Lung volume and D_{LCO} *before and during locomotion*

Locomotion had no effect on lung volume (V_L) or the pulmonary diffusing capacity for carbon monoxide (D_{LCO}) (Table 1 and 2).

Discussion

Not all varanids posses a high aerobic scope, and among their reptilian counterparts an increasing body of evidence is beginning to show that aerobic scope in this group is as varied as it is amongst mammals (Christian and Conley, 1994). The

Table 2. *The effect of activity on respiratory values for* V. mertensi

Metabolism			Ventilation			Oxygen transport		
	Exercise	Recovery		Exercise	Recovery		Exercise	Recovery
$\dot{V}_{O_2}$ kg^{-1} (ml O_2 kg^{-1} min^{-1})	14.0±4.0* (416)	10.8±2.6* (324)	P_{AO_2} (mmHg)	126±6 (104)	130±6* (107)	Pa_{O_2} (mmHg)	104±6 (104)	115±14* (114)
$\dot{V}_{CO_2}$ kg^{-1} (ml CO_2 kg^{-1} min^{-1})	11.7±2.5* (477)	11.5±2.6* (474)	P_{ACO_2} (mmHg)	31±8 (113)	29±8 (116)	Pa_{CO_2} (mmHg)	29±6 (99)	30±7 (102)
R	0.86±0.21 (122)	1.08±0.18* (152)	$\dot{V}_E$ kg^{-1} (ml kg^{-1} min^{-1})	368±100* (214)	374±260* (207)	pHa	7.378±0.166 (98)	7.275±0.198 (97)
			$\dot{V}_A$ kg^{-1} (ml kg^{-1} min^{-1})	356±86* (475)	334±74* (445)	Ca_{O_2} (mmol l^{-1})	3.7±1.1 (99)	3.8±1.1 (98)
			V_T kg^{-1} (ml kg^{-1})	38.3±17.3* (151)	37.5±15.8* (148)	$P\bar{v}_{O_2}$ (mmHg)	43±9* (72)	52±10 (85)
			f (min^{-1})	10.2±2.6* (249)	10.4±1.9* (254)	$P\bar{v}_{CO_2}$ (mmHg)	48±9* (140)	52±10* (151)
			T_I (s)	1.7±0.6* (74)	2.0±0.4 (87)	$pH_{\bar{v}}$	7.265±0.175 (97)	7.148±0.227 (95)
			T_E (s)	1.3±0.4* (76)	1.7±0.5 (100)	$C\bar{v}_{O_2}$ (mmol l^{-1})	0.5±0.3* (22)	1.2±0.6* (47)
			T_P (s)	4.3±1.8* (32)	3.2±1.1* (24)	$\dot{Q}_{tot}$ kg^{-1} (ml kg^{-1} min^{-1})	209±61* (165)	214±102* (169)
			T_{TOT}(s)	8.6±3.5* (49)	7.8±2.5* (45)	$\dot{Q}_{shunt}/\dot{Q}_{tot}$ (%)	–	–
			$\dot{V}_E/\dot{V}_{O_2}$	27.4±11.1 (102)	30.8±8.7 (115)	f_H (min^{-1})	120±14* (162)	106±11* (143)
			$\dot{V}_E/\dot{V}_{CO_2}$	35.4±11.0 (93)	35.6±12.3 (94)	$\dot{Q}_{tot}/\dot{V}_{O_2}$	15.6±5* (45)	20.6±10.2* (59)
						[La]a (mmol l^{-1})	8.5±3.2* (266)	9.7±4.4* (303)
			V_R kg^{-1} (ml kg^{-1})	114±56 (133)	–	Hct	29.3±2.9 (103)	28.6±1.6 (97)
			D_{LCO} (ml kg^{-1} min^{-1} mmHg^{-1})	0.120±0.032 (103)	–	[Hb] (g dl^{-1})	7.4±2.3 (104)	7.1±1.6 (99)

Activity was for a lizard running on a treadmill at an average speed of 0.33 m s^{-1} until exhaustion (11.8 min).
Values presented for exercise are an average of the three measurements made during the period of activity; those presented for recovery were made 2 min after exercise ceased.
Values are means ± 1 S.D.; values in parentheses are a percentage of the resting values presented in Table 1.
For metabolism and blood variables N=6; for ventilation N=5, except for P_{ACO_2} where N=3.
*Significant difference from the value at rest.
Abbreviations as in main text.

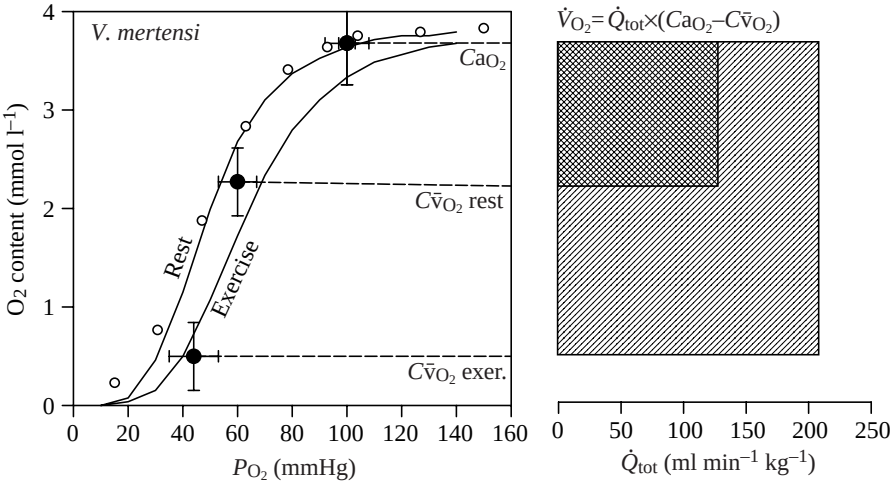

Fig. 5. Influence of exercise on arterial and venous P_{O_2} and O_2 content (solid symbols) in relation to the hemoglobin oxygen dissociation for *V. mertensi*. Values are means ± 1 S.D. (N=6). The open symbols are actual measured values of Pa_{O_2} and Ca_{O_2} determined at rest. The curve labelled 'Exercise' has been adjusted according to pHv and the Bohr effect (see Results). The right panel is a graphical representation of the Fick principle showing the relative contributions of cardiac output ($\dot{Q}_{tot}$) and arterial–venous O_2 content difference (Ca_{O_2}–$C\bar{v}_{O_2}$) to $\dot{V}_{O_2}$ (the shaded area) during rest (cross hatch) and exercise (stippled).

values reported for $\dot{V}_{O_2max}$ in this study for lizards either wearing the mask that encompassed the head or fitted with the mouthpiece (and pre- or post-surgery) were the same, and similar to that previously reported for this species (Christian and Conley, 1994). This value is approximately 20 % lower than that predicted for a similarly sized varanid by the interspecific equation for varanid $\dot{V}_{O_2max}$ (Thompson and Withers, 1997), and is possibly related to the semiaquatic lifestyle of this species.

The [La] values reported here (8.5 mmol l^{-1}) are similar to levels found in *V. exanthematicus* (7.2 mmol l^{-1}) at maximal sustainable speed (Gleeson et al., 1980). Both these values are much lower than the values (approximately 28 mmol l^{-1}) associated with high and probably unsustainable levels of activity in that species (Mitchell et al., 1981a). Obviously, accumulation of [La] implies that anaerobic metabolism is supplementing aerobic energy production. During intensive bouts of activity, anaerobic glycolysis in reptiles can account for most of the ATP produced, with aerobic metabolism playing a minor role (Bennett, 1994). The reader is referred to the reviews by Gleeson (1991, 1996); also see (Bennett, 1994) for a detailed account of anaerobic metabolism during intense activity in reptiles and the aerobic payback that accompanies recovery.

Of particular interest in this study, given the recent findings that gular movements may augment ventilation in *V. exanthematicus* (Owerkowicz et al., 1999), are the similar values in $\dot{V}_{O_2}$ achieved for lizards exercised with a mask encompassing the head or while fitted with a mouthpiece. In the study by Owerkowicz et al. (1999), lizards in which the mouth was propped open were unable to reach the level of $\dot{V}_{O_2max}$ achieved when exercised with their mouth closed and therefore capable of gular pumping. The implication from that study was that gular pumping was required to achieve appropriate levels of $\dot{V}_E$ necessary to support the high levels of $\dot{V}_{O_2}$ associated with exercise. If the presence of a functional gular pump is an attribute of all varanids during exercise, then it may be concluded that the mouthpiece used in the present experiments did not impair the gular pump. However, we did not measure buccal cavity pressure to determine if gular pumping was occurring. On the other hand, Schultz et al. (1999) studied four species of varanids (*V. spenceri, V. gouldii, V. panoptes* and *V. mertensi*) and showed that, whereas at rest all species relied on nasal breathing, when exercised they did so with their mouths open to various degrees, which could account for 11–49 % of the expired gases. This finding tends to suggest that gular-assisted ventilation is not obligatory and/or widespread amongst the varanids. Obviously, further studies are required to assess the degree to which gular pumping is a characteristic of varanids.

The finding that varanids breathe through their mouths during exercise raises some concerns about the levels of ventilation reported in previous studies, where ventilation was measured only through the nostrils. Carrier (1987a) assessed inspiratory airflows through the nostrils with a thermistor, and Hopkins et al. (1995) and Wang et al. (1997) measured airflow through a pneumotacograph attached to the nostrils. Hopkins and coworkers checked for mouth breathing at rest in an attempt to validate this approach and found none; perhaps not surprising, given the findings of Schultz et al. (1999).

Is ventilation constrained by locomotion?

It has been hypothesised that mechanical interference exists between locomotor and ventilatory function in lizards, as the intercostal muscles are required for both locomotion (lateral flexion of the trunk) and ventilation (expansion of the thorax) (Carrier, 1987a,b, 1989, 1990). Accordingly, the aerobic needs of an exercising lizard cannot be met because of the locomotory constraints on lung ventilation. Indeed, Wang et al. (1997) found no change in tidal volume during exercise in *V. exanthematicus* but a two- to fourfold increase immediately following exercise. An increase in $\dot{V}_E$ did occur, however, during exercise as a result of changes in f. The present study with *V. mertensi* also finds an increase in $\dot{V}_E$ during exercise, the result of an increase in V_T and f (primarily through a shortening in T_P), and this increase is retained in the recovery period immediately following exercise. As previously

mentioned, costal ventilation during exercise was most likely constrained in *V. exanthematicus* and gular pumping, by circumventing the constraint, contributed to the overall increase in ventilation and hence $\dot{V}_{O_2}$ (Owerkowicz et al., 1999). Gular pumping is not used in *Iguana iguana* and ventilation decreases at speeds greater than a very slow walk (Wang et al., 1997; Owerkowicz et al., 1999).

While costal ventilation may be inhibited in some lizards during exercise, the undulatory movement of the body trunk that accompanies locomotion may be of benefit to gas exchange. The highly compliant nature of the respiratory system in lizards (Perry and Duncker, 1978; Milsom, 1989) provides minimal constraint to changes in body posture and is prone to distortion. Any distortion of the lungs that occurs could enhance the effectiveness of gas exchange by improving gas mixing. However, in an experiment with *V. gouldii*, where individuals were hyperventilated to lower the $P_{a_{CO_2}}$ levels below the apneic threshold, no change was detected in the time course of arterial blood gas levels with or without lateral passive body movements (Frappell and Mortola, 1998). This suggested that lateral chest wall movements neither hindered nor facilitated gas exchange.

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

In the study of Wang et al. (1997), the air convection requirement ($\dot{V}_E/\dot{V}_{O_2}$) in *V. exanthematicus* approximately doubled during exercise at aerobically sustainable speeds, and on recovery returned to pre-exercise levels, indicating a substantial hyperventilation with exercise. In contrast, the present study finds that the increase in $\dot{V}_E$ during exercise in *V. mertensi* adequately meets the increase in $\dot{V}_{O_2}$, hence maintaining the $\dot{V}_E/\dot{V}_{O_2}$ ratio constant between exercise and pre-exercise. While the study of Bennett (1973b) reports a relative hypoventilation for *V. gouldii* immediately after activity, the results should be viewed with some degree of scepticism, as activity was achieved following electrical stimulation of restrained lizards.

While not directly measuring ventilation, Mitchell and coworkers (Mitchell et al., 1981b) indicated, from measurements of $P_{a_{CO_2}}$ and the alveolar gas equation, that during exercise *V. exanthematicus* increased $\dot{V}_A$ relatively more than $\dot{V}_{O_2}$, giving rise to an increase in $P_{A_{O_2}}$ (again calculated, not measured). Likewise, Hopkins et al. (1995) report a small hyperventilation in *V. exanthematicus*, though the associated increase in $P_{a_{O_2}}$ was not significant. The present study also revealed a small increase in $P_{A_{O_2}}$ and $P_{a_{O_2}}$ with exercise and recovery in *V. mertensi*. In the absence of any change in $\dot{V}_E/\dot{V}_{O_2}$, the increase in $P_{A_{O_2}}$ suggests a proportionally greater increase in alveolar ventilation (i.e. relative decrease in dead space). At rest, $\dot{V}_A$ accounts for approx. 70 % of $\dot{V}_E$, whereas during exercise and recovery this increased to approx. 95 % (Tables 1 and 2). In summary, the available evidence suggests that, for varanids exercised at sustainable speeds, $\dot{V}_E$ increases sufficiently during exercise to meet the increased $\dot{V}_{O_2}$, and the small increase in $P_{A_{O_2}}$ observed most likely reflects a proportionally greater increase in $\dot{V}_A$.

Does O_2 transfer across the lung limit $\dot{V}_{O_2}$ during exercise?

At rest, a substantial alveolar–arterial P_{O_2} ($P_{A_{O_2}}$–$P_{a_{O_2}}$) difference was observed in *V. mertensi* (approx. 21 mmHg), as has been previously reported for *V. exanthematicus* (approx. 14 mmHg, Mitchell et al., 1981b; approx. 27 mmHg, Hopkins et al., 1995). During exercise the increase in $P_{A_{O_2}}$ and $P_{a_{O_2}}$ in both species (this study; Hopkins et al., 1995) maintains $P_{A_{O_2}}$–$P_{a_{O_2}}$, whereas Mitchell et al. (1981b) reported an increase in $P_{A_{O_2}}$–$P_{a_{O_2}}$ during exercise to values similar to that found by Hopkins and coworkers. The level of $P_{A_{O_2}}$–$P_{a_{O_2}}$ is an indication of the efficacy of alveolar–arterial O_2 transfer and could result from right-to-left (R–L) shunts, diffusion limitation or ventilation–perfusion inhomogeneity.

At rest reptiles may possess R–L shunts. A substantial R–L shunt of between 16 % (Berger and Heisler, 1977) and 6 % (Hopkins et al., 1995) has been reported in *V. exanthematicus* at rest, though the lower value reported by Hopkins is for an intrapulmonary shunt only, as their measurements excluded intracardiac shunting. The present study reports a R–L shunt in *V. merstensi* of approx. 17 % at rest. Studies on pulmonary gas transfer must, strictly speaking, be based on blood gases sampled from the pulmonary veins (Wang et al., 1998). In the present study our measure of $P_{a_{O_2}}$ could potentially include intracardiac shunts, which conceptually are identical to that of intrapulmonary shunts. However, based on studies on *V. niloticus* (Millard and Johansen, 1974; Ishimatsu et al., 1988) and *V. exanthematicus* (Burggren and Johansen, 1982), it would appear that in varanids R–L intracardiac shunting is much reduced or absent.

During exercise *V. exanthematicus* decreased the R–L intrapulmonary shunt from about 6 % to 2 % (Hopkins et al., 1995). Further, many reptiles develop a significant L–R shunt during exercise (Hicks and Krosniunas, 1996), though in *V. exanthematicus* this was shown not to occur (Hopkins et al., 1995). A reduction in the R–L shunt may improve $P_{a_{O_2}}$, depending on $\dot{V}_{O_2}$ and blood O_2 carrying capacity. If the O_2 levels of the pulmonary veins are on the flat portion of the oxygen dissociation curve then $C_{a_{O_2}}$ and $P_{a_{O_2}}$ will be affected only marginally (Wang and Hicks, 1996). Further, changes in $C_{a_{O_2}}$–$C\bar{v}_{O_2}$ can compensate for changes in shunt fraction to produce the same $P_{a_{O_2}}$ (see Equation 9 used for determining $\dot{Q}_{shunt}/\dot{Q}_{tot}$).

Compared with those of mammals, reptilian lungs have a lower surface area for diffusion and a blood gas barrier of increased and variable thickness (Perry, 1983; Perry et al., 1994). From the relationship between $\dot{V}_{O_2}$ and $D_{L_{O_2}}$ [$\dot{V}_{O_2}$=$D_{L_{O_2}}$×($P_{A_{O_2}}$–$P_{a_{O_2}}$)], the alveolar–arterial difference (for reptiles, falveolar–pulmonary vein difference) can be determined. While reptiles have a lower metabolic rate than mammals, $D_{L_{O_2}}$ is even lower, and as a result the equivalent $P_{A_{O_2}}$–$P_{a_{O_2}}$ will be higher in reptiles than mammals (Glass, 1991). The present study measured D_{LCO} (D_{LO}=1.23D_{LCO}) in *V. mertensi* and found at rest a value about double that previously reported in *V. exanthematicus* at 35 °C (Glass et al., 1981). In *V. exanthematicus* it was concluded that diffusion limitation did not contribute significantly to the $P_{A_{O_2}}$–$P_{a_{O_2}}$ at

rest (Hopkins et al., 1995) and presumably, given a higher D_{LCO}, the same also applies to *V. mertensi*. During exercise, however, Hopkins and coworkers found that diffusion limitation accounts for a substantial part of the $P_{A_{O_2}}$–Pa_{O_2} difference in *V. exanthematicus*, a situation often reported in mammals (see Powell, 1994).

The overall ventilation perfusion ratio for the lung was 0.71 at rest and increasing to 2.13 during exercise in *V. exanthematicus*, similar to that estimated $\{\dot{V}_E/\dot{Q}_L = \dot{V}_A/[\dot{Q}_{tot}\times(1-\dot{Q}_{shunt}/\dot{Q}_{tot})]$, where $\dot{Q}_L$ is the blood flow through the lung} for *V. mertensi* (approx. 0.8 and 2.1, respectively). With exercise this reflects, in both species, a greater increase in ventilation than perfusion. Alveolar–arterial P_{O_2} difference is also enhanced by spatial ventilation–perfusion inhomogeneity, which during exercise increased in *V. exanthematicus* (Hopkins et al., 1995). A similar situation occurs in exercising mammals, though the mechanisms responsible for the development of ventilation–perfusion inhomogeneity during exercise remain unclear (Wagner et al., 1986; Schaffartzik et al., 1992; Hopkins et al., 1994). Together, the available information suggests that pulmonary gas exchange in reptiles is no more limiting in varanids than it is in mammals.

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

Oxygen delivery to the tissues depends on both $\dot{Q}_{tot}$ and Ca_{O_2}–$C\bar{v}_{O_2}$ (oxygen extraction). The reliance on both $\dot{Q}_{tot}$ and oxygen extraction by the tissues during exercise is fairly common amongst vertebrates (Gleeson et al., 1980) and occurred in *V. mertensi*; the increase in $\dot{V}_{O_2}$ during exercise is achieved through increases in both O_2 extraction and cardiac output (Fig. 5). As $P_{A_{O_2}}$ and Pa_{O_2} are maintained or slightly increased during exercise, high values of arterial saturation were preserved (Fig. 5). Likewise, *V. exanthematicus* had a high Ca_{O_2} which, in turn, permitted a greater Ca_{O_2}–$C\bar{v}_{O_2}$ during sustainable levels of exercise (Gleeson et al., 1980). Wood et al. (1977) also note that the arterial–venous O_2 content difference is large in *V. exanthematicus* compared with most other groups of reptiles, and that this was a result of low shunt flows and corresponding high values of arterial saturation (Pa_{O_2}=94 mmHg, at 35 °C), hence high Ca_{O_2}.

The ability to maximise oxygen extraction during exercise is assisted by a distinct Bohr shift in the hemoglobin saturation curve that further favours unloading of O_2, and a greater increase in Ca_{O_2}–$C\bar{v}_{O_2}$ than would otherwise be achieved (Fig. 5). Previously, Gleeson et al. (1980) noted that both *V.exanthematicus* and *I. iguana* decreased mixed venous blood O_2 content to similar levels; the difference in Ca_{O_2}–$C\bar{v}_{O_2}$ was a reflection of the varanid's greater Ca_{O_2}. Further, in that study, while the factorial scope in cardiac output was greater in *V. exanthematicus*, both the varanid and iguana reached maximum $\dot{Q}_{tot}$ at intermediate levels of $\dot{V}_{O_2}$. This implies that without further increases in O_2 extraction, transport by the circulation could be limiting. It is also interesting to note that although the air convection requirement ($\dot{V}_E/\dot{V}_{O_2}$) remained constant, the blood convection requirement ($\dot{Q}_{tot}/\dot{V}_{O_2}$) decreased with exercise in *V. mertensi*, *V. exanthematicus* (Gleeson et al., 1980; Wang et al., 1997) and *I. iguana* (Gleeson et al., 1980). A decreasing blood convection requirement during exercise and an already large arterial–venous O_2 content difference suggests that there exists limited capacity for further increases in O_2 transport by the circulatory system. More recently, Farmer and Hicks (2000) suggested that in *I. iguana* both the circulatory and ventilatory systems imposed limits to O_2 transfer at anything faster than a slow walk. However, in the iguana maximum blood flow and ventilation occur during recovery (Wang et al., 1997; Farmer and Hicks, 2000). This is in contrast to varanids, where ventilation and cardiac output reach a maximum during exercise (Gleeson et al., 1980; Wang et al., 1997; this study).

In conclusion, we have examined O_2 transfer in the varanid, *V. mertensi*, during maximal levels of sustained aerobic exercise. Ventilation and O_2 transfer across the lung would appear to be adequate to meet the aerobic needs of *V. mertensi* during exercise. This is also the case in mammals, where the lungs are built with a significant excess structural capacity (Hoppeler and Weibel, 1998). On the other hand, at $\dot{V}_{O_2max}$ the circulatory system in mammals operates at or close to the upper limit of structural capacity for O_2 transport (Hoppeler and Weibel, 1998). Together with an already large arterial–venous O_2 content difference in *V. mertensi*, a decreasing blood convection requirement with exercise suggests a physiological limit occurring with the transport of oxygen by the circulatory system.

List of symbols used

$\bar{a}$	arterial modifier
C_{O_2}	blood oxygen content
Ca_{O_2}	arterial oxygen content
Cc'_{O_2}	O_2 content of end-capillary blood
$C\bar{v}_{O_2}$	mixed venous O_2 content
D_L	diffusing capacity of the lungs
E	excurrent modifier
f	breathing frequency
f_H	heart rate
F_{CO}	fractional concentration of CO
F_{CO_2}	fractional concentration of CO_2
F_{O_2}	fractional concentration of O_2
Hb	haemoglobin
Hct	haematocrit
I	incurrent modifier
La	lactate
P_{50}	P_{O_2} at which haemoglobin is 50 % saturated
Pa_{CO_2}	arterial partial pressure of carbon dioxide
Pa_{O_2}	arterial partial pressure of oxygen
$P_{A_{O_2}}$	alveolar partial pressure of oxygen
P_B	barometric pressure
P_{CO_2}	partial pressure of carbon dioxide
$P_{H_2O(35)}$	partial pressure of water vapour at 35 °C
P_{O_2}	partial pressure of oxygen
$P\bar{v}_{CO_2}$	partial pressure of mixed venous carbon dioxide
$P\bar{v}_{O_2}$	partial pressure of of mixed venous oxygen

$\dot{Q}_L$	blood flow through the lung
$\dot{Q}_{shunt}$	shunt blood flow
$\dot{Q}_{tot}$	total cardiac output
R	respiratory exchange ratio
$\boldsymbol{R}$	gas constant
T	absolute temperature
t	time
T_b	body temperature
T_E	expiratory time
T_I	inspiratory time (does not include T_P)
T_P	inspiratory pause
T_{TOT}	total breathing time
$\bar{v}$	mixed venous blood, modifier,
$\dot{V}_A$	alveolar ventilation
V_{AIR}	volume of air
$\dot{V}_{CO_2}$	rate of carbon dioxide production
$\dot{V}_E$	minute ventilation
V_L	volume of the lung
$\dot{V}_{O_2}$	rate of oxygen consumption
$\dot{V}_{O_2max}$	maximum rate of oxygen consumption
V_R	ventilation at rest
V_{SYS}	closed circuit of known volume
V_T	tidal volume of breathing
βO_2	capacitance of blood for oxygen
τ	gas flow

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