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ACID-BASE IMBALANCE IN LIZARDS DURING ACTIVITY AND RECOVERY

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SUMMARY

1. The effects of treadmill exercise on oxygen consumption ($\dot{V}_{O_2}$), carbon dioxide production ($\dot{V}_{CO_2}$), arterial blood lactate concentration ($[L^-]_a$), arterial blood pH and arterial gas tensions (P_{aO_2} and P_{aCO_2}) were measured in 3 species of lizards (*Varanus salvator*, *V. exanthematicus*, *Iguana iguana*)

2. *Varanus salvator* was exercised 45 min at an intensity which required 85% of its $\dot{V}_{O_2\,max}$. *V. salvator* utilized supplementary anaerobic metabolism during the first 10 min of this sustainable exercise, as evidenced by a 16 mmol/l increase in $[L^-]_a$. Respiratory exchange ratios (R, where $R = \dot{V}_{CO_2}/\dot{V}_{O_2}$) exceeded 1·2 when $[L^-]_a$ and $[H^+]_a$ were maximal. One half of the accumulated lactate was removed from the blood during the remainder of the 45 min exercise period, while blood pH returned to resting levels.

3. In a second set of experiments, high intensity exercise led to exhaustion after 5 to 10 min in all three species, resulting in large lactate ($+\Delta[L^-]_a$ = 14–20 mmol/l) and hydrogen ion ($+\Delta[H^+]_a$ = 23–57 nmol/l) accumulations. R values ranged from 1·2–1·8 at exhaustion.

4. Recovery from both sustainable and non-sustainable exercise was characterized as a period of rapid lactate removal. Respiratory exchange ratios were low (0·3–0·5) as metabolic CO_2 was retained, replacing depleted bicarbonate stores.

5. We conclude that all three lizard species make ventilatory adjustments during and after exercise that minimize disturbances to resting hydrogen ion concentrations and acid-base balance. *Varanus salvator* demonstrate the ability to re-establish resting acid-base status during sustained exercise requiring 85% of their $\dot{V}_{O_2,max}$. Changes in R appear to be a useful non-invasive indicator of net blood lactate accumulation.

INTRODUCTION

The hydrogen and lactate ions produced via anaerobic metabolism can result in substantial acid-base disturbances, lowering both blood pH and plasma bicarbonate concentrations. Although considerable attention has been paid to the influence of body temperature on acid-base balance in resting lizards (Howell & Rahn, 1976; Withers,

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1978; Wood, Glass & Johansen, 1977; Wood, Johansen, Glass & Hoyt, 1981; Wood & Moberly, 1970; Ackerman & White, 1980), very little attention has been paid to how lizards cope with the acid-base disturbances associated with activity.

Anaerobic lactate production can account for 60–90% of the total energy (ATP) produced by lizards during vigorous exercise (Bennett & Licht, 1972; Bennett & Dawson, 1972; Bennett & Gleeson, 1976). The capacity of lizards to support activity aerobically is limited, and even sustainable activity may involve lactate accumulation in its initial phase (Bennett & Gleeson, 1979; Gleeson, 1979; Gleeson, Mitchell & Bennett, 1980; John-Alder & Bennett, 1981). The heavy reliance of lizards on anaerobic metabolism during exercise suggests that activity induced acid-base disturbances may be greater than temperature induced perturbations.

Mitchell, Gleeson & Bennett (1981*a*) have presented data which demonstrate that lizards make ventilatory adjustments necessary to defend blood pH and acid-base balance when faced with blood carbonic (metabolic CO_2) and non-carbonic (lactic acid) acid loads during steady-state exercise. In the present study, we describe the transient changes in acid-base status of lizards during exercise. In the first experiment, *V. salvator* were run at a speed sustainable for 45 min while metabolic and respiratory parameters were measured. The resultant data allow us to determine the role of anaerobic metabolism during the early phase of sustainable exercise. Blood pH regulation was also evaluated.

In a second set of experiments, members of three lizard species were fatigued by non-sustainable high speed running. Lactate and hydrogen ion accumulations were measured, and the acid-base imbalance resulting from the metabolic acidosis was evaluated during the recovery period which followed. Together, these experiments provide a comprehensive picture of the consequences of anaerobic metabolism on acid-base balance during sustainable and non-sustainable activity in lizards, and the mechanism by which lizards re-establish resting hydrogen ion and bicarbonate concentrations during recovery.

METHODS

Animals. Eleven lizards of three species were used in this study. Four male water monitors, *Varanus salvator*, of mean mass 458 ± 68 g (350–634 g), were captured in Thailand and obtained through a commercial animal dealer. Information on the metabolic scope and temperature preferenda of this species is available in an earlier paper (Gleeson, 1981). Three green iguanas, *Iguana iguana* (mean mass = 984 ± 243 g, 644–1456 g), and four savannah monitors, *Varanus exanthematicus* (mean mass = 1470 ± 219 g, 940–2013 g) were also used. Information on the metabolic scopes and activity capacities of these two species has also been published (Gleeson *et al.* 1980). All lizards were housed in cages equipped with a photothermal gradient (12:12 light–dark cycle). Iguanas were fed a diet of lettuce and dog food; both varanid species were fed mice. Water was available at all times.

Oxygen consumption–carbon dioxide production. Resting oxygen consumption ($\dot{V}_{O_2,rest}$) and $\dot{V}_{O_2}$ during activity were measured on masked animals using a flow-through ventilation system previously described (Gleeson, 1979, 1981). Expired gases were drawn from a clear acetate mask at 1000 to 2500 ml/min (STP) and through a colu

of silica gel to remove water vapour. An airtight diaphragm pump (Markson 6363) pumped the excurrent flow through a Brooks mass-flowmeter which regulated airflow at ±1% of flow. Downstream from the mass-flowmeter, 150 ml/min was drawn through a column of Drierite ($CaCO_2$) followed by a CO_2 analyser (Beckman LB2) and an oxygen analyser (Applied Electrochemistry S3A) connected in series. Analyser output was recorded on a dual pen stripchart recorder. Oxygen and carbon dioxide content of expired air were estimated by polar planimetry of chart recordings corresponding to periods of blood sampling (see below). Carbon dioxide production ($\dot{V}_{CO_2}$) and $\dot{V}_{O_2}$ were calculated from excurrent O_2 and CO_2 contents using equations published by Gleeson (1979). Reduction of air flow from the mask by 50% did not alter calculated rates of $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$, implying that no expired air leaked from the mask. All gas volumes are expressed at standard temperature (0 °C) and pressure (760 torr).

Cannulation and blood sampling. Lizards were cannulated at room temperature under halothane (Fluothane, Ayerst) anaesthesia. In *V. salvator*, pre-shaped polyethylene cannulae (PE 50 tapered to PE 20) were inserted 2·5 cm upstream into the left carotid artery, passed out at the top of the neck and flushed with 20 U/ml heparinized saline. *V. exanthematicus* and *Iguana* were cannulated in the left femoral artery using T-shaped flow-through cannulae fashioned from PE 50 and PE 10 tubing. Flow-through cannulae were designed to allow blood sampling without obstructing blood flow to the limb. Gas tensions of femoral artery blood did not differ from carotid arterial blood drawn from these species in earlier studies (Mitchell *et al.* 1981*a*, *b*). Blood loss from all lizards was negligible, and lizards were allowed at least 24 h to recover at 35 °C.

After 12 or more hours of equilibration in a controlled temperature cabinet (35 °C), a lizard was removed, fitted with the metabolic mask, a saline-filled cannula extension (110 cm, PE 50) and a cloacal thermistor (YSI). Instrumented *V. salvator* were then placed into a cardboard 'burrow' built onto the front of a motor driven treadmill. Burrow and treadmill were warmed by an infrared heater which cycled on and off in response to lizard body temperature. Body temperature was monitored by a Versatherm controller (Cole-Parmer Instr.) via the thermistor probe and cycled the infrared heater to maintain lizard body temperature at 35 ± 1 °C. After 2–5 h rest within the burrow, a 200 μl blood sample was drawn anaerobically as previously described (Gleeson *et al.* 1980), and stored on ice. Blood samples were generally analysed for gas tensions and pH within 2 min, and no samples were stored for more than 10 min.

Blood samples during activity were drawn in the same manner. Activity was initiated by opening the door to the burrow and drawing the lizard back onto the slowly moving treadmill. Most lizards immediately began to walk forward toward the burrow, and treadmill speed was then increased to either 0·5 km/h or 1·0 km/h. Blood samples were taken at various intervals during activity (see Figs. 1–4). *Varanus salvator* were exercised at 0·5 and 1·0 km/h on separate days. Three of four *V. salvator* appeared capable of walking indefinitely at 0·5 km/h; walking was therefore terminated after 45 min. Running at 1·0 km/h quickly exhausted all *V. salvator*, and running was terminated after 5 min. At the end of activity, the treadmill was slowed, the burrow door opened and the lizard allowed to walk into the burrow, where it remained undisturbed for 60 min while metabolic and blood gas measurements were made. This burrow system was designed after experiments with *Iguana* and *V. exanthematicus*

indicated that handling at the end of activity disturbed the lizard during the first few min of recovery.

Iguana and *V. exanthematicus* were run to exhaustion in a similar manner. *Iguana* was run 10 min at 0·4–1·0 km/h while *V. exanthematicus* required higher running speeds, 1·2–1·6 km/h. At the end of exercise, both species were placed in cloth bags and allowed to recover 60 min at 35 °C on the treadmill surface.

Blood Analysis. Blood samples were mixed by vigorous rotation of the syringe and approximately 125 μl analysed for arterial blood pH and gas tensions ($P_{a\,O_2}$ and $P_{a\,CO_2}$), using a Radiometer BMS3/PHM73 blood microsystem. Calibrations of pH, oxygen and carbon dioxide electrodes were made before and after each sample, using Radiometer buffers, precision mixed gases and air equilibrated water, all equilibrated to 35 °C. Plasma bicarbonate concentrations ($[HCO_3^-]$, mM/l) were calculated from blood pH and $P_{a\,CO_2}$ values according to Severinghaus *et al.* (1956*a*, *b*). Effective alveolar ventilation ($\dot{V}_{eff}$, ml.g^{-1}.h^{-1} BTPS) was calculated from values of $\dot{V}_{CO_2}$ and $P_{a\,CO_2}$ according to Mitchell *et al.* (1981*a*). Arterial hydrogen ion concentrations ($[H^+]_a$) were calculated from pHa assuming an activity coefficient of 1·0.

Arterial blood samples (25 μl) were mixed with 200 μl 0·6 N-$HClO_4$ and refrigerated. Acid supernatants were later analysed for lactate with enzymatic test kits (Boehringer-Mannheim, cat. 139084) on a Beckman model 25 spectrophotometer. Lactate concentrations are expressed as mmoles lactate/litre whole blood. All numerical data are presented as mean ± S.E.M.

RESULTS

Resting values of all metabolic and respiratory variables for *Varanus salvator* are given in Table 1. Respiratory gas exchange and blood lactate concentration increase rapidly in response to both exercise intensities. After the initial few minutes, the physiological response of *Varanus* to exercise differs depending on whether the activity is sustainable (0·5 km/h) or non-sustainable (1·0 km/h). The responses of *Varanus* to these two exercise stimuli are described separately. Patterns of gas exchange as described in Figs. 1–4 are the mean responses of three or more animals. All animals demonstrated qualitatively similar responses to a given exercise stimulus unless otherwise noted. Estimates of quantitative variation about mean values reported in Figs. 1–4 can be inferred from range and standard error of the mean values reported in the text and in Tables 1 and 2.

High intensity, sustainable activity. The rate of increase in respiratory gas exchange began to slow after walking 3 min at 0·5 km/h (Fig. 1*a*). Maximal rates of oxygen consumption ($\dot{V}_{O_2\,max.}$ = 1·1 ± 0·08 ml O_2.g^{-1}.h^{-1}, range 1·0–1·3 ml O_2.g^{-1}.h^{-1}) and peak carbon dioxide production ($\dot{V}_{CO_2}$ = 1·4 ± 0·18 ml CO_2.g^{-1}.h^{-1}, range 1·2–1·8 ml CO_2.g^{-1}.h^{-1}) occurred 10 min into the exercise period. Oxygen consumption decreased, and reached a plateau after 20–45 min at a value equal to 85% $\dot{V}_{O_2\,max}$. Respiratory exchange ratio (R) increased from resting values (Table 1) to peak values (R = 1·3 ± 0·07, range 1·2–1·4) after 10 min (Fig. 1*b*), and slowly declined towards R = 0·9 (range 0·77–0·98) during the duration of exercise, passing through 1·0 after 24 min. Effective alveolar ventilation ($\dot{V}_{eff}$) peaked after 10 min of exercise and declined towards the end of the exercise period. The air convection requirement ($\dot{V}_{eff}/\dot{V}_{O_2}$

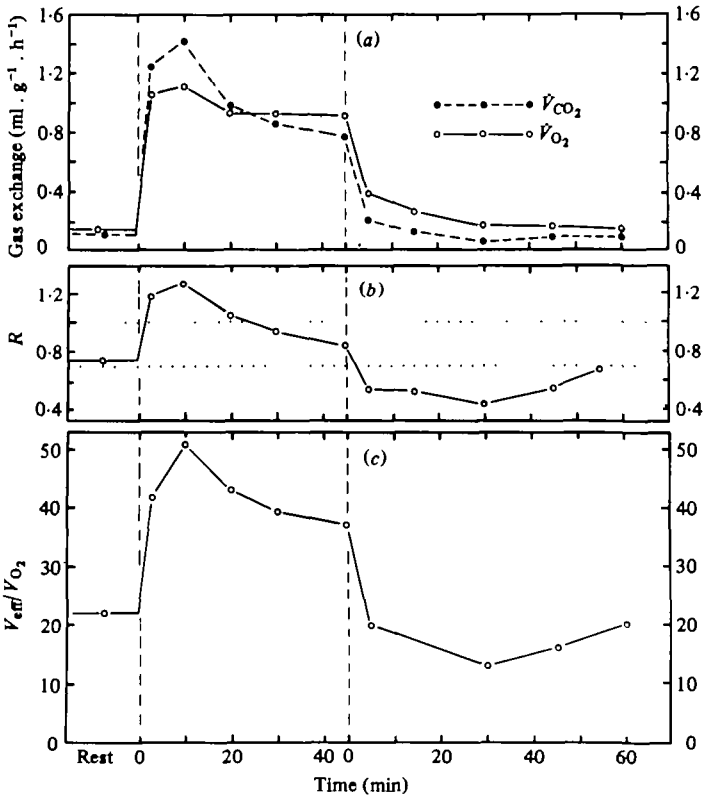

Fig. 1. Gas exchange and ventilation in three *Varanus salvator* at rest, during 45 min treadmill walking at 0·5 km/h and during 60 min of quiet recovery, all at T_B = 35 °C. See Methods for details.

showed a similar pattern (Fig. 1*c*), reaching a value of 40 at the end of exercise. This reflects a doubling of the ratio of ventilation to metabolism over the resting state (Table 1).

Fig. 2 details the changes that occur in the blood of *Varanus salvator* during this exercise and recovery period. Arterial blood lactate and hydrogen ion concentrations both peaked after 10 min of walking at 0·5 km/h, representing an increase in $[H^+]_a$ of 19·7 ± 12 nmol/l (from 28·1 nmol/l at rest) and an increase in $[L^-]_a$ of 17 mmol/l (from 0·26 mmol/l at rest) (Fig. 2*a*). Plasma bicarbonate concentration reached a minimum value (13·8 mmol/l) at the same time that $[L^-]_a$ and $[H^+]_a$ peaked, and then increased throughout the rest of the exercise period (Fig. 2*b*). Arterial CO_2 tension (P_{aCO_2}) decreased about 8 mmHg during exercise, while O_2 tension (P_{aCO_2}) remained essentially unchanged (Fig. 2*c*).

During the 60 min recovery period which followed, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ quickly returned towards resting values (Fig. 1*a*). Respiratory exchange ratio R reached minimum values of about 0·4 (range 0·32–0·60) after 30 min and then returned towards resting levels. Arterial blood became slightly alkalotic as $[H^+]_a$ decreased and $[HCO_3^-]_a$ increased relative to pre-exercise concentrations. Effective alveolar ventilation decreased in relation to metabolism (Fig. 1*c*), which resulted in a transient decline in P_{aO_2}.

Table 1. *Metabolic and respiratory variables in three* V. salvator *before, during, a after sustainable and non-sustainable activity*

	Units	Rest	0·5 km/h		1·0 km/h	
			at 45 min	60 min. recov.	at 4 min	60 min recov.
$\dot{V}_{O_2}$	ml $O_2 . g^{-1} . h^{-1}$	0·15* (0·007, 15)	0·94 (0·068, 4)	0·17 (0·014, 3)	0·93 (0·096, 3)	0·23 (0·022, 3)
$\dot{V}_{CO_2}$	ml $CO_2 . g^{-1} . h^{-1}$	0·11 (0·005, 15)	0·86 (0·006) (4)	0·11 (0·086, 3)	1·63 (0·086, 3)	0·08 (0·005, 3)
R		0·75 (0·023, 15)	0·91 (0·078, 4)	0·68 (0·085, 3)	1·77 (0·163, 3)	0·35 (0·022, 3)
$\dot{V}_{eff}$	ml (BTPS) $. g^{-1} . h^{-1}$	3·4 (0·19, 10)	34·5 (8·90, 3)	3·3 (0·46, 3)	57·2 (1·23, 3)	2·45 (0·128, 3)
$\dot{V}_{eff}/\dot{V}_{O_2}$		22·4 (1·14, 10)	36·6 (6·54, 3)	19·9 (2·66, 3)	62·5 (5·69, 3)	11·1 (1·33, 3)
P_{aO_2}	mmHg	97 (9·9, 10)	100 (1·2, 3)	97 (1·0, 3)	97 (7·0, 3)	77 (6·3, 3)
P_{aCO_2}	mmHg	28·7 (1.06, 10)	20·5 (1·93, 3)	29·1 (0·58, 3)	24·4 (0·79, 3)	27·1 (1·46, 3)
pH_a		7·54 (0·018, 10)	7·54 (0·083, 3)	7·57 (0·060, 3)	7·29 (0·107, 3)	7·56 (0·040, 3)
HCO_3^-	mmol/l	26 (8·6, 10)	19·6 (5·21, 3)	28·6 (3·72, 3)	12·7 (3·48, 3)	25·6 (3·25, 3)
[Lactate]	mmol/l	0·20 (0·55, 10)	8·1 (0·94, 3)	0·94 (0·616, 3)	17·0 (2·84, 3)	4·5 (1·02, 3)

* Mean, S.E.M. and number of determinations in parentheses.

High intensity, non-sustainable activity. All three lizard species were run under conditions which resulted in rapid fatigue. In *Varanus salvator*, running at 1 km/h led to exhaustion in 5 min. Respiratory and blood parameters reported during the exercise period in Figs. 3 and 4 were measured in *V. salvator* after 3·5–4·5 min of running.

Respiratory gas exchange increased very rapidly. Maximal $\dot{V}_{CO_2}$ was 1·6 ± 0·09 ml $CO_2 . g^{-1} . h^{-1}$ (range 1·5–1·8 ml $CO_2 . g^{-1} . h^{-1}$) and exhaustion set in before $\dot{V}_{O_2}$ had increased to maximal rates measured at the lower exercise intensity (Fig. 3*a*). Respiratory exchange ratios were higher than during sustained walking, R_{max} = 1·8 ± 0·16 (range 1·6–2·1). R = 1·0 after approximately 13–22 min of recovery. Peak respiratory responses ($\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, R, $\dot{V}_{eff}$) all occurred during the 5 min exercise period. Arterial blood lactate and hydrogen ion concentrations peaked 1·5 min after exercise was terminated and declined throughout the recovery period (Fig. 4*a*). Arterial $[L^-]_a$ increased to 20 mmol/l (range 15–23 mmol/l), while $[H^+]_a$ increased from 39 to 68 nmol/l, resulting in a pH of 7·17 (pH range 7·01–7·36).

Plasma bicarbonate and P_{aCO_2} reached minimal values ($[HCO_3]_{min}$ = 8·7 mmol/l, $P_{aCO_2,min}$ = 19 mmHg) after 5 min of recovery and increased thereafter.

Table 2 summarizes the metabolic and respiratory responses of *Varanus exanthematicus* and *Iguana iguana* to high intensity, non-sustainable exercise. The responses of *V. exanthematicus* and *Iguana* are essentially the same as those of *V. salvator* detailed in Figs. 3 and 4. Carbon dioxide production increased twice as much as did $\dot{V}_{O_2}$ in both *Iguana* and *V. exanthematicus*, resulting in R values well in excess of 1·0. Arterial $[L^-]_a$ increased 19 mmol/l in *V. exanthematicus* and 14 mmol/l in *Iguana*, resulting in pH of 7·17 and 7·05, respectively. After 60 min of recovery, R values were

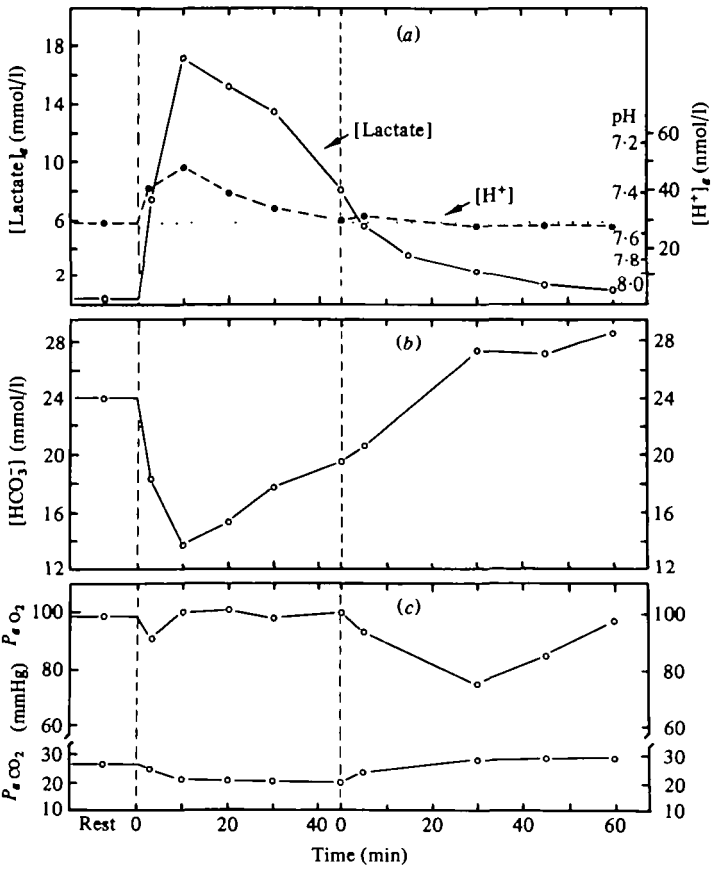

Fig. 2. Characteristics of arterial blood in three *Varanus salvator* at rest, during 45 min treadmill walking at 0·5 km/h and during 60 min of quiet recovery. T_B = 35 °C.

still low in both species (0·53–0·56), indicating that metabolic CO_2 was being retained as plasma bicarbonate, as occurred in *V. salvator* (Fig. 4*b*). Neither *V. exanthematicus* nor *Iguana* removed excess blood lactate and hydrogen ions to the same degree as *V. salvator* during the 60 min recovery period.

DISCUSSION

Physiological responses to sustained activity

Data from *Varanus salvator* walking at 0·5 km/h present the physiological responses to sustained activity at 85% $\dot{V}_{O_2\,max}$. These lizards responded to high intensity, sustainable activity by rapidly increasing oxygen consumption, supplemented initially by anaerobic energy production as indicated by lactate accumulation. Respiratory exchange ratios (R) exceeded 1·0 as bicarbonate-derived CO_2 was released, buffering the metabolic acidosis. Following this initial period, activity was sustained aerobically and there was no longer net lactate production by the animal. Rates of oxygen consumption declined 10–15% to a new steady-state value which was maintained during

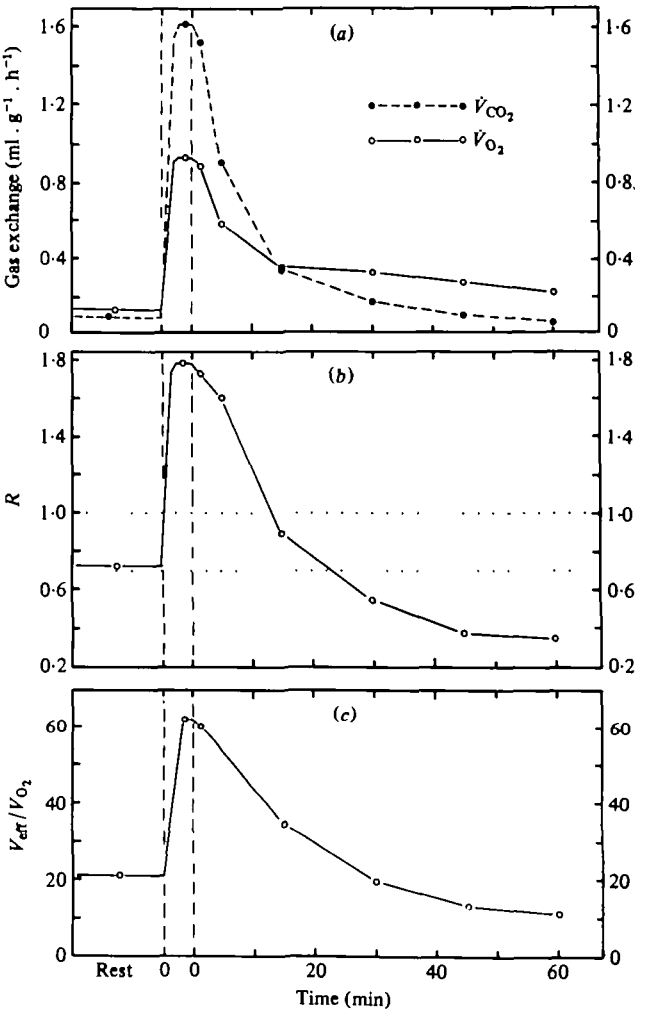

Fig. 3. Metabolic and ventilatory responses of three *V. salvator* to high intensity, exhaustive treadmill running at 1·0 km/h at 35 °C. Running time = 5 min, followed by 60 min quiet recovery. See Methods for details. Similar data for *V. exanthematicus* and *Iguana* are summarized in Table 2.

the last 20–30 min of exercise. This pattern of oxygen consumption has been demonstrated in other lizard species (Bennett & Gleeson, 1979; Gleeson, 1980). We believe that the high initial rate of $\dot{V}_{O_2}$ measured reflects the energetic cost of extra-locomotory activities by the animal before it adjusts its speed and gait to the treadmill. Rates of CO_2 production and values of R declined as the animal established a new steady-state level of gas exchange. This pattern of gas exchange is similar to that reported to occur in other exercising lizards (Bennett & Gleeson, 1979; Gleeson, 1980; Gleeson *et al.* 1980; Mitchell *et al.* 1981*a*).

There are two major events that characterize the physiological responses of *V. salvator* late in the exercise period: (1) storage of metabolically produced CO_2 as plasm

Table 2. *Summary of metabolic and respiratory responses to* 10 *min exhaustive exercise by four* Varanus exanthematicus *and three* Iguana iguana *at* 35 °C

		V. exanthematicus	*Iguana*
$\dot{V}_{O_2}$	Rest	0·17 ± 0·017 (9)*	0·26 ± 0·017 (6)
	10 min run	1·07 ± 0·059 (5)	0·98 ± 0·12 (6)
	60 min recov.	0·14 ± 0·023 (3)	0·43 ± 0·100 (5)
$\dot{V}_{CO_2}$	Rest	0·11 ± 0·013†	0·20 ± 0·022†
	10 min run	1·31 ± 0·109	1·36 ± 0·194
	60 min recov.	0·06 ± 0·013	0·24 ± 0·051
R	Rest	0·70 ± 0·062	0·77 ± 0·079
	10 min run	1·23 ± 0·067	1·38 ± 0·048
	60 min recov.	0·53 ± 0·190	0·56 ± 0·03
pH_a	Rest	7·44 ± 0·012	7·49 ± 0·026
	10 min run	7·17 ± 0·038	7·05 ± 0·066
	60 min recov.	7·39 ± 0·055	7·30 ± 0·080
P_{aCO_2}	Rest	32 ± 0·09	31 ± 1·6
	10 min run	19 ± 1·5	27 ± 6·5
	60 min recov.	23 ± 0·3	32 ± 3·0
P_{aO_2}	Rest	91 ± 4·4	97 ± 4·0
	10 min run	103 ± 10·8	106 ± 6·7
	60 min recov.	101 ± 13·0	91 ± 8·8
$[L^-]_a$	Rest	1·6 ± 0·26	2·1 ± 0·50
	10 min run	20·5 ± 0·18	16·5 ± 1·58
	60 min recov.	11·5 ± 2·03	11·1 ± 3·09

* Units as in Table 1, number of determinations in parenthesis.
† No. of determinations as for $\dot{V}_{O_2}$.

bicarbonate, and (2) lactate removal and re-establishment of resting $[H^+]_a$. This is the first report of the ability of reptiles to remove lactate from the blood during activity, and indicates that lizards are capable of utilizing a major fraction of their aerobic scope (at least 85%) during sustained activity without supplementary anaerobiosis.

The ventilatory adjustments made by *V. salvator* during exercise coupled with blood bicarbonate and non-bicarbonate buffering systems are adequate to maintain blood pH near resting levels through a wide range of lactate accumulation (Fig. 5). Arterial blood pH was maintained within ±0·1 pH unit until lactate concentrations exceeded 15 mmol/l during activity and whenever $[L^-]_a$ declined below 15 mmol/l during activity or recovery (Fig. 5).

Physiological responses to exhaustion

High speed treadmill running models a situation of burst activity during which substantial anaerobic metabolism is elicited and exhaustion occurs even before $\dot{V}_{O_2\,max}$ can be attained. The metabolic responses of *Varanus salvator*, *V. exanthematicus* and *Iguana* are nearly identical under these conditions. Respiratory CO_2 production increased dramatically, the result of both increased metabolism and lactic acid titration of the blood bicarbonate system. Respiratory exchange ratios transiently exceeded values of 2·0 in some animals. By the time these lizards reached exhaustion (in 5–10 min), blood lactate had accumulated to 15–20 mmol/l and plasma $[HCO_3^-]$ had declined a similar extent.

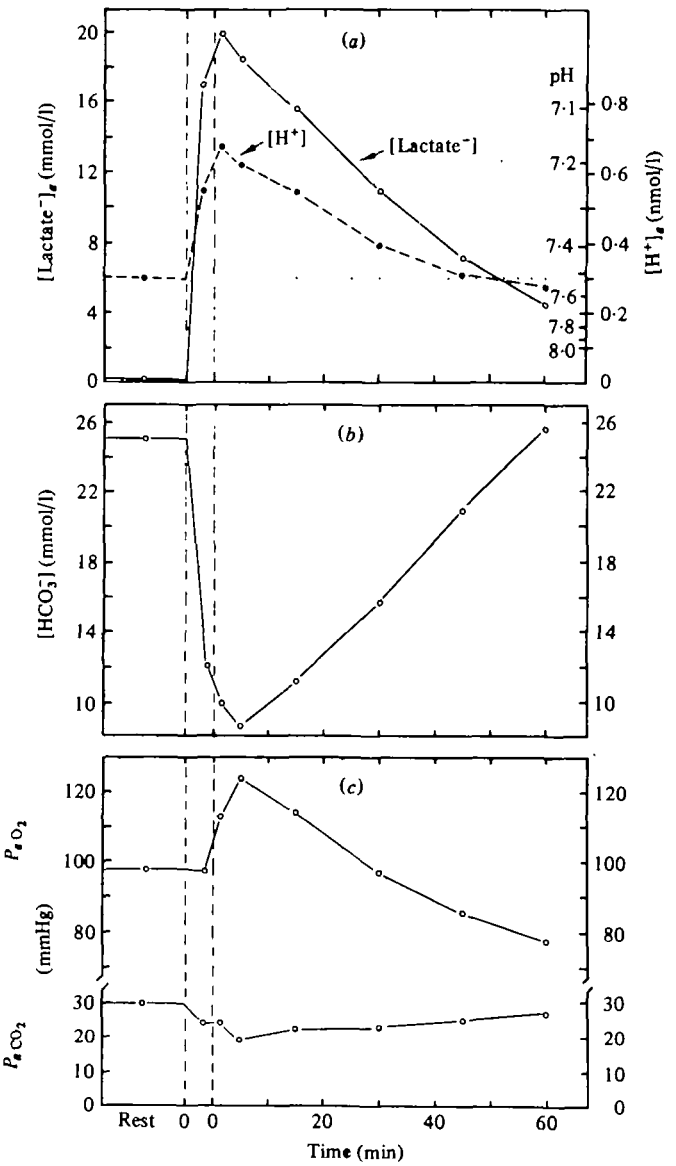

Fig. 4. Characteristics of arterial blood in three *V. salvator* before, during and after exhaustive treadmill running at 1·0 km/h. T_B = 35 °C. Similar data for *V. exanthematicus* and *Iguana* are summarized in Table 2.

The recovery process following burst activity occurs while $\dot{V}_{CO_2}$ decreases very rapidly relative to $\dot{V}_{O_2}$, resulting in decreasing values of R. Nearly 75% of the accumulated lactate was removed within the 60 min recovery period in *V. salvator* (Fig. 4*a*), compared with 60% in *V. exanthematicus* and 40% in *Iguana* (Table 2). These removal rates are similar to those found in mammals (Margaria, Edwards & Dill, 1933; Brooks, Brauner & Cassens, 1973).

There is a clear drive to re-establish resting $[H^+]_a$ and buffering capacity in *V. salv*

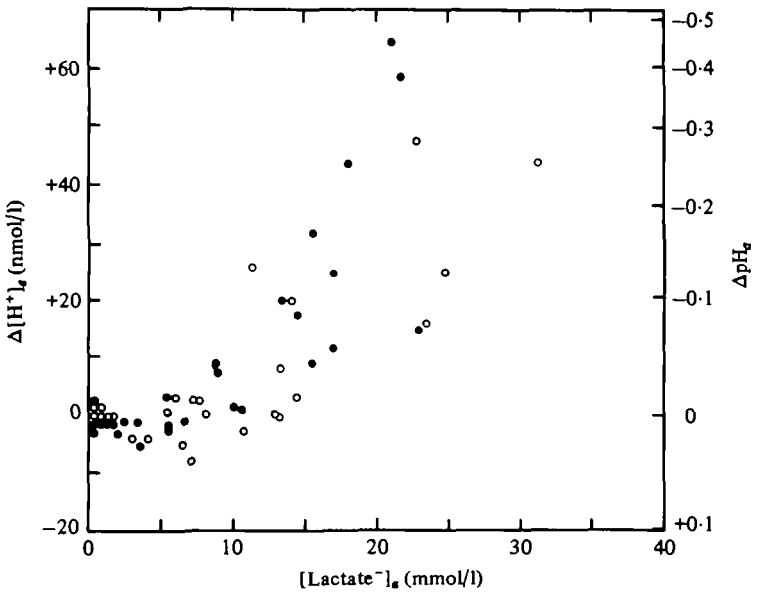

Fig. 5. Changes in arterial blood hydrogen ion concentration ($\Delta[H^+]_a$) in four *Varanus salvator* as a function of arterial lactate concentration during and after treadmill exercise. Open circles, concentrations during 60 min recovery period; closed circles, during exercise.

tor during recovery. *Varanus* hypoventilate relative to their level of metabolism (Fig. 3*c*), and a major portion of their metabolically produced CO_2 is retained, replenishing depleted plasma HCO_3^- (Fig. 4*b*). This CO_2 retention resulted in respiratory exchange ratios which were exceptionally low (0·3–0·5) during the recovery period (Fig. 3*b*). Extrapolation of the data in Figs. 3 and 4 indicates that *V. salvator* was likely to become alkalotic during later stages of recovery, with plasma HCO_3^- levels higher, and $[H^+]_a$ lower than resting values.

There are several benefits associated with this rapid replacement of plasma bicarbonate. An elevation of plasma HCO_3^- clearly improves blood buffering and acid-base balance. Elevated $[HCO_3^-]$ also speeds the rate of lactic acid efflux from muscles (Mainwood, Worsley-Brown & Paterson, 1972; Hirche *et al.* 1975; Jones *et al.* 1977). Elevated $[HCO_3^-]$ would therefore attenuate intramuscular pH disturbances during any subsequent exercise, as well as allow more lactate and hydrogen ion production to occur prior to refatiguing (Jones *et al.* 1977). Following exhaustion, recovery of muscle tension is more rapid in high $[HCO_3^-]$ than in low (Mainwood & Lucier, 1972). Therefore, rapid $[HCO_3^-]$ replenishment not only returns the acid-base status of the blood to resting conditions, but also functions to optimize subsequent muscle performance.

Predictive value of R

Respiratory exchange ratios (R) in reptiles vary considerably from traditional mammalian values of 0·7–1·0. Values of 0·4 to 8·0 have been reported (Bennett & Dawson, 1976). Recent research has demonstrated elevated values of R (1·5–2·2) associated with

treadmill activity in lizards (Bennett & Gleeson, 1979; Gleeson, 1979, 1980, 1981; Gleeson *et al.* 1980; Mitchell *et al.* 1981*a*, *b*). Data from *Varanus salvator* allow analysis of the relationships among the respiratory exchange ratio, respiratory transition (when R decreases to 1·0; Gleeson, 1980) and lactate production. We were interested in determining whether the period when $R > 1{\cdot}0$ was also indicative of the period of net supplementary anaerobic metabolism. We have used changes in arterial lactate concentration to indicate periods of net lactate production. We recognize that some lactate production probably occurs at all times in lizards. However, lactate produced by one tissue and catabolized by another or the same tissue has no net effect on the overall energetic or acid-base balance of the animal. We propose that we can estimate the time at which *net* lactate production in lizards ceases (production primarily by skeletal muscle tissue during or shortly after exercise) by determining the time at which blood lactate concentrations cease to rise. Time to peak $[L^-]_a$ may in fact overestimate the period of *net* anaerobic lactate production as diffusion gradients

Table 3. *Minutes of recovery to respiratory transition* ($R = 1{\cdot}0$) *and to peak* $[L^-]_a$ ($\Delta[L^-]_a = 0$) *in lizards after burst activity*

Species	Time to peak $[L^-]_a$ ($\Delta[L^-]_a = 0$)	Time to respiratory transition ($R = 1{\cdot}0$)
Varanus salvator	0–5	13–22
Varanus exanthematicus	0–5	10–30
Iguana iguana	0–1	15–40
*Amblyrhynchus cristatus**		
$T_B = 35$ °C	15	20–45
$T_B = 25$ °C	30–60	30–70

* From Gleeson, 1980.

from muscle to blood for lactate may exist after net muscle lactate production has ceased. When $[L^-]_a$ peaks ($d[L^-]_a/dt = 0$), lactate production has either ceased, or more likely, the rate of lactate production has declined to equal the rate of lactate removal. We are not suggesting that changes in blood lactate concentration can be used to estimate the amount of lactate produced, only the time period of *net* lactate production.

Using arterial blood lactate concentrations as discussed above, patterns of gas exchange and net anaerobic energy production can be compared. Data from *V. salvator* demonstrate that during sustained exercise and during recovery when metabolic CO_2 production is low, the period of net anaerobic energy production ceases long before respiratory exchange ratios ($\dot{V}_{CO_2}/\dot{V}_{O_2}$, R) decline to 1·0 (Figs. 1–4). The same is true of *V. exanthematicus*, *Iguana* and *Amblyrhynchus* (Table 3). There is, however, a good temporal correlation between peak $[L^-]_a$ and peak values of R. During sustainable activity by *V. salvator*, net lactate production appears to have ceased about the time R reached its maximal value (Fig. 1*b*, 2*a*). During high intensity, exhaustive exercise by the three lizard species used in this study, peak R preceded peak $[L^-]_a$ by about 3 min. Lactate accumulated so quickly under these conditions, however, that it is possible that the peak $[L^-]_a$ may have occurred earlier but was missed because of the sampling interval used (Fig. 4*a*).

We feel that the data support the use of respiratory exchange ratio R as a non-invasive indicator of net supplementary anaerobic metabolism during activity

lizards. As a general rule, increasing values of R greater than 1·0 indicate net lactate production by the animal. During this period, oxygen consumption will underestimate the total energetic expense of activity. Net lactate production appears to be terminated about the time R reaches its peak value, and decreasing values of R indicate that the lizard is no longer utilizing anaerobic lactate production for net energy production, even though R may still be greater than 1·0. In all three lizard species studied, lactate removal from the blood occurs while $R > 1{\cdot}0$. The same general relationship between R and lactate production occurs in man (Margaria *et al.* 1933) following intense treadmill running.

During periods after $[L^-]_a$ and $[H^+]_a$ had peaked but R was still > 1 ($\dot{V}_{CO_2} > \dot{V}_{O_2}$), there was excess CO_2 production that cannot be attributed to non-carbonic acid titration of the bicarbonate buffering system. From where was this excess CO_2 derived? Neither plasma bicarbonate nor dissolved CO_2 is likely to be the source of this excess CO_2 production, as P_{aCO_2} and $[HCO_3^-]$ either remained constant or rose during the periods in question. The excess CO_2 production that occurred after R and $[L^-]_a$ peaked may be due to either of two mechanisms of CO_2 washout stored in body tissues. In the first case, CO_2 produced from the rapid increase in metabolism would be stored in body tissues during the early phase of exercise. Such storage in man is thought to reduce the ventilatory load at the onset of exercise (Jones, 1980). Using data from Fig. 1 as an example, total CO_2 storage would be equivalent to 0·030 ml CO_2/g body tissue. Tissue CO_2 storage of this magnitude is reasonable, requiring a 10 mmHg increase in tissue P_{CO_2} (tissue CO_2 capacitance estimated from Cherniack *et al.* 1972). This would result in a transient increase in venous P_{CO_2} during the early phase of exercise, as occurs in man (Laug, 1934; Thomson *et al.* 1974; Jones, 1980). This stored CO_2 would then be washed out late in the exercise period, resulting in a prolonged period when $R > 1{\cdot}0$.

In the second case, no CO_2 storage is hypothesized. The excess CO_2 evolved from the tissue during this period is due to a simple washout of existing tissue CO_2. Such a washout could result from an increased flow through the tissues by arterialized blood of lower CO_2 tension. This washout hypothesis is supported by the large increase in cardiac output known to occur during exercise in these species (Gleeson *et al.* 1980), and would require a depression of tissue and venous P_{CO_2} of 10 mmHg instead of a 10 mmHg elevation as hypothesized for the CO_2 storage hypothesis above. We have no data at present that allow us to distinguish between these two theories of excess CO_2 production in lizards, although we feel that the tissue is the most likely compartment from which this CO_2 is derived. We propose that release of CO_2 from body tissues may be responsible for maintaining elevated $\dot{V}_{CO_2}$ after titration of the blood bicarbonate system with lactic acid has ceased. Thus, there are periods during activity when lizards can be considered as aerobic (not utilizing net anaerobic energy production) despite the respiratory exchange ratio being in excess of 1·0.

H^+, lactate$^-$ efflux rates

There is currently debate as to the mechanism of hydrogen ion and lactate efflux from muscle. There are data which support the hypothesis that lactic acid effluxes from skeletal muscle in the un-dissociated form (Mainwood *et al.* 1972), and other studies which indicate that H^+ efflux rates are 14 to 50 times greater than those for

lactate ions (Benadé & Heisler, 1978). A third paper suggests that hydrogen ion efflux may be slower than or equal to lactate ion efflux depending on the acid-base status of the muscle (Hirche *et al.* 1975). The implications of this controversy are that the respiratory exchange ratio R may or may not reflect lactate production by muscles, depending upon whether hydrogen ion effluxes before, with, or after lactate ions, as it is actually free hydrogen ions that drive the carbonic anhydrase reaction towards CO_2 production. Although we have no data to indicate the mechanism of lactic acid efflux from the saurian muscle cell, H^+ and lactate$^-$ efflux into the blood appeared simultaneously in exercising lizards. This does not imply that the amounts of the two ions effluxed are the same or that the efflux rates for the two ions are necessarily equal, only that the blood concentrations of these two ions rise and begin to fall in temporal synchrony. In three lizard species and under two exercise conditions, hydrogen ion concentrations and lactate concentrations in arterial blood peaked at the same point in time (Fig. 2*a*, 4*a*). This temporal synchrony may also occur in the amphibians *Bufo* and *Cryptobranchus* despite the fact that total hydrogen ion production exceeds lactate production in these animals (Boutilier *et al.* 1980; McDonald *et al.* 1980). When the exercising lizard is considered as a whole, it appears that hydrogen and lactate ion changes in the blood occur simultaneously, and that the ratio of $\dot{V}_{CO_2}/\dot{V}_{O_2}$ (R), although actually influenced by changes in $[H^+]_a$, can be used to predict qualitative changes in $[L^-]_a$.

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