Ventilation and acid-base balance during graded activity in lizards

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MITCHELL, G. S., T. T. GLEESON, AND A. F. BENNETT. *Ventilation and acid-base balance during graded activity in lizards.* Am. J. Physiol. 240 (Regulatory Integrative Comp. Physiol. 9): R29–R37, 1981.—Arterial P_{CO_2}, hydrogen ion ($[H^+]_a$), and lactate ($[L]_a$) concentrations, rates of metabolic CO_2 production ($\dot{V}_{CO_2}$) and O_2 consumption ($\dot{V}_{O_2}$), and effective alveolar ventilation ($\dot{V}$eff) were determined in the lizards *Varanus exanthematicus* and *Iguana iguana* at rest and during steady-state treadmill exercise at 35°C. In *Varanus*, $\dot{V}_{CO_2}$ increased ninefold and $\dot{V}_{O_2}$ sixfold without detectable rise in $[L]_a$ at running speeds below 1.0 to 1.5 km·h^{-1}. In this range, $\dot{V}$eff increased 12-fold resulting in decreased levels of Pa_{CO_2} and $[H^+]_a$. At higher speeds $[L]_a$ rose. Increments of 5 mM $[L]_a$ were accompanied by hyperventilation, reducing Pa_{CO_2} and thus maintaining $[H^+]_a$ near its resting level. When $[L]_a$ increased further, $[H^+]_a$ increased. Sustainable running speeds (0.3–0.5 km·h^{-1} and below) were often associated with increased $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, and $[L]_a$ in *Iguana*. Sixfold increases in $\dot{V}_{CO_2}$ and 9-mM increments in $[L]_a$ were accompanied by sufficient increase in $\dot{V}$eff (9-fold) to maintain $[H^+]_a$ at or below its control level. When $[L]_a$ increased further, $[H^+]_a$ increased. These results indicate that both lizard species maintain blood acid-base homeostasis rather effectively via ventilatory adjustments at moderate exercise intensities.

control of ventilation; treadmill exercise; reptiles; *Varanus exanthematicus*; *Iguana iguana*

THE CAPACITY OF REPTILES to maintain acid-base balance during activity has not been thoroughly investigated. Recent investigations on the regulation of acid-base balance in reptiles and other poikilothermic vertebrates have emphasized the effects of changes in body temperature at rest (cf. 13, 19). In most species studied, both the arterial carbon dioxide tension and hydrogen ion concentration increase with increasing body temperature leading several authors to suggest that maintenance of the ratio between hydroxyl and hydrogen ions is a more important consideration for acid-base homeostasis than the absolute level of hydrogen ion concentration per se (15, 18, 20). Nevertheless, at any given body temperature, maintenance of a specific level of hydrogen ion concentration defines acid-base homeostasis.

During periods of activity, most reptiles confine body temperature to a narrow, species-specific range, thermoregulating via behavioral means (cf. 25). Thus, maintenance of acid-base homeostasis in active reptiles requires the quantitative excretion or buffering of hydrogen ions generated by increased metabolism. The end product of aerobic metabolism, carbon dioxide, must be excreted by increasing ventilation in proportion to the rate of metabolic carbon dioxide production. Hydrogen ions derived from the principal end product of anaerobic metabolism in reptiles, lactic acid (cf. 6), must be buffered via the bicarbonate buffer system.

The principal aim of this study was to examine the ability of lizards to maintain acid-base balance when exercised on a treadmill at a constant body temperature. Lizard species from two genera were chosen as experimental animals, one with a high aerobic scope, *Varanus* (2, 4, 12, 32), and one with a low aerobic scope but substantial capacity to increase anaerobic metabolism, *Iguana* (12, 16, 17, 26). Additional data were collected on the physiological basis of increased oxygen consumption during activity and these are presented in another paper (12).

METHODS

Animals

Five savannah monitor lizards (*Varanus exanthematicus*) ranging from 0.94 to 1.15 kg (mean, 1.04 kg) and six green iguanas (*Iguana iguana*) between 0.60 and 0.95 kg (mean, 0.80 kg) were obtained from commercial animal dealers and housed in large cages. The varanid lizards were fed mice and iguanas were fed lettuce, bananas, and dog food. The animals were able to thermoregulate by orientation with respect to a photothermal source set on a photoperiod of 12 h.

Surgical Preparation

At room temperature, the lizards were anesthetized with a mixture of 1–3% halothane (Fluothane) in air and a catheter filled with heparinized saline was placed in the external carotid artery. The cannula was run under the skin and a length of approximately 5 cm was exteriorized on the dorsal side of the neck. A minimum of 24 h was allowed for recovery before experiments were run on any animal. Surgery did not impair oxygen consumption at any running speed tested in the animals as determined by comparison with presurgical control values.

Measurements

Oxygen consumption and carbon dioxide production. The animals were fitted with a lightweight, clear acrylic mask and gas was drawn through the mask at 1.8 to 2.6

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$l \cdot min^{-1}$. The excurrent air was passed through a column of dessicant (anhydrous calcium carbonate, Drierite) and a calibrated flowmeter. A portion of this flow was diverted through an infrared CO_2 analyzer (Beckman, model LB-2) and an oxygen analyzer (Applied Electrochemistry, model S3A). Spot checks indicated that flow rates through the mask were sufficient to prevent appreciable accumulation of CO_2 or depletion of O_2 in the inspired gas. The rates of metabolic O_2 consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) were calculated as described in a previous report from this laboratory (11) and are expressed in ml (STPD)$\cdot g^{-1} \cdot h^{-1}$.

Blood sampling and analysis. During an experiment, the arterial cannula was extended with approximately 1 m of polyethylene tubing (PE-50). Blood was sampled by withdrawing the heparinized saline in the extended cannula and about 0.3 ml of blood into a syringe and then withdrawing a second sample of blood (0.3 ml) anaerobically into a heparinized glass syringe that was capped and immediately stored on ice. The blood-saline mixture in the first syringe was then returned to the animal and the cannula was flushed with fresh heparinized saline.

Analysis of arterial P_{CO_2} and pH was begun within 5 min using a micro blood gas analyzer (Radiometer; models BMS 3, MkII, and PHM 73) maintained at 35°C. The electrodes were calibrated before and after each measurement: the P_{CO_2} electrode was calibrated with gases provided by a gas mixing pump (Radiometer, GMA2) and the pH electrode with standard Radiometer buffer solutions. Values of P_{CO_2} and pH were adjusted to the rectal temperature of the animals using the correction factors for human blood (7, 21). On a few occasions, the validity of these correction factors was confirmed by determining the effect of temperature changes on blood drawn from the animals and stored anaerobically.

A portion of each arterial blood sample was analyzed for lactate concentration ($[L]_a$). A 25-μl sample was mixed with 500 μl of 0.6 N perchloric acid and refrigerated. The supernatant solution was later analyzed for [L] by enzymatic test kits (Boehringer, Mannheim Corp.).

Calculations

Bicarbonate and hydrogen ion concentrations. The level of plasma bicarbonate ($[HCO_3^-]_a$) was calculated from $P_{a_{CO_2}}$ and pH_a with the Henderson-Hasselbalch equation. The values of pK' and CO_2 solubility chosen were those for human plasma at the appropriate temperature and pH (22, 23). The arterial hydrogen ion concentration ($[H^+]_a$) was calculated from pH_a assuming an activity coefficient of 1.0.

Ventilation. The effective alveolar ventilation ($\dot{V}$eff) was calculated from $P_{a_{CO_2}}$ and $\dot{V}CO_2$ using an equation analogous to the alveolar ventilation equation in mammals

$$\dot{V}\text{eff} = R \cdot T \cdot \frac{\dot{V}CO_2}{P_{a_{CO_2}}} \qquad (1)$$

where R is the gas constant [2.785 l·Torr·(l STPD·°K)$^{-1}$] and T is the absolute temperature (°K). In this equation $P_{a_{CO_2}}$ is taken as the ideal alveolar P_{CO_2}; consequently, $\dot{V}$eff must be regarded solely as a functional rate of ventilation without assigning a specific physical definition. Values of $\dot{V}$eff are expressed in ml (BTPS)$\cdot g^{-1} \cdot h^{-1}$.

Experimental Protocol

Several hours before experimentation, a lizard was equilibrated in a thermostated chamber at 35°C. After being fitted with the metabolic mask, the catheter extensions, and a cloacal thermistor probe, the animal was placed on the thermostated treadmill and covered until $\dot{V}O_2$ and $\dot{V}CO_2$ attained steady values, sometimes requiring several hours. A blood sample was withdrawn after these values had remained stable for more than 20 min. An experimental run was then performed at a speed of either 0.5, 0.75, 1.0, 1.5, or 2.0 $km \cdot h^{-1}$ for *Varanus* and 0.2, 0.4, 0.5, 0.7, 0.9, or 1.1 $km \cdot h^{-1}$ for *Iguana*. At speeds below those that elicited the maximal $\dot{V}O_2$ in each species, the lizards were able to sustain runs for over 1 h, but blood was usually drawn and the run terminated once it was clear that a steady state with respect to $\dot{V}O_2$ and $\dot{V}CO_2$ had been achieved (20–45 min). At higher speeds, the onset of fatigue was rather rapid and at the first signs of exhaustion, samples were drawn regardless of whether $\dot{V}O_2$ or $\dot{V}CO_2$ had achieved steady state.

After a run, the lizard was returned to the thermostated box and allowed to recover for a minimum of 4 h prior to the next trial. Seldom was an animal run more than twice in the same day. Data on any single animal were collected over a period of 4 or 5 days.

Statistical Analysis

Statistical comparisons between mean data groups were made using Student's t test. Individual measurements of $[H^+]_a$, $P_{a_{CO_2}}$, $[HCO_3^-]_a$, and $[L]_a$ were expressed as a difference from the average resting value in a given animal and $\dot{V}CO_2$, $\dot{V}O_2$, and $\dot{V}$eff were expressed as the ratio of the test value to the average resting value. Significant correlations between these variables were established by standard least squares regression and then by a two-tailed t test.

RESULTS

Resting Values

Values of the measured and calculated respiratory variables for both *Varanus* and *Iguana* are summarized in Table 1. There are no significant differences between species in any of these measurements ($P > 0.2$).

Metabolic Responses to Activity

During experimental runs, changes in body temperature were seldom more than 1°C and showed no consistent directional trend in either species.

In *Varanus*, $\dot{V}O_2$ increased progressively with increasing running speed until a maximum value was reached between 1.0 and 1.5 $km \cdot h^{-1}$. At this level of activity, $\dot{V}O_2$ had increased between 4.0 and 8.7 times the resting value (mean, 6.3 times), and $\dot{V}CO_2$ had increased between 6.2- and 12.7-fold (mean, 9.7-fold). At running speeds below that which elicited the maximum $\dot{V}O_2$, there was no

detectable rise in $[L]_a$. At higher levels of activity, $[L]_a$ increased by as much as 16.3–20.2 mM (mean, 18.2 mM). The effects on $\dot{V}CO_2$ in this range of activity were variable and seemed to depend on whether steady state had been approached before exhaustion.

In *Iguana*, $\dot{V}O_2$ reached a maximum value at slower running speeds than in *Varanus* (range, 0.2–0.5 km·h^{-1}). Maximum values of $\dot{V}O_2$ ranged between 3.5 and 6.7 times the resting value (mean, 5.0 times), and $\dot{V}CO_2$ increased 4.5- to 9.2-fold (mean, 6.5-fold). Blood lactate accumulation was variable at lower levels of activity. During more strenuous activity, the maximum levels of $[L]_a$ observed ranged from 14.9 to 30.2 mM (mean, 20.6 mM). Again, the effects on $\dot{V}CO_2$ during more strenuous exercise were inconsistent and seemed to depend on the proximity to steady state.

TABLE 1. *Values of measured and calculated variables in resting V. exanthematicus and I. iguana at 35°C*

Variable	Units	*Varanus*	*Iguana*
$\dot{V}O_2$	ml (STPD)·g^{-1}·h^{-1}	0.19 ± 0.03*	0.18 ± 0.01
$\dot{V}CO_2$	ml (STPD)·g^{-1}·h^{-1}	0.13 ± 0.02*	0.13 ± 0.01
R		0.67 ± 0.04*	0.71 ± 0.04
$\dot{V}$eff	ml (BTPS)·g^{-1}·h^{-1}	3.4 ± 0.6*	3.5 ± 0.3
$[L]_a$	mM	1.7 ± 0.6*	1.2 ± 0.1
Pa_{CO_2}	Torr	32.7 ± 0.6	31.8 ± 1.1
$[H^+]_a$	nM	32.1 ± 1.9	34.0 ± 1.9
$[HCO_3^-]_a$	mM	26.0 ± 1.8	23.8 ± 1.7
Body wt	kg	1.04 ± 0.04	0.80 ± 0.05

Mean values ± SE from 5 *Varanus* (*n = 4) and 6 *Iguana*.

Acid-Base Balance During Activity

To simplify presentation of the responses to acid loads generated by activity, the data will be expressed as a function of increased CO_2 production alone (carbonic acid load) and then as a function of lactic acid accumulation in the blood (noncarbonic acid load).

Response to carbonic acid load. The responses to increased levels of $\dot{V}CO_2$ in both *Varanus* and *Iguana* are presented in Figs. 1 and 2, respectively. In each case, only experimental runs in which the respiratory exchange ratio was less than 1.05 are plotted. Values of the respiratory exchange ratio higher than this level were interpreted as indications that substantial amounts of lactate

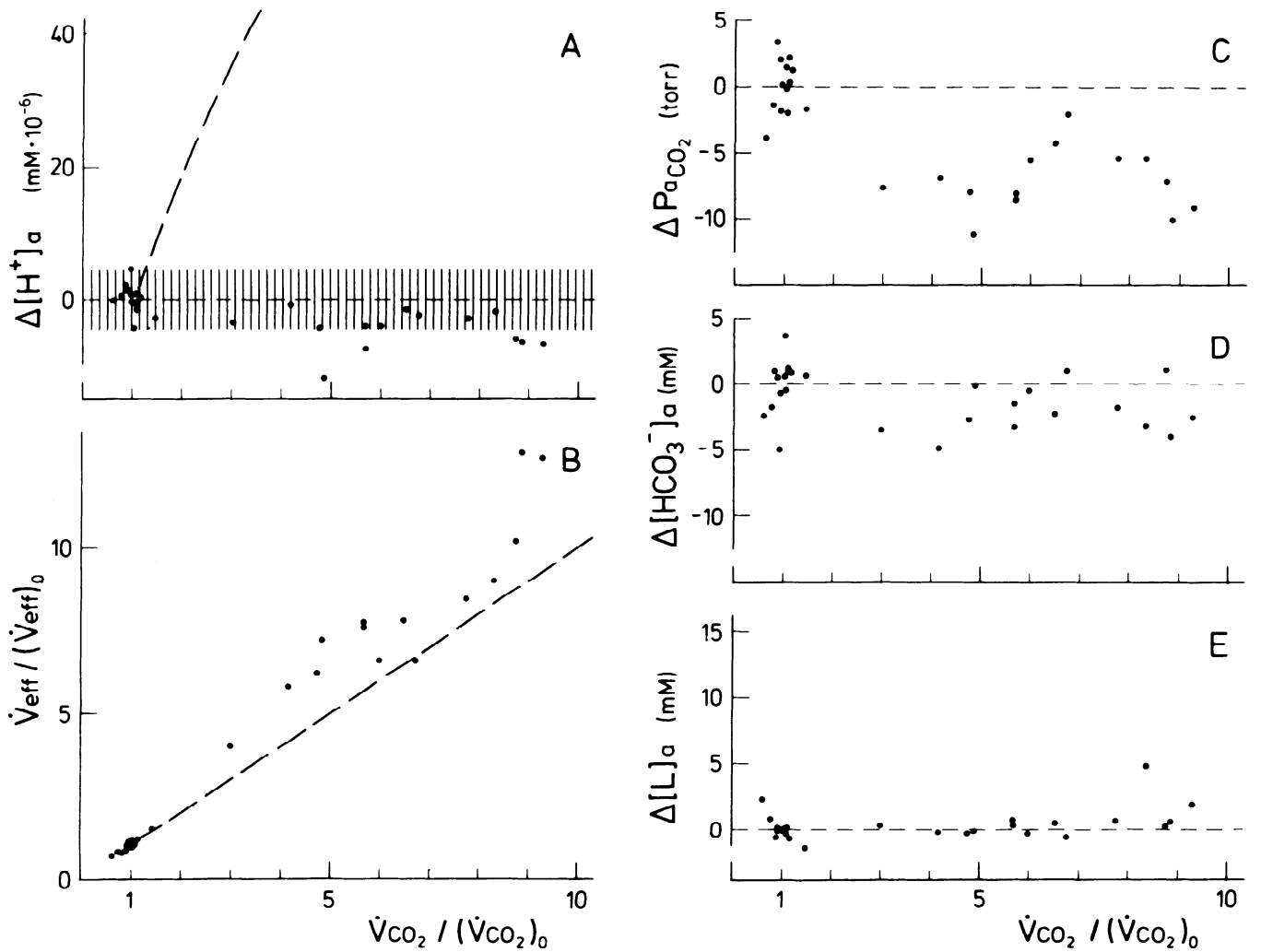

FIG. 1. Responses to increased carbonic acid production, expressed as metabolic rate ratio $[\dot{V}CO_2/(\dot{V}CO_2)_0]$, during activity in *Varanus*. Data from runs where respiratory exchange ratio was above 1.05 (due to nonsteady state and high $[L]_a$) are not plotted. *Heavy broken line* in *A* is hypothetical increase in $[H^+]_a$ if no ventilatory adjustment had occurred (see APPENDIX A). *Shaded region* is 2 standard deviations about mean resting value of $[H^+]_a$. In *B*, *heavy broken line* is line of identity.

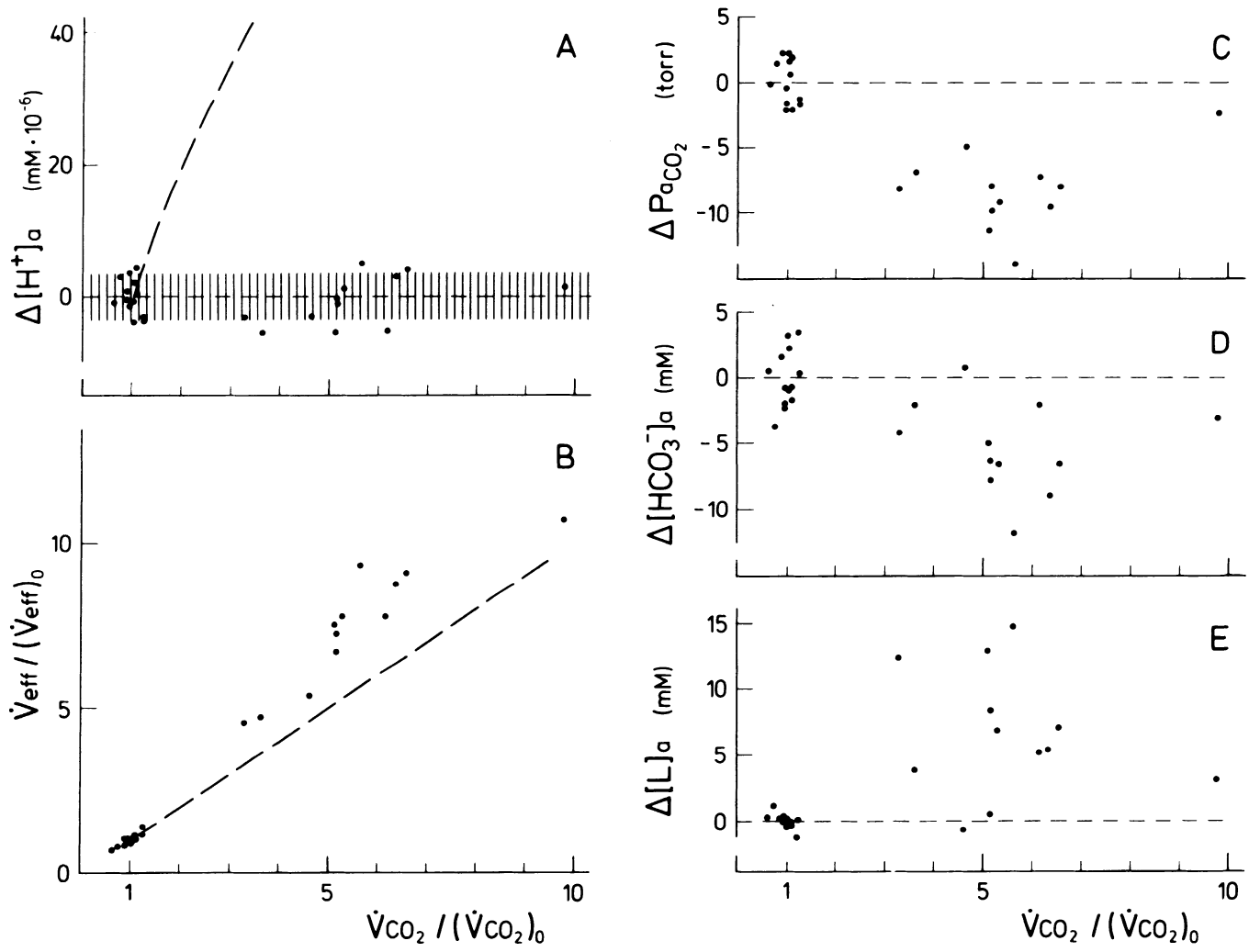

FIG. 2. Responses to increased carbonic acid production, expressed as metabolic rate ratio [$\dot{V}CO_2/(\dot{V}CO_2)_0$], during activity in *Iguana*. Only those runs where respiratory exchange ratio was below 1.05 are plotted. *Heavy broken lines* in *A* and *B* are same as in Fig. 1.

were infusing into the blood and that CO_2 exchanges were far from steady state at the time of measurement. Increased levels of $\dot{V}CO_2$ are expressed as the ratio between active and resting values (the metabolic rate ratio MRR = $\dot{V}CO_2/(\dot{V}CO_2)_0$).

In *Varanus*, $[H^+]_a$ remained at or below its resting level through values of MRR approaching 10 (Fig. 1*A*); the apparent decline in $[H^+]_a$ within this range was highly significant ($P < 0.001$), although rather small in absolute terms. The ventilatory adjustment to sustainable levels of activity overcompensated for metabolic demand as illustrated in Fig. 1*B*. The ventilatory ratio (VR = $\dot{V}eff/(\dot{V}eff)_0$) increased linearly with the MRR ($r = 0.98$) but with a slope significantly greater than unity ($m = 1.35$; $P < 0.001$), thus causing significant declines in ΔPa_{CO_2} (Fig. 1*C*; $P < 0.001$) and $\Delta[HCO_3^-]_a$ (Fig. 1*D*; $P < 0.02$) and the ensuing arterial alkalosis.

In *Iguana*, the level of $[H^+]_a$ was maintained at or below its control level through values of MRR approaching 6.5 (Fig. 2*A*). Even though VR rose faster than MRR ($m = 1.40$; $P < 0.001$), causing both the levels of ΔPa_{CO_2} and $\Delta[HCO_3^-]_a$ to decrease (Fig. 2, *C* and *D*; $P < 0.001$), there was no significant decrease in $[H^+]_a$ as found in *Varanus* since the level of $[L]_a$ was often elevated (Fig. 2*E*).

Response to noncarbonic (lactic) acid load. Once the anaerobic threshold was surpassed and the level of $[L]_a$ began to rise in *Varanus*, $[H^+]_a$ remained at or below the resting level only through 5-mM increments in $[L]_a$ above the resting level, but at higher levels it increased (Fig. 3*A*). In the low range of $\Delta[L]_a$, the lizards hyperventilated with respect to $\dot{V}CO_2$. This is illustrated in Fig. 3*B* where the ratio of the VR to the MRR is plotted as a function of $\Delta[L]_a$. Between 0 and 10 mM $\Delta[L]_a$, this ratio increased significantly ($P < 0.001$). As a result, both ΔPa_{CO_2} and $\Delta[HCO_3^-]_a$ decreased (Fig. 3, *C* and *D*; $P < 0.001$). The decrease in $\Delta[HCO_3^-]_a$ with $\Delta[L]_a$ was nearly linear ($r = 0.92$) with a slope apparently greater, but statistically indistinguishable from -1.0 below 10 mM $\Delta[L]_a$ ($m = -1.21$; $0.05 < P < 0.10$).

In *Iguana*, $[H^+]_a$ appeared to remain unchanged through a wider range of $\Delta[L]_a$ than in *Varanus* (approx 9-mM increases above rest; Fig. 4*A*). Above this level of $\Delta[L]_a$, $\Delta[H^+]_a$ increased. Again, there were significant decreases in ΔPa_{CO_2} (Fig. 4*C*; $P < 0.001$) and $\Delta[HCO_3^-]_a$ (Fig. 4*D*; $P < 0.001$) resulting from an increase in the

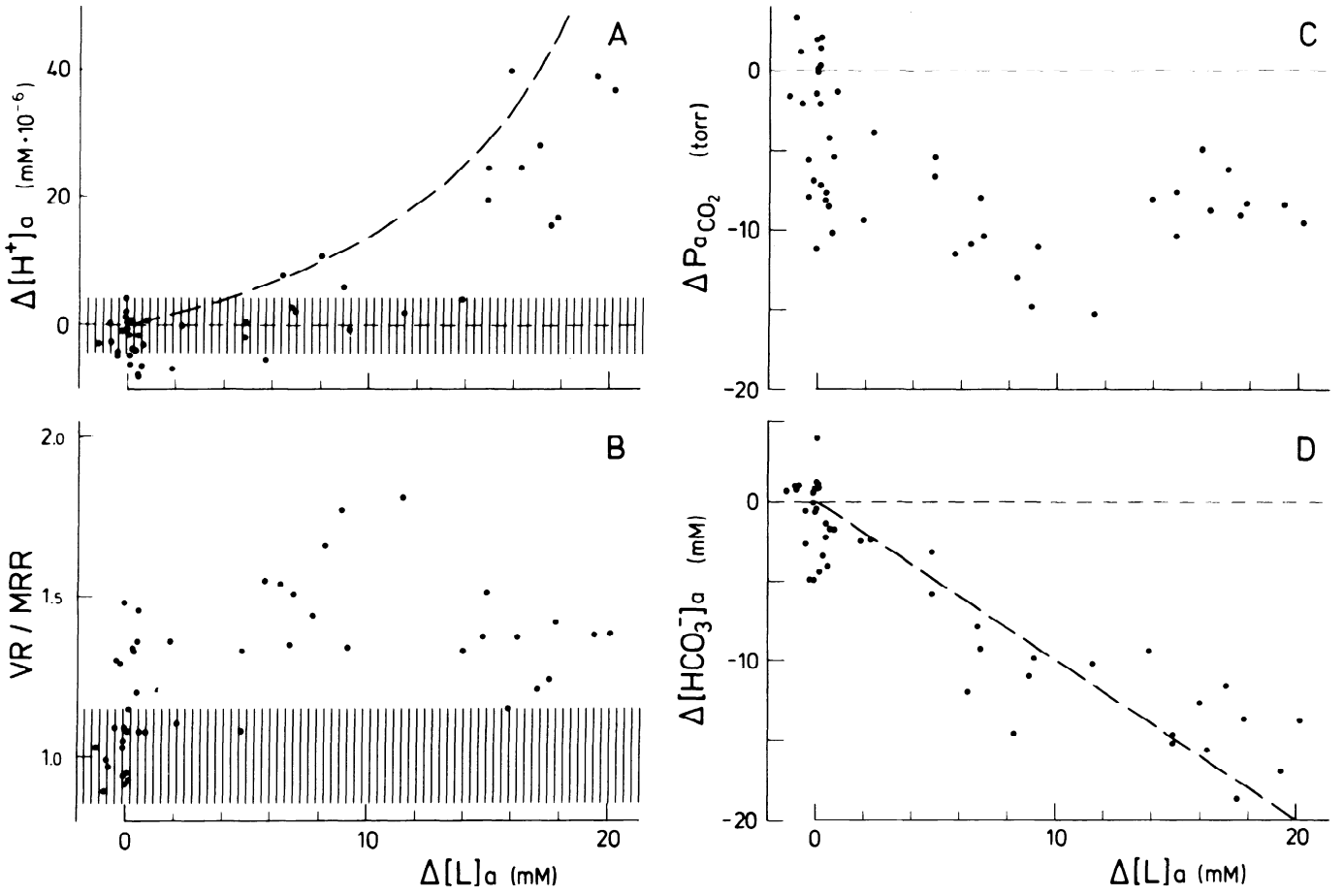

FIG. 3. Responses to increased noncarbonic acid loads, expressed as change in arterial lactate concentration ($\Delta[L]_a$), during activity in *Varanus*. *Heavy broken line* in *A* is hypothetical increase in $[H^+]_a$ without any ventilatory compensation for $\Delta[L]_a$ (see APPENDIX B). In *B*, VR is ventilatory ratio ($\dot{V}eff/\dot{V}eff_0$) and MRR metabolic rate ratio [$\dot{V}CO_2/\dot{V}CO_{2)0}$]. *Shaded bands* in *A* and *B* are 2 standard deviations about mean resting values. *Heavy broken line* in *D* is line with slope −1.0.

ratio of VR to MRR (Fig. 4*B*; $P < 0.001$) between 0 and 10 mM $\Delta[L]_a$. The decrease in $\Delta[HCO_3^-]_a$ was nearly linearly related with $\Delta[L]_a$ ($r = 0.80$) with a slope indistinguishable from −1.0 below 10 mM $\Delta[L]_a$ ($m = -0.86$; $P > 0.2$).

DISCUSSION

Acid-Base Balance

Aerobic and anaerobic metabolism generate carbonic and noncarbonic acid loads, respectively, which must be quantitatively excreted or buffered to maintain acid-base homeostasis. During periods of activity, there is insufficient time for adjustment of strong ion concentrations in the blood via renal mechanisms; thus, changes in ventilation are the only feasible means of excreting the acid loads. Both species of lizard examined in this study increased ventilation sufficiently to prevent an increase in $[H^+]_a$ during moderate activity despite substantial carbonic and noncarbonic acid loads. The required ventilatory adjustments for carbonic and noncarbonic acid loads are different and are discussed separately.

Response to carbonic acid load. Increased levels of $\dot{V}CO_2$ lead to elevated levels of arterial P_{CO_2} and, thus, $[H^+]_a$ if ventilation does not increase in direct proportion to demand. According to *Eq. 1*, $\dot{V}eff$ must increase linearly with $\dot{V}CO_2$, with a slope of RT/Pa_{CO_2} to compensate entirely for the carbon dioxide load. Ventilation increased sufficiently to prevent any increase in Pa_{CO_2} and to maintain $[H^+]_a$ at or below its control value through the highest levels of $\dot{V}CO_2$ attained in both species. In fact, *Varanus* overcompensated for the carbon dioxide load to a small degree even though $[L]_a$ had not increased, resulting in a mild alkalosis. This alkalosis in *Varanus* is similar to the response of some birds during activity (8, 14) but contrasts with the arterial isocapnic response in humans below the anaerobic threshold (cf. 1, 9, 28).

Response to noncarbonic acid load. Because the principal end product of anaerobic metabolism in reptiles is lactic acid, $\Delta[L]_a$ is used to designate the degree of noncarbonic acid load. However, it should be remembered that although the total amount of surplus H^+ is stoichiometrically equivalent to the amount of lactate formed, this does not a priori assure that the increase in arterial $[L]_a$ reflects an equivalent increase in surplus H^+ in the extracellular space. It remains possible that L^- and H^+ are not equally distributed between the intracellular and extracellular spaces. Benadé and Heisler (3) examined the relative efflux rates of L^- and H^+ ions from isolated rat diaphragm and frog sartorius muscles in vitro

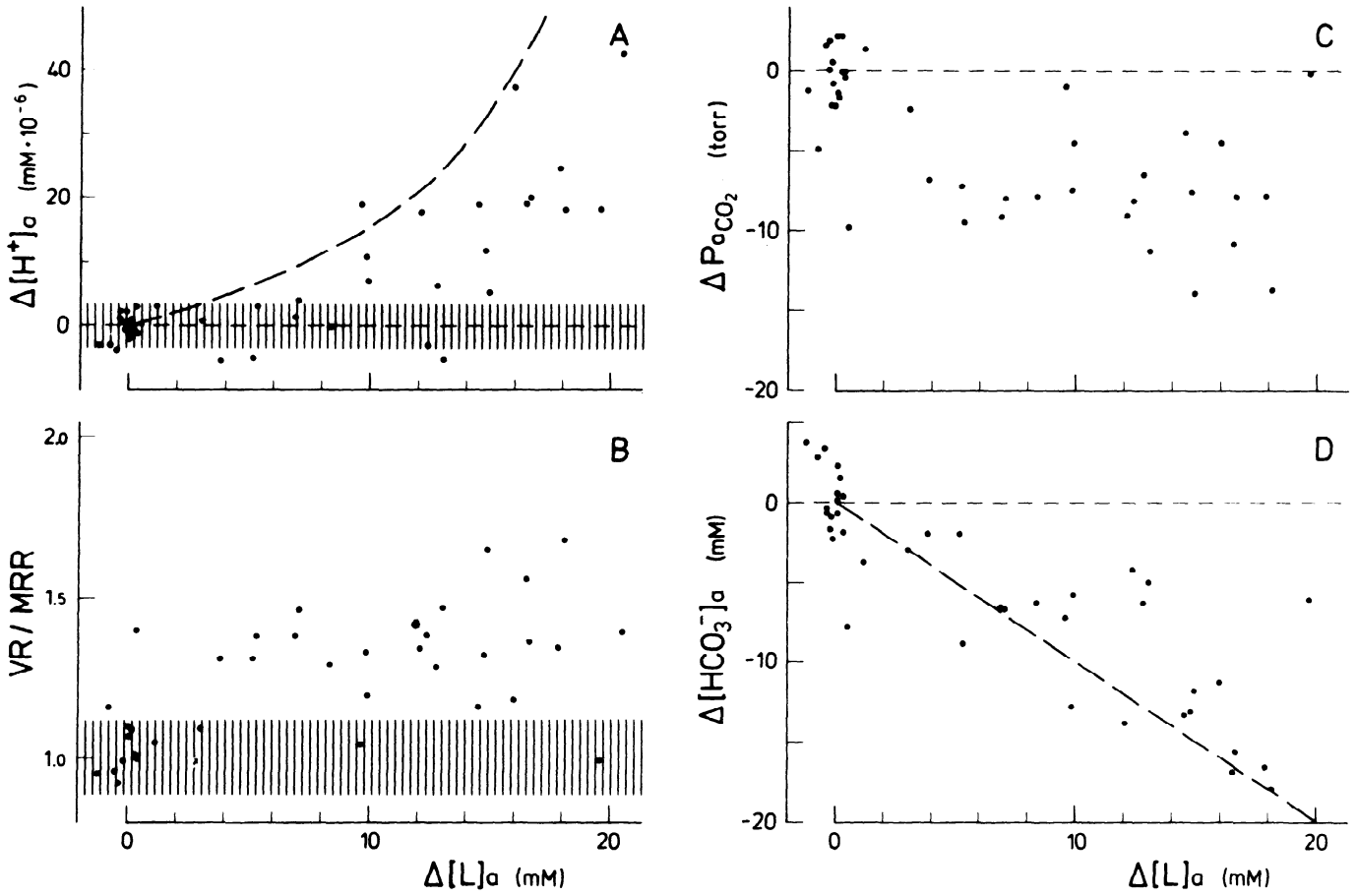

FIG. 4. Responses to increased noncarbonic acid loads, expressed as change in arterial lactate concentration ($\Delta[L]_a$) during activity in *Iguana*. *Heavy broken lines* in *A* and *B* and *shaded band* in *A* and *D* are as in Fig. 3.

and found *1*) that the rate of H^+ efflux was as much as 50 times as great as that of L^- and *2*) that the total amounts of H^+ and L^- liberated into the suspending medium after equilibration were equal. These findings indicate that, in the absence of substantial back diffusion gradients, an increase in $[L]_a$ reflects an equivalent influx of surplus H^+ into the extracellular space in steady state or that, in transient states, the amount of L^- in the extracellular space is most likely an underestimate of the total H^+ load. Thus, it is expected that $\Delta[L]_a$ at least provides a minimum estimate of the noncarbonic acid load sustained during activity.

The greatest rate of lactate accumulation in the blood occurs at exercise intensities that elicit maximal rates of aerobic metabolism in both of these species (unpublished observations). Since it has already been established that carbon dioxide loads from aerobic metabolism are quantitatively excreted, the effects of noncarbonic acid will be discussed assuming that no change in $\dot{V}_{CO_2}$ occurs simultaneously.

To compensate completely for the noncarbonic acid generated by anaerobic metabolism, influx of surplus H^+ into the extracellular space must be matched quantitatively by a decrease in HCO_3^-. Complete, or nearly complete, compensation for the influx of surplus H^+ also assures that the assumption of minimal backdiffusion gradient for either H^+ or L^- ions is valid, and that an increase in $[L]_a$ accurately reflects the influx of surplus H^+ in steady state (or represents a minimum estimate of H^+ influx in nonsteady state). Hence, assuming steady state, complete compensation for noncarbonic acid loads implies that the level of HCO_3^- decreases to the same degree that lactate increases

$$\Delta[HCO_3^-]_a = -\Delta[L]_a \qquad (2)$$

The observation that $\Delta[HCO_3^-]_a$ is nearly linearly related to $\Delta[L]_a$ with a slope indistinguishable from -1.0 below 10 mM $\Delta[L]_a$ in both species (Figs. 3*D* and 4*D*) is reassuring in this respect and suggests that $\Delta[L]_a$ is in fact a reasonably good indicator of H^+ influx.

The decrease in $[HCO_3^-]_a$ consequent to the lactic acid load can be considered in terms of two components, which are illustrated in Fig. 5. First, in the absence of ventilatory adjustment, Pa_{CO_2} would remain constant in the steady state despite increased influx of H^+ ions, since the animal is an open system. Thus, the level of $[H^+]_a$ would change in a manner essentially comparable to acid titration of blood tonometered at a constant Pco_2 as described by the Henderson-Hasselbalch equation. To restore $[H^+]_a$ to its control level, bicarbonate must be further decreased by a relative hyperventilation and the resulting decrease in Pa_{CO_2}. The decrease in Pa_{CO_2} reduces $[HCO_3^-]_a$ to an extent determined by the blood buffer capacity.

It appears that both lizard species maintain $[H^+]_a$ constant through a limited range of increased $[L]_a$, reach-

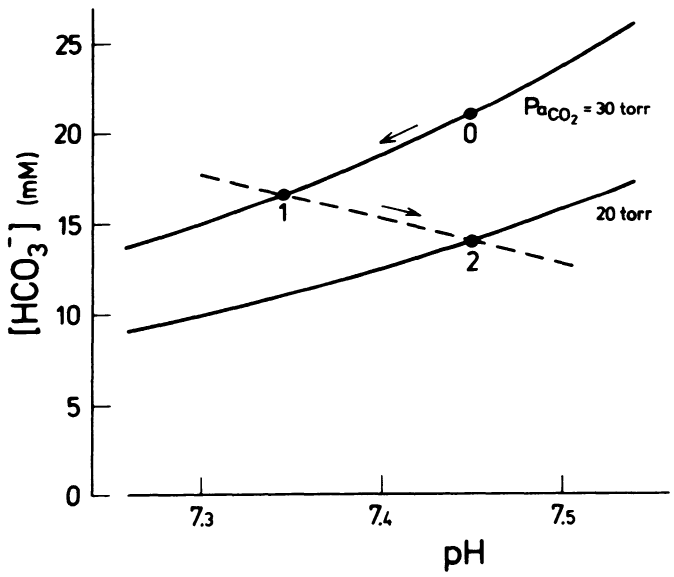

FIG. 5. Relationship between plasma bicarbonate and arterial pH calculated from Henderson-Hasselbalch equation. *Solid lines* are P_{CO_2} isobars for 30 and 20 Torr and *dotted line* is nonbicarbonate blood buffer capacity (taken to be $d[HCO_3^-]/dpH = 25$ mM). *Point* labeled 0 depicts resting condition for a representative lizard where $Pa_{CO_2} = 30$ Torr, $pH_a = 7.45$, and $[HCO_3^-]_a = 21$ mM. With infusion of lactic acid into blood and without ventilatory compensation for acid load, acid-base status of blood changes in accordance with the P_{CO_2} isobar at 30 Torr to point 1. Restoration of acid-base homeostasis entails hyperventilation, dropping Pa_{CO_2}, and proceeding from point 1 to point 2 along nonbicarbonate buffer line.

ing 5 mM $\Delta[L]_a$ in *Varanus* (Fig. 3*A*) and nearly 10 mM $\Delta[L]_a$ in *Iguana* (Fig. 4*A*). The role of a relative hyperventilation in the regulatory mechanism is illustrated by the fact that the ratio of the factorial increase in $\dot{V}eff$ to the factorial increase in $\dot{V}CO_2$ increased between 0 and 10 mM $[L]_a$ in both species (Figs. 3*B* and 4*B*).

The data of Moberly (17) in which he related the level of arterial pH with $[L]_a$ in *I. iguana* subjected to diving provides an interesting contrast to this study. During dives, the animals are essentially closed systems and are unable to exchange CO_2 at the lungs. As lactic acid diffuses into the blood during the dive, it is not effectively buffered via the bicarbonate buffer system and Pa_{CO_2} and $[H^+]_a$ rise precipitously, even at relatively low levels of $\Delta[L]_a$. In contrast, the bicarbonate buffer system is more effective during terrestrial activity, and acid-base regulation in *Iguana* is more precise.

Theoretical ventilatory response. The theoretical ventilatory adjustment (expressed as a VR, $\dot{V}eff/(\dot{V}eff)_0$) required to maintain complete homeostasis of $[H^+]_a$ with both carbonic and noncarbonic acid loads can be calculated from the metabolic rate ratio (MRR, $\dot{V}CO_2/(\dot{V}CO_2)_0$), the change in arterial lactate concentration ($\Delta[L]_a$) and the resting level of plasma bicarbonate ($[HCO_3^-]_0$)

$$VR = \frac{MRR}{1 - (\Delta[L]_a/[HCO_3^-]_0)} \qquad (3)$$

The derivation of this equation is included in APPENDIX C. The relationship between this theoretical ventilatory ratio and $\Delta[L]_a$ at four levels of the metabolic rate ratio and with $[HCO_3^-]_0 = 25$ mM is shown in Fig. 6. When $\Delta[L]_a$ increases at a constant level of the metabolic rate ratio, the ventilatory ratio hyperbolically increases with an asymptote at $\Delta[L]_a = [HCO_3^-]_0 = 25$ mM. The slope of the relationship between the theoretical ventilatory ratio and $\Delta[L]_a$ at a constant metabolic rate ratio progressively increases with MRR such that, when MRR = 1.0, very little change in $\dot{V}eff$ is required to maintain $[H^+]_a$ until $\Delta[L]_a$ exceeds 20 mM; but when MRR = 15, the ventilatory ratio must increase rapidly between 0 and 5 mM $\Delta[L]_a$.

The theoretical relationship expressed by *Eq. 3* can in part explain the relative abilities of *Varanus* and *Iguana* to maintain constancy of $[H^+]_a$ with increases in $[L]_a$. Both species are able to clear the maximal levels of CO_2 produced during activity. *Varanus,* however, has a greater aerobic scope than *Iguana,* and the level of the metabolic rate ratio is nearly 10 before lactate levels rise in the blood. In *Iguana,* $[L]_a$ is generally increased at metabolic rate ratios of five or below (Fig. 2*E*). Since *Varanus* attains a greater MRR before $[L]_a$ rises, ventilation must increase approximately two times as rapidly as in *Iguana* to maintain precise regulation of $[H^+]_a$ once $[L]_a$ begins to rise. This required increase in ventilation is in addition to the higher ventilatory rate already required to compensate for the greater carbonic acid load in *Varanus.* Thus, maintenance of acid-base homeostasis in the face of similar noncarbonic acid loads is considerably more difficult in *Varanus* than in *Iguana.*

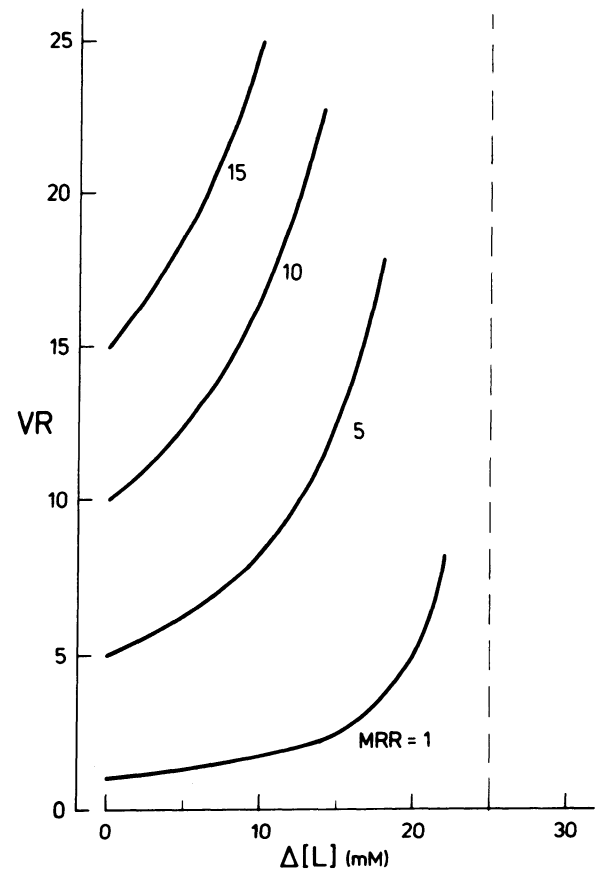

FIG. 6. Theoretical ventilatory ratio (VR; calculated from *Eq. 3*) which is required to maintain a constant level of $[H^+]_a$ when $[L]_a$ is increased at various levels of metabolic rate ratio (MRR). Resting level of $[HCO_3^-]_a$ is indicated by *dotted vertical line.*

Mechanisms of Ventilatory Control

Firm conclusions about the mechanisms controlling ventilation during activity in these two lizard species cannot be made from this study. However, it is important to note that $\dot{V}$eff increased by as much as 12-fold in *Varanus* and 9-fold in *Iguana* without detectable rise in either Pa_{CO_2} or $[H^+]_a$ and without decrease in Pa_{O_2} (unpublished observation). Thus, chemoreceptors that respond to the steady-state level of systemic arterial blood gas tensions or pH cannot account for the exercise hyperpnea. This response is similar to that in mammals (cf. 1, 9, 28) and birds (8, 14) insofar as there is no detectable stimulus at the sites of known chemoreceptors. Other, as yet undetermined, factors must control ventilation during activity in terrestrial vertebrates.

Physiological Significance

Both *Varanus* and *Iguana* regulated arterial hydrogen ion concentration rather effectively during activity while at a constant body temperature. This response contrasts with the response to changes in metabolism induced by temperature variations. *Iguana* establishes a unique $[H^+]_a$ at each body temperature in a manner that is quantitatively similar to most other poikilothermic vertebrates (10, 26). Although there has been some question as to whether such an effect occurs in *Varanus* (5, 29), recent investigations suggest that it does in fact occur, but to a lesser extent than in *Iguana* (31; N. Heisler, personal communication). These observations suggest that both lizard species adjust ventilation to establish the appropriate levels of Pa_{CO_2} and $[H^+]_a$ at the prevailing body temperature and then defend the level of $[H^+]_a$ against acid-base perturbations resulting from activity. The integrative mechanisms underlying these ventilatory responses must be rather complex inasmuch as the response elicited is dependent on the physiological state of the animal.

The central function of acid-base regulation is the preservation of an appropriate chemical environment surrounding proteins in both the intracellular and extracellular spaces. Because the level of arterial $[H^+]$ was the sole indicator of acid-base status in this study, it would be hazardous to presume that intracellular acid-base balance was maintained as effectively at moderate exercise intensities. Nevertheless, maintenance of $[H^+]_a$ should attenuate changes in the intracellular $[H^+]$ to some extent and assure a relatively constant environment in the metabolizing tissues. Maintenance of $[H^+]_a$ may also be important in preventing acid-base perturbations from spreading to tissues not directly involved in the muscular exercise, such as the central nervous system.

In conclusion, these data indicate that during activity, when most lizard species maintain elevated and constant body temperatures, acid-base regulation is rather precise, protecting the chemical environment of extracellular and intracellular proteins. Thus, although changes in body temperature are undoubtedly an important determinant of acid-base balance and ventilatory control in poikilothermic vertebrates, it apppars that the concepts of respiratory control in homeotherms, emphasizing maintenance of a constant arterial pH, also apply to at least some reptiles.

APPENDIX

A. Effects of Increased $\dot{V}_{CO_2}$ Without Ventilatory Adjustment

If the rate of metabolic carbon dioxide production had increased without altering the rate of effective ventilation (i.e., VR = 1.0), arterial P_{CO_2} would have increased in direct proportion to the MRR. From *Eq. 1*

$$\frac{\dot{V}\text{eff}}{(\dot{V}\text{eff})_0} = \frac{\dot{V}_{CO_2} \cdot (Pa_{CO_2})_0}{(\dot{V}_{CO_2})_0 \cdot Pa_{CO_2}} \tag{4}$$

where the subscript 0 refers to the resting state. When VR = 1.0

$$Pa_{CO_2} = \text{MRR} \cdot (Pa_{CO_2})_0 \tag{5}$$

As the level of arterial P_{CO_2} increases with MRR, both $[H^+]_a$ and $[HCO_3^-]_a$ will increase according to the in vivo blood buffer capacity. Values of the in vitro blood buffer capacity are available for both *Varanus* ($d[HCO_3^-]/dpH$ = 23.5 mM; Ref. 30) and *Iguana* ($d[HCO_3^-]/dpH$ = 25 mM; Ref. 33). Using the equation of Visser and Maas (27), these values for $d[HCO_3^-]/dpH$ can be converted to a similar expression relating the d log (Pa_{CO_2}) with dpH

$$\frac{d \log P_{CO_2}}{dpH} = -\left(1 - \frac{dpK'}{dpH}\right) + \frac{1}{2.3 \cdot [HCO_3^-]_p} \cdot \left(\frac{d[HCO_3^-]}{dpH}\right) \tag{6}$$

where dpK'/dpH is taken for mammalian blood at pH = 7.4, Pa_{CO_2} = 40 Torr, temperature = 37°C, and protein concentration = 70 $g \cdot l^{-1}$ as 0.04 (24). Thus, for *Varanus* d log (P_{CO_2})/dpH = −1.43, and for *Iguana*, the value is −1.50 for whole blood in vitro. These values are expected to be higher than comparable values in vivo, however, they should provide a reasonable minimum estimate of the change in pH (24). Along with *Eq. 5*, the change in pH and, thus, the change in $[H^+]_a$ can be calculated using these values.

B. Effects of Increased $[L]_a$ Without Ventilatory Adjustment

By conservation of mass, $[HCO_3^-] = K' \cdot \alpha \cdot P_{CO_2}/[H^+]$. Thus

$$\Delta[HCO_3^-] = [HCO_3^-] - [HCO_3^-]_0 = \frac{K' \cdot \alpha \cdot P_{CO_2}}{[H^+]} - \frac{K' \cdot \alpha \cdot (P_{CO_2})_0}{[H^+]_0} \tag{7}$$

and since $([H^+]_a)_0 = [H^+]_a$ with complete compensation

$$\Delta[HCO_3^-] = \frac{K' \cdot \alpha}{[H^+]_0}[P_{CO_2} - (P_{CO_2})_0] \tag{8}$$

Substituting *Eq. 2* into *8* this becomes

$$P_{CO_2} = (P_{CO_2})_0 - \frac{\Delta[L][H^+]_0}{K' \cdot \alpha} \tag{9}$$

Thus, the change in log (Pa_{CO_2}) necessary to maintain homeostasis of $[H^+]_a$ can be calculated and then converted to a change in pH using the values of d log (P_{CO_2})/dpH determined in APPENDIX A. This change in pH is that which would have occurred without hyperventilation and can be converted to a change in $[H^+]$.

C. Ventilatory Adjustment Required for Complete $[H^+]_a$ Homeostasis

Homeostasis of $[H^+]_a$ is defined by

$$\Delta[H^+] = [H^+] - [H^+]_0 = \frac{[HCO_3^-]}{K' \cdot \alpha \cdot P_{CO_2}} - \frac{[HCO_3^-]_0}{K' \cdot \alpha \cdot (P_{CO_2})_0} = 0 \tag{10}$$

or, rearranging

$$[HCO_3^-] = [HCO_3]_0 \cdot \frac{P_{CO_2}}{(P_{CO_2})_0} \tag{11}$$

Substituting *Eq. 11* into *2* one finds that

$$\Delta[L] = [HCO_3^-]_0 \cdot \left[1 - \frac{P_{CO_2}}{(P_{CO_2})_0}\right] \quad (12)$$

Since *Eq. 4* also holds in this condition

$$\frac{P_{CO_2}}{(P_{CO_2})_0} = \frac{\dot{V}_{CO_2} \cdot (\dot{V}eff)_0}{(\dot{V}_{CO_2})_0 \cdot \dot{V}eff} = 1 - \frac{\Delta[L]}{[HCO_3^-]_0} \quad (13)$$

This can be written as *Eq. 3*

$$VR = \frac{MRR}{1 - (\Delta[L]/[HCO_3^-]_0)}$$

Equation 3 expresses the factorial increase in $\dot{V}eff$ required to compensate for an increase in $\dot{V}_{CO_2}$ (of magnitude MRR) and an increase in $[L]_a$, thus maintaining $[H^+]_a$ precisely at the resting level. The critical assumption in the derivation of this equation is that $[L]_a$ is a suitable indicator of surplus H^+ ions infusing into the extracellular fluid (see above).

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