Contribution of Gular Pumping to Lung Ventilation in Monitor Lizards

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A controversial hypothesis has proposed that lizards are subject to a speed-dependent axial constraint that prevents effective lung ventilation during moderate- and high-speed locomotion. This hypothesis has been challenged by results demonstrating that monitor lizards (genus *Varanus*) experience no axial constraint. Evidence presented here shows that, during locomotion, varanids use a positive pressure gular pump to assist lung ventilation. Disabling the gular pump reveals that the axial constraint is present in varanids but it is masked by gular pumping under normal conditions. These findings support the prediction that the axial constraint may be found in other tetrapods that breathe by costal aspiration and locomote with a lateral undulatory gait.

When lizards walk and run, they generally use a lateral undulatory gait in which their bodies flex from side to side with each stride. It has been proposed that these lateral flexions prevent effective lung ventilation during moderate- and high-speed locomotion (*1*). When lizards breathe at rest, intercostal muscles are active bilaterally to rotate the ribs, thereby expanding the thorax and aspirating air into the lungs (*2*). During locomotion, however, the intercostal muscles cannot produce adequate lung ventilation because they are active unilaterally to flex the trunk (*2, 3*). These results support the hypothesis that lizards are subject to a speed-dependent axial constraint on lung ventilation. According to this hypothesis, just when a lizard needs more oxygen, because it is running faster, it is actually able to breathe less effectively (*1*).

The axial constraint hypothesis has remained controversial for over a decade. Metabolic studies of green iguanas (*Iguana iguana*) and monitor lizards (genus *Varanus*) have found that oxygen consumption increases and blood oxygen concentrations remain elevated as speed increases during locomotion (*4, 5*). These observations suggest that ventilation in lizards does not decrease as speed increases, although ventilation volume was not measured directly in these studies.

Predictions about the relation between ventilation volume ($\dot{V}_E$, milliliters per minute per kilogram) and speed of locomotion can be made from the axial constraint hypothesis (*6*). If the constraint is not present, $\dot{V}_E$ should increase with speed until the maximum rate of oxygen consumption ($\dot{V}_{O_2max}$) is reached and then remain constant at this maximum value ($\dot{V}_{Emax}$) as speed increases (Fig. 1, blue line). Above the speed at which $\dot{V}_{O_2max}$ is reached, lizards rely on their well-developed capacity for anaerobic metabolism to supply the additional energy required for locomotion (*5*). During the recovery period immediately after exercise, $\dot{V}_E$ does not exceed the value for $\dot{V}_{Emax}$ recorded during exercise, and, in most situations, $\dot{V}_E$ is expected to decrease rapidly after cessation of exercise (*5*).

If a speed-dependent axial constraint is present (Fig. 1, red line), $\dot{V}_E$ increases above resting values at low speeds because the intercostal muscles are not constrained when lizards are walking slowly (*1, 2*). However, at moderate and high speeds, the mechanical constraint on the axial musculature begins to exert its influence, and $\dot{V}_E$ decreases with increasing speed. Maximum ventilation is not reached during exercise, but rather $\dot{V}_{Emax}$ is reached immediately after exercise when the mechanical constraint due to locomotion is lifted, but the animal's oxygen requirements remain high to pay back the oxygen debt incurred during anaerobic activity (Fig. 1, red line).

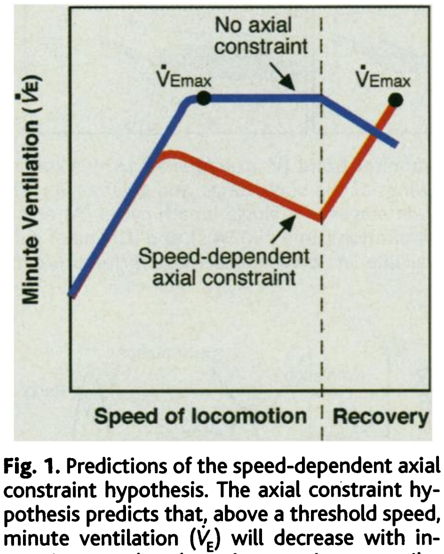

Fig. 1. Predictions of the speed-dependent axial constraint hypothesis. The axial constraint hypothesis predicts that, above a threshold speed, minute ventilation ($\dot{V}_E$) will decrease with increasing speed and maximum minute ventilation ($\dot{V}_{Emax}$) will be reached during the recovery period immediately after exercise.

Recent experiments performed to test these predictions yielded conflicting results for two different species of lizards (*6*). Results from green iguanas were consistent with the predictions of the axial constraint hypothesis: $\dot{V}_E$ decreased with increasing speed, and $\dot{V}_{Emax}$ was reached during the recovery period (Fig. 1, red line). Results from savannah monitor lizards (*Varanus exanthematicus*), however, agreed with the predictions of no axial constraint (Fig. 1, blue line). Ventilation increased with increasing speed up to 2 km hour^{-1} and leveled off between 2 and 3 km hour^{-1}, and $\dot{V}_{Emax}$ was reached during locomotion rather than during recovery. These findings cast doubt on the generality of the axial constraint hypothesis; green iguanas appear to be subject to the constraint, but varanids do not, even though both lizards use lateral undulatory gaits (*6*).

To determine whether differences in breathing mechanics might explain the conflicting results obtained from varanids and iguanas, we used videoradiography to observe lung ventilation in savannah monitors (*V. exanthematicus*) and green iguanas (*I. iguana*) during locomotion on a treadmill at speeds between 1 and 3 km hour^{-1} (*7*). In savannah monitors, a breathing cycle begins with exhalation (Fig. 2A), followed immediately by a costal inspiratory phase that does not completely inflate the lungs (Fig. 2B). During costal inspiration, the hyobranchial apparatus is depressed to expand the gular cavity (Fig. 2B) and then elevated to com-

Table 1. Locomotor endurance times (*20*) of six monitor lizards (*V. exanthematicus*) with functional (control) and impaired (experiment) gular pumps (values ± SEM). Endurance times of gular-impaired lizards were statistically significantly shorter at 2 and 3 km hour^{-1} than the endurance times of the same animals under control conditions (paired t test; $P < 0.01$).

Treadmill speed (km hour^{-1})	Endurance (seconds before exhaustion)	
	Control	Experiment
1*	900	900
2	346 ± 55	225 ± 25
3	140 ± 14	89 ± 5

*At 1 km hour^{-1}, exhaustion was not reached in either treatment during the 15-min exercise period.

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press the gular cavity and pump additional air to the lungs (Fig. 2C). We observed that savannah monitors perform between one and eight accessory gular pumps per breath, with a mean of three pumps per breath when locomoting at 2 km hour^{-1} (*8*). Simultaneous pressure recordings from the gular cavity and lungs (*9*) demonstrate that air is pumped from the gular cavity into the lungs under positive pressure, rather than being aspirated into the lungs from the gular cavity by subatmospheric pressure (Fig. 3). While locomoting on a treadmill and videoradiographed under the same conditions as *V. exanthematicus* (*7*), green iguanas were never observed to use gular pumping for lung ventilation.

To evaluate the contribution of gular pumping to minute ventilation and gas exchange in varanids, we impaired the gular pump by propping the lizards' mouths open with a plastic retainer (*10*). A sealed mask was then placed over the mouth and nares, and minute ventilation ($\dot{V}_E$) and the rate of oxygen consumption ($\dot{V}_{O_2}$) were compared in the same individuals under the intact (control) and impaired (experimental) conditions (Fig. 4). In the experimental condition, $\dot{V}_E$ increased above the rest value during slow locomotion (1 km hour^{-1}) but then decreased significantly ($P < 0.05$) between 1 and 2 km hour^{-1}. Relative to the control condition, impairment of the gular pump resulted in a significant decrease in $\dot{V}_E$ of 23% at 1 km hour^{-1} and of 54% at 2 km hour^{-1}. This decrease, in turn, limited aerobic capacity under experimental conditions, as reflected by a reduction of $\dot{V}_{O_2}$ by 18% at 1 km hour^{-1} and 22% at 2 km hour^{-1}, relative to control conditions. The experimentally induced decrease in $\dot{V}_{O_2}$ was less dramatic than the decrease in $\dot{V}_E$, indicating that oxygen extraction efficiency must have increased. In the control conditions, $\dot{V}_{O_2}$ increased between 1 and 2 km hour^{-1} ($P < 0.05$), indicating that $\dot{V}_{O_2\mathrm{max}}$ was reached at a speed greater than 1 km hour^{-1}. Under experimental conditions, $\dot{V}_{O_2}$ was not significantly different at 1 and 2 km hour^{-1} ($P > 0.05$), indicating that $\dot{V}_{O_2\mathrm{max}}$ was reached at or below 1 km hour^{-1}. Under control conditions, $\dot{V}_E$ decreased rapidly after cessation of exercise at 2 km hour^{-1} (Fig. 4). In contrast, under experimental conditions, $\dot{V}_E$ increased during recovery, and $\dot{V}_{E\mathrm{max}}$ was reached during the recovery period. In addition, the length of time that the lizards could locomote before becoming exhausted also decreased when the gular pump was impaired (Table 1).

Comparison of our $\dot{V}_E$ measurements (Fig. 4) with predictions of the axial constraint hypothesis (Fig. 1) indicates that varanid lizards are subject to the speed-dependent axial constraint but have circumvented this constraint by using an accessory gular pump to augment costal ventilation. When the gular pump is impaired, varanids exhibit a speed-dependent decrease in lung ventilation, and the highest rates of ventilation occur after cessation of exercise (Fig. 4). These results are consistent with the predictions of the axial constraint hypothesis and are consistent with results obtained for green iguanas (*6*). In contrast, intact varanids show increasing ventilation with speeds up to the maximum aerobic speed, indicating that their ventilation is not limited by the speed-dependent axial constraint.

These findings may also provide a solution to the apparent conflict between the axial constraint hypothesis and results from metabolic studies of locomotion. The metabolic studies (*4, 5*) were performed on varanids locomoting at speeds up to 2 km hour^{-1} and on green iguanas at speeds up to 1.5 km hour^{-1} (*11*). We found that in varanids, the effects of the constraint are masked by gular pumping at speeds up to 3 km hour^{-1}, and others have found that in green iguanas, the constraint only begins to exert its influence at speeds between 1 and 2 km hour^{-1} (*6*). Therefore, in the conditions under which the metabolic studies were conducted, we would not expect the speed-dependent axial constraint to have

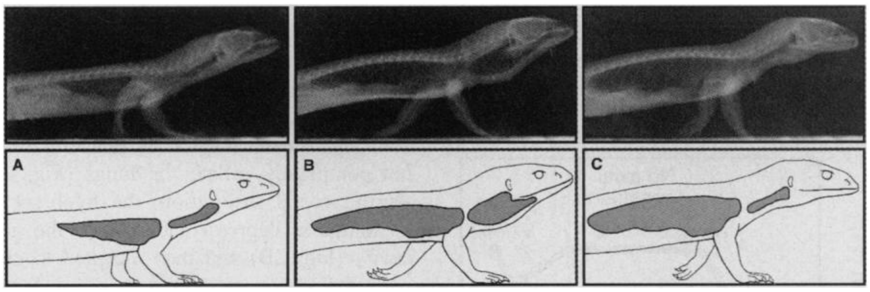

Fig. 2. X-ray negative video images of a savannah monitor lizard (*V. exanthematicus*) walking on a treadmill at 1 km hour^{-1}, with corresponding drawings of the body, lungs, and gular cavity. The lizard (body mass = 200 g) is shown at three different stages of a single breath cycle: (**A**) end of exhalation (time = 0 s), (**B**) end of costal and gular inspiration (time = 0.94 s), and (**C**) end of gular pump (time = 1.08 s). The full video clip is available at www.sciencemag.org/feature/data/982779.shl

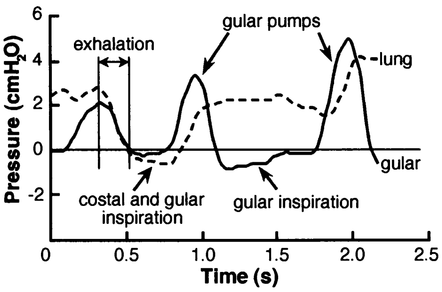

Fig. 3. Pressure measured simultaneously in one lung and in the gular cavity of *V. exanthematicus* (body mass = 100 g) locomoting on a treadmill at 1 km hour^{-1}. This representative pressure trace shows one exhalation, one costal inhalation, and two accessory gular pumps. Solid trace, gular pressure; dashed trace, lung pressure.

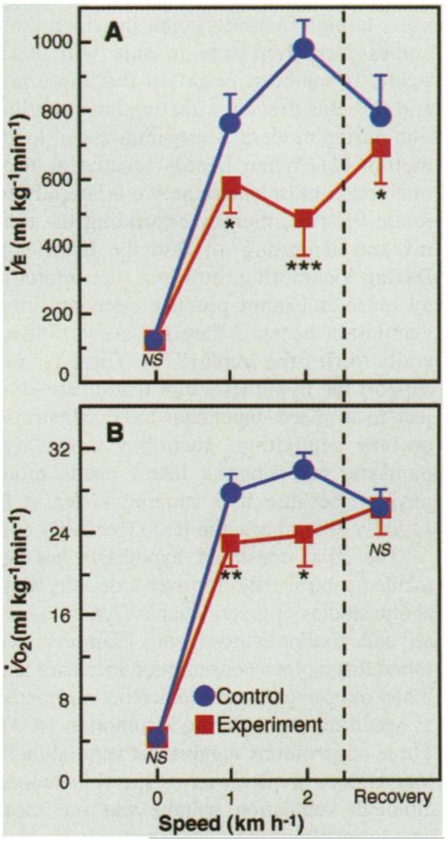

Fig. 4. (**A**) Minute ventilation ($\dot{V}_E$) and (**B**) rate of oxygen consumption ($\dot{V}_{O_2}$) in savannah monitor lizards with functional (●, control) and nonfunctional (■, experiment) gular pumps. Compare the measured changes in $\dot{V}_E$ with the predicted patterns in Fig. 1. Data for recovery were recorded during the first minute after cessation of locomotion at 2 km hour^{-1}. Values presented are means ± SEM for six individuals (n = 6). Statistical significance of differences between control and experimental treatments was assessed for each speed by a t test for paired comparisons (one tail). *NS*, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

had a measurable effect. However, in non–gular-pumping lizards locomoting at moderate to high speeds, the constraint does have a measurable effect on lung ventilation and oxygen consumption (*6*) (Fig. 4).

Varanids and iguanas are distantly related members of the clade Squamata [lizards and snakes (*12*)]. The finding that these distantly related lizards are both subject to the speed-dependent axial constraint suggests that this constraint was probably present in the common ancestor of all squamates. Furthermore, on the basis of the underlying biomechanics, it is possible that early amniotes, which are thought to have locomoted with a lateral undulatory gait and relied on costal aspiration for lung ventilation, were subject to the constraint (*1*). The evolution of erect gaits, which reduced the role of lateral flexion in locomotion, may have allowed some lineages, such as mammals, birds, and possibly bipedal dinosaurs, to circumvent the constraint and evolve greater locomotor endurance (*1*). Similarly, varanids exhibit greater aerobic capacity than other lizards (*5, 13*), and it is likely that gular pumping, coupled with other adaptations such as a functionally divided heart and a large surface area for gas exchange in the lungs (*14*), contributes to the high locomotor stamina of varanids observed in the wild (*15*).

Gular pumping or gular flutter has previously been shown to play a role in defense and threat behavior, olfaction, and thermoregulation in many groups of lizards (*16*). Preliminary videoradiographs of six other species indicate that gular pumping for lung ventilation is present in some species that naturally exhibit high amounts of locomotor activity and is absent in other species that do not (*17*). A more complete comparative analysis is required to determine whether gular pumping evolved within Squamata or whether it might be primitive for Squamata and even perhaps for Amniota. It has previously been assumed that early amniotes completely abandoned the buccal pump in favor of costal aspiration breathing (*18*). However, the gular pump of lizards is similar to the buccal pump that is used for lung ventilation in air-breathing fishes and amphibians (*19*), and our discovery of gular pumping in lizards suggests that buccal pumping and aspiration breathing may have coexisted throughout most of amniote history.

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7. We recorded x-ray videos of three juvenile *I. iguana* (70 to 100 g) and six *V. exanthematicus* (two adults, 1500 and 2000 g, and four juveniles, 120 to 200 g) while they were locomoting at speeds of 1 to 3 km hour^{-1} on a motorized treadmill at 32° to 35°C. Videos were recorded in lateral projection with a Siemens cineradiographic apparatus and a Sony DCR VX1000 Digital Camcorder at 30 frames per second and 1/250 s shutter speed. Each individual ran at the maximum speed that it would maintain steadily on the treadmill for between 1 and 3 min. Videos were reviewed frame by frame to look for evidence of gular pumping. Gular-pumping motions and associated lung inflations were obvious during locomotion and in a few breaths immediately after locomotion in all six *V. exanthematicus* (Fig. 2); no gular pumping was seen in the three *I. iguana*.
8. The number of gular pumps per breath was measured from recordings of air flow through the nares and mouth (*10*). Gular pumps appear on flow traces as a series of small inspirations between much larger expirations. When locomoting at 1 km hour^{-1}, savannah monitors used a mean of 2.82 ± 0.85 gular pumps per breath, and at 2 km hour^{-1}, they used a mean of 3.04 ± 0.65 pumps per breath (means ± SEM, $n = 6$; these means are not significantly different). Flow traces of breathing in *I. iguana* during locomotion show no evidence of gular pumping (C. Farmer, unpublished data).
9. Polyethylene cannulas (PE 160; 1.6-mm outer diameter O.D.) were surgically implanted into the gular cavity and one lung of four juvenile *V. exanthematicus* (100 to 200 g) and connected to Omega PX-138 pressure transducers sutured to the skin above the pectoral and pelvic girdles. Pressures were recorded during locomotion at treadmill speeds between 1 and 3 km hour^{-1} and synchronized with videoradiographs of the animals to correlate pressures with the gular and lung volume changes visible in the x-ray images (Fig. 2).
10. Six savannah monitor lizards (body mass, 305 to 480 g) were trained to locomote on a motorized treadmill in an environmental chamber at 36°C. During gular compression in varanids, the mouth is closed and the internal nares are sealed by ridges of tissue (sublingual plicae) that lie lateral to the tongue (T. Owerkowicz, unpublished data). We disrupted the oral seal by propping the mouth open with a retainer made from polyethylene tubing. A lightweight plastic mask was then epoxy glued around the snout to enclose the mouth and nostrils. For the control condition, the same individuals were tested with just the plastic mask and no retainer. A bias flow of humidified air (1200 ml min^{-1}) was drawn through the mask, and a pneumotachograph (H. Rudolph, 8421 series 0-5 LPM) connected to a differential pressure transducer (Validyne MP 45-1-871) measured air flow. Oxygen and CO_2 analyzers (Applied Electrochemistry, S-3A and CD-3A) monitored the gas composition. Instruments were calibrated by injection of known volumes of mixed gases before data collection each day. Expired gas volume ($\dot{V}_E$) and rate of oxygen consumption ($\dot{V}_{O_2}$) were measured during the last minute of locomotion and first minute of recovery at each speed. Four out of six of the animals tested would not locomote steadily in control and experimental conditions above 2 km hour^{-1}; two locomoted steadily up to 3 km hour^{-1}.
11. The maximum running speeds measured for these lizards on a trackway in the lab are 15 km hour^{-1} for *I. iguana* and 6.6 km hour^{-1} for *V. exanthematicus* (*1*). Thus, the maximum treadmill speeds used in the metabolic studies represent only about 10% of the maximum speed of *I. iguana* and 30% of the maximum speed of *V. exanthematicus* (*6*).
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17. Single individuals from each of six additional species were videoradiographed to look for gular pumping during and immediately after locomotion. Gular pumping was observed in *Uromastyx maliensis*, *Eublepharis macularius*, *Tupinambis teguixin*, and *Heloderma suspectum*. No gular pumping was observed in *Chamaeleo jacksonii* or *Tiliqua scincoides*.
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20. To obtain an estimate of locomotor endurance, we timed six savannah monitor lizards (0.640 to 0.900 kg) (up to 15 min) while they were locomoting to exhaustion on a motorized treadmill once per day, over the course of a week. Exhaustion was assumed after an animal failed to respond to repetitive prodding and slid to the end of the treadmill (length = 2.5 m) [after H. B. John-Alder and A. F. Bennett, *Am. J. Physiol.* **241**, R342 (1981)]. The procedure was repeated for each individual with the animal's gular region wrapped with 5-cm-wide Elastoplast tape to prevent gular pumping. The tape did not interfere directly with air flow in any way; the mouth was free to open, the cartilaginous rings of the trachea prevented tracheal compression, and the glottis in varanids is located in the front of the oral cavity below the internal nares, well cranial to the position of the tape. The animals used for these endurance tests were larger than those used for ventilation and oxygen consumption measurements (*10*); therefore, it is not unexpected that they were able to sustain locomotion at higher speeds than would be predicted from Fig. 4.
21. All animal experiments were conducted in accordance with institutional guidelines. We thank W. Bennett, D. Carrier, A. Crompton, T. Hiebert, N. Kley, T. Landberg, J. O'Reilly, R. Simons, and A. Summers for valuable comments on the manuscript. We are very grateful to L. Meszoly for the line drawings used in Fig. 2. This study was supported by NSF grants IBN-9875245 and IBN-9419892 and a University of Massachusetts Executive Area for Research grant to E.L.B., NSF grant IBN-9630807 to J.W.H., NIH grant 1F32-HL09796-01 to C.G.F., and a traveling fellowship (Company of Biologists Limited) and a Chapman Fellowship to T.O.

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